I declare that this thesis was composed entirely by me, and that with the exceptions detailed below, the work for this thesis was performed by me.

Approximately half the observations of haemoglobin concentration, haematocrit, mean corpuscular haemoglobin concentration and body weight were made by Drs. R.M. Bannerman and R. G. Cooper, who kindly permitted me to analyse their data in conjunction with my own. In teaching me the technique of measuring free erythrocyte protoporphyrin concentration, Dr. Martha Kreimer-Birnbaum performed two of the estimates used in this thesis.

A collaborative study of haemopoietic stem cell behavior in X-linked anaemia was carried out with Drs. M. Bennett, G. Cudkowicz and R.M. Bannerman; only those parts of the study undertaken by me are included in this thesis.

Due acknowledgement of technical help is made in the introduction to the thesis.

ProQuest LLC.
789 East Eisenhower Parkway
P.O. Box 1346
Ann Arbor, MI 48106 – 1346

X-Linked Anaemia of Mice: An Hereditary Disorder of Iron Absorption

Summary of Thesis Submitted for the Degree of Doctor of Medicine in the University of Glasgow.

Peter H. Pinkerton

The X-linked anaemia of mice (gene symbol, sla) is hypochromic and microcytic in character. Typically, the mean corpuscular haemoglobin concentration, the mean cell volume and the mean cell diameter are reduced. Anaemia is moderately severe in young hemizygous male and homozygous female mice, and regresses spontaneously with age. Heterozygous female animals show little or no haematological abnormality, and duality of the red cell population is not seen.

The iron stores of anaemic mice, as determined both chemically and histochemically, are depleted at all ages. The clearance of iron from the plasma is rapid and the appearance of radio-iron in the red cells, after parenteral injection, is both greater and more rapid than normal. The serum iron concentration is low and the total serum iron binding capacity is elevated, as is the free erythrocyte protoporphyrin concentration. The anaemia responds well to parenteral iron dextran. Together, these findings are characteristic of iron deficiency.

In contrast to the usual situation in iron deficiency in man and other mammals, intestinal iron absorption is not increased in X-linked anaemia of mice; indeed a significantly low level of absorption of inorganic iron at three dose levels (0.1, 1.0 and 10 ug) has been found. Histochemical studies reveal abnormal accumulation of stainable iron in the mucosal epithelium of the small bowel of anaemic and heterozygous mice.

These findings imply that the deficiency of iron is a consequence of malabsorption of iron by the small bowel, probably as a result of a primary, genetically controlled, fault in the metabolic processes of iron transfer from the mucosal cell to the plasma. The histochemical findings in the mucosal epithelium of heterozygous carriers are consistent with,

although they do not constitute proof of, the provisions of the Lyon hypothesis of X-chromosome inactivation.

X-linked mouse anaemia does not, at present, seem to be a model for any known form of heritable human anaemia, but is potentially a useful tool in the investigation of the still mysterious processes whereby iron is transferred from the mucosal epithelium to the plasma. A hypothetical model explaining known features of iron absorption, and incorporating possible sites of action of the sla gene, is presented and discussed. It is postulated that the sla gene controls the production either of an enzyme ("iron transferase") necessary for the transfer of iron to the plasma from the mucosal epithelial cell of the small bowel, or of a carrier substance ("cytoferrin") to which iron must be bound for this transfer to be achieved.

X-LINKED ANAEMIA OF MICE:

AN HEREDITARY DISORDER OF

IRON ABSORPTION

THESIS SUBMITTED FOR THE

DEGREE OF DOCTOR OF MEDICINE

IN THE UNIVERSITY OF GLASGOW

PETER HARVEY PINKERTON

MARCH 1968

CONTENTS

VOLUME 1

VOLUME 2

PREFACE

A variety of inherited anaemias of mice has been described in the last fifty years and some already from valuable experimental tools for research in haematology, immunology and developmental biology. Others are less well known and have been as yet little studied.

The existence of such a series of mutants exhibiting anaemia through direct or indirect interference with the complicated processes of red cell formation at different points provides an opportunity to identify and analyse single steps. Each abnormal mutant gene must, perforce, have a normal allele as its counterpart, subserving a single normal function, and comparison of the expression of the mutant and normal genes may permit definition of the normal gene action. In such a way has the study of the hereditary coagulation disorders in man led to better understanding of the normal coagulation mechanism.

In some of the inherited mouse anaemias, the haematological changes represent but one of a group of features, or pleiotropic effects, of the action of a single gene, and detailed analysis of these different features may offer insight into the control of developmental processes. Some mouse anaemias closely mimic human disorders and are exploited as models of human disease.

It is the purpose of this thesis to describe in detail the haematological and histopathological findings in the sex-linked or, preferably, X-linked anaemia of mice, to present data indicating that the fundamental disturbance concerns the intestinal absorption of iron, and to assess the value of X-linked mouse anaemia as a model for human disease.

The thesis begins with a description of the normal haematological picture in the laboratory mouse and a review of the heritable mouse anaemias; previous published work on X-linked anaemia (gene symbol, sla) is considered in a separate chapter. The investigations forming the body of the thesis are then reported, followed by discussion of the mechanism of X-linked anaemia and its relationship to the process of iron absorption, and comment on its resemblance to superficially similar human anaemias. In presenting this thesis, the relevant literature up to the end of 1967 has been considered, except for a few references to work, of which I had prior knowledge, published in 1968. The detailed results of the various investigations are contained in the appendices which together with the tables constitute volume 2.

Some of the work reported here has been presented at Annual Meetings of the Federation of American Societies for Experimental Biology (Pinkerton, Kreimer-Birnbaum and Bannerman, 1966) and the American Society of Haematology

(Pinkerton and Bannerman, 1966). A review of the heritable mouse anaemias (Pinkerton and Bannerman, 1968), and detailed reports of the haematological findings (Bannerman and Pinkerton, 1967) and pathological appearances (Pinkerton, 1968) have been published, together with a preliminary report of some investigations of iron metabolism in X-linked mouse anaemia (Pinkerton and Bannerman, 1967).

The work was carried out between September 1965 and August 1967 in the Department of Medicine of the State University of New York at Buffalo, in association with Dr. R. M. Bannerman, Associate Professor of Medicine and Head of Medical Genetics. I wish to record my debt of gratitude to Dr. Bannerman for creating the opportunity for me to carry out this work, for his stimulating criticism and advice and for his kind hospitality during my stay in Buffalo. I am further indebted to him and also to Dr. R. G. Cooper, for encouraging me to incorporate into this thesis some of the preliminary data on the haematological picture accumulated by them prior to my arrival in Buffalo. I am grateful to Drs. Michael Bennett, and Gustavo Cudkowicz of Roswell Park Memorial Institute, Buffalo, and to Dr. Bannerman for permitting me to include the results of experiments performed by me, as part of a collaborative study; and to Dr. Martha Kreimer-Birnbaum, Research Associate in the Department of Medicine, for her interest, and for assistance with estimations of the free erythrocyte protoporphyrin concentration.

With the exceptions stated above the practical work described here was carried out by me, or by technicians in the Laboratories of the Division of Medical Genetics, working under my direct tuition and supervision.

Miss Margaret Porter rendered much painstaking technical assistance and her concientious care of the colony of mice was invaluable. Mrs. Betty Murphy and Mrs. Margarete Peutsch also contributed valuable technical help. The colour prints illustrating Chapter 6 were made by Kodak, Ltd., Rochester, N. Y. Miss Susan Kozmycz kindly prepared the diagrams which have been used to illustrate the thesis. In most, British spelling is used, but in some the American form has been employed. This is because some of the diagrams were prepared with a view to publication in British journals and some in American journals; I apologize for this inconsistency.

While in Buffalo, I held a Buswell Research Fellowship from the State University of New York at Buffalo with an appointment as Visiting Assistant Research Professor in the Department of Medicine. The research work was supported by grants from the Buswell Foundation, the United Health Foundation of Western New York and the National Institutes of Health and I am indebted to these organizations.

Finally, I would like to record my deep gratitude to my wife for her patience in typing and the final script of this thesis, and for her encouragement and forbearance during its preparation.

CHAPTER 1

The Normal Haematological Findings in the House Mouse

A considerable volume of information has accumulated over many years concerning the haematological picture in the normal mouse. This chapter reviews the previous data, presenting the information from the recent literature in greater detail, and omitting much from earlier published work, which has been reviewed by Petri (1933), Fekete (1941) and Gardner (1947). An extensive and recent review by Russell and Bernstein (1966) is found in the second edition of "The Biology of the Laboratory Mouse", and the haematology of the normal mouse has also been described by Pinkerton and Bannerman (1968) in considering the heritable mouse anaemias.

a) The red-cell indices and appearances.

The haemoglobin concentration, packed cell volume and mean corpuscular haemoglobin concentration (M.C.H.C.) in adult mice generally resemble the values found for these indices in the human (Table 1.1). The haemoglobin concentration is maximal at the time of sexual maturity (Grüneberg, 1952) and during adult life a slight and very gradual decline is found (Francis and Strong, 1938; Strong

and Francis, 1940; Grüneberg, 1952; Ewing and Tauber, 1964).

The haemoglobin concentration and haematocrit may also be influenced significantly by genetic background, and this has been demonstrated (Russell, Neufeld and Higgins, 1951) in a study of 18 different inbred strains (Table 1.1); these differences between strains are constant over long periods of time and many generations within the same inbred strain (Russell and Bernstein, 1966).

In contrast to the similarity between human and mouse haemoglobin concentrations and packed cell volumes, the red cell count in mice is very much higher than in the human, and hence the mean cell volume (M.C.V.) and mean cell haemoglobin (M.C.H.) are very much lower. This is illustrated in Table 1.2 where the strain differences described for the haemoglobin concentration and packed cell volume are also evident (Russell et al, 1951). A gradual fall in the red cell count with advancing age also occurs (Ewing & Tauber, 1964).

The reticulocyte count usually lies between 1 and 6 per cent (Scarborough, 1931; Russell et al, 1951; Albritton, 1952; Russell and Bernstein, 1966) although a wider range of up to 17.3 per cent has been quoted (Petri, 1933).

The erythrocyte is a non-nucleated biconcave disc

and numerous estimates of the mean cell diameter have been reported between 5.5 and 6 μ (Goodall, 1910; Scarborough, 1931; Petri, 1933; Fekete, 1941; Albritton, 1952; Russell and Bernstein, 1966). Polychromasia is often a feature of Romanowsky-stained blood films (Simonds, 1925; Petri, 1933; Fekete, 1941); normoblasts are rare in the peripheral blood (Scarborough, 1931; Andrew, 1965) but Howell-Jolly bodies may be found in normal mice (Pinkerton and Bannerman, 1968).

Studies of red cell osmotic fragility are few. The first estimate was made by Kato (1941) who found lysis to commence at 0.535 per cent NaCl and to be complete at 0.33 per cent NaCl. More recent estimates of the median corpuscular fragility (M.C.F.) gave values of 0.40 ± 0.02 per cent NaCl for C_3H mice and 0.45 ± 0.02 per cent NaCl in AKR mice (Wiadrowski and Metcalf, 1963).

Antigenic differences in the red cells of various strains of house mouse and various individuals of a wild population have been demonstrated using heteroimmune rabbit antisera (Gorer, 1936a; Singer, Foster, Petras, Towling and Sloane, 1964) and human sera (Gorer, 1936b). However, I have been unable to find a report of naturally-occurring isoagglutinins in the house mouse, and McDowell and Hubbard (1922) failed to demonstrate their presence in several strains.

b) The leucocytes.

The total leucocyte count shows wide variation between and within different strains, (Table 1.3), and at different times in the same animal (Parsons, 1936). It is at least partly under genetic control (Tchakotine, 1938; Gowen and Calhoun, 1943; Russell et al, 1951; Weir and Schlager 1962, a and b) and is influenced by the sex of the animal, since granulocyte counts are significantly higher in males (Russell et al, 1951). Striking diurnal variation is seen in the total leucocytes, lymphocytes and eosinophils with peak values in the morning (Panzenhagen and Speirs, 1953; Halberg, Visscher and Bittner, 1953; Brown and Dougherty, 1956). Tail vein blood contains many more leucocytes than blood taken by cardiac puncture (Fekete, 1941). Thus, many and varied are the factors which affect the leucocyte count.

In general, lymphocytes, predominantly small, outnumber the granulocytes (Petri, 1933; Fekete, 1941; Gowen and Calhoun, 1943; Andrew, 1965; Penny, 1967). The neutrophil granulocytes show wide variation in nuclear form, from the relatively immature ring, or "doughnut", to the "broken ring" and ultimately to the segmenting or multilobed form (Simonds, 1925; Gardner, 1947) and, unlike the human, granules are scanty and difficult to stain (Goodall, 1910; Bunting, 1922; Endicott and Gump, 1947).

The granules of eosinophils on the other hand are prominent and easily stained; basophils are extremely scanty, (Russell and Bernstein, 1966; Penny, 1967). The monocyte is a large cell with a convoluted nucleus, closely resembling its human counterpart. Representative values for total and differential white cell counts are given in Table 1.3.

c) Platelets and coagulation.

The range of platelet counts reported for the house mouse is large as may be seen from Table 1.4, and mostly, they lie between 150,000 and 600,000/cumm. The survival of platelets in the peripheral circulation is 4-5 days (Odell and McDonald, 1961). Age and sex apparently do not affect the platelet count.

Meier and his associates (Meier, Hoag and Allen, 1961; Meier, Allen and Hoag, 1961; Meier, Allen and Hoag, 1962; Allen, Meier and Hoag, 1962) during an investigation of an acquired, ethylene-oxide-induced, haemorrhagic diathesis in their colony of mice, carried out extensive tests of the coagulation process in mice of various strains. They describe the following normal values:- clotting time 2-10 mins.; partial thromboplastin time 55-100 secs.; prothrombin time 7-19 secs.; prothrombin consumption - less than 10 per cent remains after 1/2 hour; Russell Viper Venom time 8-14 secs.; thromboplastin

generation test - subsampling tube at 6 minutes clots in 11-18 secs. No sex or strain differences were noted. The various coagulation factors, which behave in vitro in a similar fashion to those in man, also showed a distribution pattern in the plasma proteins similar to that seen in man.

d) Mouse haemoglobin.

The overall architecture of haemoglobin molecules in various vertebrates is remarkably alike (Ingram, 1963; Lehmann and Huntsman, 1966) and it appears that the predominant adult haemoglobin of mammals, including the mouse, is a tetramer made up of two pairs of chains resembling the α and β chains of human haemoglobin A.

Using paper electrophoresis, Ranney and Gluecksohn-Waelsch (1955) noted two different haemoglobin patterns in different mouse strains, which they called "single" and "diffuse" on account of the appearances of the haemoglobin band on the electrophoretic strip; these characteristic patterns were found to be inherited as simple Mendelian autosomal characteristics. (Gluecksohn-Waelsch, Ranney and Sisken, 1957). The use of more refined techniques soon revealed that the diffuse pattern consisted of several discrete bands (Popp and St. Amand, 1958; Rosa, Schapira, Dreyfus, de Grouchy, Mathé and Bernard, 1958; Gluecksohn-Waelsch, 1960). One of these

bands was shown by ultracentrifugation studies to result from polymerization (Gluecksohn-Waelsch, 1960) and this was subsequently confirmed by the use of the depolymerizing agent, iodacetamide (Riggs, 1965). The polymer increases in quantity with storage (Rosa et al., 1958; Ranney, Marlow-Smith and Gluecksohn-Waelsch, 1960). It was suggested that gene reduplication might account for the other bands, a situation similar to that seen in man in respect of haemoglobins A and A_2 (Gluecksohn-Waelsch, 1960).

The symbols $\underline{Hb}^1$ and $\underline{Hb}^2$ were allotted to the allelic pair of genes giving rise to "single" and "diffuse" patterns (Ranney, Marlow-Smith and Gluecksohn-Waelsch, 1960). Close linkage of the $\underline{Hb}$ locus to albinism in linkage group I has been demonstrated (Popp and St. Amand, 1958, 1960; Hutton, Bishop, Schweet and Russell, 1962a). In wild mouse populations, both single and diffuse forms are seen, with a predominance of the diffuse type suggesting a selective advantage for this genotype (Heinecke and Wagner, 1964). In this connection it has also been noted that more inbred strains show the diffuse than the single type (Russell and Gerald, 1958).

Although the "single" haemoglobin patterns from various strains of mice are indistinguishable electrophoretically, these haemoglobins show variability in their solubility in phosphate buffer, and at least four

degrees of such solubility have been detected among inbred mouse strains (Popp, 1963). Inheritance of this characteristic also follows Mendelian principles (Popp and St. Amand, 1960; Popp, 1962a) and it segregates independently of Hb: it has been identified by the symbol Sol. While the linkage group for this locus has not yet been established, it has been shown not to be sex-linked (Popp, 1962b) and not to be in linkage groups III, IV, XI, XIII or XVI (Hutton, Schweet, Wolfe and Russell, 1964).

The independent assortment of the genes controlling the electrophoretic and solubility characteristics of mouse haemoglobin suggested that the structure of this molecule is controlled by two entirely separate loci (Popp, 1965). Using a "finger-print" technique, Hutton, Bishop, Schweet and Russell (1962b) compared the separated polypeptide chains of the haemoglobin of C57Bl/6J mice with the α and β chains of human haemoglobin A, and found sufficient points of similarity to allow them to identify the analogous α and β chains of the mouse haemoglobin molecule. Alleles at the locus Sol were found to control α-chain structure (Hutton et al, 1964; Rifkin, Rifkin and Konigsberg, 1965) and those at the Hb locus to control β-chain structure (Hutton et al, 1962a), confirming the earlier suggestions of Popp (1962c; 1962d; Popp, Popp and Webb, 1963).

In all inbred strains of mice so far examined with the Hb^1 ("single") constitution, only one type of non-α-chain is found (β-chain) and although these β-chains have similar electrophoretic properties, differences in tryptic peptide pattern can be shown between various strains (Hutton et al., 1962a). In mice with Hb^2 ("diffuse") constitution a major and a minor fraction can be distinguished, besides the polymer described above, and these fractions have been shown to differ in their non-α-chains. It has been suggested (Russell, personal communication) that the non-α-chain in greater concentration should be designated "β" and that in lower concentration, "δ". Thus, adult mice with the "single" pattern have a single haemoglobin $_{2}{}_{2}$, and those with the "diffuse" pattern, two types of haemoglobin, $\alpha_2\beta_2$ and $\alpha_2\delta_2$. The allele controlling β and δ chain structure segregates as a unit (Hutton et al., 1962b) indicating close linkage between the respective structural genes, as is seen with the β and δ chain genes in man (Weatherall, 1965). It has been shown for the AKR and FL strains of mice that the α-chains of both the major and minor components, although different between strains, are the same within each strain (Hutton et al., 1962b), a situation analogous to that for human haemoglobins A and A_2, where the α-chains are identical (Ingram and Stretton, 1961).

With the emergence of much new information on the

structure of mouse haemoglobin, it is desirable to revise the nomenclature of the loci controlling haemoglobin structure and devise a notation for the various types of mouse haemoglobin. No definite system of nomenclature has as yet been arrived at, but the suggestions of Russell (1966) are outlined in the reviews of mouse anaemias published by Russell and Bernstein (1966) and Pinkerton and Bannerman (1968). A similar system has been proposed by Popp (1965). Details of these notations are beyond the scope of this thesis. A statement of the features of the haemoglobins in 48 inbred strains of mice is given by Russell and Bernstein (1966).

Foetal Haemoglobins in the Mouse

A foetal haemoglobin component in the CBA mice was first detected by electrophoresis (Barrowman and Roberts, 1961). It was not apparent after the 16th day of gestation and could not be distinguished from adult mouse haemoglobin by the alkali denaturation technique. Further studies with the same strain revealed two foetal haemoglobins, one disappearing on the 15th gestational day and the other on the 16th day (Barrowman and Craig, 1961), and the presence of multiple foetal haemoglobins in mice has subsequently been confirmed (Craig and Russell, 1963). A relationship to the presence

of primitive nucleated erythrocytes of yolk sac origin was suggested, and Craig and Russell (1964) showed in C57Bl/6J mice, that the 3 foetal haemoglobin components disappeared pari passu with the nucleated erythrocytes. Further studies of foetal blood cells, separated by differential centrifugation, have provided direct demonstration that the primitive nucleated yolk sac erythroid cells synthesise foetal haemoglobins while the liver erythroid cells synthesise adult haemoglobins only. Thus at the 12th-13th day of gestation peripheral blood contains a mixture of yolk sac cells synthesising foetal haemoglobins and liver erythroid cells synthesising adult haemoglobin (Kovach, Russell and Marks, 1966).

Structural studies on foetal mouse haemoglobin have recently been reported; three haemoglobins, made up of $\varkappa$ and φ, α and γ and $\varkappa$ and $\varkappa$ chains, respectively, are produced by the yolk sac cells; it should be noted that none of the mouse foetal haemoglobins contain β - chains (Fantoni, de la Chappelle, Rifkind and Marks, 1967).

e) Serum iron and Transferrin.

Estimates of serum or plasma iron concentration in the mouse are few. During a study of "meat anaemia" in mice, apparently a result of copper deficiency, Ilan, Guggenheim and Ickawitz (1963) found a mean serum iron

concentration for 7 normal mice of 296 µg/100 ml with a standard error of 22 µg/100 ml. The mean total iron binding capacity (T.I.B.C.) was 427 µg/100 ml. Baker and Wilson (1965) found similar values for the serum iron - 308 µg/100 ml with a range of 253 - 360 µg/100 ml. Studying the Swiss Webster strain of mice, Brodsky, Dennis, Kahn and Brady (1966) obtained a mean plasma iron level of 247.4 µg/100 ml. with a standard deviation of 107.2 µg/100 ml. in 55 mice.

Thus, the serum iron in mice appears to be higher than in the human and subject to considerable variability even within an inbred line. The degree of saturation of the T.I.B.C. also appears rather higher than in the human.

The iron binding beta-globulin, transferrin, is present in the mouse in two forms, inherited as co-dominant alleles, thus producing 3 phenotypes (Cohen, 1960; Shreffler, 1960). The alleles for the two types of transferrin are designated by the symbols $\underline{Trf}^{a}$ and $\underline{Trf}^{b}$ and the three phenotypes by the notation Trf-a, Trf-ab and Trf-b (Cohen and Shreffler, 1961). The locus controlling transferrin in the mouse has been shown to be in linkage group II (Shreffler, 1963).

Mouse transferrin has slower electrophoretic mobility in starch gel than its human counterpart; the type a has the faster mobility and is found in the CBA

strain. Other lines so far examined, representing the majority of the well-known mouse strains, showed the slower, or b, transferrin type. Minor components associated with these alleles have also been demonstrated (Cohen, 1960; Shreffler, 1960).

Plasma iron clearance rates were estimated by Brodsky et al (1966) in 166 animals. The mean half-time for disappearance of intravenously administered tracer-doses of ^{59}iron was 61.7 mins. with a standard deviation of 17.7 mins.

f) Iron absorption and utilization for erythropoiesis in the normal mouse:

(i) Iron absorption.

Using 10 week old mice of the CF1 strain, Krantz, Goldwasser and Jacobson (1959) measured the absorption of iron in normal animals as part of a study of the relationship of humoral stimulation of erythropoiesis and iron absorption. Four groups of normal "control" animals were given a low iron diet followed by 14 µg of iron in the form of ^{59}ferric chloride and retention of iron studied at 4-7 days after administration. The results are summarized below:

No. of mice	Per cent absorption	Days after dose
10	7.4 ± 1.4	4
9	9.7 ± 5.2	7
5	8.4 ± 3.5	4
8	7.7 ± 2.2	4

Mendel (1961) using mice of the same strain and similar age, gave 7.3 µg of elemental iron in the form of ^{59}ferrous sulphate. He obtained the following estimates of iron absorption (means ± 1 standard error of the mean) at 3 days after dosing in 4 groups of 9, 10, 10 and 8 normal mice respectively - 28.8% ± 5.03%, 15.3% ± 2.4%, 24.8% ± 2.22% and 15.5% ± 3.98%.

Gitlin and Cruchaud (1962) investigated the effect of variation in the dose of iron on absorption in the mouse. ^{59}Iron-tagged ferrous sulphate was given orally over a wide range of doses and the proportion of iron retained determined by counting the radioactivity remaining in the whole mouse at daily intervals. With doses of 0.6 µg of iron, approximately 18 percent of the dose was retained by normal animals 5 days after administration. About 11 per cent of 5 µg and 10 per cent of 10 µg were retained after 5 days. Little difference in percentage retention (or absorption) is seen between the 3rd and 8th days. Doses of 1000-3000 µg of iron gave iron absorption values of between 2-3 percent. Beutler, Kelly and Beutler (1962) also measured iron absorption over a

wide dose range using ^{59}ferrous sulphate in 0.01 N hydrochloric acid. The doses employed ranged from 0.08 µg to 15 mg. The mean absorption of iron at the lowest dose was 14.18%; at 0.5 µg, 9.64%; at 5 µg, 10.47% and at 50 µg, 7.41%. A group of 14 mice were also given 10 µg doses, and the range of variation in absorption was from 4.5-37 per cent.

Harriss (1962) studied iron absorption in normal mice as part of an investigation of iron metabolism in experimental pyridoxine deficiency. ^{59}Ferric chloride (10 µg, 1 µc) was given orally to fasting animals, and the absorption of the dose 3 days after administration was estimated. Absorption was very variable from mouse to mouse and lay between 2 and 17 per cent of the administered dose with a mean value of about 8 per cent. These figures can only be approximate, since they are based on diagrams published by Harriss (1962), rather than on precise data.

(ii) Utilization of iron for haemopoiesis.

Estimates of the utilization of parenterally administered doses of iron for erythropoiesis in the mouse have been reported by several authors. Mendel (1961) assumed a blood volume of 0.6 ml per 10G body weight, and obtained a mean value of 38.8 per cent with a standard error of 2.5 per cent for 8 mice. Harriss (1962),

assuming a blood volume of 0.7 ml per 10G body weight found a wide range of variation in normal mice at 3 days after administration of tracer doses of ^{59}iron. The mean value was about 50 per cent with a range of 17-80 per cent. Again these results are taken from diagrams and are regarded as approximations. Konitzer and Michalke (1965) found that the reappearance of tracer doses of ^{59}iron in the peripheral blood exceeded 50 per cent within 24 hours of intraperitoneal injection into normal white mice.

More recently, Brodsky et al. (1966) have estimated the reappearance of tracer doses of radioiron in the peripheral blood on the first and second day after administration. At 24 hours 62.4 per cent of the iron had been used for haemopoiesis (standard deviation 18.1 per cent, results obtained in 90 mice); at 48 hours 82 per cent had been so utilized (±17 per cent, 57 mice).

g) The body iron content of the mouse.

Little information is available on the total body iron content of the mouse. The first estimates were made by Spray and Widdowson (1950) who studied young albino mice. Thirty-one animals aged 15 days (both sexes) with a mean weight of 9.3G had a mean iron content of 4.63 mg/100G mouse, and 39 mice aged 30 days (both sexes) with a mean weight of 21G had a mean iron content of

4.89 mg/100G mouse. The dietary iron content is not stated. Chappelle, Gabrio, Stevens and Finch (1955) found a mean total body iron of 9.4 mg/100G for a group of nine white Swiss mice. More recently, Eickholt and White (1964) obtained results in the range 4.7-6.3 mg/100G of mouse for six B4BC mice, weighing 26-27G. The results of Spray and Widdowson (1950) and of Eickholt and White (1964) are thus in good agreement, but those of Chappelle et al (1955) indicate a total body iron content of approximately twice that found by the other authors. It may be that dietary iron content and, perhaps age and strain differences play a part in determining the total amount of iron in the mouse carcass. Since details of the dietary content of iron and other factors are not available for the studies of Spray and Widdowson (1950) and of Eickholt and White (1964), no assessment of the influence of diet on the total body iron content can be made.

h) The blood volume of the mouse.

The earliest estimates of the blood volume of the mouse were based on determination of the total body haemoglobin and its concentration in the peripheral blood (Dreyer and Ray, 1910). It was suggested that the blood volume could be calculated from the formula -

blood volume in ml = $W^{2/3}/K$ where W is the body weight in grams and K is a constant (6.7 for the mouse). Subsequently, by a similar technique, Oakley and Warrack (1940) evolved the formula - Blood volume in ml = $0.09 \times W^{0.88}$. Also, employing this method Grüneberg (1941) obtained values of 50.9 - 65.3 ml/Kg body weight.

Taylor (1945) using an exsanguination method found the blood volume for C57 mice to be 4.9 ± 0.17 ml/100G, and for DBA mice to be 5.23 ± 0.31 ml/100G. Furth and Sobel (1946) using a similar technique obtained a mean value of 5.2 ml/100G with a range of 3.5 - 6.6 ml/100G, for first generation AK x Rf hybrids; higher values in the range 9 - 12.7 ml/100G (mean 10.9 ml/100G) were found when a dye dilution method (T1824 - Evans Blue) was used. Further estimates using dilution techniques with T1824, ^{131}iodine and ^{32}phosphorus gave results in the range of 7.8 - 8.6 ml/100G, or 6.44 - 8.41 per cent of body weight (Wish, Furth and Storey, 1950).

With radio-iodinated protein, a range of 4.6 - 8.3 ml/100G was obtained for the plasma volume of AKm mice by Kaliss and Pressman (1950); the mean was 6.7 ml/100G with a standard error of 0.4 ml/100G. The total blood volume was estimated to be 12.1 ± 0.8 ml/100G. Keighley, Russell and Lowy (1962) found a blood volume of 5.4 - 5.7 ml/100G for first generation WBB6 hybrids

using ^{59}iron-labelled red cells.

The highest values seem to be obtained with the dye and radio-iodinated protein dilution techniques; this is presumably due, at least in part, to over-estimation consequent upon loss of tracer material into extravascular compartments. Exsanguination, on the other hand, is liable to give an underestimate since complete removal of the blood and blood pigments is difficult. However, the results with the exsanguination techniques agree fairly well with those obtained with radioisotope-labelled erythrocytes, and the blood volume of the mouse seems to lie in the region of 5 - 7 ml/100G.

i) Red cell survival.

Red cell survival has been determined by various radioisotopic methods and the estimates obtained by various authors are listed in Table 1.5. The absolute survival ranges from 40-55 days with a pattern of random cell destruction (Burwell, Brickley and Finch, 1953). Estimates of the radiochromium half-time show a wide variation, between 8.6 and 20.2 days.

j) The normal haematology of embryonic and young mice.

The earliest red cell form is large and nucleated with a diameter of about 12 μ (Grüneberg, 1952). The proportion of these cells in the foetal blood falls

steadily from 94 - 100 per cent on the 12th gestational day, to 50 per cent on the 13th day and 7 per cent on the 15th day; none are apparent on the 16th day (Craig and Russell, 1964; Kovach, Russell and Marks, 1966). These primitive erythrocytes at 11 days have many polyribosomes and are biosynthetically highly active, producing foetal haemoglobin. By the 14th day, although they retain pyknotic nuclei, they have lost ribosomes and have ceased synthesising haemoglobin (Kovach, Russell and Marks, 1966).

The disappearance of the nucleated erythrocytes occurring about the 13th gestational day, coincides with the appearance of smaller erythrocytes with a diameter of about 8 μ. Although these cells are non-nucleated in the foetal blood, they retain ribosomes, and synthesise adult haemoglobin only (Kovach, Russell and Marks, 1966). Shortly before birth, erythrocytes characteristic of adult mice begin to be produced (Grüneberg, 1942a; 1952). Blood findings in embryonic mice are given in Table 1.6.

In the newborn mouse the haemoglobin concentration and haematocrit are somewhat lower than in the adult, but the red cell count is much reduced, and erythrocytes are substantially larger than in the adult. During the first few weeks of life, after an initial fall, the haemoglobin concentration and haematocrit increase. The red cell count increases relatively more than the other indices,

and the mean cell volume accordingly decreases. The high mean cell volume at birth is reflected in an increase in red cell diameter as compared with the adult (Guzman and Briones, 1946), and steady decrease in mean cell volume to adult values is seen during the first few weeks of life (Grüneberg, 1939, 1942a; Grewal, 1962). The reticulocyte count in neonatal and young mice is elevated when compared with the adult; siderocytes are present, forming up to 5 per cent of red cells at birth and disappearing during the ensuing week (Grüneberg, 1952). Representative data from the literature illustrating these changes is presented in Table 1.7.

k) Haemopoiesis, tissue iron stores, and the histological appearances of various organs.

Three stages of haemopoiesis can be recognized in the developing mouse embryo (Grüneberg, 1952).

(i) The primitive, yolk sac stage

(ii) Hepatic haemopoiesis

(iii) Definitive medullary and splenic haemopoiesis

The primitive yolk sac generation of red cells is large (12 μ in diameter) and nucleated, and is produced between the 8th and 11th days of gestation. As these cells disappear from the circulation, they are replaced by smaller, non-nucleated red cells (about 8 μ in diameter)

which are produced in the liver. Hepatic, or "intermediate" haemopoiesis commences about the 12th gestational day and until the 16th day, this is the principle site of haemopoiesis (Russell and Bernstein, 1966). The activity is almost exclusively erythropoietic but evidence of granulocyte production can also be found (Borghese, 1959a).

Definitive splenic and medullary haemopoiesis occur just before or at the time of birth. Blood production in the spleen is evident at about the 15th gestational day and increases greatly between the 17th day and birth; the spleen is the first major site of myelopoiesis, which becomes prominent at about 17 days of gestation (Borghese, 1959a). The spleen shares in the formation of all the cellular elements of the blood throughout life (Dunn, 1954). Haemopoietic activity in the spleen is mainly localized to the red pulp in which erythroblasts, granulocyte precursors and megakaryocytes can all be readily identified (Dunn, 1954). In the adult, the spleen varies in size depending on age and strain, but in general it weighs between 60 and 100 mg, being rather lighter in females than in males (Hummell, Richardson and Fekete, 1966). Stainable iron is normally present in the spleen of mice, its distribution in the white and red pulp varying from strain to strain (Dunn, 1954), as do the relative proportions of red and white pulp.

Medullary haemopoiesis commences on the 16th - 17th day of gestation and is at first predominantly granulopoietic. Over the next few days erythropoietic cells appear in increasing numbers (Borghese, 1952a and b, 1959a). The bone marrow remains the main post-natal site of blood formation throughout life and most of the bony cavities contain red marrow (Russell and Bernstein, 1966). The bone marrow of the femur and vertebral column normally occupies between 90 and 96 per cent of the available medullary space (Endicott and Gump, 1947) and fat spaces are very seldom seen (Dunn, 1954). Few cytological examinations of the bone marrow have been reported, perhaps at least partly on account of difficulty in identifying the various cell types. Endicott and Gump (1947) employed the peroxidase stain to overcome this difficulty; they were able to recognize the maturation phases of promyelocyte, myelocyte and metamyelocyte up to mature granulocyte formation. Erythroblasts were identified by their nuclear characteristics. The results of their differential marrow counts are presented in Table 1.8. Similar values have been obtained by Petri (1933) and Penny (1967).

The adult mouse liver is made up of typical cords of parenchymal cells, which are only very indistinctly divided in lobules, with portal tracts and central veins. Kupffer cells are present. The liver in young mice is

frequently a site of extramedullary haemopoiesis (Hummel et al., 1966) and foci of haemopoiesis may occasionally persist into adult life (Dunn, 1954). In DBA mice, minute amounts of stainable iron are sometimes present but frequently none is found (Morris, Dunn and Wagner, 1954).

The histological appearances in the digestive tract of the mouse have been extensively described by Malewitz (1965) and Hummel et al., (1966). The glandular stomach and upper small intestine only will be described. In the fundic region of the stomach the "test-tube" glands of the stomach are long, with mucus-secreting cells, chief or zymogenic cells and the acid secreting parietal cells. Towards the pylorus, the glands are shorter, and tend more to mucus secretion, with less evidence of zymogenic and acid-secreting activity.

The duodenum, in its upper part, shows the presence of Brunner's glands. The villi of the duodenum are tall and pyramidal, or leaf shaped, whereas those of the jejunum are more slender and cylindrical. Goblet cells are comparatively infrequent in the duodenum and increase in number towards the more caudal part of the small bowel; Paneth cells are found in both duodenum and jejunum but are more frequent in the latter. The lamina propria of the villi is vascular, and contains lacteals, lymphocytes and granulocytes. Stainable iron

may be found in the duodenal mucosal epithelial cells (Morris et al., 1954), but this is by no means a constant feature (Hochhaus and Quincke, 1896; Cappell, 1930).

The heart consists of the three main layers - endocardium, myocardium and epicardium - the myocardium showing the usual syncytial appearance of cardiac muscle. I can find no report of examination of the heart for stainable iron in the normal mouse.

The lungs exhibit small, fine-walled alveoli with networks of fine capillaries. The bronchioles are lined by low columnar epithelium and the bronchi by columnar ciliated epithelium. No report of stainable iron in the lungs of mice has been found.

The kidneys show the usual cortical zone of glomeruli and convoluted tubules, encased in a fine connective tissue capsule. The medulla is composed of collecting tubules mainly supported by fine connective tissue. Morris et al., (1954) were unable to find stainable iron in the kidneys of DBA mice.

1) Summary.

The haematological findings in embryonic, young and adult mice are described. The values for haemoglobin concentration and haematocrit are similar to those for humans. The red cell, like the human, is a biconcave disc; it is, however, very much smaller, having a mean

cell volume about half that of man. The white cells and platelets are briefly discussed. The process of blood coagulation in the mouse is also briefly reviewed and is seen to resemble closely that in man.

The structure of the mouse haemoglobin molecule and its genetic control is described.

Available data from the literature on the metabolism of iron in the mouse, is summarised, together with information on blood volume and red cell survival. The development of haemopoiesis is traced from early embryonic to adult life, and the histological appearances of the adult viscera are briefly described.

CHAPTER 2

The Heritable Mouse Anaemias

These may be classified in three main groups - the macrocytic anaemias, the haemolytic anaemias and the hypochromic anaemias. A fourth, minor, group of miscellaneous and, in the main, poorly understood anaemias is discussed briefly at the end of the chapter (see Table 2.1).

In the macrocytic anaemias, anaemia is only one feature in a group of syndromes wherein the underlying, genetically-controlled defect lies in the processes of cell differentiation both at an intra-uterine stage of development and subsequently, and recent work on these anaemias has yielded information on the mechanism of the erythropoietic stimulus.

The second main group of heritable mouse anaemias consists of two forms of congenital spherocytosis, one in the Deer mouse (Peromyscus maniculatus) and the other in the house mouse, and two non-spherocytic haemolytic disorders which both cause neonatal jaundice. The precise nature of the metabolic disturbance in these anaemias is not known. A fifth form of haemolytic anaemia, of an apparently heritable nature is the well-known auto-immune haemolytic anaemia of the NZB/Bl mice which bears many resemblances

to human auto-immune haemolytic anaemia and systemic lupus erythematosus.

The best known of the hypochromic anaemias is that found in "flex-tailed" mice which, typically, show a transient hypochromic anaemia in foetal and early postnatal life. The characteristic morphological feature is the presence of siderocytes, which persists throughout life in spite of the absence of anaemia. A disturbance in the enzymic control of haem synthesis is believed to be responsible. Sex-linked anaemia also falls into this category, but will be described in more detail in the following chapters. A third anaemia ("inherited microcytic anaemia") of this type has been recently described, but no detailed information is yet available about the nature of the basic defect.

The following sections review these various genetically determined anaemias:

a) The macrocytic anaemias

(i) Anaemia associated with dominant spotting (Gene symbol, W; linkage group XVII).

The W series of genes, in the homozygous state, produce a triad of pleiotropic effects - macrocytic anaemia, failure of pigmentation of the coat, and defective gonadal development (Russell, 1954).

The W allele, first described by Little, is lethal in the homozygote, and in the heterozygous state, a white spotting effect, "dominant spotting", is seen in the coat (Little, 1915, 1917). Anaemia in homozygotes was noted by Detlefsen (1923), and these animals died within 3 days of birth. However, in a few instances, they have survived into adult life, developing into black-eyed white mice, devoid of coat pigment (Russell and Fondal, 1951). Animals kept alive by intra-peritoneal transfusion of whole blood (Gowen and Gay, 1932) also develop into black eyed whites.

A number of other mutants at the W locus have been described and effects of some are contrasted in Table 2.2. The first to be described was W^v, originally called W^1 (Little and Cloudman, 1937), which was also studied by Gruneberg (1939) who used the notation W^2. Homozygous W^v/W^v animals survive into adult life and hence the symbol, W^v, was subsequently adopted, the superscript, "v" standing for "viable". A further mutation, W^J, results in anaemia in the homozygote, similar in severity to that produced by W, but the extent of white spotting in the heterozygote is greater (Russell, Lawson and Schabtach, 1957). A further mutation, W^b, in the heterozygous state produces still more extensive spotting, together with a marked decrease in pigment granules at the base of the shaft of coloured hairs. Detailed assessment of the anaemia has not been reported (Ballantyne, Beck, Strong and Quevedo, 1962).

W^s, causes anaemia, lethal before birth in the homozygous animal, and a white belly streak, with a large frontal blaze and white patches on the dorsum and shoulders when present in single dose (Strong and Hollander, 1953). Other less well described mutants have been reported but it is not clear whether they are in fact alleles of W (Keeler, 1931; Fortuyn, 1939).

The Anaemia

While the severity of the anaemia produced by the homozygous and doubly heterozygous states for the various mutant alleles at the W-locus varies, the anaemia in each case is of the same macrocytic type. The first assessment of the anaemia was carried out by deAberle (1924, 1925, 1927a, 1927b) who described the haematological findings in embryonic and newborn mice of constitution W/W. Anaemic embryos are clearly recognizable at the 16th day of gestation by their pallor and small size (deAberle, 1924, 1925). In the 16-17 day old foetus, the red cell count is reduced to less than one-fifth and the haemoglobin to less than one-third of normal; at birth these differences are even more obvious (see Table 2.3). The red cells are larger than normal and the colour index is elevated (deAberle, 1927a). The white cell count is also strikingly reduced, but the platelet count is similar in anaemic and non-anaemic animals. A high proportion of polychromatophilic and reticulated erythrocytes persists in anaemic foetal and

newborn mice, inversely related to the haemoglobin concentration (deAberle, 1927b). However, in spite of this, the absolute reticulocyte count is reduced in anaemic mice, implying an underproduction of new erythrocytes (Grüneberg, 1939). Anaemia was postulated as the cause of death and of failure of growth in these animals and the demonstration that whole blood, injected intraperitoneally, prolonged life, supports this suggestion (Gowen and Gay, 1932).

The haematological changes in $\underline{W/W}$ anaemia have been compared with those in the milder, viable form $\underline{W^V/W^V}$, and with those in the hybrid $\underline{W/W^V}$ (Grüneberg, 1939; Russell and Fondal, 1951). $\underline{W^V/W^V}$ showed a less severe anaemia than $\underline{W/W}$, with $\underline{W/W^V}$ occupying an intermediate position (see Table 2.3). These three genotypes show a similar relationship in respect of viability and growth. The macrocytic nature of the anaemia was also confirmed in these genotypes, the colour index being raised and the Price-Jones curves for anaemic animals showing a clear increase in mean cell diameter. Although the heterozygote $\underline{W/w}$ appears indistinguishable from the normal $\underline{w/w}$ haematologically, $\underline{W^V/w}$ mice show significant macrocytosis and a reduction in the red cell count (Grüneberg, 1942b; Russell, 1949). This difference has been confirmed by direct measurement of red cells in photomicrographs: a small but puzzling difference in the

opposite direction was noted for erythrocytes of W/w animals which were smaller than those of normal W/w animals (Attfield, 1951).

While most haematological investigations of the W/W series of anaemias have been carried out on embryos or young mice, it has been shown (Grüneberg, 1939; Russell and Fondal, 1951) that the macrocytosis associated with the W^V/W^V and W/W^V types of anaemias persists into adult life. Table 2.4 presents the red cell counts and mean cell volumes of 28 day old mice and Table 2.5 shows the haematological findings in young and adult mice. The results of red cell counts and packed and mean cell volume determinations confirm the presence of macrocytosis in adult W/W^V mice (Bernstein, 1962a). The low white cell count seen in anaemic newborn mice persists into adult life (Grüneberg, 1939; Fekete, Little and Cloudman, 1941).

Haemopoiesis

The primitive generation of red cells derived from the yolk sac are not affected in homozygous W/W mice, since they are morphologically normal and the anaemia does not appear until their production ceases (Grüneberg, 1952).

Attention has, therefore, been focused on the hepatic and definitive (splenic and medullary) phases of haemopoiesis. Borghese (1959a) found noticeably less erythropoietic activity in the livers of W/W embryos at the 13th and 14th

days of gestation and this is in keeping with the earlier observation that the first indication of abnormal haemopoiesis in W and W^v homozygotes is appreciable in the liver of 12 1/2-day embryos (Mirsky, 1949). Histological examination of the liver of 15-day anaemic embryos shows severely deficient haemopoiesis (Borghese, 1951) and smears contain a relative excess of hepatic parenchymal cells (Borghese, 1952c). All members of the erythropoietic series are reduced in number and the eosinophilic erythroblasts are unduly large (Borghese, 1954; Gorini, Rondanelli and Ferrata, 1957). These changes are even more conspicuous by the 17th gestational day (Borghese, 1952c). Granulopoiesis is also reduced in the livers of anaemic embryos, but megakaryocytes are apparently present in normal numbers (Borghese, 1959a).

Splenic haemopoiesis in the mouse normally commences at the 14-15th day of gestation; in W-anaemic embryos it commences at the same gestational age, but erythroblasts are fewer in number and show delay in maturation; this difference persists throughout the rest of embryonic development. Leucopoiesis is later in appearing and very much reduced in anaemics, but megakaryocytes are present in similar numbers in normal and anaemic embryos (Borghese, 1959a, 1959b). In newborn mice, erythropoietic foci are fewer and smaller than normal in the anaemic group (Gelmetti, 1952).

Bone marrow haemopoiesis, which normally commences in the mouse on the 16th-17th day of gestation, is at first primarily leucopoietic. At this time, in the anaemic embryo, while ossification has proceeded normally and the medullary cavity is well defined, only scanty leucopoietic elements can be found in a fine mesenchymal stroma. Erythropoietic cells make their appearance over the next few days, but, in anaemic animals, these remain extremely small in number (Borghese, 1952a, 1952b, 1959a). Sections of bone marrow, from W/W animals kept alive by injections of whole blood, show wide sinusoids and severe reduction in the amount of haemopoietic tissue (Russell and Fondal, 1951; Russell, Snow, Murray and Cornier, 1953). Significant delay in maturation in the erythroid series in the anaemic mice, prior to the pyknotic normoblast stage has also been demonstrated (Russell, Snow, Murray and Cornier, 1953). Some evidence of arrest in the myeloid series was also found, and the absence of alteration in the myeloid/erythroid ratio is in keeping with the suggestion that both elements are affected in the W/W anaemias.

The changes in the bone marrow are less severe in the W/W^v and W^v/W^v genotypes than in W/W mice, as in the peripheral blood, and in the adult individuals of the former two genotypes the cellularity of the marrow is more or less normal (Russell, 1963).

Erythropoietic foci are normally found in the adrenals

of about 80 per cent of normal mice at a late stage of intra-uterine life (Borghese, 1952d), but these are absent in homozygous anaemic embryos (Borghese, 1959a).

The effect of therapeutic agents:

A variety of therapeutic agents has been used without success. Iron ammonium citrate (Gowen and Gay, 1932), liver extract (Grüneberg, 1939; Bianchi, 1951), and folic acid (Bianchi, 1951) all failed to produce any significant improvement. A slight rise in red cell count and haemoglobin concentration was reported following the administration of anterior pituitary extract (Bianchi, 1951), but the number of animals treated was small, and the significance of this observation doubtful.

As mentioned previously, whole blood injections keep anaemic animals alive (Gowen and Gay, 1932), and it has been shown that this effect is a function of the injected red cells, and not the plasma (Searle, 1952).

Haemopoietic transplantation studies

Russell and her associates (Russell, Smith and Lawson, 1956; Russell, Bernstein, Lawson and Smith, 1959) showed that w/w and W/W^v haemopoietic cells, transplanted into irradiated host animals behave according to their original genotype, and have found that w/w cells can colonize non-irradiated W/W^v hosts to produce a normal blood picture (Bernstein and Russell, 1959). The ability of haemopoietic

cells from anaemic $\underline{W/W^v}$ mice to form spleen colonies in irradiated host animals is reduced two hundred-fold as compared with cells from normal $\underline{w/w}$ mice (McCulloch, Siminovitch and Till, 1964). This confirms that the defect in the $\underline{W/W}$ anaemias is inherent in the haemopoietic tissue and suggests a defect of stem-cell function. Severe depression of the development of red cell forming colonies derived from haemopoietic cells from $\underline{W/W^v}$ mice has been reported (Lewis, O'Grady, Bernstein, Russell and Trobaugh, 1965). The rate of differentiation and growth of the granulocyte series and megakaryocytes are also reduced.

Thus, the anaemia caused by mutations at the $\underline{W}$-locus appears to result at least in part from a failure of differentiation in the early stages of haemopoietic maturation, and this affects the erythroid, myeloid and platelet elements.

The effect of erythropoietin

Grüneberg (1939) exposed anaemic mice to hypoxia and obtained a rise in red cell count and haemoglobin, to about 75 per cent of the levels found in normal control animals. Rapid reversion to pre-hypoxic levels followed return to normal oxygen tension. This has been amply confirmed by other workers (Keighley, Russell and Lowy, 1962; Mirand and Gutman, 1963; Mirand and Prentice, 1964), and has led to extensive investigation of the effect of erythropoietin in the $\underline{W}$ series of anaemias.

The effect of exogenous erythropoietin from anaemic human, rabbit and mouse sources, and from hypoxic rabbits and mice has been investigated (Keighley et al, 1962; Niece, McFarland and Russell, 1963; Thompson, Russell and McFarland, 1963; Mirand and Gutman, 1963; Mirand and Prentice, 1964). Even with quite large doses, no response, or a negligible increase in erythropoietic activity in W-anaemic mice has usually been found, although normal (w/w) hypertransfused mice respond well, and untreated normal mice show a definite response to these preparations. Even plasma from hypoxic isologous w/w mice has been without apparent effect in anaemic animals. Recently, however, it has been shown conclusively that W/W^V mice are capable of responding to exogenous erythropoietin in massive doses (Keighley, Lowy, Russell and Thompson, 1966), 150 times as much erythropoietin being required by W/W^V animals as by w/w animals to produce the same response. The observation that W/W^V mice can also respond to erythropoietin from animals of the same genotype (Mirand, personal communication; Mirand and Prentice, 1964) has also been confirmed (Keighley et al, 1966), although the response is less than that obtained by erythropoietin from W/W^V mice in normal (w/w) recipients.

The defective capacity to respond to erythropoietin is an inherent characteristic of W/W^V blood forming tissues, since w/w:W/W^V chimeras (produced by transplantation of

W/W bone marrow into *W/W^V* animals), respond well to the administration of this factor (Keighley et al, 1962).

The effects of hypoxia and erythropoietic activity in *W/W^V* mice have been compared (Keighley et al, 1966) and the former is clearly more effective, in keeping with the early observation of Grüneberg (1939) who found a substantial haemopoietic response to hypoxia. On this basis, it has been suggested that hypoxia may, in some circumstances at least, have a more direct effect on erythropoiesis in addition to that mediated through erythropoietin (Keighley et al, 1966).

The effect of X-irradiation

Differences in X-ray sensitivity of mice of different genotype at the *W*-locus were reported by Bernstein (1962, 1963a), who also showed by transplantation techniques, that these differences are a property of the haemopoietic tissue itself and not of the host environment. Anaemic animals of the genotype *W/W^V* are extremely sensitive to irradiation when compared to *w/w* mice, and even a single dose of the *W* or *W^V* genes produces an increase in radiosensitivity. The increased susceptibility to X-irradiation in *W/W^V* animals is related to a slow rate of regeneration of haemopoietic tissue in the post-irradiation phase (Russell, Bernstein, McFarland and Modeen, 1963).

Haemoglobin biosynthesis

A disturbance in the process of haem synthesis has been demonstrated by Altman and co-workers (Altman, Russell, Saloman and Scott, 1953; Altman and Russell, 1964). Following administration of ^{14}C-glycine, ^{14}C-delta-aminolaevulinic acid (ALA) or ^{14}C-porphobilinogen (PBG) to groups of W/W, W/w and W^v/w, W^v/W and W^v/W^v mice, they measured the labelling of globin and the protoporphyrin of haem at intervals up to 33 days. In homozygous anaemic mice there was a marked delay in the appearance of the label from glycine, ALA or PBG in the haem, whereas the ^{14}C label of glycine appeared promptly in the globin. Furthermore, the haem specific activity was lower than normal, particularly when PBG was the source. The authors postulated the presence of an abnormality of the haem synthetic pathway somewhere between ALA, or PBG, and haem. Their results could also be interpreted as indicating accumulation of a pool of precursors (e.g. of PBG) diluting the label. From this pool, the pathway must be maintained such that haem is available to attach to the normally-labelled globin, as tends to be confirmed by the findings that the rate of ^{59}Fe incorporation into haem was within normal limits. (Altman and Russell, 1964). There is as yet no direct evidence of altered enzyme activity and ALA dehydratase activity, for instance, is probably normal (Russell and Coleman, 1963).

Sterility

A germ cell defect in W^V/W^V animals was reported by Fekete and associates (Fekete, Russell and Cloudman, 1941) who found absence of oogenesis and virtual aspermia. Similar changes are present in W^V/W and W/W mice (Russell, Coulombre and Fekete, 1952; Coulombre and Russell, 1954; Russell, 1954). Sterility is an inherent property of the gonad itself. Ovaries from isologous w/w mice, were transplanted to the ovarian capsule of W^V/W^V mice whose own ovaries had been removed; these anaemic mice were then able to support the function of the transplanted ovaries and successfully concluded pregnancies. The offspring showed the genetic characters of the donor of the ovaries (Russell and Russell, 1948). Gonads transplanted from 12-16 day embryos to the spleens of adult castrate mice develop germ cells according to their original genotype at all embryonic ages, thus suggesting the presence of defective gonadogenesis prior to the development of the first manifestation of disordered haemopoiesis (Russell, Murray, Small and Silvers, 1956). This suggestion was confirmed by Borghese who was able to distinguish between fertile and sterile gonads from 12 day embryos in culture (Borghese, 1955, 1956, 1957). Further confirmation of defective germ cell development, preceding anaemia in time, was provided by Mintz and Russell (Mintz and Russell, 1955, 1957; Mintz, 1957; Russell, 1963). They showed that in W/W, W/W^V, W^V/W^V and W^J/W^J embryos, the

number and location of primordial germ cells was abnormal. They demonstrated defective multiplication during migration of these cells from the yolk sac to the germinal ridges at the 8th day of gestation. Thus, there is clear evidence of disordered germ cell behaviour at least 4 days before any sign of disordered haemopoiesis, and it is improbable therefore that sterility is a consequence of anaemia *per se*.

Pigment Changes

The changes in coat pigment in the anaemias associated with dominant spotting have been summarized in Table 2.2. All surviving mice homozygous for one of the mutant genes for dominant spotting, or heterozygous for two, develop into black-eyed whites. Animals heterozygous for one of the *W* alleles show a variable degree of white spotting, with or without dilution.

Deficiency in numbers of melanoblasts underlies the lack of pigment, and this abnormality is evident at the 10th day of gestation (Russell, 1963), that is, prior to the development of anaemia. It is, therefore, unlikely to be secondary to anaemia, and is taken to represent a further failure of cell development.

Discussion

"The physiological basis of the *W*-series of anaemias is still obscure". This statement of Grüneberg's made in 1952 is still largely true 16 years later, despite intensive

study, and the nature of the primary gene action of the W alleles remains an enigma.

The mutants which in single dose produce minor effects, in double dose profoundly, and sometimes fatally, disturb the function of at least three tissues. It is now generally accepted that the various effects of the homozygous or doubly heterozygous state on different tissues are independent. Sterility and failure of pigmentation are unlikely to be the result of anaemia since evidence of dysfunction of germ cells and melanoblasts precedes the development of anaemia in the W/W embryo. Furthermore, transfusion does not prevent the development of sterility or the failure of pigmentation. The defects seem to be an inherent property of the individual cells, since they behave according to their original genotype when transplanted into normal congenic hosts. The tissue effects in the W series of mutants may be more widespread than has been appreciated hitherto, since defective function of antibody-forming cells has recently been observed (Cudkowicz, 1967; Shearer and Cudkowicz, 1967).

While the precise mechanism of the anaemia is not clear, disturbance of primitive cell maturation and differentiation is involved, as borne out by haemopoietic transplantation studies (McCulloch et al, 1964; Lewis et al, 1965). This is consistent with the anomalous response to erythropoietin, as it is known that stem cell behaviour

is at least partially controlled by this agent (Erslev, 1959; Gurney, Lajtha and Oliver, 1962; Lange and Pavlovic-Kentera, 1964). The relative inability of W-anaemic mice to respond to various erythropoietin preparations is a function of the haemopoietic cell itself, and leads to the speculation that a defect exists in the cell receptor mechanism for the stimulus to differentiation. Such a receptor mechanism might be similar in the primitive cells of several tissues and might require a common metabolic pathway which is defective in the W-mutants.

Further detailed study of the haemopoietic disturbance in the W anaemias may be expected to throw light upon the control of tissue differentiation in general.

(ii) Steel series of anaemias (Gene symbol, Sl; linkage group IV)

The dominant mutation "steel" (Sl) arose spontaneously in the C3H strain of mice and since its first description in 1956 (Sarvella and Russell, 1965) a considerable number of further mutations at this locus have been observed, both spontaneously occurring and following X-irradiation (Russell and Bernstein, 1966).

Heterozygotes are characterized by dilution of coat colour, moderate anaemia and reduction in gonad size. The Sl/Sl homozygotes die at, or, more usually before birth, and show severe anaemia, deficiency of germ cells and

defective pigment production (Bennett, 1956). The triad of pleiotrophic effects thus closely resembles that produced by the W-series of genes; however, the Sl and W genes assort independently (Savella and Russell, 1956) and their loci lie within different linkage groups (Green, 1966).

The anaemia

The first account of the anaemia of steel was published by Bennett (1956). It is evident in embryos at the 13 - 14th day of gestation and the red cell count falls progressively to 16 per cent of normal on the 15th gestational day. The erythrocytes are larger than normal with a mean red cell diameter of 9.84 μ as compared with 7.45 μ in normal embryos. Persistence of a high proportion of nucleated (primitive) erythrocytes during the development of anaemia suggests a failure of production of the intermediate and definitive generations of red cells; this is confirmed by histological examination of the liver and bone marrow which show very marked reduction in haemopoietic activity.

The embryonic heterozygote (Sl/+) shows moderate reduction in the red cell count (to 61-82% of normal) and the erythrocytes are slightly, but significantly larger than normal. These differences persist throughout life (Russell and Bernstein, 1966).

For haematological investigations in young and adult mice, extensive use has been made of the allelic mutant, steel-Dickie (<u>Sl</u>^{d}). Homozygous <u>Sl</u>^{d}/<u>Sl</u>^{d} mice have a survival of about 20-25 per cent at 30 days of age. Such mice develop into sterile black-eyed whites (Russell and Bernstein, 1966).

Russell and Bernstein (1966) have recently reviewed the steel anaemias and include much hitherto unpublished data. The anaemia is macrocytic at all ages from birth to adult life, the MCV in <u>Sl</u>/<u>Sl</u>^{d} animals being about 70 per cent greater than normal. The white cells are apparently unaffected. Reticulocyte counts, both totally and proportionally, are raised, in spite of normal red cell survival times and evidence of bone marrow hypoplasia. Thus, it seems that reticulocytes in anaemic mice must have a prolonged survival in the peripheral circulation, but it is not clear whether this is due to disturbed maturation or premature release, or both.

Marrow cells from anaemic (<u>Sl</u>/<u>Sl</u>^{d} and <u>Sl</u>^{d}/<u>Sl</u>^{d}) mice form spleen colonies in X-irradiated host mice with the same facility as cells from normal mice (McCulloch, Siminovitch, Till, Russell and Bernstein, 1965), implying that the defect in the steel anaemias is not inherent in the haemopoietic cells themselves, but is rather a function of their environment. This thesis is supported by the relative failure of proliferation of normal (+/+) isologous

haemopoietic tissue to cure the anaemia, and by the success of parabiosis in producing a normal haematological picture in both animals (Bernstein, 1966). Thus, the anaemia of steel appears to result, at least in part, from a defect which is extrinsic to the erythron, but which interferes with differentiation and/or proliferation, and this is in sharp contrast to the W anaemias.

The possibility of deficiency of the commonly used haematinic agents is excluded by their failure to produce remission of the anaemia. Vitamin B12, folic, folinic and ascorbic acid, pyridoxal phosphate, iron, testosterone and vitamin E have all proved ineffective (Russell and Bernstein, 1966).

The response to erythropoietin, even in large and repeated doses, is poor (Russell and Bernstein, 1966; Kales, Fried and Gurney, 1966), but some improvement can be obtained with exposure to hypoxia (Keighley, 1966; Kales et al, 1966). However haemopoietic tissue from steel anaemic mice, transplanted into W anaemic mice, responds normally to erythropoietin (Bernstein and Newberger, 1966), confirming the impression of an extra-erythroid defect in steel anaemia, which affects the ability of homozygous Sl cells to respond to erythropoietin. Blood loss may also make a contribution; Kales and associates (Kales et al, 1965) report gastro-intestinal blood loss amounting to about 3.5 per cent of the blood volume per day in a further

mutant at the steel locus (Sl^m/Sl^m), but the source of bleeding has not yet been found. Its quantitative significance in the causation of the anaemia also remains uncertain, particularly in view of the report of normal red cell survival in Sl^d/Sl^d homozygotes (Russell and Bernstein, 1966).

Sterility:

The gonads of male and female Sl/Sl 14-15 day embryos are totally lacking in germ cells (Bennett, 1956). The heterozygotes show active oogenesis and spermatogenesis although the testes and ovaries are smaller than normal. In 8-9 day Sl/Sl embryos, there is defective migration of primordial germ cells, and a progressive deficiency in numbers with age is seen. It is not clear whether this is the result of generation of cells with poor migrating ability or a failure of division or both (Bennett, 1956).

Pigmentation:

Skin grafts from 10 day and 14-15 day Sl/Sl embryos to isologous animals fail to produce pigment and Bennett (1956) has suggested that the basic disturbance is a failure of migration of primitive melanoblasts from the neural crest area. An abnormality of the biochemical pathway for pigment synthesis is unlikely as steel-anaemic adult mice (e.g. Sl^d/Sl^d) are capable of forming eye pigment. Interference with germ cell and pigment cell

function is evident 3-5 days prior to the first sign of anaemia, and hence it is unlikely that these phenomena are secondary to the haemopoietic effects of the Sl gene.

Discussion

The close similarity in the phenotypic effects of the Sl and W genes is striking. However, these genes are not allelic (Sarvella and Russell, 1956) and Russell (1963) has suggested that they produce their effects by acting on different sites in the same metabolic pathway. This metabolic pathway would probably involve some aspect of erythropoietin function as both Sl and W anaemic mice show abnormal responses to this factor. In steel anaemia, the anomalous response appears to be a function of the environment of the haemopoietic cells, whereas in W anaemia, it seems to result from an intrinsic property of the stem cell (McCulloch, et al, 1964; McCulloch, et al, 1965).

Thus, one might speculate that steel anaemia results from deficiency of some extra cellular "co-agent" for erythropoietin and that the W-anaemias are a consequence of a defective or relatively insensitive "receptor" mechanism for the haemopoietic stimulus.

Too little is known of germ cells and pigment cell production to postulate where the defect in their control may lie in W and Sl anaemia.

(iii) Hertwig's Anaemia (Gene symbol, an; linkage group, VIII)

Hertwig's anaemia was discovered in the descendants of a heavily X-irradiated male mouse. Inheritance is as an autosomal recessive (Hertwig, 1942a).

The homozygote (an/an) shows severe embryonic and neonatal anaemia usually fatal within a few days or weeks of birth. Occasional animals live for some months and survival appears to be influenced by modifying genetic factors (Russell and Bernstein, 1966). Sterility and failure of gonadal development are seen in an/an mice reaching adult life, but there is no effect on coat colour (Hertwig, 1942a). Affected mice weigh less than their littermates at all ages and are recognizable from the 14th gestational day by their pallor.

The anaemia is of variable severity with reduction in the red cell count, usually to between 30 and 50 per cent of normal. The effect on the haemoglobin concentration is less striking. Macrocytosis and anisocytosis are seen in blood smears (Hertwig, 1942b). Polychromasia is conspicuously absent and the reticulocyte count is reduced, both relatively and absolutely (Kunze, 1954), suggesting defective red cell output. The white cell count is also low (Kunze, 1954).

Erythropoiesis is quantitatively decreased in the livers of anaemic mice, and this is appreciable as early

as the 12th gestational day. Complete regression of hepatic haemopoiesis occurs at about 4 days after birth in an/an homozygotes, compared with 10 days in normal animals. The bone marrow is fatty, with decreased haemopoietic activity (Kunze, 1954). Maturation arrest of the erythroid series, with "megaloblastic" forms, is seen (Thoms, 1951). The spleen is very small in young anaemic mice, but becomes larger than normal in animals which survive into adult life (Hertwig, 1942a; Kunze, 1954).

Therapy with liver extract has proved ineffective (Kunze, 1954).

Discussion

The presence of macrocytosis and sterility in Hertwig's anaemia is reminiscent of the W and steel series of anaemias. However, it is clearly different from the other two types of macrocytic anaemia since its linkage group is different and there is no effect on coat colour.

Decreased haemopoietic activity in the bone marrow and liver, and the low reticulocyte count have led Kunze (1954) to suggest that Hertwig's anaemia should be regarded as "aplastic" or "aregenerative". Clearly, there is interference with maturation of the red cell series, and some effect is also seen on the white cells. The presence of an unduly small spleen in young animals is in keeping

with a hypoplastic type of anaemia, but the enlargement of this organ in older animals is hard to explain, and it has been suggested that it represents an attempt at compensation for the anaemia (Kunze, 1954). Alternatively, it may result from an accumulation of haemopoietic cells at an arrested stage of development, insufficiently mature for release into the peripheral circulation.

The similarity to the W-series of anaemias is borne out by the recent observations of Thompson and co-workers (Thompson, McCulloch, Siminovitch and Till, 1966a). When spleen or marrow cells fron an/an donors were transplanted into lethally irradiated hosts, the spleen colonies formed in these hosts were small in size and deficient in erythropoiesis particularly, but also in granulopoiesis and stem cell renewal. Evidence to suggest increased red cell destruction was also found. Although an is clearly different from the W and steel series of anaemias, sufficient points of similarity exist to permit the speculation that the same, or a closely related, metabolic pathway is affected in all three macrocytic anaemias. Cross-transplantation experiments with haemopoietic tissue into and from W-anaemic, steel-anaemic and an/an mice would also be of interest.

b) The Haemolytic Anaemias

(i) Intrinsic red cell defects

The four mutants to be described here produce congenital haemolytic disease and show their main effects in the neonatal period. All are the result of intrinsic red cell defects. In two the characteristic feature is spherocytosis; these syndromes resemble human hereditary spherocytosis except in their mode of inheritance which is autosomal recessive in the mouse and dominant in man. However, some human pedigrees have been reported which do not conform to this pattern, and the human disease is probably heterogeneous.

Hereditary Spherocytosis in the Deer Mouse (gene symbol, sp)

The disease was first described as a form of neonatal jaundice (Huestis and Anderson, 1954). Examination of the blood revealed the typical findings of spherocytosis and increased haemolysis as the basis for jaundice and the haematological and pathological findings have been reported in detail by Motulsky and associates (Motulsky, Huestis and Anderson, 1956; Huestis, Anderson and Motulsky, 1956; Anderson, Huestis and Motulsky, 1960). The peripheral blood findings in normal control mice of this species and mice with spherocytosis are given in Table 2.6.

Anaemia, if present, is slight; the M.C.H.C. is a little higher in the animals with spherocytosis than in the controls, and this has also been noted in man (Dacie, 1960).

The slightly raised M.C.V. in the sp/sp animals is probably a consequence of reticulocytosis. The blood film shows spherocytosis with reduced red cell diameter, and rouleaux formation is poor. Characteristically, osmotic fragility is increased. The type of haemoglobin is qualitatively similar in normal and abnormal animals. Red cell survival, as measured by a ^{51}Cr technique, is reduced; cross transfusion experiments show normal survival of normal cells in animals with spherocytosis, and reduced survival of spherocytes in normal animals, confirming the intrinsic nature of the defect.

There is gross splenic enlargement, the pulp being stuffed with erythrocytes; splenectomy is effective in reducing the reticulocyte count and lengthening the red cell survival time. The bone marrow shows gross red cell hyperplasia. Mild hepatomegaly is frequently present and the incidence of gallstones in the animals with spherocytosis is four times that in normal animals.

The neonatal jaundice is evanescent, and although jaundice is not present in the adult mouse, a well compensated haemolytic state persists. Fertility and viability appear to be normal (Anderson et al, 1960).

Inheritance is as a Mendelian autosomal recessive (Huestis and Anderson, 1954) and the heterozygous state for this gene (sp/+) is indistinguishable from the normal. The mutation arose spontaneously and its linkage group

has not been established, but linkage to albinism, pink-eye, flex-tailed and silver pelage has been excluded.

This condition very closely resembles hereditary spherocytosis in the human in almost every haematological and pathological aspect (Table 2.7).

The principal difference is in the mode of inheritance, and this has been discussed at some length by Anderson et al (1960). They have suggested that, if the underlying biochemical defect is identical in the dominant hereditary spherocytosis of man, and in the recessive form in the mouse, modifying genes, brought out by the pressures of natural selection in the mouse, might be responsible for the different mode of inheritance. For instance, it is known that elevation of the ambient temperature to 35°C leads to an increased severity of anaemia and increased mortality in sp/sp mice (Anderson and Motulsky, 1966), and this might be an example of a selective pressure, influencing the mode of inheritance of an analagous mutation in different species. However, human spherocytosis is probably a heterogeneous condition, on both genetic and biochemical grounds. Pedigrees have been reported in which both parents (and other relatives) of affected propositi were haematologically

normal (Young, 1955). Furthermore, metabolic differences between different cases of hereditary spherocytosis have been observed: for example, differences in sodium ion transport in red cells have been found between the "typical" cases with a clearly dominant pattern of inheritance, and the cases with neither parent affected (Bertles, 1957). Thus, spherocytosis probably represents the phenotypic expression of a number of different disorders of the same metabolic pathway, and comparison of Deer mouse spherocytosis with the less usual, possibly recessive, forms of the human disease might be more appropriate.

Hereditary Spherocytosis (gene symbol sph: linkage group unknown)

The other form of hereditary spherocytosis arose spontaneously in the C3H strain of the house mouse (Joe, Teasdale and Miller, 1962). Inheritance is also an autosomal recessive.

The homozygous state is lethal within 24 hours of birth. The newborn mice are pale and acquire a jaundiced tint within a few hours of birth. The haemoglobin and haematocrit values are reduced to about one-fourth of

normal, and the reticulocyte count varies between 60 and 80 per cent (mormals 10-35 per cent). The plasma bilirubin concentration is raised to between 3 and 5.9 mg/100 ml compared with the normal range of 0.5-0.9 mg/100 ml. The blood film shows marked microspherocytosis with poikilocytosis and occasional elliptocytes. Nucleated red cells are frequent in anaemic animals, which also show erythrophagocytosis and faulty rouleaux formation. The mean cell diameter is much reduced. Howell-Jolly bodies and siderocytes are numerous in both control and anaemic mice, but Heinz bodies are found in neither group.

The most striking pathological changes are seen in the liver, which is enlarged and dark, and shows active erythropoiesis, congestion, and dilation of the sinusoids; the bile canaliculi are plugged with bright yellow pigment, which is also present in the intestines. The spleen is not enlarged. The brain is pale but kernicterus is not seen.

This disorder differs from deer mouse spherocytosis in being lethal, and in not showing splenomegaly. As in deer mouse spherocytosis the heterozygote is indistinguishable from the normal and the mode of inheritance differs from the usual human form of the disease.

"Jaundiced" (gene symbol, ja, linkage group unknown)

Another mutation causing neonatal jaundice in the house mouse was described by Stevens, Mackensen and Bernstein (1959). Inheritance is as a Mendelian autosomal recessive. The homozygotes, which are anaemic at birth, rapidly become jaundiced and die, usually within 24 hours. The anaemia is severe, the red cell count being about half normal in the newborn anaemic animals. The mean cell volume is also much reduced, to 40-70 cu/μ, compared with 100-120 cu/μ in their normal sibs. The blood film shows hypochromia, anisopoikilocytosis, schistocytes, leuco-erythroblastosis and polychromatophilia. The reticulocyte count is increased, and basophilic stippling is also a feature. Target cells or spherocytes are not present and no increase in siderocytes has been seen.

Studies with ^{51}Cr-labelled red cells have demonstrated very severe reduction in survival of cells from homozygous (ja/ja) mice in normal (+/+) recipients and cross transfusion experiments with labelled cells confirm that the abnormality is intracorpuscular (Russell and Bernstein, 1966).

Heterozygotes are viable and fertile, and although originally believed to be unaffected are now known to show a mild, well compensated form of the disorder, manifest as a moderate shortening of red cell survival, which is not apparent when labelled ja/+ red cells are

transfused into splenectomized recipients (Russell and Bernstein, 1966). However, splenectomy does not cure the severe anaemia of the homozygote, or increase viability. Homozygous ja/ja mice show hyperplasia of the haemopoietic system, icterus and hepatosplenomegaly.

The nature of the underlying defect is unknown. No evidence of qualitative alteration in the haemoglobin molecule has been found, and there is nothing to suggest a materno-foetal immunological basis. Metabolic abnormalities in the red cells of ja/ja mice have not yet been demonstrated (Russell and Bernstein, 1966).

"Haemolytic anaemia" (gene symbol, ha; linkage group unknown)

Bernstein (1963b) has described a similar condition in the house mouse, called "haemolytic anaemia". Inheritance, again, is as an autosomal recessive, and the homozygotes (ha/ha) resemble those for neonatal jaundice (ja/ja) in almost every detail except viability. Genetic evidence, however, shows ha and ja to be non-allelic (Bernstein, 1963).

Affected individuals are recognizable before birth by their anaemia and become jaundiced within a few hours of birth. The majority of ha/ha homozygotes die within a week of birth. Red cell survival is severely reduced and, as in ja/ja, cross-transfusion experiments show the

defect to be intracorpuscular. There is again no positive evidence to suggest an abnormal haemoglobin or an immunological disturbance as the cause, and the red cell enzymes glucose-6-phosphate dehydrogenase and phosphokinase are not deficient (Russell and Bernstein, 1966). Heterozygous carriers (ha/+) show a mild and well compensated form of the disorder with moderate reduction in red cell survival.

The aetiological basis of these nonspherocytic neonatal haemolytic anaemias is unknown. While both show some similarities to human erythroblastosis foetalis, the absence of any positive evidence of an immunological disorder reduces the validity of this analogy. The possibility of a purely quantitative abnormality of haemoglobin synthesis has not yet been excluded, and the resemblance to the thalassaemias requires further investigation. The genetic linkage of ja and ha is unknown, and further study of this in relation to haemoglobin loci in mice might help to clarify the possibility of a thalassaemia-like syndrome.

(ii) Autoimmune Haemolytic Anaemia of NZB/BL mice

This form of heritable haemolytic anaemia arose, apparently spontaneously, in the 11th generation of an inbred line derived from a mixed strain, and is characterized by jaundice and splenomegaly. A detailed report

on the findings in this disease was published by Helyer and Howie (1963a) following the first description given by Bielschowsky, Helyer and Howie, (1959).

In the early stages of the disease, the affected animals show a hunched posture with narrowed palpebral fissures and sparse coat; peri-orbital and urinary infections are common. Jaundice is usually mild and intermittent, although in the later stages, it is often severe. While the onset is commonly between 6 and 12 months, in a small proportion it may be delayed as long as 15 months; in the end, however, all animals in the strain manifest the syndrome. Progressive anaemia develops, with a sudden onset in some mice, and more insidiously in others. The former occurs more commonly in males at about 6-9 months age, with moderate anaemia and gross reticulocytosis and splenomegaly. Gradual onset is found largely among rather older female mice (at about 12 months), in which the anaemia is usually profound, but splenomegaly is slight or absent and reticulocytosis is moderate. Blood films show anisocytosis and polychromasia, and the Coombs test always becomes positive as discussed below. Osmotic fragility is variable, some animals being normal, some showing increased resistance and others increased fragility. Consistent changes in the white cell counts, total and differential, are not found, and platelet counts are within the normal range.

Lindsey, Donaldson and Woodruff (1966) determined red cell survival using ^{51}Cr-labelled autologous erythrocytes. In a group of severely affected animals the mean $T\frac{1}{2}$ ^{51}Cr was 2.1 days, as compared with as yet unaffected animals in which the mean value was 13.3 days; in a further group, of intermediate severity, the $T\frac{1}{2}$ ^{51}Cr was 8.2 days. The reduction in red cell survival is related to the degree of positivity of the Coombs test and the magnitude of the reticulocytosis.

When isogeneic cells are used in cross transfusion experiments, cells from normal mice are rapidly destroyed by affected recipients, and cells from affected donors survive normally in unaffected mice. This finding, paralleled to some extent in auto-immune haemolytic anaemia of man (Dacie, 1962), implies either that mere coating of the red cell with autoantibody is not in itself sufficient to lead to shortened red cell survival, or that elution of the coating antibody may occur in the normal circulation; there is some evidence to support the latter suggestion (Selwyn and Hackett, 1949; Dacie, 1962). The destruction of red cells in NZB/Bl mice occurs both in the liver and the spleen (Barnes and Tuffrey, 1966).

Serological findings

Incomplete red cell agglutinating antibodies active at 37°C are demonstrable in red cell eluates and free in

the serum. The titre varies from just detectable levels to about 1/2000. The higher titres tend to be found in the mice showing sudden onset of the syndrome, and the lower titres in those in which onset is more insidious. Chronologically, the appearance of such antibodies precedes the appearance of anaemia or reticulocytosis (Bielschowsky, Helyer and Howie, 1959). The antibody is also demonstrable by enzyme and albumin techniques, and in 50 per cent a weak "complete" antibody can be detected. Activity is present over a wide thermal range, being constant between 18 and 37°C and weak at 4°C. The free and eluted antibody have the same characteristics and neither is complement dependent. Antibody appears earlier in females than in males (Helyer and Howie, 1963a). It has also been shown that the antibody coats the red cells of other strains of mice and gives a very weak positive indirect Coombs test with rat erythrocytes; negative reactions were found with human, sheep, rabbit, guinea pig and chicken red cells (Long, Holmes and Burnet, 1963). The antibody coating the red cells is a 7S gamma-globulin as demonstrated by immunoelectrophoresis (Norins and Holmes, 1964a). A further antibody has recently been detected in the NZB/Bl mice (Holborrow, Barnes and Tuffrey, 1965). It is complement-fixing and agglutinates both mouse and human red cells. It also appears before the incomplete antibody described above.

Both antibodies are transmissable to neonatal and 3-5 week old NZB/Bl mice by intraperitoneal injection of spleen cells from NZB/Bl mice with a positive Coombs test (Holmes, Gorrie and Burnet, 1961; Holborrow et al, 1965), but not by cells from lymph notes, thymus, bone marrow and buffy coat (Holmes, 1965); this is consistent with the suggestion that the spleen is the main site of antibody production in this disease (Mellors, 1965).

Positive lupus erythematosus (L.E.) tests were found in 41.2 per cent of female and 13.6 per cent of male mice; 70 per cent of the positive tests were in females and 30 per cent in males (Helyer and Howie, 1961). A 7S gamma globulin with nucleoprotein specificity (antinuclear factor, A.N.F.) is present in 30-45 per cent of NZB/Bl mice after 2 months of age. It is detectable at a younger age in females than in males, but the ultimate incidence does not differ significantly when the sexes are compared (Norins and Holmes, 1964b). The presence of A.N.F. in NZB/Bl mice is associated with severe renal disease. A.N.F. has also been found in normal C_3H and C57 mice with an incidence of less than 15 per cent: in normal HI mice, the frequency of A.N.F. increases steadily with age to 85 per cent but ill effects are not seen and the Coombs and L.E. tests remain negative.

Antibodies to renal glomeruli have also been demonstrated in NZB/Bl mice. Immune globulin extracted

from renal tissue, shows affinity for glomerular tufts but not for red cells or cell nuclei, and a circulating antibody with similar characteristics can also be detected (Mellors, 1965). Deposition of these immune globulins commences at birth and increases until it is present in almost all glomeruli at 12 months of age (Sagel, Treser, Ty, Wachstein and Lange, 1965). The deposits contain albumin, α- and β - globulin, in addition to γ-globulin as their main constituent (Nairn, McGiven, Ironside and Norins, 1966).

Pathological changes

These have been described by Helyer and Howie (1963a) and Miyasato, Manaligod and Pollak (1967). The bone marrow shows gross hyperplasia, or, occasionally, marrow failure. There is myeloid metaplasia in the lymph nodes, and frequent plasma cells are seen; a few show "neoplastic features" (Helyer and Howie, 1963a). Hemosiderosis is present in the liver, which is enlarged, and patches of focal necrosis may be found, especially in association with jaundice. Single cell necrosis and regeneration, with inflammatory changes resembling those of human lupoid hepatitis have been described (Horowitz, Dubois and Channing, 1965). Gall stones are frequent. Splenomegaly is largely due to extramedullary haemopoiesis, and is most marked in male mice and least obvious in breeder females (Helyer and Howie, 1963a). Abundant haemosiderin

is present and, in some instances, perivascular fibrosis and hyaline changes in the arteriolar walls (Horowitz et al, 1965; Miyasato et al, 1967).

The renal lesions in NZB/Bl mice and hybrid crosses with other strains have been extensively studied, on account of their close resemblance to those of human systemic lupus erythematosus (S.L.E.) (Helyer and Howie, 1963a; Miyasato et al, 1967). The changes are more obvious and appear earlier in females. After 6 months of age, thickening of the capillary basement membrane and "wire-looping" of the capillaries appears followed by increased lobulation of the tufts and fibrinoid necrosis (Aarons, 1964; Horowitz et al, 1965). Fusion of the "foot processes" is seen on electron microscopy (Horowitz et al, 1965). In an outbred stock Wigley and Couchman (1966) found lesions in the kidneys resembling those of human polyarteritis nodosa.

Effect of therapy

a) Splenectomy: Splenectomy before 3 months of age results in lower antibody titres and reticulocyte counts, and morbid anatomical changes are less severe (Helyer and Howie, 1963a). However, an increased frequency of lethal nephritis follows this procedure (Holmes and Burnet, 1963a). Late splenectomy (after 6-8 months) has little effect, except in severely affected animals, in which death rapidly ensues.

b) Steroids: Administration of A.C.T.H. is followed by gain in weight, rise in haematocrit, fall in reticulocyte count, regression of splenomegaly and haemosiderosis, and a fall in antibody titre. Remission is never complete and relapse consistently follows the cessation of treatment (Helyer and Howie, 1963a).

Cortisone acetate in doses of 2 mg/wk per mouse produces reversion of the Coombs test to negative, and, in younger animals, prevents the development of a positive Coombs test. The reticulocyte count falls but no effect is seen in the haemoglobin concentration or haematocrit, as compared with untreated controls. Relapse occurs when treatment is stopped, but the Coombs test becomes negative again following further steroid administration. An unduly high incidence of infection is found in the treated group (Giltinan, Holmes and Burnet, 1965). Beta-methasone phosphate produces improvement in anaemia, weakening or reversion to negative of the Coombs test and diminution in splenomegaly (Casey and Howie, 1965). It has been pointed out that NZB/Bl mice would form a useful model for the trial of drugs for treatment of human autoimmune disease (Casey and Howie, 1965).

c) Immunosuppressive drugs: Burnet and his colleagues (Russell, Hicks and Burnet, 1966) have reported a favorable effect of cyclophosphamide in halting or preventing the pathological changes in the kidneys of NZB/NZW F_1 hybrid mice, and in prolonging life. The lesions of animals

treated after the onset of renal disease show evidence of scarring and fibrosis, interpreted as healing. They suggest that the NZB/NZW F_1 hybrid may prove to be a useful system for testing the value of immunosuppressive drugs prior to their use in man; and that further studies with cyclophosphamide may help to elucidate the nature and mechanism of the disease process itself. Alteration in the course of the disease does not follow the use of azothioprine or actinomycin C, as reflected by the Coombs test, reticulocyte count and haemotocrit (Stickel and Woodruff, 1966).

Increased liability to neoplasia

While the presence of neoplastic changes in the lymphoid tissue of NZB/Bl mice has been regarded by some as coincidental, other evidence does not support this contention. NZB mice have an increased liability to develop lymphoma, compared with controls, when given carcinogenic agents (Bielschowsky and Bielschowsky, 1962), and a high incidence of malignant reticulosis in NZB mice has been reported. Out of 20 mice aged between 9 and 11 months, 2 developed reticulosarcoma and 2 malignant lymphoma. All showed evidence of the immunological disturbance before they developed lymphoma (Mellors, 1966).

Effect of differences in genetic background

Considerable differences in the incidence of the various autoimmune manifestations in NZB mice can be

produced by crossing NZB with other strains. Helyer and Howie (1961, 1963b) demonstrated that F_1 and F_2 hybrids, derived by crossing NZB/Bl with NZY/Bl, showed a higher incidence of renal lesions and a lower incidence of anaemia than the original NZB/Bl strain, and this effect is even more marked in crosses with NZW. Hybrids from matings of NZB with C_3H show a delayed onset of positivity of the Coombs test (Holmes and Burnet, 1964); effects of crossing with other strains are described by Burnet and Holmes (1965) and Wigley and Couchman (1966).

Differences between male and female mice may be genetic in origin, or may be humoral. There is at present no evidence to confirm or refute either possibility.

An autosomal dominant pattern of inheritance for the autoimmune disease of NZB mice has been suggested (Bielschowsky and Bielschowsky, 1964), but the inevitable development of autoimmune lesions in the F2 back-cross generations of NZB/Bl with NZY/Bl mice (Helyer and Howie, 1963a and b) implies a more complex form of inheritance. The recent discovery of virus-like particles in the organs of NZB/Bl mouse tissues, together with the pattern of inheritance, raises the possibility of vertical viral transmission of the disease from generation to generation through the sperm and ovum (Mellors and Huang, 1966).

Resemblance of the autoimmune disturbance in NZB/Bl mice, to human S.L.E. with haemolytic anaemia

The points of similarity and difference between human autoimmune disease with S.L.E. and haemolytic anaemia, and the findings in NZB mice are shown in Table 2.8. This comparison to human autoimmune disease has been considered by several authors (Holmes and Burnet, 1963b; Channing, Kasuga, Horowitz, Dubois and Demopoulos, 1965; Dubois, Horowitz, Demopoulos and Teplitz, 1966).

c) The Hypochromic Anaemias

(1) The anaemia of flex-tailed mice (gene symbol, f; linkage group XIV)

"Flex-tailed" arose as a spontaneous mutant with an autosomal recessive pattern of inheritance (Hunt and Permar, 1928). The first feature to be reported was deformity of the axial skeleton, manifested as kinks in the tail; the severity of the condition appeared to depend on modifying genetic factors, since segregation for degree of deformity was apparent (Hunt, Mixter and Permar, 1933). The presence of a white belly spot in the homozygous (f/f) mice was also noted (Clark, 1934). The first assessment of the transient anaemia in flex-tailed (Mixter and Hunt, 1933) showed significantly low haemoglobin concentrations in affected mice at birth and one week of age; these differences disappeared by 2 weeks. No haematological abnormality was found in heterozygous animals.

The pathology of the deformities in the axial skeleton was described by Kamenoff (1935), who found defective development of the annulus fibrosus of the vertebral column commencing about the 14-15th gestational day; this resulted in unilateral or bilateral fusion of the vertebrae. Where unilateral fusion had occurred, differential growth of the two sides of the vertebral column produced deformity.

Anaemia was also shown to be established by the 14th day of gestation, thus preceding the earliest evidence of disordered development of the intervertebral discs. The possibility that the skeletal abnormalities, belly spot and anaemia might be due to different genes has been extensively investigated and discussed elsewhere (Mixter and Hunt, 1933; Clark, 1934; Kamenoff, 1935; Grüneberg, 1952) and it has been concluded that this triad of features results from the action of a single gene.

In embryo there is marked reduction of the red cell count, which is about 50 per cent of normal at 14 days gestation (Kamenoff, 1935), associated with significant reduction in hepatic haemopoiesis. Earlier haematological findings (Mixter and Hunt, 1933; Kamenoff, 1935) were confirmed and extended by Grüneberg (1942a), who found a moderately severe reduction in haemoglobin at birth, with the packed cell volume and red cell count less affected.

The haemoglobin concentration was normal after 4 weeks and the red cell count by 1 week of age. The resulting reduction in the M.C.H.C. had disappeared by 14 days of age, but that in the mean corpuscular haemoglobin was still apparent at 28 days. The effect on the mean cell volume was surprisingly slight. A mild increase in reticulocytes was seen in anaemic mice up to 2 weeks after birth, and osmotic resistance was described as "at least normal" (Grüneberg, 1952). The disappearance of the anaemia coincided, more or less, with the disappearance of hepatic erythropoiesis, and its replacement by intra-medullary blood formation.

Siderocytes

In a further study, using the Prussian Blue reaction, Grüneberg (1942c) demonstrated the presence of excessive numbers of erythrocytes containing stainable iron granules (siderocytes) in flex-tailed anaemia (Fig. 2.1).

In the primitive erythropoietic cells (yolk sac generation) of both normal and homozygous f/f embryos, siderotic granules were present in large numbers. Although this feature was slightly more marked in the anaemic mice, their primitive erythrocytes showed abundant haemoglobinization, and significant anaemia was not present at this stage of development. Thus, it seems unlikely that the f gene has much effect on the yolk sac

Fig. 2.1: Blood film from a newborn mouse homozygous for "flex-tailed" (f/f), showing numerous siderocytes. The dark staining inclusions give a positive reaction with the Prussian Blue reaction. Perl's stain, X1500.

stage of erythropoiesis. However, with the emergence, on or about the 13th-14th gestational day, of the intermediate (hepatic) generation of red cells, a clear and significant excess of siderocytes was seen in the anaemic mice, coinciding with the development of anaemia. The frequency of siderocytes was related to the severity of the anaemia, and the decline in their numbers occurred pari passu with its spontaneous regression. At birth 70-92 per cent of erythrocytes in anaemic mice contained siderotic granules, as opposed to 2-6 per cent in normal control animals. Siderocytes could not be found in normal mice after first week of life; in flex-tailed anaemia they declined in number to 1-8 per cent, at which level they persisted throughout life in spite of the normal haematological indices; this suggests the persistence of the underlying abnormality.

Grüneberg concluded that the siderotic granules represented "unused" iron. Their presence certainly excludes iron deficiency as a cause of the anaemia, and suggests the presence of an intrinsic abnormality of the red cell series, concerned with the synthesis of haemoglobin.

Nature of the defect

Thompson and her associates (Thompson, McCulloch, Siminovich and Till, 1966b) have shown that cells from

embryonic liver and from the bone marrow of 8 week and 7 month old homozygous f/f mice, transplanted into irradiated hosts form smaller spleen colonies than do cells from normal mice. This finding confirms that the defect is inherent in the erythroid cells and that it does persist in latent form in adult life, as Grüneberg (1942c) had suggested. The spleen colonies of f/f origin were also found to incorporate ^{59}iron poorly at 6-10 days after injection. By 30 days, however, no difference in ^{59}iron incorporation existed between f/f and normal cell colonies, indicating that the defect is only manifested under conditions of rapid proliferation.

Accumulation of iron within the abnormal red cell suggests a defect in globin and haem synthesis. A direct effect on the structure of globin is rendered unlikely by studies of linkage. "Flex-tailed" is not linked to the structural gene for α-chain synthesis (Hutton, Bishop, Schweet and Russell, 1962a), and the structural gene for β-chain synthesis is known to be closely linked to albinism (Popp and St. Amand, 1960), whereas that for flex-tailed anaemia is not (Hunt, Mixter and Permar, 1933).

There is more positive evidence of interference with haem synthesis, since impaired activity of the enzyme δ-aminolaevulinate dehydratase (ALAD) has been demonstrated (Margolis, 1965; Margolis and Russell, 1965). This enzyme catalyses the condensation of two molecules of δ-amino-

laevulinic acid to form porphobilinogen (Granick and Levere, 1964). Its level of activity in mouse liver varies from strain to strain and is under genetic control, probably by a single factor, Lv (Russell and Coleman, 1963). In adult mice, both homozygous f/f and heterozygous f/+, ALAD levels in liver and spleen are reduced to about one third of normal (Margolis, 1965). Phenylhydrazine induced haemolysis leads to a considerable increase in ALAD activity in normal mouse haemopoietic tissue, but has only a slight effect in homozygous f/f animals; the f/+ heterozygotes show an intermediate increase, and thus the genotypes can be distinguished (Margolis and Russell, 1965).

The metabolic abnormality concerns the early stages of haem synthesis, manifested as hypochromic anaemia during a period of rapid growth, and detectable throughout life as persistence of siderocytes and failure on the part of the haem synthetic pathway to react adequately to stress. The intermediate degree of augmentation of ALAD activity by haemolysis in heterozygous (f/+) animals suggests a higher potential for haem synthesis than is possessed by the homozygous (f/f) mice, and this higher potential is apparently adequate to prevent the appearance of haematological or morphological abnormalities in the heterozygous group.

The deficiency of ALAD activity in the adult f/f

mouse is probably less than complete since it results in comparitively minor morphological abnormalities in a system which is synthesizing haem in relatively large quantities. Since enough haem can be synthesized to support a normal haemoglobin concentration in the peripheral blood, it is not necessarily surprising that no apparent effect of the f gene is obvious in other haem synthesizing systems.

The cause of the skeletal deformities is not a present clear. Anaemia precedes the earliest evidence of skeletal maldevelopment leading to the suggestion that abnormal differentiation of the intervertebral discs might be a consequence of anaemia (Kamenoff, 1935). This aspect is further discussed by Grüneberg (1952, 1963) who feels that it is unlikely to be the whole explanation, as skeletal anomalies are not present in the WW anaemias where the haemopoietic defect is apparent at about the same stage of development. Whether the degree of deficiency of ALAD in f/f mice can influence the development of axial skeleton is not known at present. The explanation of the belly spot is also not clear.

(ii) Inherited Microcytic Anaemia (Gene Symbol, mk; linkage group unknown).

Microcytic anaemia is inherited as an autosomal recessive and affected newborn (mk/mk) mice are recognizable by their pallor and reduction in body weight.

Viability is impaired, only 50 per cent remaining alive at the time of weaning, but the fertility of of survivors is normal (Nash, Kent, Dickie and Russell, 1964).

Blood smears from anaemic mice show extreme microcytosis, hypochromia and leptocytosis. The red cell count in newborn anaemic mice is only slightly lower than in their normal sibs, and during the first 8 weeks of postnatal life it increases greatly, until it is 50 per cent higher than normal. Thus, there appears to be a compensatory increase in red cell production in the anaemic mice and this is reflected in an increase in reticulocytes to 11-13 per cent compared with 3-4 per cent in control animals. The mean corpuscular volume and mean cell volume are decreased to about 60 per cent of normal (Russell and Bernstein, 1966). The metabolic disturbance producing this anaemia is as yet unknown.

d) Miscellaneous Anaemias

(1) Anaemia associated with "diminutive" (gene symbol, dm: linkage group, V).

The mutation dm is inherited as an autosomal recessive, and in the homozygous condition gives rise to retarded growth, multiple skeletal abnormalities and

severe neonatal macrocytic anaemia, which improves somewhat with increasing age (Stevens and Mackensen, 1958).

At birth the red cell count is reduced to about one third of normal in the anaemic (dm/dm) animals, while the mean cell volume is increased by up to 40 per cent, to 137.5-144.5 cu.μ. As the anaemia improves, the differences between the dm/dm mice and the normal become less, but the macrocytic picture is still clearly present at 250 days of age. An increase in siderocytes is not present, unlike the anaemia of "flex-tailed", and some homozygous animals are fertile, in contrast to the W and steel series of anaemias.

The presence of severe anaemia at birth implies that the hepatic generation of erythrocytes is affected as well as the adult medullary and splenic phases of haemopoiesis but whether the earlier yolk sac generation is also involved is not at present known, and further investigation of the anaemia does not appear to have been undertaken.

The mechanism of the skeletal anomalies is also uncertain and Grüneberg's suggestion (1963) that they might be secondary to anaemia in foetal life, as in "Tail-short" (Deol, 1961), is unlikely to be the whole explanation since the original heterozygous carrier (dm/+) showed fusions of sacral and caudal vertebrae

and heterozygotes are not anaemic (Stevens and Mackensen, 1958).

(ii) Anaemia associated with "Tail-short" (gene symbol Ts; linkage group unknown)

The dominant gene for "Tail-short" (Ts) arose as a spontaneous mutation in a highly inbred colony of strain C mice. In the homozygous state it is lethal and in the heterozygous state produces multiple defects of the skull, and axial and limb skeleton, including vertebral fusions, fewer caudal vertebrae, extra thoracic vertebrae, ribs and sternebrae, and rib fusions (Morgan, 1950, Deol, 1961).

Severe anaemia is seen in embryos between the 9th and 14th days of gestation and while this is still appreciable at 16 days, by birth the haematological findings are not significantly different from normal. On the 8th day of gestation, there is clear reduction of the number of blood islands in the yolk sac (Deol, 1961). Delay in maturation of the cartilagenous skeleton is appreciable at 14-17 days, and it has been suggested that the primary defect in the development of the blood islands leads to anaemia which in turn leads to the retardation of growth and differentiation of the skeleton.

(iii) The anaemia of luxoid mice (gene symbol, lst; linkage group unknown)

Several genes producing a syndrome of luxation

of the limbs and polydactyly have been described (Carter, 1951; Green, 1955; Strong and Hardy, 1956). "Strong's luxoid" (gene symbol, lst) (Strong and Hardy, 1956) in which anaemia sometimes occurs, arose in a CBA/N/JK hybrid which was receiving methylcholanthrene. The nature of the anaemia was clarified by Forsthoefel and Kuharcik (Forsthoefel and Kuharcik, 1961; Kuharcik and Forsthoefel, 1963) who found it to be present in about a third of newborn homozygous lst/lst mice, but not in the heterozygous animals, nor in adults, nor in the foetal stage of development. The anaemia is normocytic in character and mild in degree, with normal hepatic and medullary hemopoiesis. Tearing of the abdominal wall between the umbilicus and genital tubercle of the newborn luxoid mice, and quite prolonged bleeding from this site in some instances, was noted. There was a significant correlation between the presence of such tears and the presence of anaemia. Thus it appears that the transient normocytic neonatal anaemia of Strong's luxoid is not a primary gene effect, but a post-haemorrhagic complication of damage to the abdominal wall and umbilical vessels at the time of separation of the umbilical cord.

(iv) Foetal erythroblastic anaemia (no gene symbol allotted, inheritance uncertain)

Foetal erythroblastic anaemia, described by Hertwig (1956), occurs during embryonic life and is lethal

between the 14th and 17th gestational days. It appears to differ from the other mouse anaemia, with which Hertwig's name is associated (1942a,b) and which has been discussed already.

The nature of the inheritance is not clear, but it is found in the offspring of matings which have produced mice with hydrops, microcephaly and other congenital malformations.

The peripheral blood in anaemic embryos shows a virtual absence of non-nucleated erythrocytes of the hepatic generation, and as the embryos mature, the nucleated erythrocytes show progressively less and less haemoglobinization with the development of profound anaemia.

The association with hydrops has led Hertwig (1956) to postulate that the explanation of the anaemia may lie in materno-foetal serological incompatibility. The failure of hepatic haemopoiesis and haemoglobinization of the erythroblasts do not seem entirely consistent with this suggestion. The co-existence of hydrops and failure of haemoglobinization invites comparison with human α -thalassaemia (Lie-Injo Luan Eng, 1962). It is clear that further study is required before the true nature of this anaemia can be established.

e) Summary

This chapter has surveyed the variety of hereditary

anaemias which have been described in mice, and summarized the main haematological features of the various syndromes.

Their study has already proved valuable towards the elucidation of the processes of control of cellular and tissue differentiation, as exemplified by the work on the W and steel series. The presence of apparently independent gene effects in a variety of tissues suggests that a common mechanism may exist for the control of their development. The response of the W and steel anaemias to erythropoietic factor implies that such a common mechanism involves both humoral and cellular components, identical in the various affected tissues.

Disturbance of red cell formation by single gene mutations, which affect single steps in the whole chain of processes, allows analysis of the normal working of such steps and of their genetic control. Investigation of the congenital haemolytic anaemias of mice should yield further information on the mechanisms for maintenance of red cell shape and functional integrity.

As models of human disease, the hereditary mouse anaemias already offer some useful analogies. Thus, the autoimmune disease of NZB/Bl mice provides an opportunity to study the failure of control of the immunological mechanisms, and of "self-recognition" on the part of

the immunologically competent cell. It further provides a model of human systemic lupus erythematosus for investigation of pathogenic mechanisms and treatment. Many of the other syndromes require further investigation before their value as disease models can be assessed. For example, several of those which so far have been little studied resemble the thalassaemias, a group of human diseases for which an animal model would be most useful.

CHAPTER 3

X-Linked Anaemia of Mice: Previously Reported Data

a) Origin and genetic assessment:

Sex-linked mouse anaemia (gene-symbol, sla) or, more properly, X-linked mouse anaemia, was discovered during experiments in the production of X-linked mutations by irradiation with x-rays. The mutant was first noted in the offspring of a daughter of a male mouse which had received 500 rads of x-rays. When mated with an unrelated wild-type male, she produced six anaemic and ten normal male offspring (Falconer and Isaacson, 1962).

Anaemic animals were so classified by inspection at or soon after birth, and pedigree studies clearly indicated an X-linked recessive pattern of inheritance. Four females from matings which were not expected to produce homozygotes, were classified as anaemic. Two of these proved to be heterozygotes on subsequent mating tests; the other two were apparently not tested. If all four were heterozygotes then the penetrance of the sla gene amounted to 6 per cent (4 out of 65 expected heterozygotes). There was also a suggestion from linkage data, that occasional normal males might have been classified as anaemic and this could be taken

to reflect the inaccuracy of inspection of neonatal mice as a method of classification, and, further, throws doubt on the estimate of penetrance in the heterozygous females.

Falconer and Isaacson (1962) also performed three-point linkage studies of sla with "Tabby", (Ta), (Falconer, 1952, 1953) and "Brindled" (Mo^{br})(Fraser, Sobey and Spicer, 1953; Falconer, 1953); both these X-linked genes affect the coat colour of mice. Recombination frequencies of 4.2 per cent between Mo^{br} and Ta and of 3.2 per cent between Ta and sla were found and the three-point linkage tests established the order of genes on the X-chromosome as Mo^{br} - Ta - sla. Other X-linked genes, the positions of which were known at that time, are "jimpy" (jp), (Phillips, 1954) and "Bent-tail" (Bn)(Garber, 1952). The former lies about 21 crossover units from Ta on the side remote from sla, and the latter about 13 units from Ta on the same side as sla. Thus, sla lies between Bn and Ta and Falconer and Isaacson (1962) gave the following linkage map of the X-chromosome of the mouse (figures represent the number of crossover units between each locus):-

Bn —10— sla —3— Ta —4— Mo^{br} —17— jp

A more recent and complete map of the X-chromosome of the mouse, taken from Green (1966), is given below, together with an explanation of the symbols.

Symbol**	Name	
Bn	"Bent-tail"	
Str	"Striated"	
sla	"sex-linked anaemia"	
Ta	"Tabby"	allelic
Gs	"Greasy"	allelic
Blo	"Blotchy"	allelic
Mo	"Mottled"	allelic
To	"Tortoiseshell"	allelic
jp	"jimpy"	
sf	"scurfy"	

* Precise location uncertain.

** Capital letters in the gene symbol and name indicate dominance, and lower case, recessive inheritance.

b) Haematological investigations:

The first haematological assessment of the anaemia produced by the sla gene was given by Grewal (1962), who studied mainly newborn and young mice. The animals studied were the descendants of a single male from the colony of Falconer and Isaacson (1962). This mouse was mated with first generation (F_1) females from a cross between the inbred strains, CBA/Gr and C57Bl/Gr, to produce animals for study.

The results obtained by Grewal (1962) for haemoglobin, concentration, haematocrit, M.C.H.C., red cell count, mean cell volume (M.C.V.) and mean cell haemoglobin (M.C.H.) at various ages are given in Table 3.1 and the anaemic:normal ratios for each measurement or index in each age group are given in Table 3.2. As can be seen from the latter table, there was little difference in the extent of reduction of haemoglobin concentration, haematocrit and red cell count and consequently comparitively little deviation from normal is evident in the M.C.H.C., M.C.V. and M.C.H. Thus, the degree of microcytosis and hypochromia appeared to be slight. Little change in the anaemia was seen with age up to 72 days, but animals examined at 240 days of age were less anaemic than the younger mice. Since serial studies were not reported, it is not clear whether this represented a true improvement, or merely a differential

survival of less anaemic mice. The extent of variation in the haematological findings within the various age groups studied is not stated.

Blood films showed mild anisocytosis and poikilocytosis and hypochromia; anisocytosis and microcytosis were reflected in the red cell diameter measurements, in which there was more variation in anaemic animals at all ages, with an appreciable "shift" in the direction of microcytosis. The extent of change seemed to decrease with increasing age. Anaemic animals had a slightly raised reticulocyte count, and during the first week of life, they are reported to have shown the presence of siderocytes in slightly greater than normal numbers. Insufficient data were made available for critical assessment of this. Red cell osmotic fragility in anaemic mice was found to lie in the same range as the normals. Examination of blood films showed no differences in the white cells suggesting that the sla gene does not affect the leucocyte series.

Ten heterozygous carrier females were examined, and compared with their normal brothers. No difference was found between the two groups and the gene was thus regarded by Grewal as being fully recessive.

The haemopoietic organs (liver, spleen and bone marrow) were examined histologically, after staining with haematoxylin and eosin. In the liver, anaemic mice

showed fewer haemopoietic foci than the normals at birth, but more at age 7 days. No haemopoietic foci were found after 14 days in anaemics or normals. No differences were found when the spleens were compared, but tibial bone marrow from anaemic mice showed defective erythropoiesis at ages up to 70 days. The extent of the iron stores was not examined.

Thus, the haematological picture which emerged was of a moderate anaemia with comparatively slight morphological changes and alterations in the red cell indices. The histological findings in the bone marrow implied that failure of erythropoiesis may have had a part to play in the pathogenesis of the anaemia, but otherwise there was little to indicate the possible mechanism of X-linked mouse anaemia.

The haemoglobin and plasma proteins were investigated electrophoretically by Cohen (1962) and did not show any differences from established normal patterns.

The X-linked inheritance of sla clearly demonstrated its non-identity with the other forms of heritable mouse anaemia described in Chapter 2, and also implied the presence on the X-chromosome of the normal mouse of a gene concerned, directly or indirectly, with the maintenance of normal haemopoiesis.

Grewal considered briefly a possible analogy between X-linked mouse anaemia and thalassaemia (Cooley's

anaemia) in the human; this will be discussed in more detail in a subsequent chapter.

Bannerman and Cooper (1964) followed up Grewal's (1962) suggestion of an analogy to thalassaemia. Working with a mixed strain of mice descended from Falconer's original colony, they were able to confirm the hypochromic nature of the anaemia and the X-linked pattern of inheritance. They, too, were unable to demonstrate an abnormality on haemoglobin electrophoresis, nor could they demonstrate significant alterations in the absorption or utilization of iron. They suggested a resemblance to the human X-linked hypochromic anaemia described by Cooley (1945) and Rundles and Falls (1946), a suggestion also put forward by McKusick (1964) and Ohno (1967). However, unlike this human X-linked hypochromic anaemia (Bishop and Bethel, 1959, 1960) the mouse anaemia proved unresponsive to pyridoxine. The anaemia did, however, respond to parenteral iron dextran.

In a subsequent communication Bannerman and Cooper (1966) reported in more detail on their findings in X-linked anaemia. The anaemia was more severe in their colony than in Grewal's, with haemoglobin concentrations in the range of 4 - 8G/100 ml. at age 30-40 days. The reduction in haematocrit was proportionately less than in haemoglobin and the M.C.H.C. was thus low. The

reduction in red cell count was stated to be moderate and the mean cell volume to be normal or slightly reduced. This is somewhat at variance with their finding of hypochromia, microcytosis and red cell fragments in the peripheral blood smears, but could perhaps be explained by the presence of larger, polychromatophilic cells and large target cells. They also found minor morphological changes in blood smears in some heterozygous female carriers. This is consistent with Falconer and Isaacson's (1962) finding that sla was incompletely recessive, but is in disagreement with Grewal's (1962) statement that the heterozygotes are apparently normal haematologically.

Spontaneous improvement in the anaemia with age was noted and the previously reported response to intraperitoneal iron-dextran was confirmed.

Preliminary estimates of inorganic iron absorption and utilization for haemopoiesis did not show significant differences from normal controls.

Determinations of total body iron gave similar results in normal and anaemic mice.

It was concluded that the anaemia was probably a consequence of an unusual primary defect in iron metabolism on the basis of the favourable response to iron dextran in the absence of apparent iron deficiency and in the presence of apparently normal iron absorption.

It seems surprising, however, that the absorption and utilization of iron for haemopoiesis can both be normal in the presence of a hypochromic and microcytic anaemia and clarification of this point seemed necessary.

The differences in the haematological findings in X-linked anaemia in the colonies investigated by Grewal (1962) and by Bannerman and Cooper (1964, 1966) may be a consequence of variable expressivity of the sla gene on different genetic backgrounds, or perhaps of variations in environmental factors such as diet, or, of course, environment and inheritance may both have a part to play.

In a very recent brief comment on X-linked mouse anaemia Ohno (1967) reports that the serum iron is apparently elevated, but that siderocytosis and haemochromatosis are not observed. Data supporting these statements are not presented.

c) Summary

The origin of X-linked mouse anaemia is described. The mutant (sla)gene arose as a consequence of X-irradiation, and shows the typical X-linked pattern of inheritance. The position of this locus on the X chromosome of the mouse has been determined.

Data reported by others on the nature of the anaemia is presented. The earliest assessment showed a mild or moderate anaemia with slight alterations in the red cell

indices in the direction of hypochromia and microcytosis. Subsequent investigations suggested that the anaemia and the associated morphological changes were more severe than was at first thought, and several possible explanations for this discrepancy exist.

Preliminary studies with tracer doses of radio-iron, and chemical estimates of body iron content, have led to the suggestion that an unusual disturbance of iron metabolism underlies the anaemia.

CHAPTER 4

Materials and Methods

a) The animals studied

The origin of the mutant gene, sla, has been described in Chapter 3. Through the generosity of Dr. D. S. Falconer of the Institute of Animal Genetics in Edinburgh, Dr. Bannerman was able to obtain a small breeding stock of mice of the original mixed strain; it is from these that the animals studied here are descended.

In the convention that will be used for recording genotypes, "sla" represents the gene for anaemia carried on the X-chromosome, "+" is the normal, wild-type allele, and "-" indicates the absence of either gene on the Y-chromosome of the male. Thus, the normal female is "+/+", the normal male "+/-", the heterozygous carrier female "sla/+", the hemizygous anaemic male, "sla/-", and the homozygous anaemic female, "sla/sla".

The breeding plan shown in Fig. 4.1 was followed at first, using brother x sister matings in an attempt to produce an inbred strain. The gene for "Brindled" (Mo^{br}), which causes alterations in coat colour (Fraser, Sobey and Spicer, 1953) and is closely linked to sla (Falconer and Isaacson, 1962), was present in the original stock,

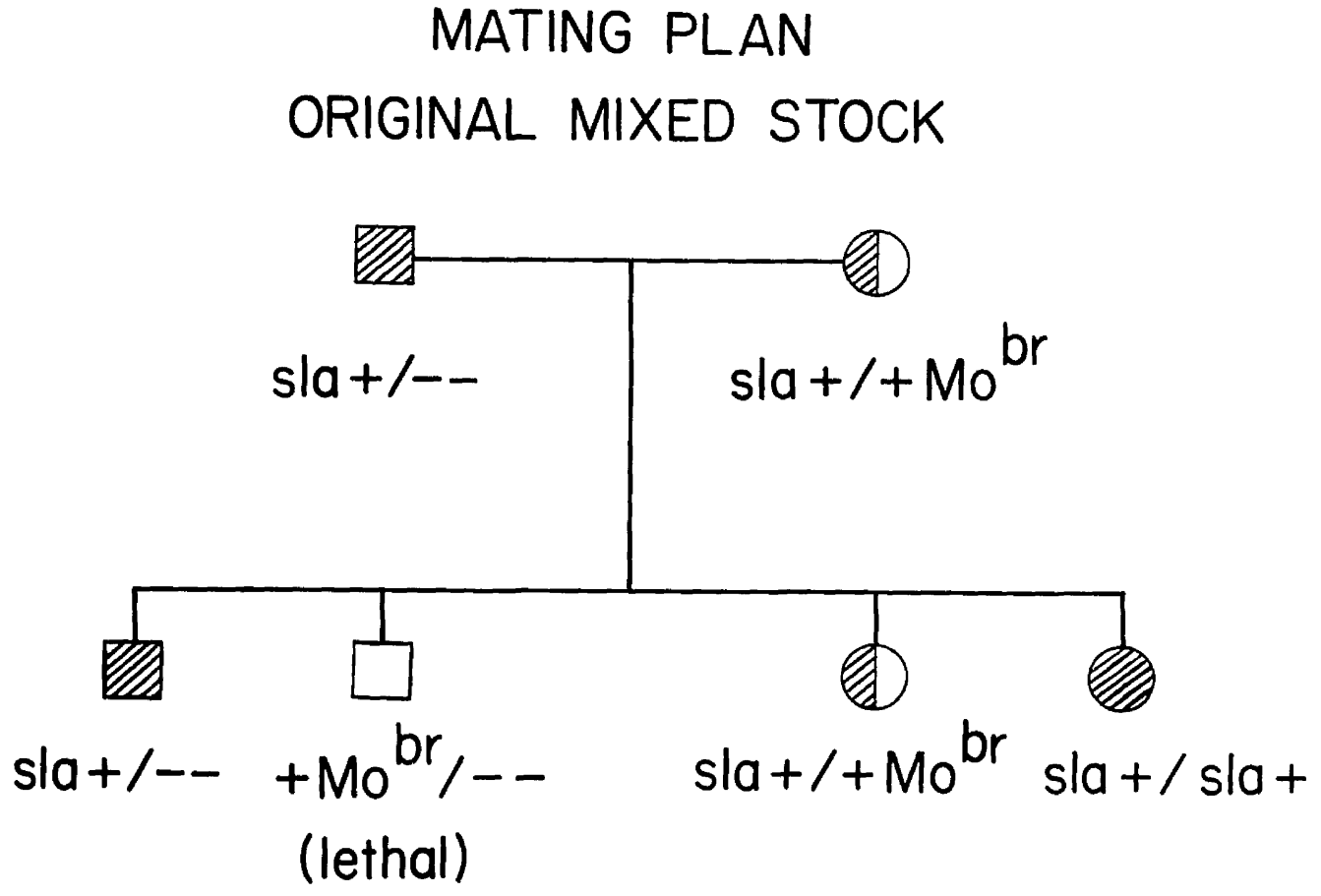


Fig. 4.1: The mating plan used in breeding the original mixed stock obtained from Edinburgh. The cross-hatched squares represent hemizygous anaemic males; the open squares, hemizygous Brindled males; the cross-hatched circle, a homozygous anaemic female, and the half open, half cross-hatched circles, doubly heterozygous females with the genes for X-linked anaemia (sla) and Brindled (Mo^{br}) in repulsion. (See text).

carried in repulsion to sla. Thus the offspring of a brindled carrier for sla (+Mo^{br}/sla+) would, in the absence of crossing-over, inherit a gene either for "Brindled" or for X-linked anaemia. When such a double heterozygote is mated to a non-Brindled anaemic male (sla+/--), two types of daughters result - another double carrier (+Mo^{br}/sla+) and a homozygous anaemic female (sla+/sla+). Carriers could be distinguished from homozygous anaemic females by coat colour alone, a feature of value in the early assessment of the anaemia before it had been clearly established that carriers show little or no change in the red cells. The ease of recognition of carriers for sla was also helpful in the continuation of the breeding programme.

"Brindled" (Mo^{br}) is usually lethal in the male between birth and 2 weeks (Fraser, Sobey and Spicer, 1953) and hemizygous (Mo^{br}/-) males do not survive to breed. This has resulted in a severe shortage of normal non-anaemic, male mice of the original mixed stock, and accounts for the relatively small numbers of normal mice available for comparison with anaemics in the haematological assessment of X-linked anaemia in Chapter 5. Such normal male animals as were available in the original mixed stock resulted from crossovers between Mo^{br} and sla in carriers, or were descendents of males and females resulting from such crossovers. However, Mo^{br} and sla are closely linked, and crossing over between them is a rare event.

Since genetic differences between strains of mice may affect the haematological picture (Russell et al., 1951), the use of mice of other strains, or hybrids, as controls has been avoided as far as possible in the haematological assessment of X-linked anaemia. However, preliminary observations suggested that there was no significant difference in the haematological findings in normal mice of the original mixed stock and first generation hybrids of the C57Bl/6J strain and the original mixed stock (Bannerman, 1965a).

Since we had been warned (Falconer, 1965) that attempts to in-breed a mixed stock with wild ancestry might well fail, an alternative breeding programme was also set up, to put the sla gene onto the background of the already inbred strain C57 Black/6 Jax (Abbreviation - C57Bl/6J) as supplied by the Jackson Laboratory, Bar Harbor, Maine. The C57 Black/6 Jax strain originated from a collection owned by a Massachusetts mouse fancier, Miss Lathrop, from whom C.C. Little obtained a littermate pair of mice in 1921. Progeny of this pair segregated for black and brown coat colour. These two lines were then inbred to give, ultimately, the C57 Black and C57 Brown strains. The C57Bl strain was subsequently divided into the C57Bl/6 and C57Bl/10 substrains (Staats, 1966). The Jackson Laboratory substrains are identified by the suffix "Jax" or "J".

The breeding plan employed in transferring the gene for X-linked anaemia into the C57Bl/6J strain is shown in Fig. 4.2. In the parental generation (P), an anaemic male of the original mixed stock was crossed with a C57Bl/6J female. All daughters of this mating are heterozygous carriers of sla (sla/+). These F_1 (first generation hybrid) females were in turn bred to C57Bl/6J males, and the offspring (second generation hybrids, F_2) of this mating are of the following genotypes: normal male (+/-), anaemic male (sla/-), carrier female (sla/+) and normal female (+/+); since carrier and normal females cannot be clearly distinguished without breeding experiments, the animals of these genotypes from this mating were generally destroyed. However, occasionally, a few females with low body weights of the F_2 and F_4 generations were selected for test matings with C57Bl/6J males, since it had been shown (see Chapter 5) that carrier females tended to be rather lighter than their normal counterparts. In consequence, occasional F_3 and F_5 normal and anaemic male animals have been available for study.

The degree of congeneity of the hybrids of each generation with the C57Bl/6J strain is given below:

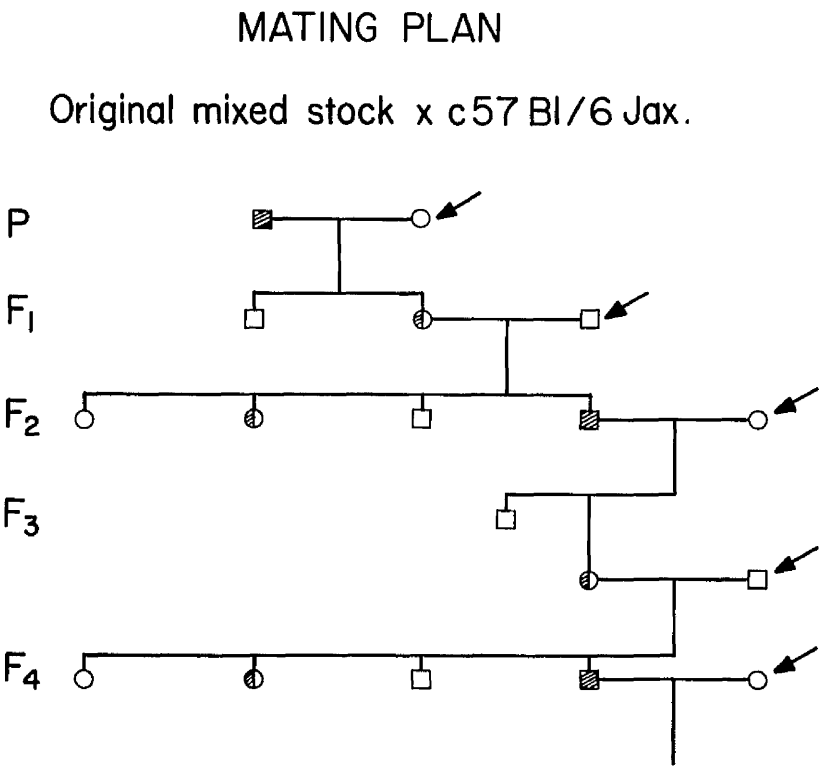


Fig. 4.2: The mating plan employed in transferring the gene for X-linked anaemia onto the inbred C57B1/6J background. The arrows indicate C57B1/6J animals and the anaemic male in the parental (P) generation is of the original mixed stock. The closed squares indicate anaemic males; the open ones, normal males. The half closed circles represent heterozygous carrier females, the open ones, normal females.

The symbol F_1 indicates the first hybrid generation; F_2, the second (back-cross) generation; F_3, the third (back-cross) generation to C57B1/6J; and so forth.

F_1	50%
F_2	75%
F_3	87.5%
F_4	93.75%
F_5	96.88%

The animals were kept in a thermostatically controlled room, with a temperature of 70-72°F. Polypropylene cages, 11½ x 7½ x 5 inches, were used, and two to six mice kept in each. Clear material was employed except for the breeding cages, which were of white opaque polypropylene. The lids were made of zinc-plated steel, with wires 7/16 inch apart, and containing a food hopper and separate water bottle compartment. The cages were stored on large racks, containing up to 40 cages each. The mice remained in the animal room at all times, except when removed to the laboratory for experimental procedures.

The bedding was commercially available dried, ground corn-cob ("San-i-Cel", Paxco, Paxton, Ill.) and the feed, Rockland rat and mouse diet, the constitution of which is given in Table 4.1. Tap water was given ad libitum. Repeated attempts to estimate the iron content of this water revealed an undetectable concentration of iron.

Animals were identified by numbers according to a conventional earmarking method (Dickie, 1966) and cage

identification cards of different colours were used for each hybrid generation, and for the original stock. Each cage card showed the parentage, sex, date of birth and number of each animal in that cage. In addition, detailed records of each animal were kept on a separate card, filed in the laboratory (see Fig. 4.3) and containing details of the individual animal's parentage, date of birth, haematological findings, weight record, and an indication of other experiments carried out on that animal. A detailed mating register was also kept and it has been possible to trace the ancestry of any mouse in the colony back to the original breeding stock obtained from Edinburgh.

b) Haematological methods

Haematological methods were, in general, similar to those used in human haematological practice and blood was obtained by severing a tail vein with a scalpel blade, or by cardiac puncture. Haemoglobin concentrations were estimated by a cyanmethaemoglobin method (Dacie & Lewis, 1963) using a Coleman Junior Spectrophotometer at 540 mμ. Packed cell volumes were measured on an International Equipment Company micro-capillary centrifuge, model M.B. Red cell counts were performed on a Coulter model B particle counter; the lower threshold was set at 19 and the reciprocals of the amplitude and aperture current at 1/8 and 2, respectively.

517 ♂ MIXED STRAIN

Date.	Wt.	HGB	PCV	MCHC	Age	
						Born of ♀ 32' × 81 ♂
16. 7. 65					0	
12. 8. 65					27	Weaned
16. 8. 65	14.5	7.2	26.5	27.2	31	
3. 10. 65	26.3	8.5	39	22	79	Smear B 92
26. 10. 65						Mated with ♀ (sister) 180
10. 11. 65			35		117	Smear B 96
13. 12. 65			36.5		150	
10. 2. 66			42.5		209	R.C.C. 13.9 m./cu. mm.
						M.C.V. 30.5 cu. μ.
						Mated to ♀ (littermate) 521
1. 3. 66						
16. 5. 66	31.0	11.8	39.5	29.9	304	Smear B 106
23. 1. 67					556	Died during night
						Decomposed, no post-mortem

Fig. 4.3: Photostat copy of a specimen record card, incorporating data on parentage, date of birth, age, haematological findings, body weight, blood smear identification numbers, and other experiments. Mouse number 517 of the original mixed stock.

Red cell volume distribution was analysed on the Coulter Model J automatic particle size analyser with the reciprocals of the amplitude and aperture current at 1/2 and 4 respectively. Mean cell volumes were estimated from the red cell counts and packed cell volumes, or calculated from the red cell volume distribution histograms according to the procedure outlined in the next paragraph. Typical red cell volume histograms are given in Appendix A, which contains the detailed results of the haematological assessment of X-linked mouse anaemia, described in the next chapter.

The total area of the histogram drawn by the model J particle counter is calculated in arbitrary units and the vertical line dividing this area into two halves is drawn. The location of this line on the threshold axis (abcissa, calibrated in units of red cell volume) is referred to as the "mean threshold", i.e., the lower threshold setting on the instrument at which 50% of the cells will be detected and counted at the larger end of the distribution curve of red cell size. This quantity had previously been related to the mean cell volume obtained from packed cell volume estimation and red cell counts in the laboratory mouse, and from this relationship the mean cell volume could be calculated from the red cell size plot. The mean cell volume/mean threshold ratio for the mouse obtained with the instrument used in this laboratory is 4.63, for the amplitude and

aperture current settings described above. (Bishop, 1965). Two assumptions involved in this calculation are: a) that there is a linear relationship between cell volume and mean threshold and: b) that the red cells do not change size in the saline in which they are suspended during analysis; in order to minimize the risk of this change, red cell plots were performed on suspensions of cells within 5 minutes of obtaining the sample from each individual mouse under examination.

Estimates of the mean corpuscular haemoglobin were not made.

Red cell diameters were measured, using a Leitz eyepiece micrometer, in blood smears stained with Wright's stain. One hundred cells were counted in each smear. A semi-quantitative estimate of the degree of morphological abnormality in the red cells in stained films was obtained by a visual scoring method. Five types of abnormality (hypochromia, microcytosis, anisocytosis, poikilocytosis and target cells) were scored separately as absent (0), slight (1) moderate (2), severe (3) or very severe (4); thus the total film "score" ranged from 0 to 20. This method was found to give good agreement for a series of films scored independently by two observers (Dr. R. M. Bannerman and me) without foreknowledge of the genotype, the haematological findings, or the other's assessment.

Only the results obtained by me are included here, however.

The percentage of polychromatophilic cells was estimated by counting and assessing 500 cells in each blood film. Reticulocytes were counted using brilliant Cresyl blue as described by Dacie and Lewis (1963). Siderocytes were sought in blood films after staining with the Prussian Blue reaction (Dacie and Lewis, 1963) and counterstaining with weak eosin. By this method, siderocytes were easily demonstrated in this laboratory in blood films from mice with flex-tailed anaemia (Grüneberg, 1942c) and from patients with thalassaemia and splenectomy.

White cell counts and platelet counts were performed by hand according to Dacie and Lewis (1963). Differential white cell counts on blood films stained with Wright's stain were also performed.

Osmotic fragility curves were plotted and the mean corpuscular fragility estimated employing an Elron Electronic Industries "Fragiligraph" Model D2. This apparatus records continuously the extent of erythrocyte lysis as the salt concentration of their ambience is gradually decreased by dialysis against distilled water. The salt concentration of the medium is also recorded. The details of this technique are given by Danon (1963, 1967).

Body weights were recorded at arbitrary time intervals to the nearest 0.1G.

c) Histological examination of the tissues, including the cytological examination of the bone marrow

Material taken for histological examination was immediately fixed in 10% formol saline and sections cut after embedding in paraffin wax. These were stained with haematoxylin and eosin and by the Prussian Blue reaction counterstained with 1% neutral red. Smears of femoral bone marrow and imprints of the cut surface of the spleen were stained by Wright's stain and by the Prussian Blue reaction. With each batch of histological sections stained for haemosiderin, a section of human spleen, containing plentiful stainable iron, was included, and with each batch of splenic imprints or marrow smears, a blood film, containing numerous siderocytes, from a man with β-thalassaemia who had undergone splenectomy was included. On each occasion, the expected Prussian Blue positive material was seen in these "control" preparations.

At the time of sacrifice the total body weight, to the nearest 0.1G., and the spleen weight, to the nearest mg, were recorded to obtain an estimate of splenic enlargement in anaemic animals. The proportion of splenic tissue occupied by red pulp was assessed by microscopic examination, and visually graded in 4

arbitrary categories - less than 25%, 25-50%, 51-75% and more than 75% of the spleen. Where doubt existed as to which category was appropriate, the result was recorded in the lower percentage group.

The amount of stainable iron in the spleen was recorded semi-quantitatively according to the following definitions:

Spleen iron "score"	0	Stainable iron absent
Spleen iron "score"	1	Less than one iron containing cell in 5 high power fields (Field diameter 0.37 mm)
Spleen iron "score"	2	One iron containing cell in 1 - 5 high power fields
Spleen iron "score"	3	1 - 5 iron containing cells in each high power field
Spleen iron "score"	4	More than 5 iron containing cells in each high power field

In assessing erythropoietic activity in the spleen imprints and bone marrow smears, the number of erythroblasts has been compared with the number of metamyelocytes and mature polymorphs; these cells are easily recognized by their nuclear characteristics, whereas the more primitive granulopoietic cells are difficult to identify with certainty on account of the lack of granules in the neutrophil series (Endicott and Gump, 1947). In each smear, two hundred erythroblasts, metamyelocytes and mature polymorphs were counted, and 400 erythroblasts were examined for siderotic granules.

Sections of sternal marrow were examined. Since the available bone marrow space of the normal mouse is almost entirely occupied by haemopoietic tissue (Endicott and Gump, 1947), and since no differences in cellularity of the marrow were appreciable when normal (+/-) and anaemic (sla/- and sla/sla) mice were compared on simple microscopic examination, detailed measurements of bone marrow cellularity have not been made.

Stainable iron in the epithelial cells of the duodenal villi was recorded according to the following definitions:

Epithelial iron "score"	0	Stainable iron absent
Epithelial iron "score"	1	Scanty, small haemosiderin granules
Epithelial iron "score"	2	Obvious haemosiderin granules in occasional cells
Epithelial iron "score"	3	Haemosiderin granules in many cells
Epithelial iron "score"	4	Haemosiderin granules in all or nearly all cells

The quantity of stainable iron in other tissues proved to be extremely small, if present at all, and is merely recorded as present or absent.

d) Radio-isotopic Techniques:

Investigations of the utilization of tracer doses of radio-iron for haemopoiesis have been carried out. For the calculations involved in these determinations, a measure of the blood volume of the mice under investigation

is required, and this has been obtained by using ^{51}chromium-labelled red cells and ^{131}iodine-labelled human serum albumin. The rate of clearance of tracer doses of ^{59}Fe from the plasma has been measured and a preliminary assessment of the excretion of iron over a prolonged period has also been made.

The intestinal absorption of iron at 3 dose levels has been determined by radio-isotopic methods, and preliminary studies carried out of the absorption of isotope-labelled fat, copper, zinc and cobalt.

Details of the isotope solutions used in each experiment and their source are included in Appendix C and will not be repeated here.

In all presentations of data in Appendix C, and in all data used for the calculations of results of experiments involving radioisotopes, the background counts for the instruments used for radioactive counting have been previously subtracted by the use of an automatic "Background Subtract" mechanism built into these instruments.

1) Blood volume

This has been determined by two isotope dilution methods, employing ^{51}chromium (^{51}Cr)-tagged mouse red cells and ^{131}I-labelled human serum albumin. Mouse erythrocytes from donors of the third and fourth backcross generations described above were tagged with Na $^{51}CrO_4$ using a dose containing 2 µg of chromium and 300 µc per

millilitre of packed mouse red cells (Lajtha, 1961). Nineteen normal and 9 anaemic recipient mice each received 0.1 ml of labelled red cells intravenously. Red cell incompatibility was not sought prior to these experiments, since naturally-occurring iso-agglutinins are not found in the mouse (McDonald & Hubbard, 1922), and none of the animals used had received prior injections of foreign erythrocytes. ^{131}I-labelled albumin was administered similarly to 7 normal and 5 anaemic mice, each animal receiving 0.1 ml of a 6 per cent solution, containing 2 μc. Each animal was weighed prior to injection and the weight recorded to the nearest 0.1G.

Injections of labelled red cells or albumin were made into a lateral tail vein, and, 10 minutes after injection, a 20 μl blood sample was obtained from the contralateral tail vein; this blood was diluted in 2 ml of water for counting. A 10 minute interval between injection and sampling was arbitrarily selected as sufficient to allow adequate mixing, but probably insufficient to permit undue loss of label from the circulation, (Wish et al., 1950; Kaliss and Pressman, 1950). Counting standards were prepared by diluting 0.1 ml of labelled red cells or albumin to 50 ml with water; duplicate 2 ml aliquots of the standards were counted. Counting was performed for one minute in an automatic gamma scintillation well counter (Nuclear

Chicago, Model 1085). The counts obtained for all samples were in excess of 10,000 c.p.m. An additional blood sample was taken for microhaematocrit determination, after the sample for radioactive counting had been secured.

The total blood volume was determined from the formula:

$$\text{Blood volume (ml)} = \frac{\text{c.p.m. of standard}}{\text{c.p.m. of sample x 2}}$$

The red cell and plasma volumes were derived from the blood volume and haematocrit, using a correction factor of 0.88 for conversion of venous to whole body haematocrit (Wish et al., 1950). Allowance has not been made for trapped plasma, nor has correction been made for the injected volume of labelled red cells or albumin. All results have been recorded as ml of blood, or plasma, or red cells, and as ml of blood, plasma or red cells per 100G. of body weight.

ii) The utilization of tracer doses of ^{59}Fe for haemopoiesis:

The appearance or reappearance of tracer doses of radio-iron in the peripheral blood of normal and anaemic (sla/-) mice has been studied at varying intervals following intraperitoneal or intravenous injection of ^{59}Fe as ferrous citrate. The dose of radio-iron given has varied between 0.065 and 0.14 µg, and 0.6 and 1.7 µc,

the original isotope solution, supplied commercially, being diluted where necessary with normal saline prepared from de-ionized water, to supply the desired dose of ^{59}Fe in a volume of 0.1 ml. Immediately following injection of the radio iron, the animals' whole body radioactivity was measured in a TOBOR (Nuclear-Chicago) large volume gamma scintillation counter with 4 inch crystals, and compared with a standard, prepared by adding the same amount of isotope solution as was given to each animal, to 25 ml of distilled water.

Blood samples of 20 µl, or occasionally 40 µl, were taken from a lateral tail vein after incision with a scalpel blade at varying periods after injection for determination of the proportion of the injected dose of radio-iron in the circulating blood. In some instances samples were taken serially for up to 11 days, in others on the 5th day after injection only, and in others again on first and 5th days after injection. The whole blood sample was diluted to 25 ml with water and counted, together with the standard, in the TOBOR counter. The radioactivity in the whole blood sample was taken to represent the radioactivity in the circulating red cells, since preliminary experiments had shown that, on the first and subsequent days after injection, the radioactivity in the peripheral blood was entirely in the saline-washed red cells, with none

detectable in the washings.

Hence, the proportion of the administered radioactivity reappearing in the peripheral blood on any day is hereafter referred to as the utilization of iron for haemopoiesis on that day, or as the percentage uptake of ^{59}Fe by the peripheral blood on that day. This index is calculated as follows:

The utilization of iron for haemopoiesis on day n after injection.

$$= \frac{C_b \times W \times B.V.}{C_a} \times \frac{S_o}{S_n} \times 100 \text{ per cent.}$$

Where C_b = counts per minute/ml blood on day n

W = weight in grams

B.V. = blood volume in ml/gm body weight

C_a = counts per minute for the whole mouse on day of injection (i.e. - the dose administered)

S_o = counts per minute for standard on the day of injection

S_n = counts per minute for standard on day n after injection

Since it is difficult to administer the small volumes of isotope solution (0.1 ml) used in this study accurately it has been considered inadvisable and inaccurate to use the standard isotope solution as a

reference for expressing the utilization of iron for haemopoiesis. The standard has therefore only been used to correct for radioactive decay and for the day-to-day vagaries of the TOBOR counter. Furthermore, since the mouse tends to lose a small, but appreciable, proportion of the injected radioactivity in the first few days after injection (Chappelle, Gabrio, Stevens and Finch, 1955), it was considered inappropriate to calculate the utilization of iron on any day after injection as a percentage of the retained radioactivity on that day. Hence, all estimates of iron utilization have been calculated as a percentage of the dose actually administered, as reflected by the counts per minute for the whole mouse immediately after injection. In the case of animals used first for plasma iron clearance studies and then for iron utilization investigations as part of the same experiment, the whole body radioactivity was assessed after removal of the samples for plasma iron clearance studies; these latter investigations are described in the next section.

When the blood volumes were determined as described earlier, it was found, as will be reported in detail in Chapter 7, that the ^{131}iodine-labelled albumin method gave significantly higher results that did the ^{51}chromium-labelled red cell technique. In an attempt to determine which of these methods gave the

more reliable estimate of blood volume, the utilization of iron for haemopoiesis in each of 5 normal mice was calculated separately using the mean blood volume determined by the two methods. In each instance, the ^{131}iodine-labelled serum albumin method gave values for the utilization of iron in excess of the dose administered (see Table 4.2). It was therefore concluded that the ^{51}chromium red cell method gave the more accurate estimate of the blood volume, and hence this estimate has been used for all calculations of the utilization of iron for haemopoiesis.

In principle, the method described above for the determination of the utilization of iron for haemopoiesis is standard, and similar to that described by Lajtha (1961) for investigations of iron utilization for haemopoiesis in man.

(iii) Plasma iron clearance rate:

Again, the principles involved in assessing the plasma iron clearance rate are adapted from those commonly used in man (Lajtha, 1961). Groups of normal (+/-) and anaemic (sla/-) male mice were given tracer doses of ^{59}iron as ferrous citrate intravenously (0.05-0.14 µg, 1.05-1.9 µc) into a lateral tail vein. As in the investigations of utilization of tracer doses of ^{59}Fe for haemopoiesis, the commercially supplied isotope solution was diluted with normal

saline, prepared with de-ionized water, to supply the desired dose of ^{59}iron in a volume of 0.1 ml. Blood samples were taken from the contralateral tail vein (to avoid contamination with ^{59}iron from the injection site) into heparinized microhaematocrit tubes, at 10, 20 and 30 minutes after injection. These samples were then centrifuged, in a high speed centrifuge, and broken in two on the plasma side of the plasma-cell interface. The plasma was then blown out onto clean, sterile waxed paper, from which a 10 µl, sample of plasma was taken, placed in 2 ml of distilled water in a clean plastic disposable tube, and counted in an automatic gamma scintillation well counter (Nuclear-Chicago, model 1085) for 5 minutes. Counts were corrected to counts per minute per 10 µl of plasma, and, for each mouse examined, the c.p.m. per 10 µl plasma were plotted against time on semi-logarithmic graph paper, with the c.p.m. on the logarithmic scale. For each animal the points fell on, or very close to, a straight line. The line most closely fitting the three points obtained was drawn and extrapolated back to zero time. The theoretical level of radioactivity at zero time in c.p.m. per 10 µl of plasma was read from the graph, and the time for the plasma radioactivity to decline to half this theoretical level estimated. This value is described as the plasma iron clearance half-time or plasma T $\frac{1}{2}$ ^{59}Fe.

(iv) Iron excretion:

A small group of mice, composed of both normal and anaemic animals, was given tracer doses of ^{59}iron as ferrous citrate (0.074 - 0.25 µg, 1.6 - 2.5 µc) intraperitoneally, the radioisotope solution having been previously diluted with normal saline prepared from de-ionized water, to supply the desired dose of ^{59}iron in a volume of 0.1 ml; the retention of radioactivity was followed for varying periods up to 6 months. The radioactivity of each mouse was counted in a TOBOR (Nuclear-Chicago) large volume gamma scintillation counter, together with a standard, immediately after injection. The standard for each experiment was prepared by placing the same dose of isotope solution as was given to the mice in 25 ml of water. At arbitrary intervals the retained radioactivity in each animal was counted together with the standard, and the percentage retention of the administered trace dose of iron calculated from the formula:

Retention of iron on day n = $\frac{C_n}{C_o} \times \frac{S_o}{S_n} \times 100$ per cent

where

C_n = counts per minute of animal on day n.

C_o = counts per minute of animal on day of injection.

S_o = counts per minute of standard on day of injection.

S_n = counts per minute of standard on day n.

(v) Iron absorption:

The intestinal absorption of iron was studied at three dose levels, viz. 0.1 µg, 1 µg and 10 µg of elemental iron. The radioisotope solution was prepared for oral administration by dilution of commercially supplied ^{59}ferrous sulphate with appropriate quantities of a solution of non-radioactive ferrous sulphate, to provide the chosen doses of elemental iron in 0.10 - 0.16 ml, with 0.5 - 1.6 µc of radioactivity. Each dose of iron contained 1 - 2 µg of ascorbic acid, which was present in the original isotope solution. The dose of ^{59}iron-tagged ferrous sulphate was administered to each mouse stomach tube, fashioned from a fine plastic tube designed for use as an intravenous catheter in man ("Intracath", Bardic). The mice were deprived of food and water for six hours prior to administration of the tagged dose of iron. The whole body radioactivity of each animal was counted immediately after dosing in the TOBOR large volume gamma scintillation counter, together with a standard, prepared by putting the same dose of ^{59}iron as was given to each mouse in 25 ml of water in a plastic container. As described in greater detail in Chapter 7, the retention of radioactivity was followed daily for 5 or 6 days in some animals, and was measured in others on the fifth day after administration only. The proportion of radioactivity,

and hence of the oral dose of iron, retained on the fourth and fifth days after administration in one group of experiments, and on the fourth, fifth and sixth days in another group, was similar; faecal elimination of unabsorbed iron by the animal was therefore assumed to be virtually complete on the fifth day, and the proportion of iron retained on this day has been used as a measure of iron absorption. The bedding in the cages was changed repeatedly during these experiments to reduce the risk of erroneous results arising from coprophagia. The percentage of the dose of iron retained, or absorbed, by each animal was calculated from the formula:

$$\text{absorption} = \frac{C_n}{C_o} \times \frac{S_o}{S_n} \times 100 \text{ per cent}$$

where C_o = c.p.m. of whole mouse immediately after administration of ^{59} iron.

C_n = c.p.m. of whole mouse in days after administration of ^{59} iron.

S_o = c.p.m. of standard at commencement of experiment.

and S_n = c.p.m. of standard on day n of experiment.

(vi) Absorption of fat:

The absorption of radio-iodinated triolein ("^{131}I - Trioleotope", Squibb) was assessed by a faecal collection method.

Ten mice, 5 normal (+/-) and 5 anaemic (sla/-) males, fasted for 6 hours, received 6.25 mg of radioiodinated triolein suspended in 0.1 ml of commercial margarine, administered in a similar manner to the oral doses of ^{59} iron-labelled ferrous sulphate described in the previous section, and containing 1 μc.

The mice were then counted in the TOBOR large volume gamma scintillation counter to measure whole body radioactivity (i.e., the dose administered), and a standard was also counted following preparation by injecting 0.1 ml of labelled margarine into a cotton wool swab which was then placed in a plastic container for counting. Each mouse was subsequently placed in a separate cage and the faeces were collected and the bedding changed daily for 5 days. The five day collection of faeces was then counted in the TOBOR counter and the retention or absorption of radioiodinated triolein calculated from the following formula:

percentage absorption of triolein

$$= 100 - \left(\frac{C_f}{C_m} \times \frac{S_o}{S_5} \times 100\right)$$

where C_f = c.p.m. of 5 day collection of faeces.

C_m = c.p.m. of whole mouse immediately after dosing with radioiodinated triolein.

S_o = c.p.m. standard on day of administration of radioiodinated triolein.

S_5 = c.p.m. standard 5 days after administration of radioiodinated triolein.

(vii) Absorption of metals other than iron :

The absorption of zinc, cobalt and copper by normal and anaemic mice has been studied. For each element five normal (+/-) and five anaemic (sla/-) mice, fasted for 6 hours, were used and the methods employed were similar to those for the investigations of iron absorption. The isotope solution in each instance was diluted with de-ionized water to give the smallest dose of elemental metal which would provide adequate whole body counts after faecal elimination of unabsorbed zinc, cobalt or copper was completed. For zinc and cobalt, whole body counting was performed 5 days after administration of isotope, and in the case of copper, 4 days after administration, since the half-life of ^{64} copper is very short (12.8 hours: The Radio Chemical Manual, 1966) and the residual activity at 5 days would be too low for adequate counting rates to be achieved. The doses of elemental zinc, cobalt and copper were 3.5 µg (2 µc), 0.006 µg (2 µc) and 10 µg (100 µc) respectively. The percentage absorption of these metals was calculated from the formula

$$\text{Absorption} = \frac{C_n}{C_o} \times \frac{S_o}{S_n} \times 100$$

where C_n = c.p.m. whole mouse on the 4th (copper) or 5th (zinc and cobalt) day after administration.

C_o = c.p.m. whole mouse immediately after administration of zinc, cobalt or copper.

S_o = c.p.m. standard on day of administration.

S = c.p.m. standard on 4th (copper) or 5th (zinc and cobalt) day after administration.

e) Intestinal transit time:

The time required for complete faecal elimination of an oral dose of carmine red has been measured in 4 normal and 4 anaemic male mice. The animals received 0.15 ml of carmine red suspension orally, administered in a manner similar to that used for oral dosing with ^{59}iron in the investigations of iron absorption. They were then placed in separate cages and the faeces collected daily for 4 days for inspection. Carmine red was recorded as being "obviously" present, present in "traces" or not present.

f) Chemical estimations of total body iron content, serum iron and serum total iron binding capacity (T.I.B.C)

The total body iron content of normal and anaemic mice has been estimated by a modification of an ashing method devised for the measurement of the iron content of urine (Padowetz, 1965). Since this technique is not generally available in the literature it will be described here in some detail. The previously weighed mouse carcass is ashed with concentrated sulphuric and nitric acids, following which the iron concentration is determined colormetrically in the decomposition solution

with ortho-phenanthroline. The mouse carcass is placed in a 250 ml Kjeldahl flask; 50 ml each of concentrated sulphuric and nitric acids are added, and the flask placed on an incineration stand. The mixture is heated gradually until the sulphuric acid boils and all the nitric oxides are removed; it is then allowed to cool and 1 ml of 30% hydrogen peroxide is added; ashing is continued until the mixture begins to smoke. Further 1 ml aliquots of 30% hydrogen peroxide are added until a clear and colourless solution is obtained. This is allowed to cool and 2 ml of 20% w/v sodium pyrosulphite solution is added. The mixture is again heated until all the water has evaporated, and then it is again allowed to cool. Deionized water is then added to bring the mixture to 250 ml volume, and a 2.5 ml aliquot is removed, neutralized with 20% ammonia using para-nitrophenol as an indicator, and the resultant yellow colour removed by adding, drop by drop, half normal sulphuric acid. Hydroquinone (4% solution in 0.05 normal sulphuric acid) and ortho-phenanthroline (1% solution in 0.05 normal sulphuric acid) are added (0.5 ml of each) and the mixture is left to stand for one hour. The solution is transferred to a 20 ml flask, with 2 ml of alcohol and made up to the mark with de-ionized water. After thorough mixing, the coloured solution is read at 510 mμ against water in a 10 mm cell in a Coleman Junior Spectrophotometer.

The "blank" solution is prepared by processing 25 ml of de-ionized water in the same manner as the mouse carcass and the reading for the blank against water deducted from the readings for the decomposed mouse carcasses. With every batch of mouse carcasses, a "blank" solution was prepared since the reagents used cannot be completely guaranteed to be free of traces of iron.

Calibration curves are constructed from a standard solution of ferric oxide, 2.86mg. being dissolved in a few millilitres of concentrated hydrochloric acid, the mixture then being made up to 50 ml with de-ionized water. Of this solution, 0.5, 1.0, 1.5, 2.0 and 2.5 ml (corresponding to 0.02, 0.04, 0.06, 0.08 and 0.10 mg of iron) are transferred into 20 ml measuring flasks, and, where necessary additional de-ionized water is added to bring the volume up to 2.5 ml. These aliquots of standard iron solution are then treated in the same manner, described above, as decomposition solution derived from the whole mouse carcasses. The calibration curve of iron concentration against photometer reading is linear within the range used.

From the readings obtained, the total body iron content was estimated, and the concentration of iron per 100 G. body weight calculated. The total body iron content is equal to mg iron in the 2.5 ml aliquot of

decomposition solution (from calibration curve), times 100, and the body iron concentration in mg/100G

$$= \frac{\text{total body iron content x 100}}{\text{body weight in G}}$$

Serum iron concentration and total iron binding capacity (T.I.B.C.) have been determined on the serum from whole blood samples obtained by cardiac puncture under ether anesthesia. A measured volume (between 0.2 and 0.6 ml) was placed in a test tube and diluted to 2 ml with de-ionized water. Estimations of serum iron concentration and T.I.B.C. were then performed according to Caraway (1963) with appropriate correction for the dilution of the serum sample. Since differences between the results obtained from fasting and non-fasting animals were not found, results from both groups of animals have been analyzed together. Samples showing visible hemolysis were rejected, and all samples were taken between 11 AM and 12 Noon to avoid the well known variability in the serum iron due to diurnal fluctuation. Glassware was prepared according to Caraway (1963) and only very slight variation was seen from day to day in the absorbance readings of the standard. Readings of absorbance were made using a Coleman Junior Spectrophotometer at 590 mμ.

g) Free erythrocyte protoporphyrin concentration

Free erythrocyte protoporphyrin concentration was determined by the method of Grinstein and Wintrobe

(1948). The quantity of packed red cells used was 0.2 ml - 0.4 ml and appropriate corrections were made in the calculations.

h) Electrophoretic studies:

Qualitative examination of the haemoglobin type was performed by starch gel electrophoresis according to Hutchison, Pinkerton, Alton and Cassidy (1963), employing the continuous buffer system recommended by Huehns and Shooter (1965). Transferrin was examined qualitatively by vertical starch gel electrophoresis of serum followed by Amido Black staining of the sliced starch gel strip. (Smithies, 1959). In some instances, 100 µl of serum was incubated for 1 hour with 5 µl of a solution of ferric citrate labelled with ^{59}Fe, prior to electrophoresis. After electrophoresis, the stained starch gel strips were each cut into sections containing the various protein bands, and each piece of starch gel was counted individually either in the TOBOR large volume gamma scintillation counter for 10 minutes, or in the Nuclear-Chicago Model 1085 automatic well type gamma scintillation counter for 5 minutes.

i) Effects of treatment:

The effect of administration of various haematinic agents was studied. These were administered by intra-peritoneal injection under light ether anaesthesia.

Estimations of the haematocrit were made before, and at varying intervals after, injection of the therapeutic agents, according to the method given earlier in this chapter.

j) Transplantation studies:

As part of a wider study (Bennett, M., Pinkerton, P. H., Cudkowicz, G., Bannerman, R. M. in preparation) of the behaviour of haemopoietic cells transplanted from X-linked anaemic mice to normal, lethally-irradiated hosts, a number of the irradiated animals which had received transplants of normal and sla/- haemopoietic tissue were preserved to see if such transplanted tissue from sla/- donors could, in a normal (+/-) environment from which endogenous haemopoietic tissue had been ablated, produce morphologically normal erythocytes. Preliminary experiments indicated that the dose of X-rays (see below) given to recipient mice was sufficient completely to destroy the endogenous haemopoietic tissue, and that the erythrocytes subsequently produced in the recipient stemmed from the grafted donor haemopoietic cells. In these preliminary experiments, donor haemopoietic cells from fourth generation backcross donors (original mixed stock to C57Bl/6 Jax strain), producing red cells with a "single" haemoglobin pattern on starch gel electrophoresis (Hb^1/Hb^1) were grafted into irradiated B10.129 (5M) and

129 RR mice, selectively bred for the "diffuse", haemoglobin pattern ($\underline{Hb}^2/\underline{Hb}^2$) (see chapter 1). The red cells subsequently produced exhibited a "single" haemoglobin electrophoretic pattern, showing that the erythrocytes produced in recipients after irradiation were indeed of donor, and not of endogenous (recipient), origin. The belief that the red cells in irradiated recipients were of donor, and not endogenous, origin received further confirmation from the demonstration that the electrophoretic pattern of red cell esterases in donor and recipient animals differs, and that the red cells produced in the irradiated recipients exhibited the enzyme pattern of the donor animals (Edwards, 1967).

In the experiments described here, donor male animals of normal (+/-) and anaemic ($\underline{sla}$/-) genotype from the fourth backcross generation of the original mixed stock to C57Bl/6 Jax mice were used. The recipients were 10-12 weeks old lethally irradiated C57Bl/10 Jax mice which had previously been shown to accept readily marrow and spleen grafts from such donors. (Bennett et al., in preparation).

Cell suspensions were prepared from donor mice bone marrow by severing the ends of bones and flushing the bone marrow cavity with chilled Eagle's medium; spleen cells were harvested by piercing the capsule, and then expressing the cells by gently applying pressure with blunt forcepts while flushing the spleen with Eagle's

medium. Particulate matter was removed by filtering cell suspensions through stainless steel gauze (200 mesh/inch). Nucleated cell counts were performed with Coulter particle counter (model B) and the cell suspensions adjusted to the desired concentration by dilution with Eagle's medium.

Prospective recipient mice were exposed to 750-780 R of X-rays. Within four hours they were grafted with marrow or spleen cells from normal or anaemic (sla/-) mice by intravenous injection. Four groups of 10 recipient mice received a) 7.2×10^6 normal bone marrow cells, or b) 1.9×10^6 anaemic (sla/-) bone marrow cells, or c) 8.96×10^6 anaemic spleen cells, or d) 9.0×10^6 normal spleen cells. In group a) 5 animals survived, in group b) all 10 survived, in group c) 2 mice survived and in group d) all 10 mice died. One hundred and sixteen days after grafting of bone marrow and spleen cells, haemoglobin and haematocrit estimations, and blood film examination were carried out, by methods described earlier in this chapter, on the 17 surviving recipients.

In the experiments described above, Dr. Bennett was responsible for irradiation of the hosts and, together, we prepared and injected the donor cell suspensions. The haematological assessment of the surviving grafted recipient animals was performed by me 116 days after transplantation of haemopoietic tissue.

k) Statistical analyses have been carried out according to Bradford Hill (1966).

l) Summary:

The methods and materials employed in the investigation of the X-linked anaemia of mice have been described. Where these methods have been standard and widely used, little detail is given, other than to describe any modifications which have been made to adapt them for use in mice. In other instances, full descriptions of the techniques employed are included.

The origin of the animals investigated has also been described in detail.

CHAPTER 5

The Peripheral Blood in X-Linked Mouse Anaemia

The haematological picture of X-linked mouse anaemia will now be described in detail. Measurements have been made of haemoglobin (Hb) concentration, packed cell volume (P.C.V.), mean corpuscular haemoglobin concentration (M.C.H.C.), red cell count, mean cell volume (M.C.V.), extent of morphological abnormality of the red cells in blood films, red cell diameter, reticulocyte and polychromatophilic cell counts, and red cell osmotic fragility. Total and differential white cell counts and platelet counts have also been performed and body weights have been recorded. The individual measurements used in assessing the anaemia are recorded in detail in Appendix A. As described more fully in the previous chapter, standard techniques in human haematology have been employed or adapted for use on mice.

Serial observations of Hb concentration, P.C.V., M.C.H.C. and body weight have been made at arbitrary intervals from 3-4 weeks of age onwards, until about 400 days of age, in 64 anaemic male (sla/-), 45 anaemic female (sla/sla), 69 heterozygous female (sla/+) and 28 normal (+/-) male mice of the original mixed stock. Approximately half of the observations of Hb concentration, P.C.V., M.C.H.C. and body weight were obtained by Drs. R. M.

Bannerman and R. G. Cooper and they have kindly permitted me to analyse them in conjunction with the remaining data used in this chapter which have been accumulated by me. All the observations presented in this section with the exception of some of the red cell counts, and estimates of mean cell volume and median corpuscular fragility have been obtained on mice of the original mixed stock. These exceptions are indicated in appendix A. The necessity of using hybrid animals for some investigations, and the reasons for the small number of normal (+/-) animals of the original mixed stock available, have been explained in the preceding chapter.

For the purposes of presentation the results of measurements of Hb concentration, P.C.V., and M.C.H.C. have been summarized over 10 day periods for the first 100 days of life and for 50 or 100 day periods thereafter. The anaemia in young hemizygous (sla/-) and homozygous animals is severe at 30-40 days of age; the Hb concentration usually lies between 5 and 8 G/100 ml. Thereafter the Hb level tends to rise, so that by 300 days of age, the normal values are approached. In the normal animals by contrast, there is a gradual decline in the haemoglobin concentration over the same time; this has been observed by others in normal mice (Strong and Francis, 1940; Ewing and Tauber, 1964). Young heterozygous (sla/+) females may show an Hb concentration rather lower than that of normal males,

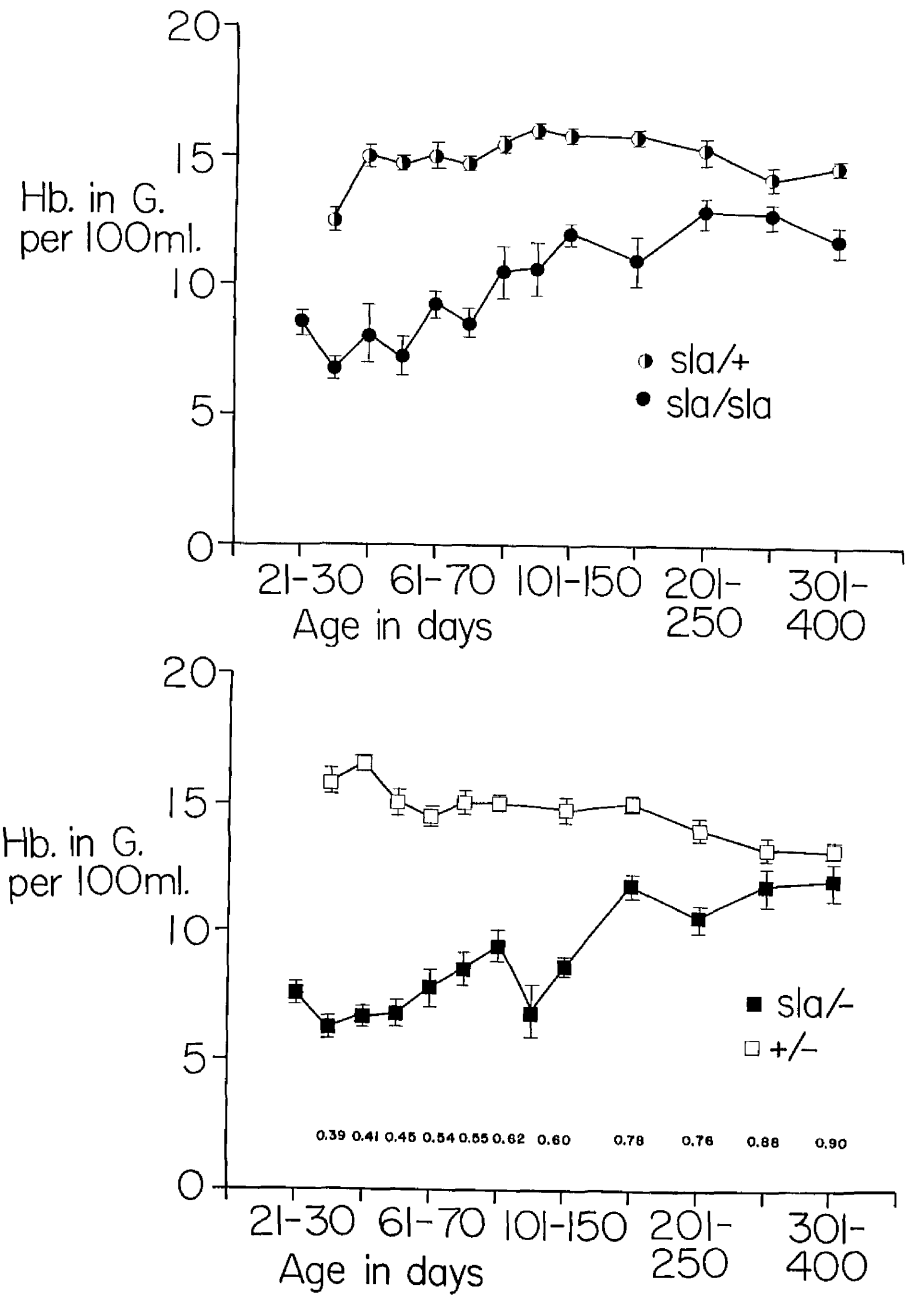


Fig. 5.1: Changes in haemoglobin concentration with age in normal (+/-) and anaemic male (sla/-) mice (lower half) and heterogygous carrier (sla/+) and anaemic (sla/sla) female mice. Each point represents the mean value ±1 standard error of the mean (S.E.), and the ratio of the anaemic/normal means for males is given below each pair of results.

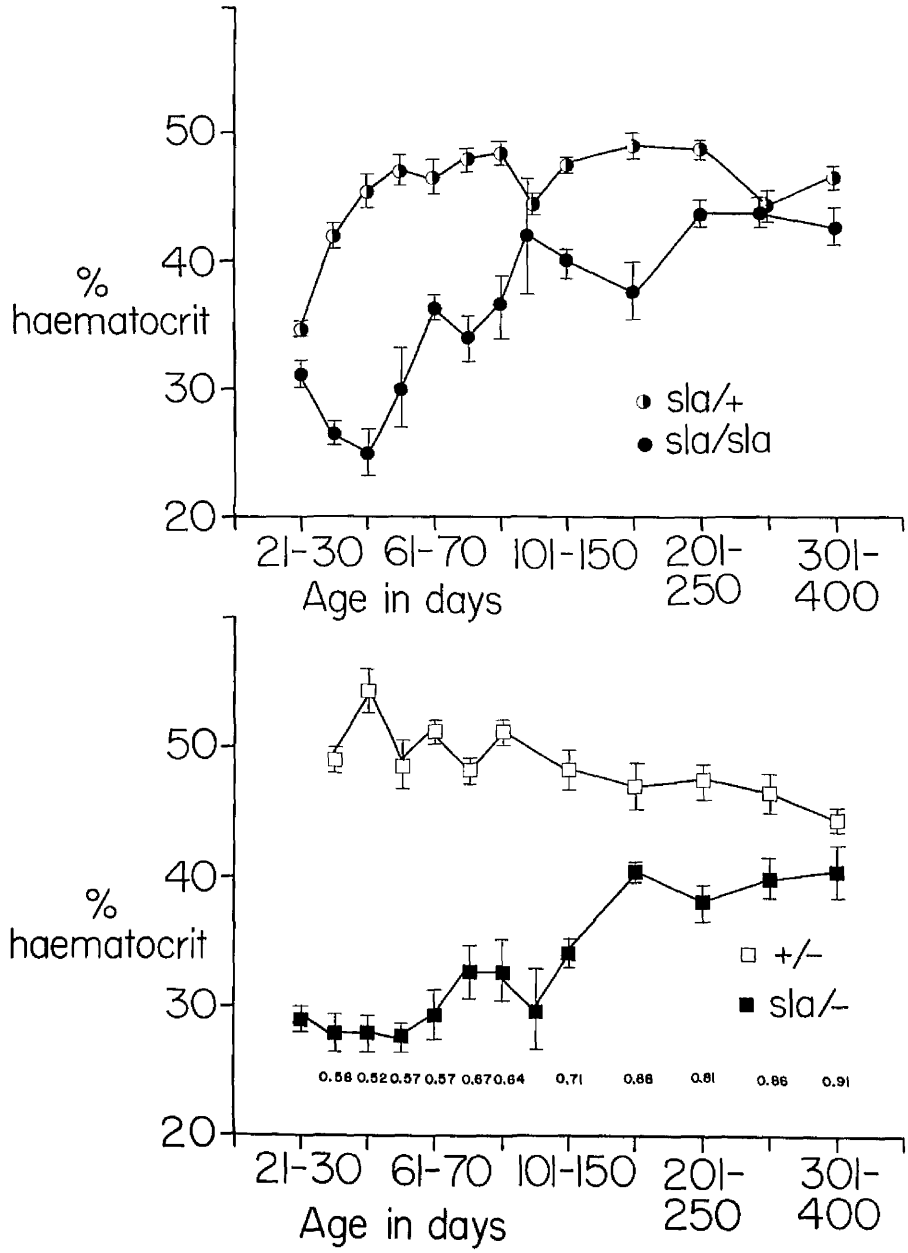


Fig. 5.2: Changes in packed cell volume (P.C.V.) with age in normal (+/-) and anaemic (sla/-) male mice (lower half), and heterozygous carrier (sla/+) and anaemic (sla/sla) female mice. Each point represents the mean value ± 1. S.E. and the ratio of the anaemic/normal means for males is given below each pair of results.

but this difference is not apparent after the age of 40 days. These features are illustrated in Figure 5.1. The improvement in the anaemia with increasing age, seen here for mice presented in groups, can, of course, be observed to occur in individual animals. This is typified in the record card illustrated in Chapter 4 (Figure 4.3).

Parallel changes occur in the P.C.V. with age (Figure 5.2). However, the reduction in the P.C.V. is proportionately less than that in the Hb concentration, as may be seen by comparing the ratios of these two parameters for anaemic and normal mice at various ages. The values obtained for the M.C.H.C. are accordingly low (Table 5.1), but these, too, tend to return to normal with advancing age. No change in the M.C.H.C. has been detected in heterozygous female (sla/+) carriers.

The results of red cell counts, grouped over 100 day age periods, are given in Table 5.2; the reduction in young anaemic mice is comparatively slight and is proportionately much less than that in the Hb concentration or in the P.C.V. This, again, is reflected in ratios of the red cell counts of anaemic and normal mice. In older mice, over 200 days, no difference is apparent between normal and anaemic mice. The mean cell volume is reduced in anaemic mice, but this index increased with age. Values for the M.C.V. are illustrated, again grouped over 100 day

periods, in Figure 5.3. Carrier (sla/+) females show no detectable deviation from normal in respect of the red cell count or M.C.V.

The results obtained for the red cell indices in anaemic male (sla/-) and female (sla/sla) animals are typical of hypochromic, microcytic anaemia, with reduction in the M.C.H.C. and M.C.V.

Inspection of blood films reflects the changes described above. The red cells show hypochromia, microcytosis, anisocytosis, poikilocytosis, leptocytosis and fragmentation. These changes are illustrated in Figures 5.6 - 5.9; blood films from a young normal male and young carrier female are included for comparison (Figures 5.4 and 5.5). An assessment of the degree of morphological change in the red cells is presented in Figures 5.10 and 5.11. The "film-score", derived as described in Chapter 4, is higher, the more abnormal the appearance of the erythrocytes. This index is plotted against age in Figure 5.10 and the trend of improvement with age is again seen; blood smears from older anaemic male and female mice may show little or no abnormality. Occasionally, young carrier female (sla/+) mice show film scores above the upper limit of normal, but the changes are inconstant, and diagnosis of the carrier state from blood films has not proved possible. The majority appear normal and the few which show minor

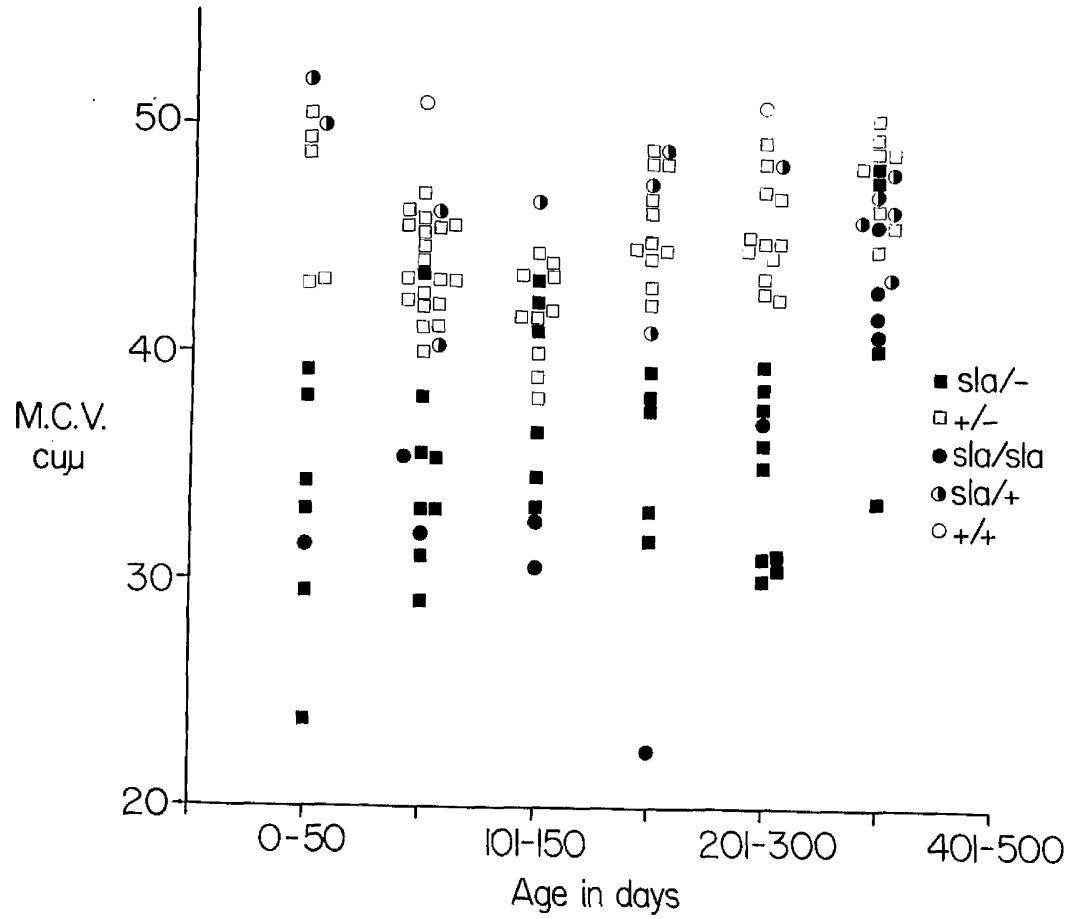


Fig. 5.3: Mean cell volumes (M.C.V.) in anaemic, carrier and normal mice, showing reduction of the M.C.V. in young anaemic mice, with progressive increase towards normal with age. M.C.V.s were estimated from either a) red cell size plots, or b) red cell counts and P.C.V. (see Chapter 4).

Fig. 5.4: Blood film from a normal male mouse at age 33 days, showing uniform size and haemoglobinization of the red cells. Wrights stain x 1400.

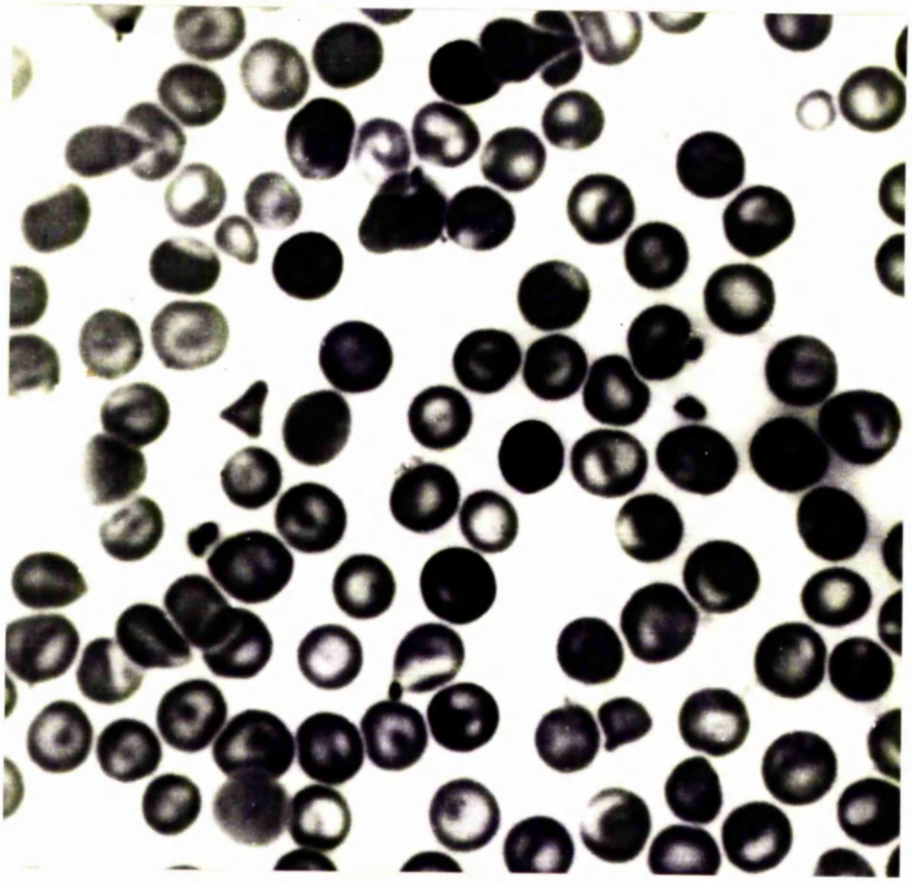


Fig. 5.5: Blood film from a female carrier (sla/+) at age 33 days showing very mild anisocytosis and hypochromia with occasional small mishapen cells and poorly developed target cell formation. Wrights stain x 1400.

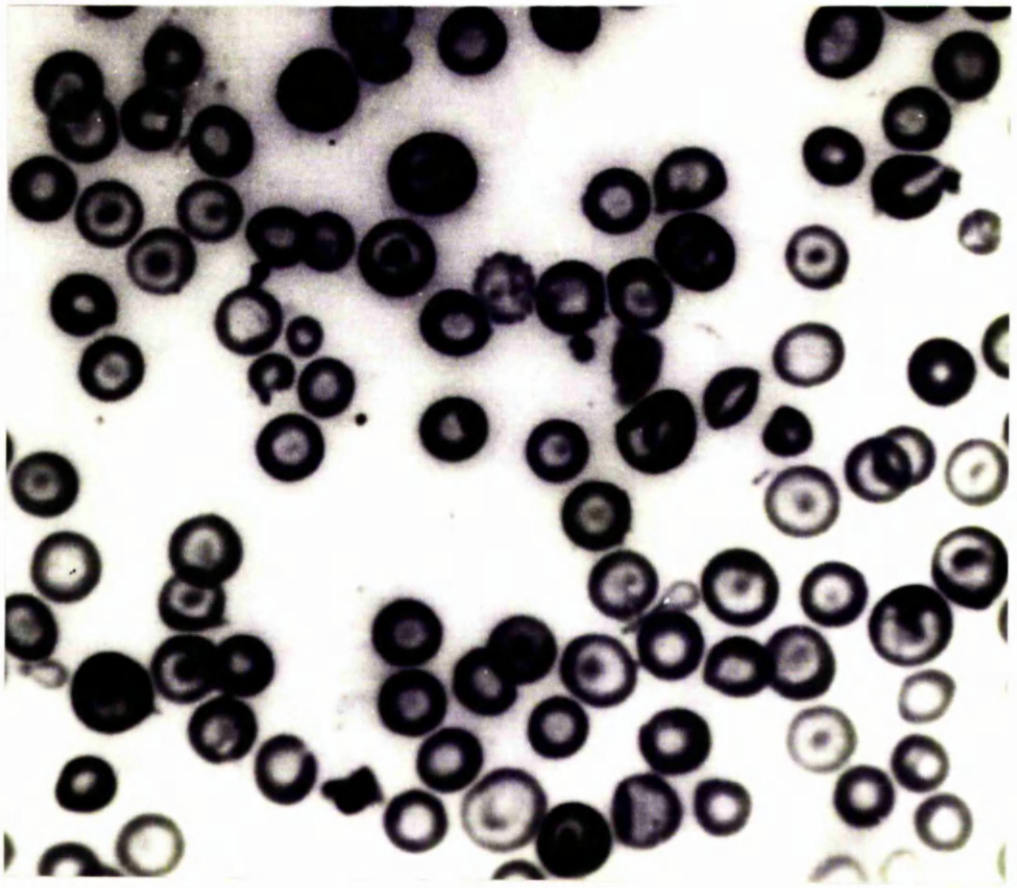


Fig. 5.6: Blood film from an anaemic male (sla/-) mouse at age 33 days, showing hypochromia, anisocytosis, target cells and small mishapen cells. The intensely staining cells are polychromatophilic. Wrights stain x 1400.

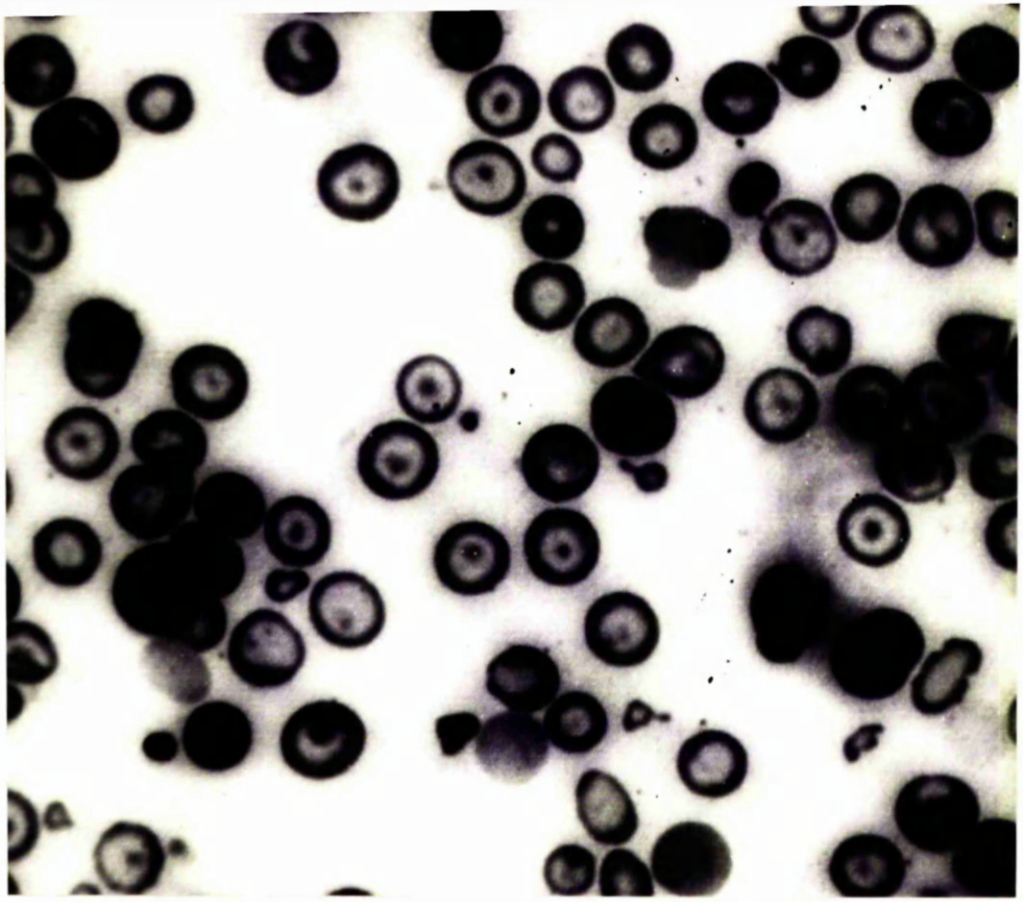


Fig. 5.7: Blood film from an anaemic male (sla/-) mouse showing changes similar to those seen in Fig. 5.6. Wrights stain x 1400.

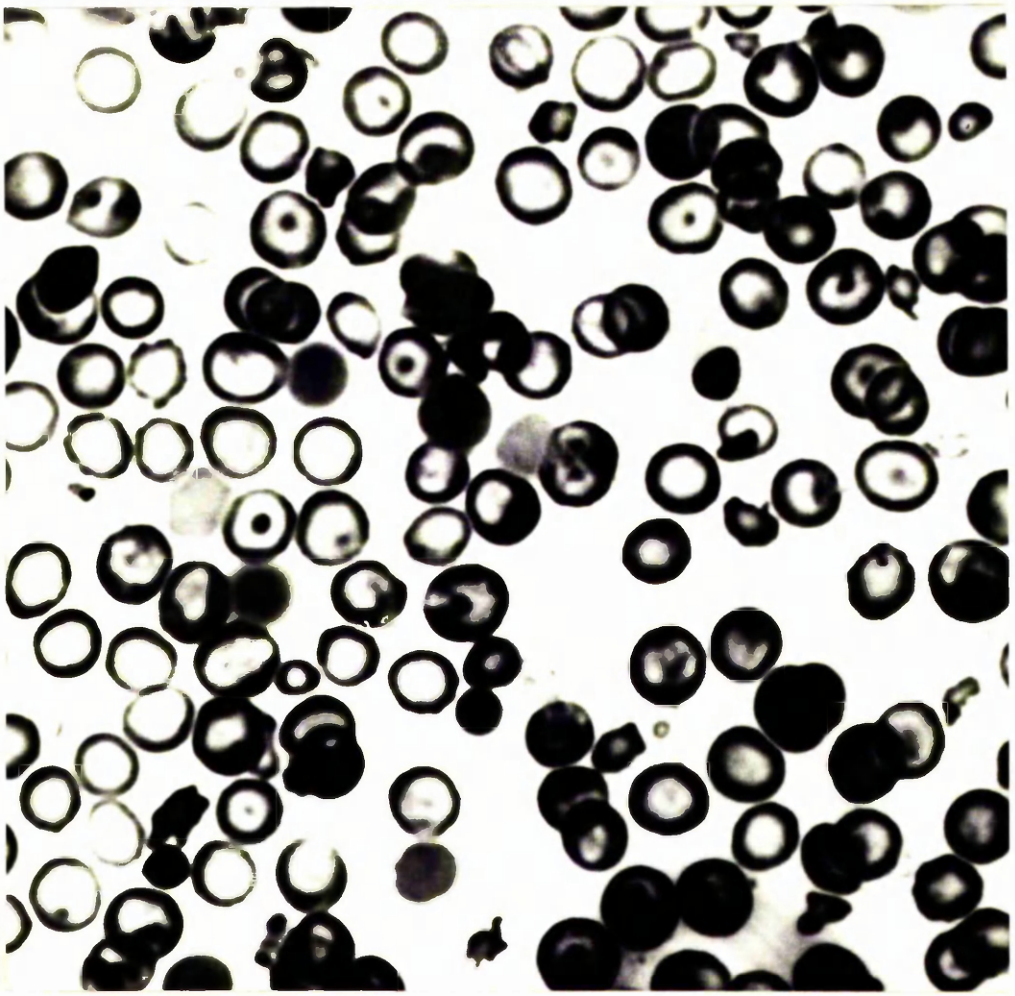


Fig. 5.8: Blood film from an anaemic male (sla/-) mouse, aged 100 days, again showing similar changes to those seen in Figs. 5.6 and 5.7. Wrights stain, x 1400.

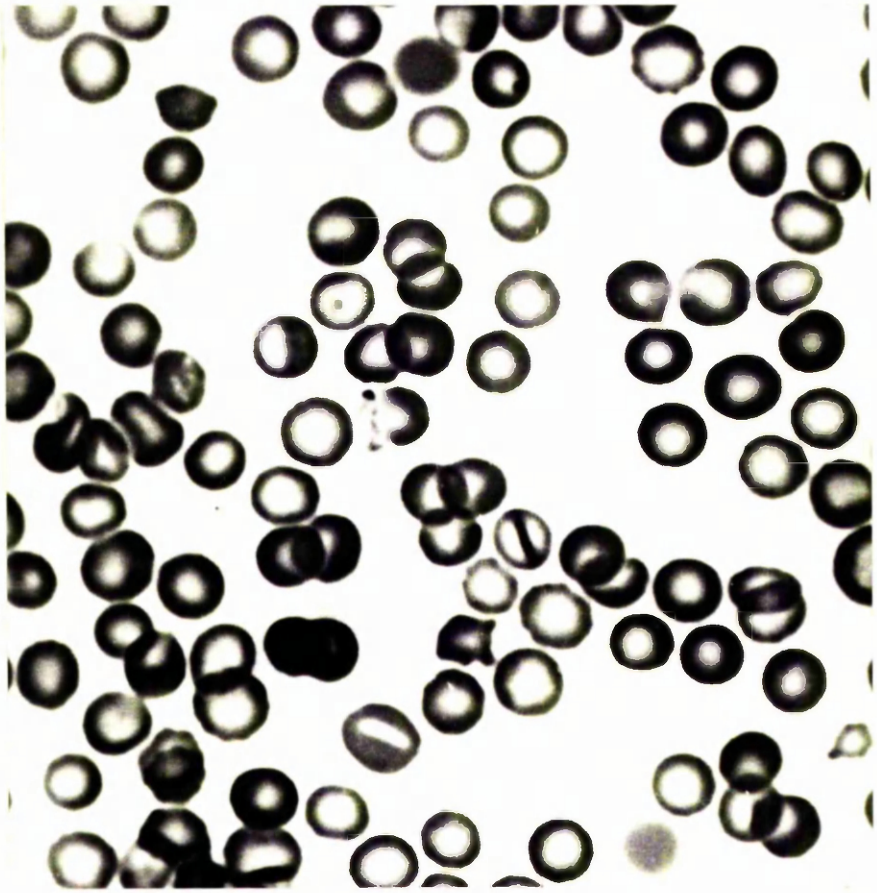


Fig. 5.9: Blood film from an anaemic male (sla/-) mouse aged 300 days, showing only comparitively mild abnormalities. Wrights stain x 1400.

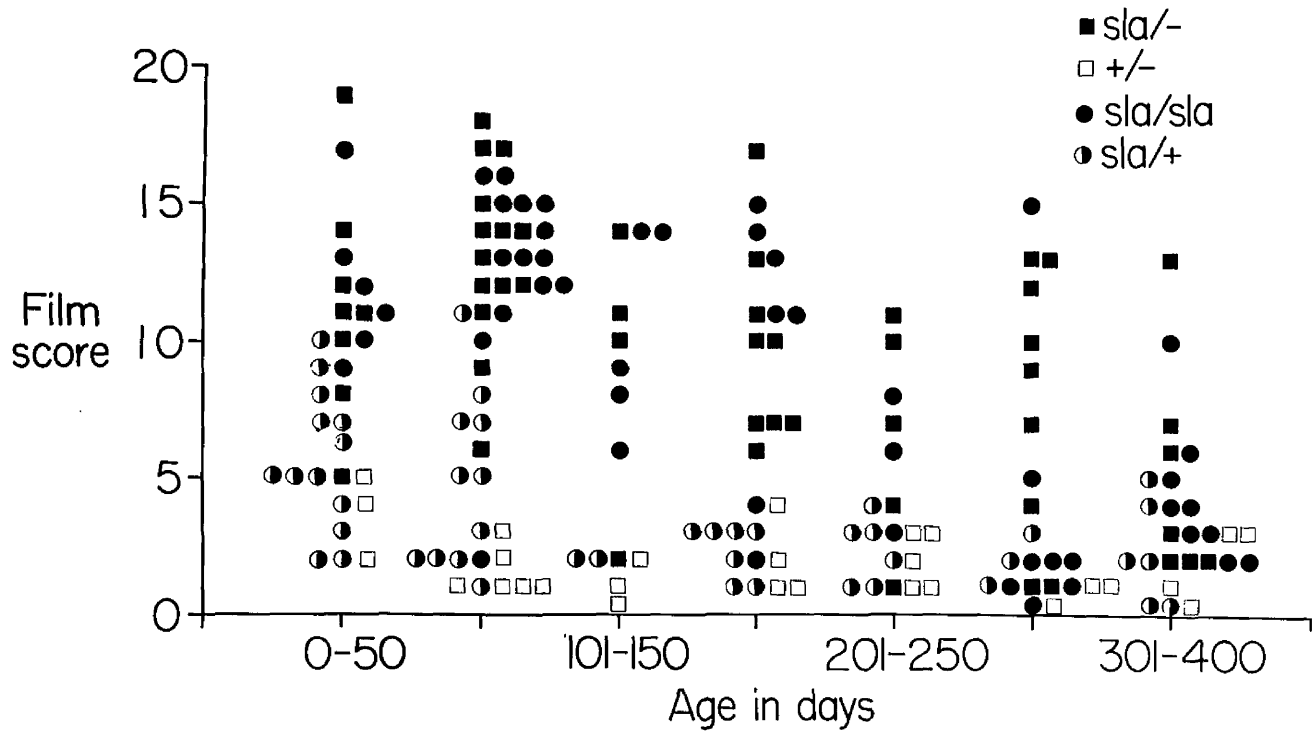


Fig. 5.10: The effect of age on the degree of red cell abnormality by film "score"; a trend of decreasing severity of morphological change is seen with increasing age.

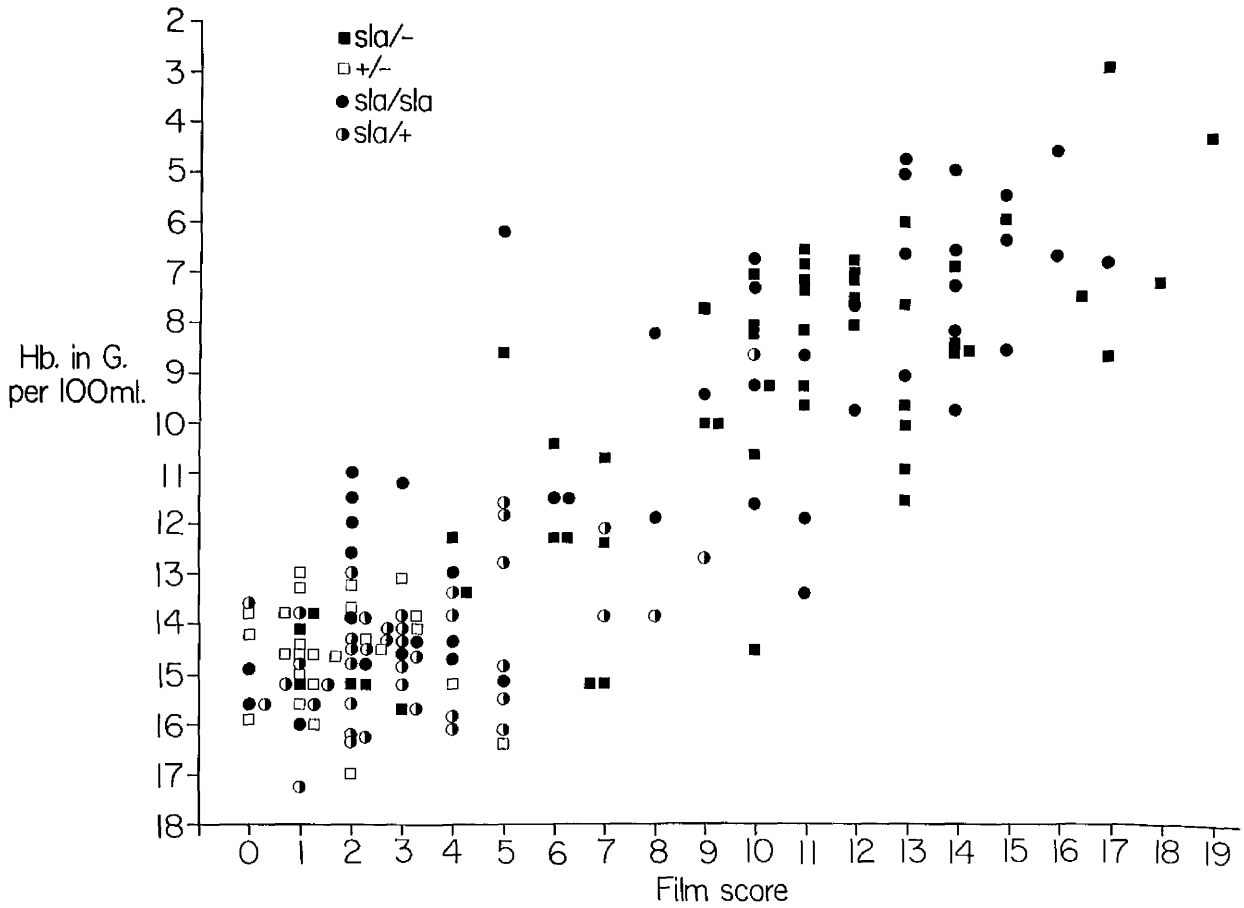


Fig. 5.11: Correlation of the severity of anaemia and the degree of red cell abnormality observed in the blood film.

morphological changes (Figure 5.5) are indistinguishable from mildly affected (usually older) anaemic animals in respect of red cell morphology (Figure 5.9). In no instance did any carrier (sla/+) female mouse exhibit a clear duality of red cell population.

In general, there is good correlation between the severity of the anaemia and the degree of red cell abnormality in the blood film, as may be seen in Figure 5.11, where the film score has been plotted against the degree of anaemia as reflected by the Hb concentration. The details of the individual film scores are given in appendix A.

Measurements of the red cell diameters in anaemic male and female, carrier female and normal male mice is shown in Table 5.3, and the presence of microcytosis is confirmed in anaemic animals. While occasional microcytes may be seen in heterozygous (sla/+) carriers, the distribution of the red cells generally conforms to that seen in normal animals and there is nothing to suggest a double, microcytic and normocytic, red cell population.

Typical examples of red cell volume distribution curves derived from red cell volume "plots" made with the Coulter model J particle size analyser are given in Figure 5.12, and a clear difference is seen between normal and anaemic mice; microcytosis is again demonstrated in the latter. Figure 5.13 shows curves obtained from a normal male (+/-) mouse, anaemic female (sla/sla) mouse

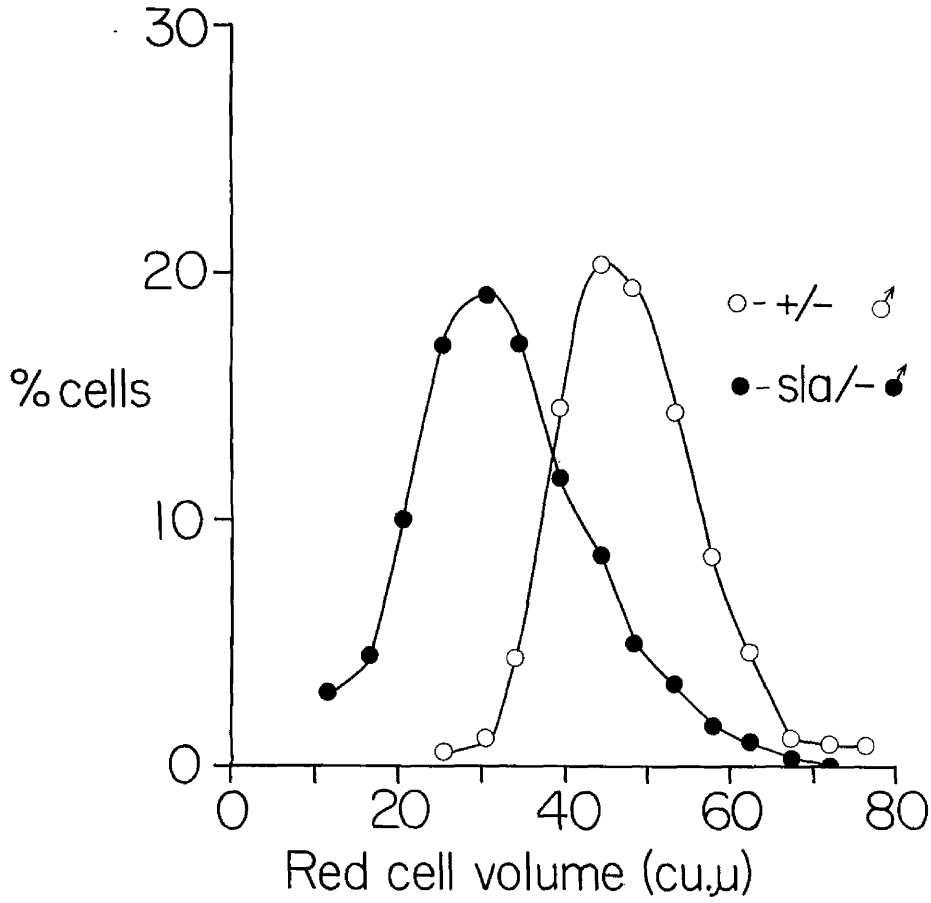


Fig. 5.12: Distribution of the red cell population in relation to the red cell volume. There is clear reduction in anaemic (sla/-) mice, as compared with normal (+/-) animals.

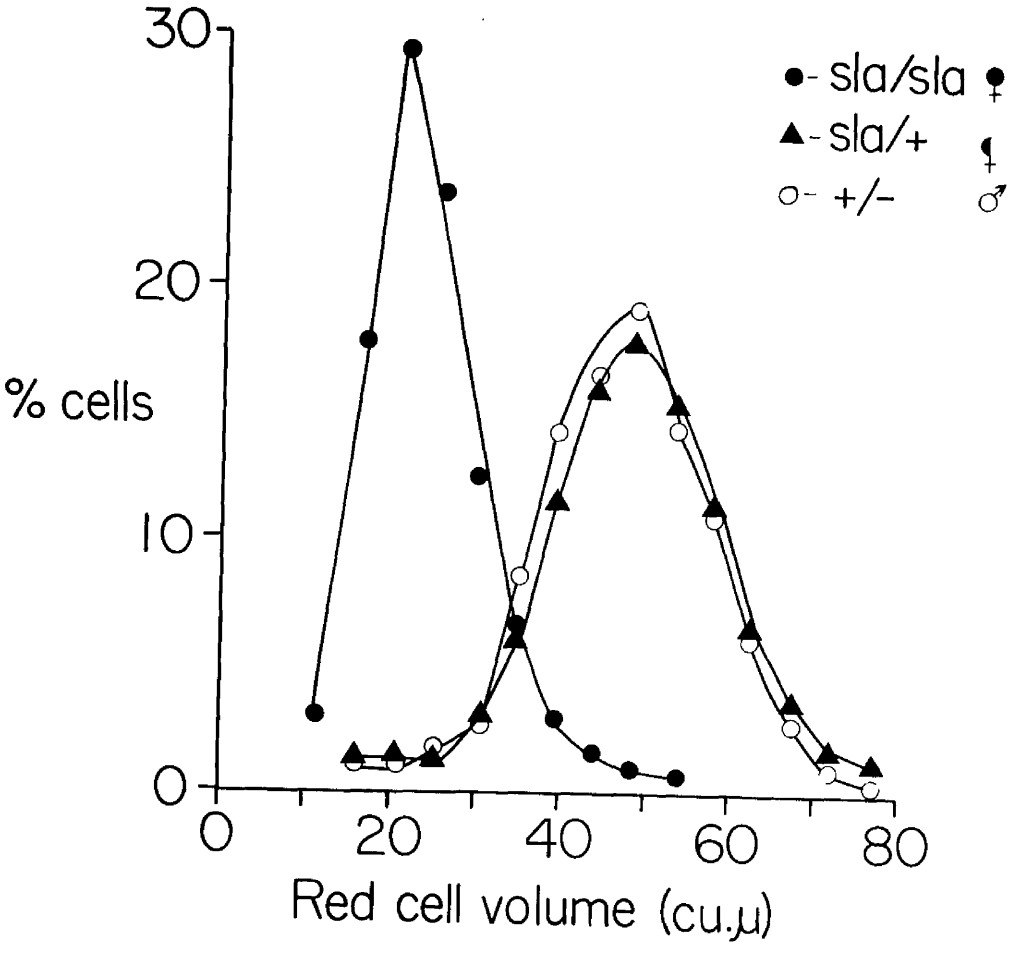


Fig. 5.13: Distribution of the red cell population in relation to the red cell volume. There is again clear reduction in anaemic mice (sla/sla) with no differences appreciable when normal (+/-) and heterozygous carrier female (sla/+) mice are compared. No evidence to suggest a dual population of red cells in the heterozygous carriers is seen.

and a heterozygous carrier female (sla/+) animal. The curve obtained for the heterozygote closely resembles that obtained for the normal animal and again evidence suggesting a dual population of red cells is not present. Photo-copies of representative examples of the original plots of red cell volume distribution obtained from the particle size analyser are included at the end of Appendix A. These are taken from two normal male, two anaemic female and two carrier female mice, and from one old male mouse of anaemic genotype, in which the anaemia had undergone spontaneous remission.

The results of reticulocyte counts in normal, carrier female, and anaemic mice are summarised in Table 5.4, and given in detail in Appendix A. The majority of anaemic mice give results within the normal range. However, a moderate reticulocytosis is quite common, and occasionally the increase in reticulocytes may be considerable, and accompanied in the blood film by a degree of polychromatophilia far exceeding that normally seen in mice. The counts of polychromatic erythrocytes frequently exceeded the number of reticulocytes counted in the same sample, but a general correlation (correlation coefficient, $r = +0.66$) was found between the numbers of reticulocytes and polychromatophilic cells (see Appendix A). There was also a significant correlation between the extent of the reticulocytosis in anaemic animals, and the degree of anaemia. This is reflected in the negative coefficient of correlation (r)

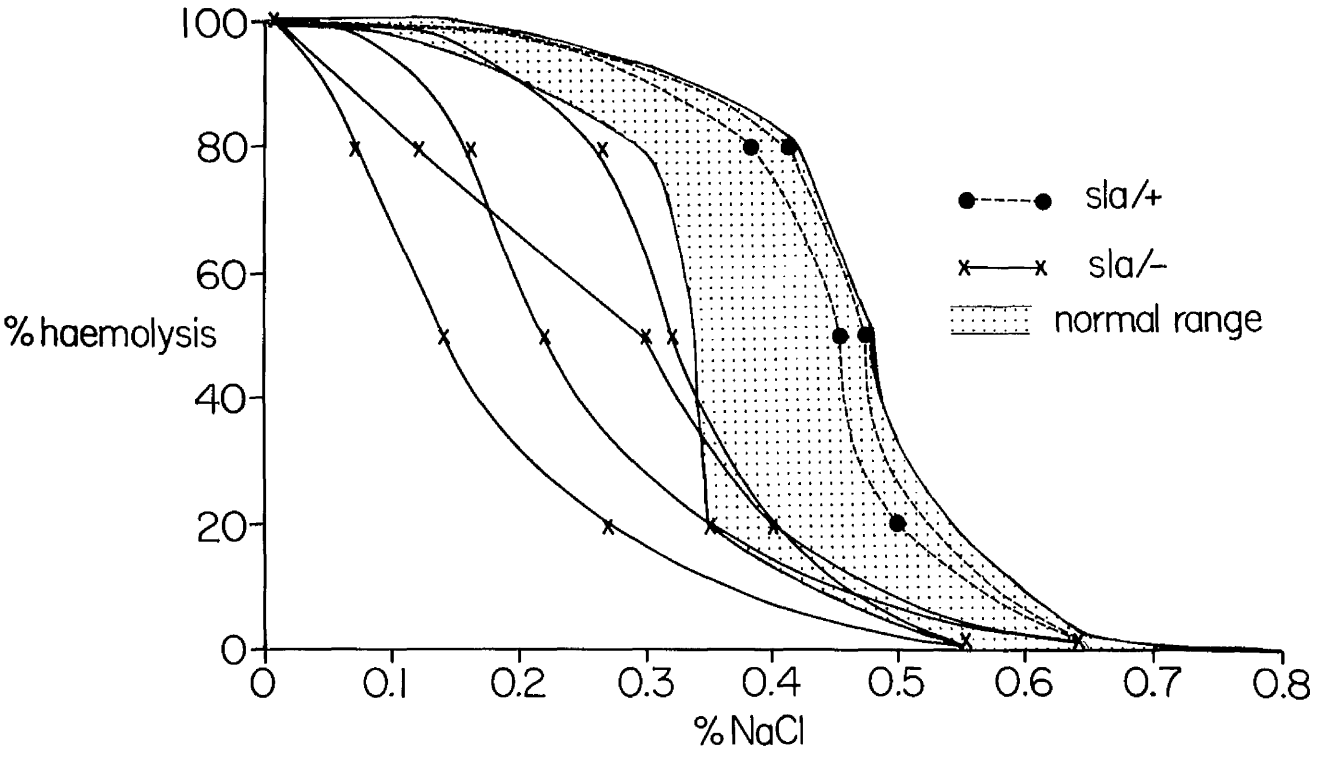


Fig. 5.14: Red cell osmotic fragility curves. Anaemic male (sla/-) mice show a "shift" in the direction of increased osmotic resistance. Curves for heterozygous carrier animals (sla/+) conform to the normal pattern.

between the reticulocyte count and the packed cell volume in anaemic mice ($r = -0.48$).

Siderocytes were sought by examining 1000 red cells in each of 20 anaemic (sla/- and sla/sla), 12 heterozygous carrier (sla/+), and 9 normal (+/-) adult mice; none was found.

Estimates of median corpuscular fragility (M.C.F.) were made in 9 normal (+/-), 7 anaemic (sla/-) and 2 heterozygous (sla/+) carrier female mice. The range for normal mice was 0.34 - 0.46% NaCl with a mean value of 0.41% NaCl, and a standard error of 0.013. In the anaemic animals the range was 0.14 - 0.38% NaCl with a mean of 0.27% NaCl and a standard error of 0.031. The difference of the means is significant ($p < 0.005$). The two heterozygous female mice gave values within the normal range. Representative osmotic fragility curves are presented in Figure 5.14. The shift to the side of increased osmotic resistance is clearly seen in the anaemic animals. The curves for heterozygous carrier females conform to the normal pattern and do not show a "tail" of osmotically resistant cells; thus, by this criterion, evidence of heterogeneity of the red cell population in sla/+ mice is lacking. Fuller details of the estimates of osmotic fragility are given in appendix A.

Total and differential white cell counts were performed in 8 anaemic and 10 normal mice, and platelet

counts in 8 anaemic and 9 normal mice. Significant differences were not found when the two groups were compared (Table 5.5).

Weight gain and growth are retarded in anaemic male mice which are lighter than normal at all ages (see Figure 5.15), but no obvious correlation has been detected between the severity of the anaemia and the degree of growth retardation ($r = -0.157$). Heterozygous (sla/+) and homozygous (sla/sla) female mice show little difference in their rate of weight gain (Figure 5.15); insufficient normal (+/+) females of the same genetic background could be definitely identified for valid comparison with the sla/+ and sla/sla animals. Details of the recorded data on body weight are available in appendix A.

SUMMARY:

The haematological features of X-linked mouse anaemia are described. The findings are typically those of a microcytic, hypochromic anaemia, with reduction in haemoglobin concentration proportionately greater than that in the packed cell volume; the red cell count is only slightly reduced in younger anaemic mice and is normal in older anaemic animals. The morphological appearances of the red cells are in keeping with the estimates of the red cell indices. Anaemia may occasionally be accompanied by a mild or moderate reticulocytosis and an increase in the number of polychromatophilic cells seen in blood films.

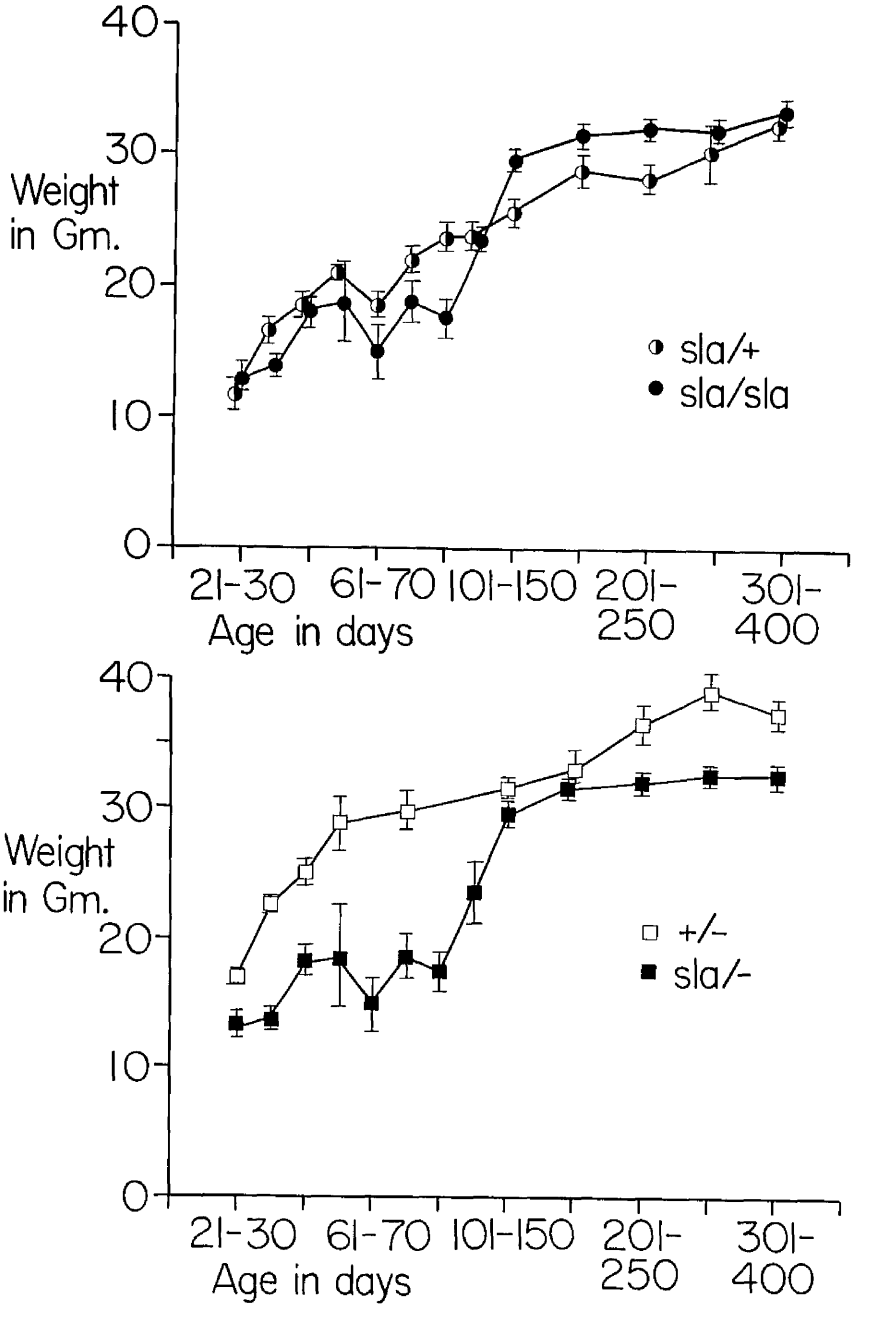


Fig. 5.15: Change in body weight with age in normal (+/-) and anaemic (sla/-) male mice (lower half), and in carrier (sla/+) and anaemic (sla/sla) female mice. Means ± 1 S.E. Anaemic male mice show retardation of growth in comparison with normal, but little difference is seen when carrier and anaemic females are compared.

Siderocytes are not found and resistance to osmotic lysis is increased in anaemic animals. Spontaneous improvement in the anaemia occurs with age.

Total and differential white cell counts and platelet counts are normal in anaemic mice.

While heterozygous female (sla/+) animals may occasionally show minor morphological alterations in the red cells and may have a slightly lower than normal Hb concentration and packed cell volume early in life, in general the haematological findings in animals of this (sla/+) genotype conform to the normal and evidence of a duality of red cell population has not been detected.

The anaemia is associated with retarded growth and weight gain. This feature is probably also present in the heterozygous female animal.

CHAPTER 6

Pathological Appearances and the Stainable Iron Stores

Histochemical examination of the extent of the stainable tissue iron stores has been undertaken, and tissue changes which might indicate directly or indirectly, the site of action of the primary, genetically controlled, defect in X-linked mouse anaemia have been sought. Tissues were studied from 43 mice with X-linked anaemia (sla/- and sla/sla), from 14 heterozygous carriers (sla/+) and from 41 genetically normal (+/-) control animals; spleen, liver, bone marrow, stomach, small bowel, pancreas, heart, lung, kidney and testes were examined, and smears of femoral bone marrow and imprints of the cut surface of the spleen were taken from some. The size of the spleen, as absolute weight, and as a percentage of body weight was recorded at the time of sacrifice in 66 mice. The proportion of splenic tissue occupied by red pulp, the extent of the splenic iron stores and the quantity of iron deposition in the duodenal mucosa have been recorded as defined in Chapter 4. The amount of stainable iron in other tissues has proved to be small, if present at all, and it has simply been recorded as present or absent. Full details of the histological examination of the tissues from each of the 98 mice examined are included in Appendix B.

Spleen:

Splenomegaly is a frequent but inconstant finding in X-linked mouse and the enlargement of this organ may occasionally be considerable (Fig. 6.1). The results of analysis of spleen weights are given in Table 6.1, expressed both as a percentage of body weight and in absolute measurements; detailed results for individual animals are given in Table 6.2. There is a good correlation between the degree of splenic enlargement and the severity of the anaemia as reflected by the haematocrit ($r = -0.60$) (Figure 6.2). The increase in splenic size appears to result from hyperplasia of the red pulp; the proportions of red pulp in anaemic, carrier and normal mice are shown in Tables 6.3 and 6.4 and are plotted against the splenic weight, as a percentage of body weight, in Figure 6.3. There is a tendency towards an increasing proportion of red pulp with increasing spleen weight. No significant differences are seen when the spleen sizes of normal (+/-) and carrier female (sla/+) mice are compared (Table 6.1).

Histologically, the spleens of normal (+/-), carrier (sla/+) and anaemic (sla/- and sla/sla) mice all show active erythropoiesis and granulopoiesis; megakaryocytes are present and are sometimes numerous. Haemopoiesis is confined to the red pulp, which, as described above, is hyperplastic in the anaemic group. Differences in

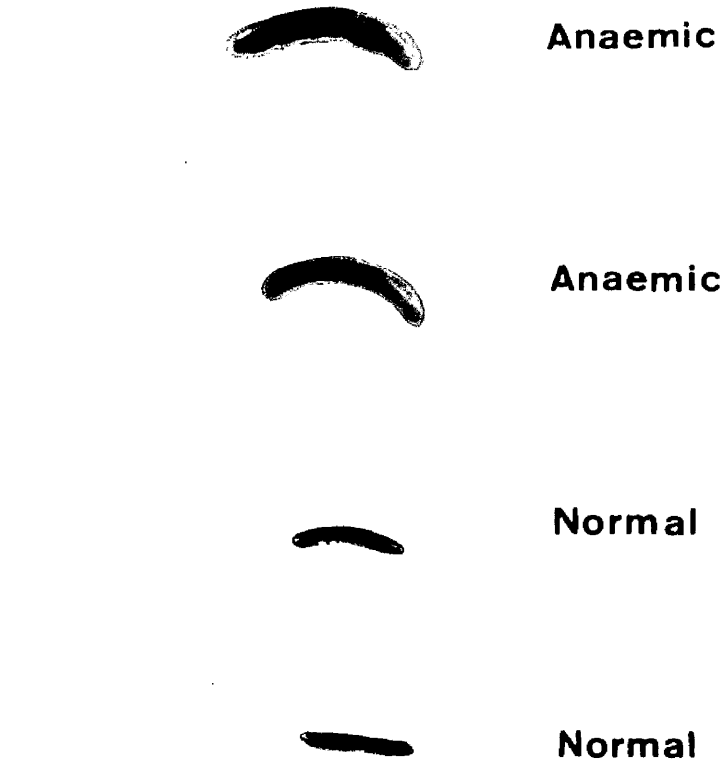


Fig. 6.1: Splenomegaly in X-linked anaemia of mice. The spleen from 4 male littermate mice are shown. The upper 2 spleens are from anaemic (sla/-) mice, and the lower 2 from normal (+/-) animals.

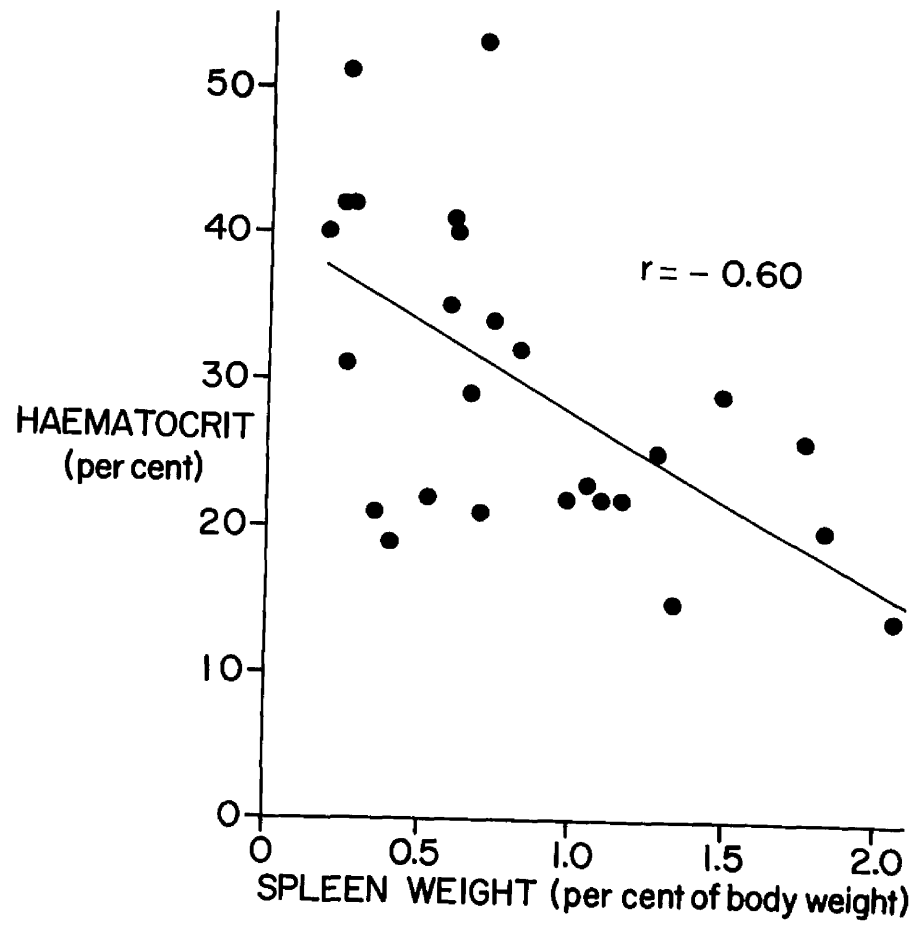


Fig. 6.2: Spleen weights of anaemic mice of genotype sla/sla, or sla/-, expressed as percentage of total body weight, and related to the degree of anaemia as reflected by the haematocrit. There is a negative correlation ($r = -0.60$) between the level of the haematocrit and the size of the spleen, and the line represents the regression of y (haematocrit) on x (spleen weight).

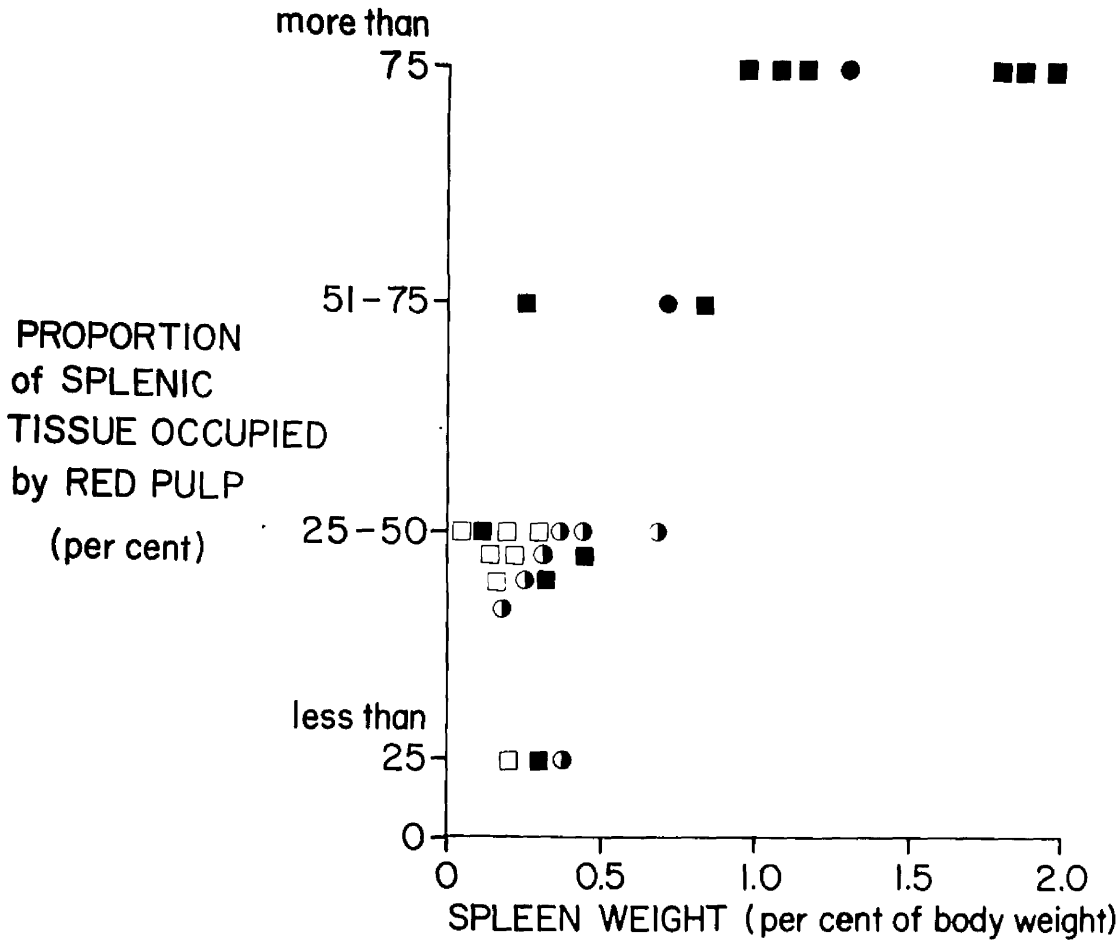


Fig. 6.3: The proportion of splenic tissue in the form of red pulp, as assessed visually (see Chapter 4), related to the weight of the spleen as a percentage of total body weight, for normal male (+/-), carrier female (sla/+) and anaemic male (sla/-) and female (sla/sla) mice. The open squares represent normal male mice, the closed ones anaemic male mice; the half-closed circles indicate heterozygous carrier females, and the closed circles, anaemic females.

the white pulp could not be detected when normal and anaemic mice were compared.

Spleen imprints from 11 anaemic (sla/-) and 22 normal (+/-) mice were examined and the ratio of normoblasts to metamyelocytes and granulocytes estimated. In the anaemic mice the mean ratio was 5.6 with a range of 1 - 17.2 and a standard error of 1.32; for normal animals, the mean ratio was 3.3 with a range of 0.7 - 9 and a standard error of 0.34. The difference of the means is significant at the 5% level. In none of these animals, anaemic or otherwise, were iron-containing erythroblasts (sideroblasts) identified.

The stores of stainable iron in the spleen of anaemic (sla/- or sla/sla) mice are depleted (Table 6.5). The majority show no stainable iron at all (Figure 6.4), and the remainder show only a very few reticulo-endothelial cells containing haemosiderin. All the normal animals had abundant stainable iron in their spleens (Figure 6.5), and no appreciable effect of age was seen. The iron in the spleens of normal mice was distributed in both the red pulp and the malpighian bodies; in 15 the former was more heavily laden, and in 6 the latter; in the remaining 4, haemosiderin was equally concentrated in these two sites. In the anaemic mice, the stainable iron in the spleen, when present, was located in the red pulp in 2, in the malpighian bodies in 5 and in both sites in one.

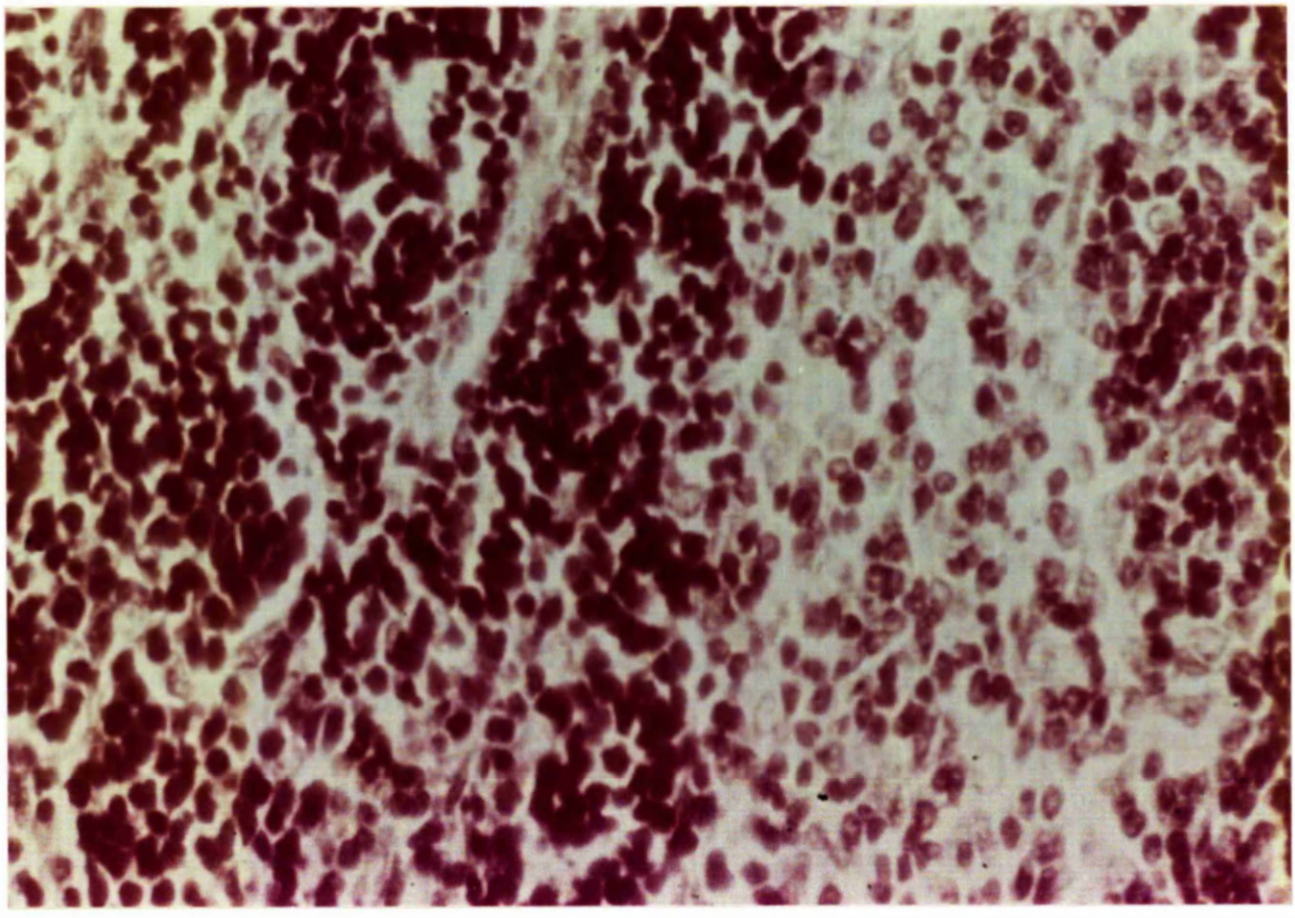


Fig. 6.4: Section of spleen from an anaemic male (sla/-) mouse showing the absence of stainable iron. (Prussian Blue reaction, counterstained with 1% neutral red, x 1200).

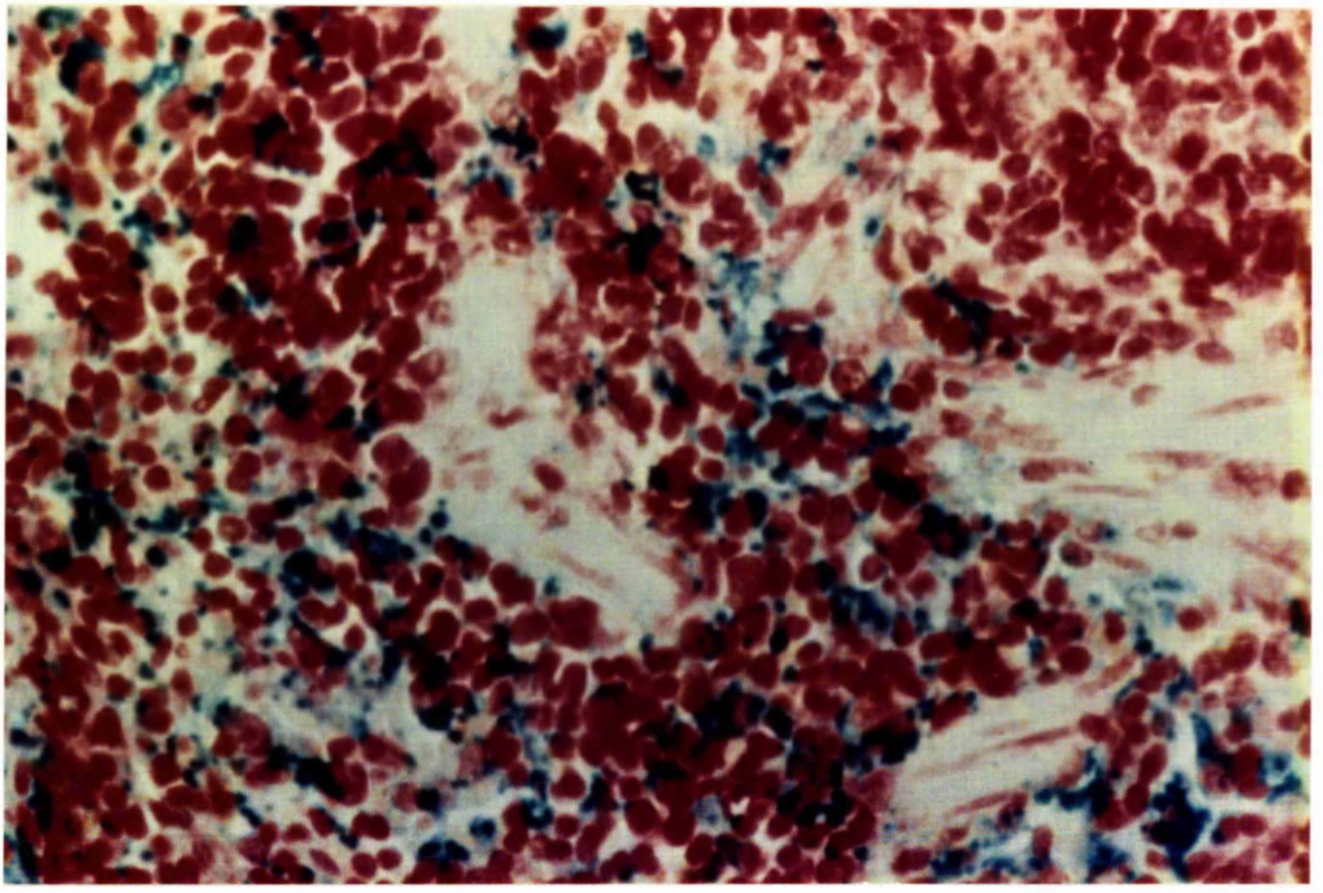


Fig. 6.5: Section of spleen from a normal (+/-) mouse, showing the presence of abundant stainable iron stores (Prussian Blue reaction, counterstained with 1% neutral red, x 1200).

These differences in distribution of stainable iron between the red and white pulp of both anaemic and normal mice are probably a consequence of genetic heterogeneity, (Dunn, 1954).

In view of the quite striking effect of age on the anaemia described in the previous chapter, an effect of age on the iron stores in the anaemic animals was sought. The presence or absence of stainable iron in the spleen of anaemic mice is shown in Table 6.6, along with the haematocrit and age at the time of sacrifice. While those 8 anaemic animals which do show stainable iron are all over 300 days of age, there are 6 of similar age which show total absence of stainable iron. Furthermore, some anaemic mice with normal or near normal haematocrits (e.g. numbers 17, 46 & 729) still show an absence of stainable iron in the spleen, and the amount of iron present in any of the spleens from anaemic mice is minute compared with normal. Thus, although the anaemia tends to improve with age, there is only the most trivial effect on the histochemically demonstrable splenic iron stores.

Bone marrow:

Sections of sternal marrow from 22 normal (+/-), 10 heterozygous (sla/+) and 18 anaemic (sla/- and sla/sla) mice were examined. In all instances, the available medullary space was almost completely occupied by actively haemopoietic tissue in which erythroblasts,

polymorphonuclear leucocytes and megakaryocytes could be easily identified. Vascular channels were similarly prominent in anaemic and normal or carrier mice, and evidence of hypoplasia was seen in neither group; fat spaces were found only in two carrier female mice. Very scanty deposition of stainable iron was present in reticulo-endothelial cells in the sternal marrow of two normal (+/-) animals and none was seen in any anaemic or carrier animal.

Smears from the femoral marrow were examined in 11 anaemic (sla/-) and 23 normal (+/-) mice, and the ratio of erythroblasts to metamyelocytes and granulocytes estimated. In anaemic animals, the mean ratio was 0.60 with a range of 0.20 - 1.39 and a standard error of 0.12; in the normal mice the mean ratio was 0.43, with a range of 0.15 - 1.04 and a standard error of 0.04. While the mean erythropoietic activity in anaemic mice appears greater, the difference is not statistically significant ($0.1 > p > 0.05$). Marrow smears were also stained by the Prussian Blue method in 20 normal mice and all 11 anaemic mice. Of the normal animals, 15 showed the presence in the femoral marrow, of scanty stainable iron either within reticuloendothelial cells or apparently lying free; none of the anaemic mice showed any stainable iron. Sideroblasts were not found in any of the 31 mice (20 anaemic, 11 normal) examined.

Liver:

The liver was examined in 26 anaemic (sla/- or sla/sla) mice, 14 heterozygotes (sla/+) and 14 normal (+/-) mice. The appearance of the hepatic parenchymal cells was normal in all animals. Evidence of hepatic haemopoiesis was seen in 13 anaemic mice, varying in extent from a few small foci or scattered haemopoietic elements, to considerable numbers of well-defined aggregates of a dozen or more cells. Only one normal mouse and 3 carriers showed any evidence of hepatic haemopoiesis. When the incidence of haemopoietic foci in the livers of normal and of anaemic mice is compared, the difference is statistically significant ($0.02 > p > 0.01$).

Stainable iron was absent from the livers of all anaemic animals; 5 carriers showed stainable iron in the parenchymal cells, and of these 2 also had stainable iron in the Kupffer cells. Five normal mice had stainable iron in the parenchyma and a further 2 showed scanty deposition of iron in both hepatic epithelial and Kupffer cells.

Thus, there is an abnormally high frequency of hepatic haemopoiesis in the anaemic group, and total absence of stainable iron in the liver.

Stomach:

The secretory portion of the stomach was examined in 9 anaemic (sla/-), 7 heterozygous (sla/+) and 10 normal

(+/-) mice. No abnormality was noted in any of the stomachs examined, and in particular there was no apparent deficiency of chief or parietal cells, nor evidence of gastric atrophy in the anaemic mice. Only one mouse, of normal genotype showed any stainable iron, and this was confined to a few iron-containing macrophages in lamina propria.

Small intestine:

Duodenum

Sections of the duodenum were taken between at 0.5 cm. from the pylorus. Seventeen anaemic (sla/- and sla/sla), 6 heterozygous (sla/+) and 22 normal mice were examined. On staining with haematoxylin and eosin, all three groups showed, without exception, the normal mucosal pattern of this area of the small bowel. No abnormality of the tall leaf-shaped villi, of the Paneth cells, of the crypts of Lieberkühn, of the columnar epithelial cells or their striated "brush" border could be detected in the anaemic or carrier mice.

However, striking deposition of Prussian Blue positive, haemosiderin granules was found in the epithelial cells of the distal halves of the duodenal villi of anaemic animals. The iron deposits were located toward the luminal border of the cells and this is illustrated in Figure 6.6. The appearance of a similarly treated section of normal duodenal mucosal

epithelium is shown in Figure 6.7; stainable iron is not seen in the epithelial cells. The results of semi-quantitative assessment of the epithelial iron deposition in animals of the various genotypes is shown in Table 6.7; the definitions of the grading categories, 0 - 4, are given in Chapter 4. The majority of anaemic mice show obvious accumulations of iron whereas it is unusual for the normal animals to exhibit more than a few small granules. Carrier (sla/+) females appear to occupy an intermediate position, with usually patchy, but quite distinct, iron granulation in the epithelial cells.

Eight normal and 3 carrier mice showed the presence of iron containing macrophages in the villous core. In none of the anaemic mice was this feature present.

Since the material from the small intestine thus far had been taken from non-fasting animals, an additional 6 normal (+/-), 5 carrier (sla/+) and 7 anaemic (sla/-) animals were killed after 6 hours fasting and sections were taken from the duodenum at 0.5 cm. from the pylorus. None of the normal mice showed the presence of any stainable iron in the epithelium of the duodenal mucosa, whereas haemosiderin could be demonstrated in varying amounts in all the anaemic mice (see Table 6.7). Carriers again appeared intermediate between normal and anaemic mice and exhibited patchy

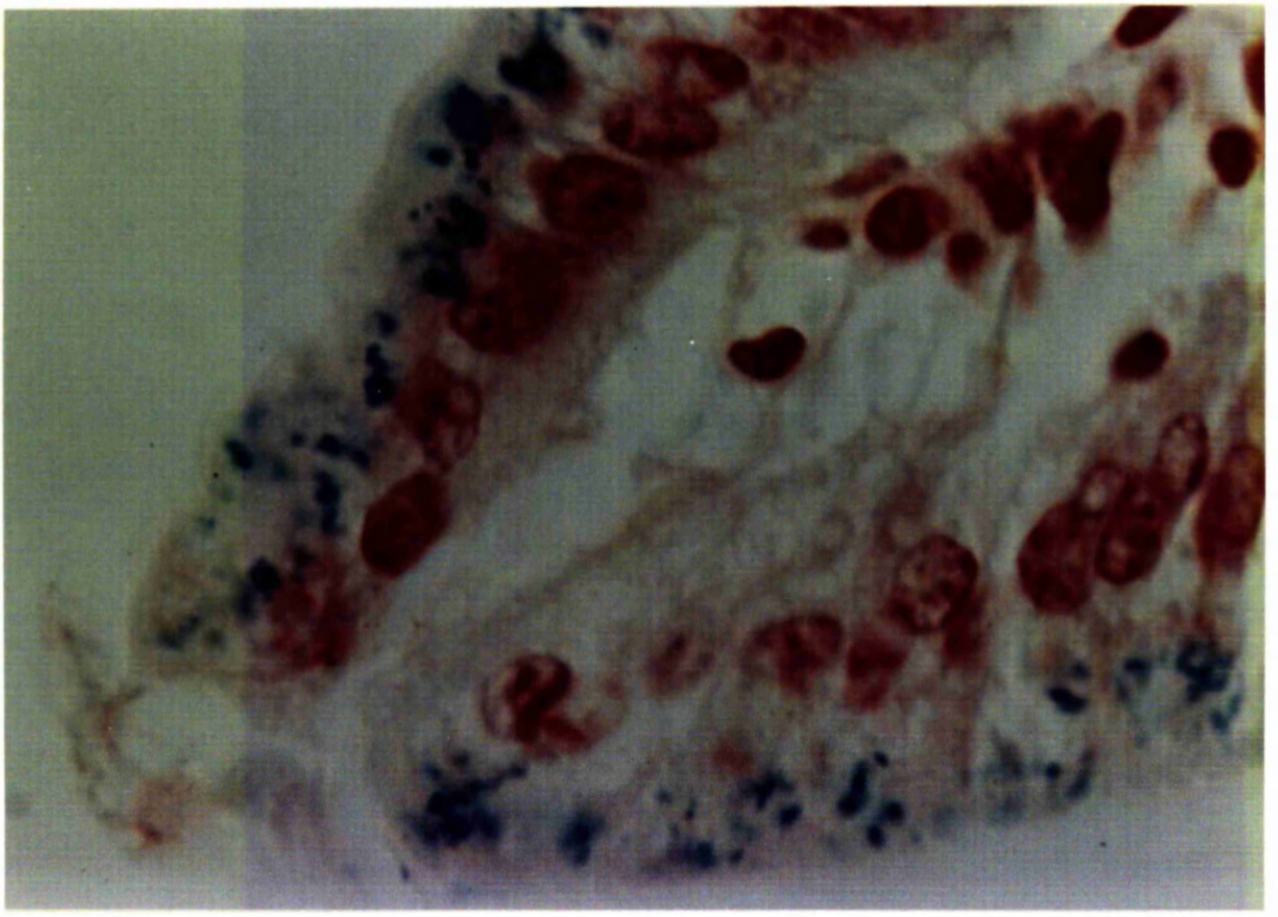


Fig. 6.6: Epithelium of the villous tip from the duodenal mucosa of a fasting anaemic (sla/-) mouse. Cytoplasmic haemosiderin deposits are present on the luminal side of the epithelial cells (Prussian Blue reaction, counterstained with 1% neutral red, x 2500).

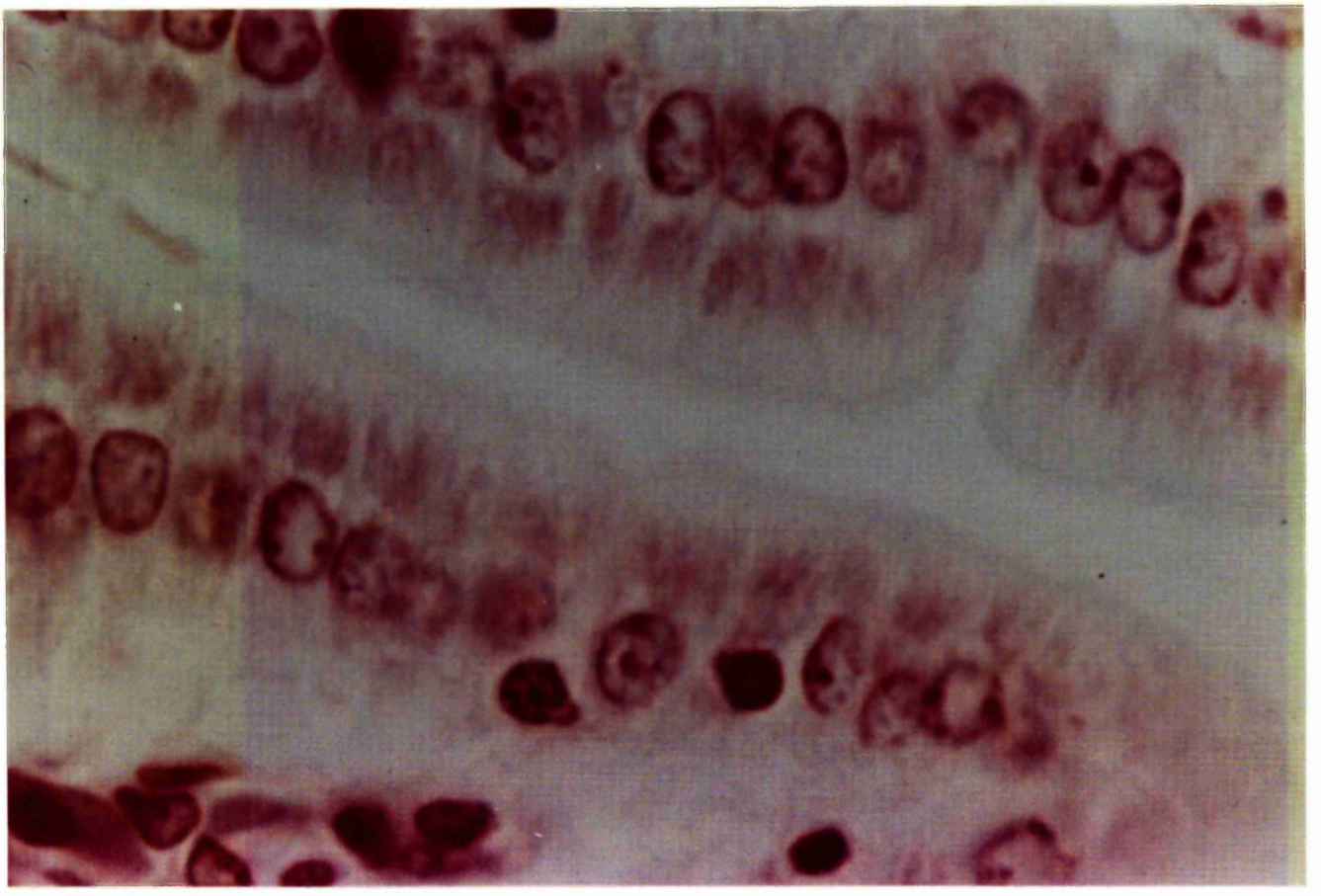


Fig. 6.7: Epithelium of the villous tip from the duodenal mucosa of a non-fasting normal (+/-) mouse. Stainable iron is not seen. (Prussian Blue reaction, counterstained with 1% neutral red, x 2500).

iron granulation in the mucosal epithelium in 4 and widespread deposition of mucosal haemosiderin in the fifth.

Although the anaemic mice with lesser degrees of iron deposition in the mucosal epithelium tended to be older (e.g. Nos. 395, and 453) this was by no means always the case (e.g. Nos. 729 and 887), and several quite old mice still showed heavy deposition of iron (e.g. Nos. 14, 345). There is thus no clear-cut effect of age (see Table 6.8). There is, however, a correlation, although a rather poor one, between the degree of anaemia as reflected by the haematocrit and the amount of iron laid down in the mucosal epithelium; the correlation coefficient of the haematocrit and the duodenal mucosal iron "score" is -0.48. (Table 6.8.) Thus, while age has no obvious effect on the deposition of iron in the duodenal epithelium it appears that there may be some relationship between the accumulation of iron in the mucosal epithelium and the severity of the anaemia.

Jejunum:

The upper jejunum was examined in 11 normal mice. No abnormality was detected on staining with haematoxylin and eosin and none showed the presence of stainable iron, either in the mucosal epithelium or in the lamina propria. Of 10 anaemic mice, 7 showed stainable iron in the

Jejunal mucosal epithelium, but none showed any in the lamina propria. Mucosal epithelial deposits of stainable iron were present in none of 7 carrier female mice, but 3 showed macrophages containing stainable iron in the lamina propria.

Pancreas:

This organ was examined histologically in 29 normal male (+/-), 18 anaemic male (sla/-), 1 anaemic female (sla/sla) and 10 heterozygous female (sla/+) animals. The appearances were normal in every instance and none showed the presence of stainable iron.

Lungs:

Ten anaemic (sla/- and sla/sla), 8 carrier (sla/+) female and 11 normal (+/-) mice were examined. Pathological changes were not seen in the bronchial tree, alveoli or blood vessels in anaemic or carrier mice.

Eight of the normal mice and all the carriers showed the presence of iron-containing macrophages in the peribronchial connective tissue. Such iron deposition was not seen in the connective tissue of the lungs of the anaemic mice. In 6 normal and 6 carrier female mice, iron-containing macrophages were found in the alveoli and bronchioles; six anaemic mice also showed such cells, and in one instance this feature was very obvious. No relationship to the severity of the anaemia could be

discerned and their significance in normal, heterozygous and anaemic mice is not clear.

Kidney:

The kidney was examined in 13 anaemic (sla/-, sla/sla), 7 heterozygous (sla/+) and 12 normal (+/-) mice. No histological abnormality of the glomeruli or renal tubules was found in any of these animals. Of the 12 normal mice, 8 showed occasional small deposits of haemosiderin in the capsular connective tissue, 5 had stainable iron in the perivascular connective tissue and 3 showed minute iron granules in the epithelial cells of the convoluted tubules. Four of the heterozygous females showed stainable iron in the capsular and perivascular connective tissue and in the epithelial cells of the convoluted tubules, and a further carrier had stainable iron in the capsule only. None of the 13 anaemic mice showed any stainable iron in the kidney.

Testis:

This organ was examined in 4 anaemic (sla/-) and 8 normal (+/-) mice. There was no detectable deficit of spermatogenesis or of interstitial cells in the anaemic group. Stainable iron was found in none of the 12 animals examined.

Summary:

Organs from anaemic, carrier and normal mice have been examined histologically. An increase in proportion of the red pulp of the spleen has been noted and this can be related to the degree of splenomegaly, which in turn is related to the severity of the anaemia. Increase in erythropoietic activity has been found in anaemic mice in both spleen and bone marrow, but only in the former is the increase clear cut. Excessive numbers of haemopoietic foci are present in the liver of anaemic mice. Other pathological tissue changes have not been detected in anaemic or carrier female mice by haematoxylin and eosin or Romanowsky staining.

Histochemical studies of the iron stores have shown depletion in anaemic animals, most obvious in the spleen. By contrast, excessive deposition of haemosiderin in the epithelium of the mucosa of the small intestine has been detected in anaemic mice, even after 6 hours fasting. Carrier female mice have apparently normal tissue iron stores, although rather low amounts of haemosiderin have been found in the spleen in a few; the duodenal mucosal epithelium in these mice exhibits a degree of iron deposition, intermediate between the normal and anaemic groups.

CHAPTER 7

Studies with Radio-isotopes

The findings described in Chapters 5 and 6 indicate disordered iron metabolism in X-linked mouse anaemia, and for this reason studies of the fate of oral and parenteral doses of iron, labelled with ^{59}iron, have been carried out in normal and anaemic mice.

The utilization of tracer doses of iron for erythropoiesis has been estimated after intra-peritoneal and intravenous injection. The clearance rate of intravenously administered tracer doses of ^{59}iron has been studied and the loss of parenterally administered tracer has been followed in a small group of animals for up to 6 months.

The intestinal absorption of iron has been determined following the oral administration of inorganic and organic iron compounds labelled with ^{59}iron, and preliminary observations on the absorption of ^{131}iodine-labelled triolein, ^{64}copper, ^{65}zinc and ^{57}cobalt are also included. Details of the methods employed are presented in Chapter 4.

The utilization of iron for erythropoiesis:

As explained in Chapter 4, the utilization of iron for erythropoiesis is taken to be that fraction of a parenterally administered dose of radio-iron which reappears in the circulating red cells. The calculations

of the utilization of iron for haemopoiesis depend on knowledge of the blood volume of the animals under investigation, and estimates of the blood volume of anaemic and normal mice were made using two radio-isotope dilution techniques, with ^{51}chromium-labelled mouse erythrocytes and with ^{131}iodine-labelled human serum albumin (RISA). Nineteen normal and 9 anaemic mice were investigated with ^{51}chromium, and 7 normal and 5 anaemic mice with the RISA technique. The results of the estimates of whole blood volume are given in Table 7.1 and shown diagrammatically in Figure 7.1, where it can be seen that the anaemic mice, by both methods, have a significantly increased blood volume, and that in both normal and anaemic animals, the RISA method gives significantly higher estimates of blood volume than does the ^{51}chromium-labelled red cell technique.

The red cell mass and plasma volume have been calculated from the whole blood volume and the haematocrit, using a correction factor of 0.88 to convert venous, to whole body, haematocrit. (Wish et al., 1950). The results are given in Tables 7.2 and 7.3, respectively, and in Figure 7.1. The reduction in red cell mass in anaemic mice, although significant, is comparitively small, and the increase in plasma volume is correspondingly greater in anaemic mice. It is clear that the anaemia produced by the sla gene is, at least in part, a dilution phenomenon due to an absolute increase in plasma volume.

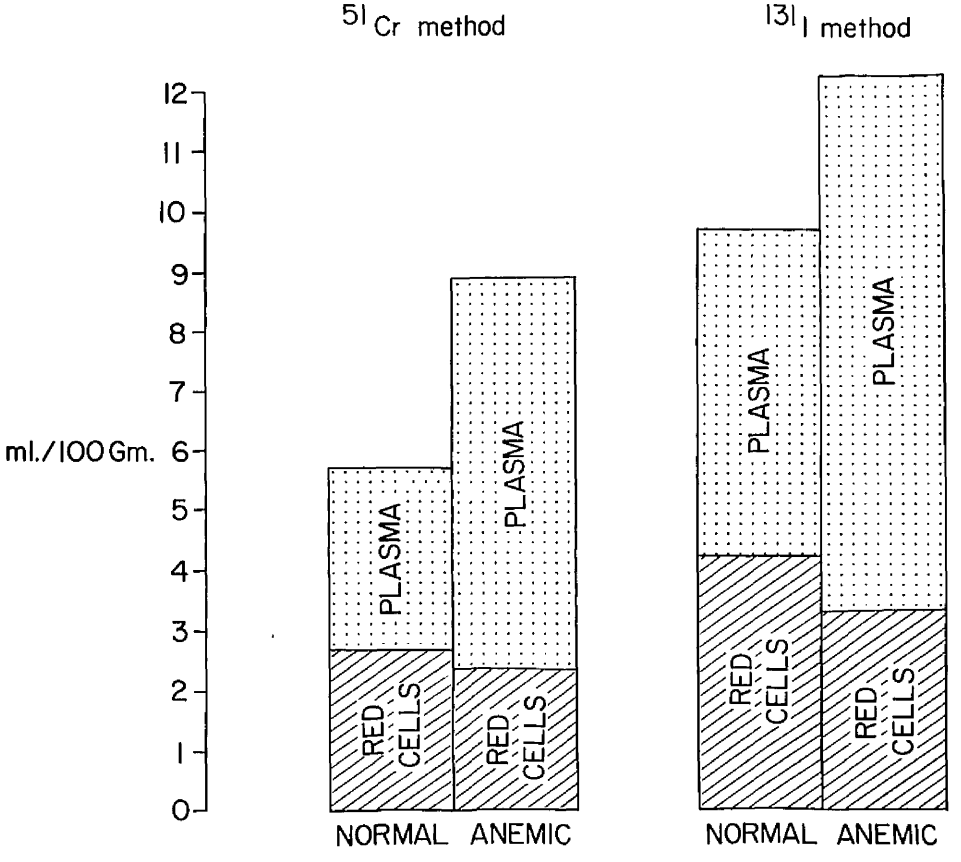


Fig. 7.1: The blood volume, red cell mass and plasma volume of normal (+/-) and anaemic (sla/-) mice, by dilution techniques employing ^{51}chromium-labelled red cells (left) and ^{131}iodine-labelled albumin (right).

As noted above, the RISA method gives significantly greater estimates of blood volume in the mouse than does the ^{51}chromium-labelled red cell method. In an attempt to assess which method provides the more reliable value, five normal animals were given an intravenous tracer dose of ^{59}iron, and its reappearance in the peripheral blood was measured 5 days later. The utilization of iron for haemopoiesis was estimated, using the mean blood volume (as a volume/weight percentage of total body weight) obtained by the two methods. The results are shown in Table 7.4; with the blood volume as measured by ^{51}chromium-labelled red cells, the utilization of ^{59}iron for haemopoiesis was in the range 63-75% of the administered dose, whereas, with the RISA method, the apparent utilization of ^{59}iron for haemopoiesis exceeded the original dose given. Hence, for the calculations of the utilization of iron reported below, the estimate of blood volume obtained by the ^{51}chromium-labelled mouse red cell technique has been used. Full details of these experiments are given in Appendix C.

Utilization of iron for erythropoiesis has been studied both serially over several days, and on the first and fifth days, following intraperitoneal or intravenous injections of ^{59}iron. The results of serial studies of the appearance of radioiron in the peripheral blood for 5 days following intraperitoneal injection of tracer

doses of ^{59}iron are depicted in Figure 7.2 and analyzed in Table 7.5. While the values for anaemic (sla/-) mice are higher on all 5 days, the differences are significant on the first day after injection only. However, in a further experiment, when a single estimate was made on the 5th day after injection of ^{59}iron, the utilization of iron was significantly greater in anaemic animals (Table 7.6). Further studies of iron utilization for erythropoiesis were performed following intravenous injection of tracer doses of ^{59}iron. The reappearance of radio-iron in the peripheral blood 24 hours after injection is very much greater in anaemic than in normal mice (Table 7.7, Figure 7.3) and 5 days after injection, anaemic mice still showed significantly greater iron utilization. The differences between normal and anaemic mice seen with the intravenous method are similar in pattern to, but greater in extent than, those seen with the intraperitoneal method. Thus, the utilization of tracer doses of ^{59}iron for haemopoiesis is both more rapid and greater than normal. Details of the data from which the results described above were obtained are presented in Appendix C.

The clearance of iron from the plasma:

The rate of clearance of intravenously injected tracer doses of ^{59}iron from the plasma is increased in anaemic mice (Figure 7.4). The mean plasma iron clearance

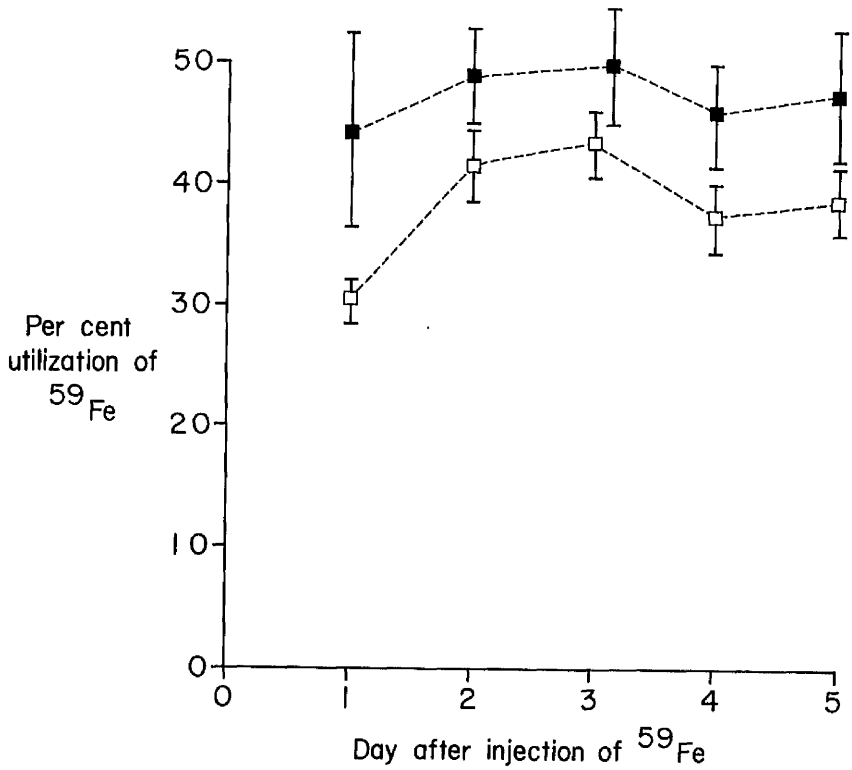


Fig. 7.2: Serial estimates of the utilization for haemopoiesis of tracer doses of ^{59}iron, given intraperitoneally to normal (+/-) and anaemic (sla/-) mice. The open squares represent the means for normal mice, and the closed squares, the means for anaemic mice; the vertical bars represent the limits of ± 1 standard error of the mean. (See also Table 7.5).

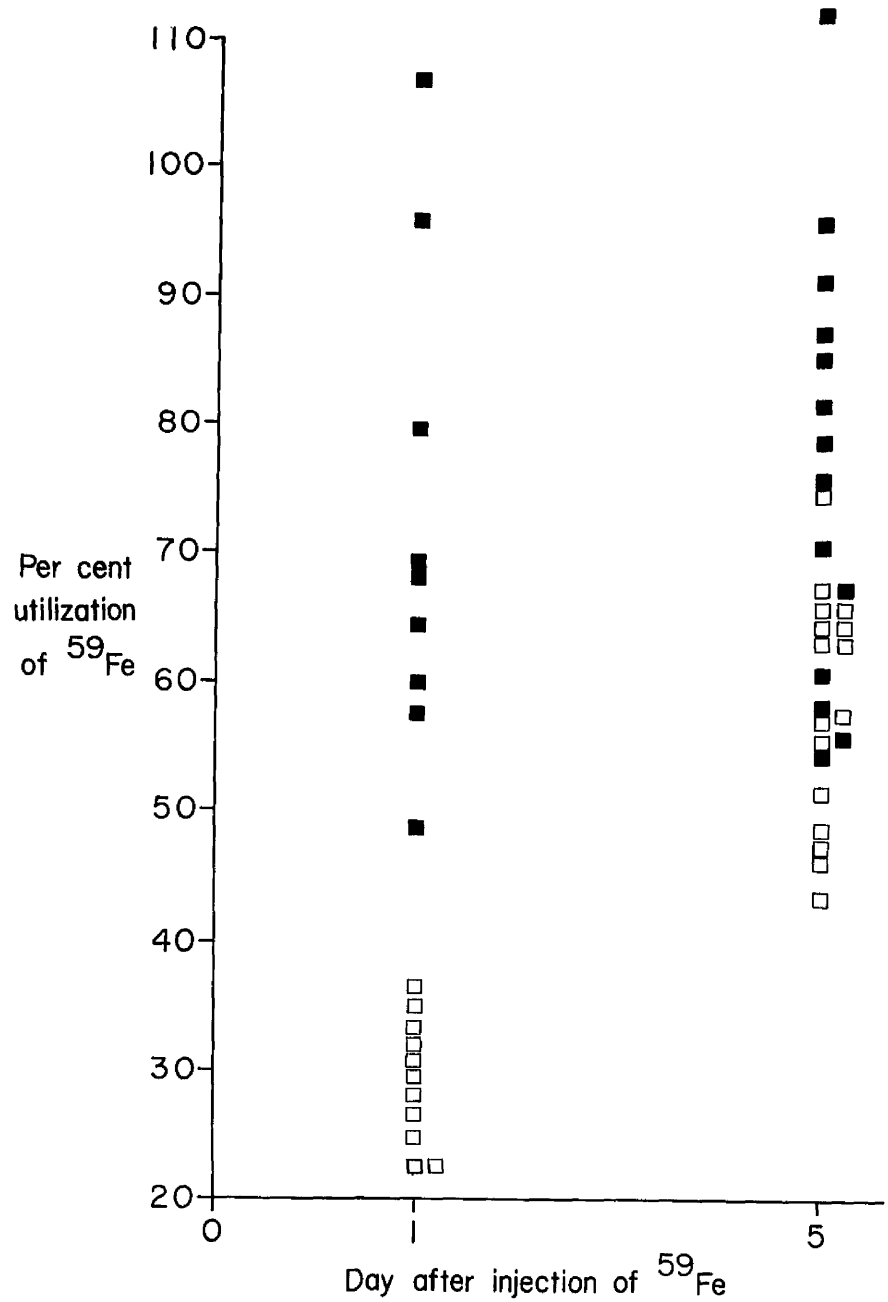


Fig. 7.3: Utilization of tracer doses of ^{59}iron for haemopoiesis by normal (+/-) and anaemic (sla/-) mice, one and five days after intravenous injection of isotope. Open squares represent the individual estimations for normal mice, and closed squares, those for anaemic mice (see also, Table 7.7).

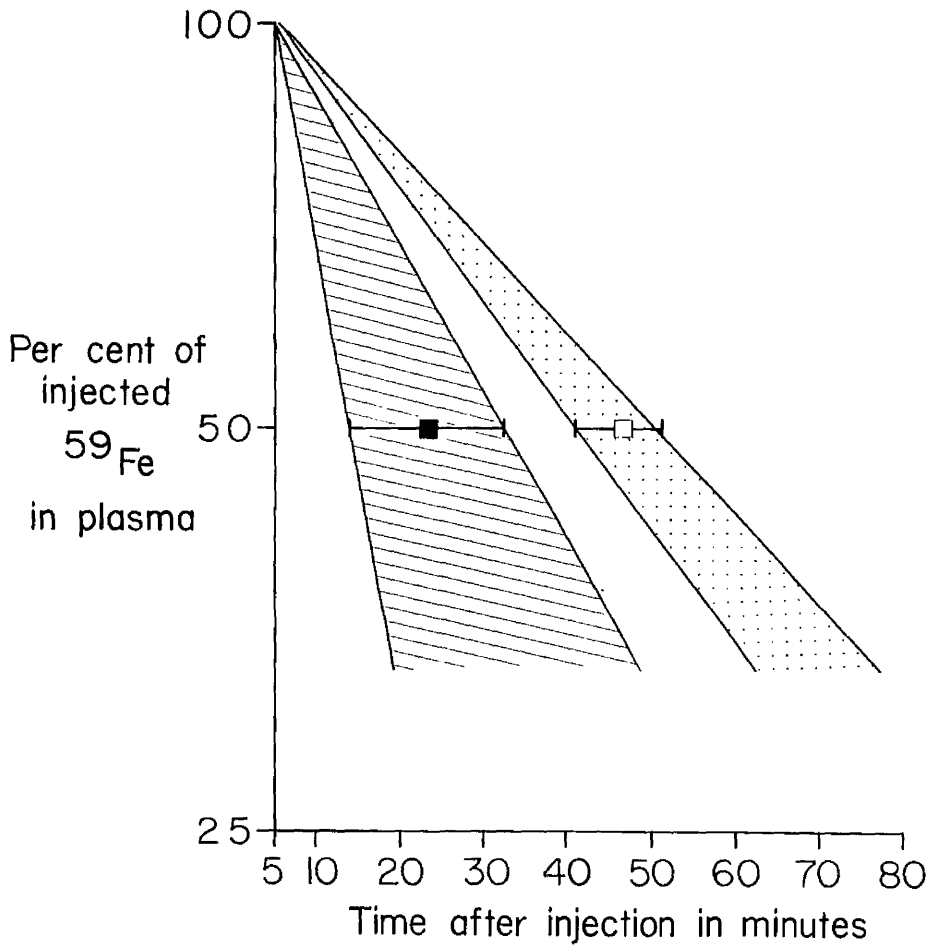


Fig. 7.4: The clearance of intravenously administered tracer doses of ^{59}iron from the plasma. The means of the plasma iron clearance half-times are shown for normal (+/-) mice by an open square and for anaemic (sla/-) mice by a closed square; the limits of 2 standard errors of the mean are shown by the transverse bars. The cross-hatched area represents plasma iron clearance in anaemic mice, and the stippled area, clearance in normal mice.

half time ($T\frac{1}{2}$ ^{59}Fe) in normal mice is 46.1 minutes, and in anaemic (sla/-) mice, 23.4 minutes. The difference of the means is highly significant (Table 7.8). The complete results of the experiments providing these conclusions are given in Appendix C.

The excretion of parenterally administered ^{59}iron:

Following intraperitoneal injection of radio-iron, the whole body radioactivity of normal and anaemic mice has been followed over a period of approximately six months. The numbers of animals involved is small, and full details are given in Appendix C. The decline in body radioactivity, after correction for decay, is shown in Figure 7.5. The range of variability is wide, especially in anaemic animals, and, since the numbers of animals involved is so small, statistical analysis would not be valid. However, anaemic mice show a rate of iron loss similar to normal animals.

The absorption of iron:

The absorption of iron in normal (+/-) mice has been assessed by measuring the retention of ^{59}iron-tagged oral doses of ferrous sulphate in doses containing 0.1 µg, 1.0 µg and 10 µg of elemental iron, and ^{59}iron-tagged mouse haemoglobin containing 10 µg of elemental iron. Serial measurements of retained radioactivity have been made at daily intervals for up to 6 days, with doses of 0.1 µg

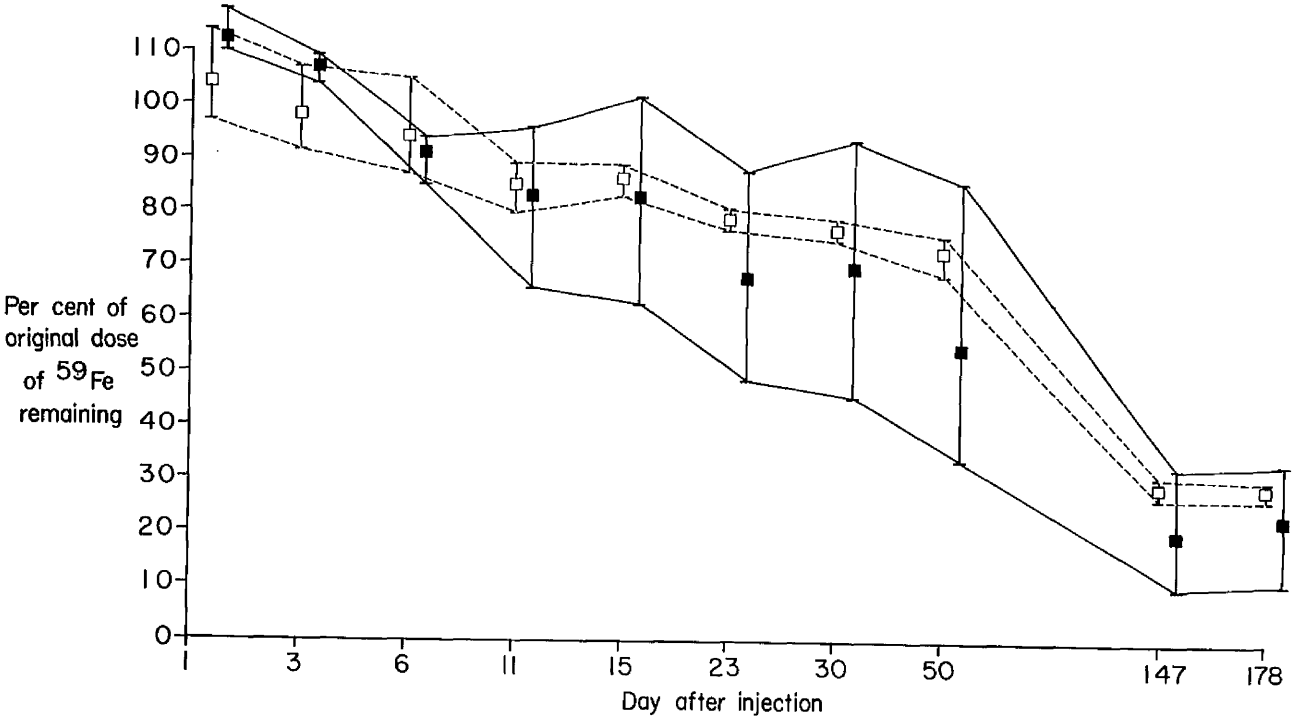


Fig. 7.5: The loss of parenterally administered ^{59}iron by normal (+/-) and anaemic (sla/-) mice. The closed squares represent the means for anaemic mice and the open squares, the means for normal mice. The vertical bars represent the range of values obtained.

and 1.0 μg of elemental iron. With the latter dose, the amounts retained by normal mice on the 4th, 5th and 6th days are similar, and the same is true for anaemic mice (Figure 7.6). With the smaller dose, only the amounts retained on the 4th and 5th days after dosing have been measured, but a similar pattern is seen (Figure 7.7). These findings suggest that the period of 5 days is sufficient to permit elimination of all unabsorbed faecal radioactivity and in addition, to permit the faecal elimination of any radio-iron which may be absorbed into, and retained in, the intestinal mucosal cells, which are exfoliated from the villous tips within 48-72 hours of their formation in crypts of Lieberkühn (Creamer, 1967). Thus, the retained proportion of radioactivity 5 days after oral dosing is taken to represent the proportion of iron absorbed through the intestinal mucosa.

The amount of inorganic iron absorbed at the three dose levels by normal (+/-) and anaemic (sla/-) mice is shown diagrammatically in Figure 7.8 and the analysis of these data is given in Table 7.9. Anaemic mice absorb significantly less of oral doses of 0.1 μg, 1 μg and 10 μg of elemental iron than do their normal counterparts. The proportion of iron absorbed falls as the dose administered is increased, although the absolute amount of iron absorbed is, of course, smaller, the smaller the dose administered (Table 7.10).

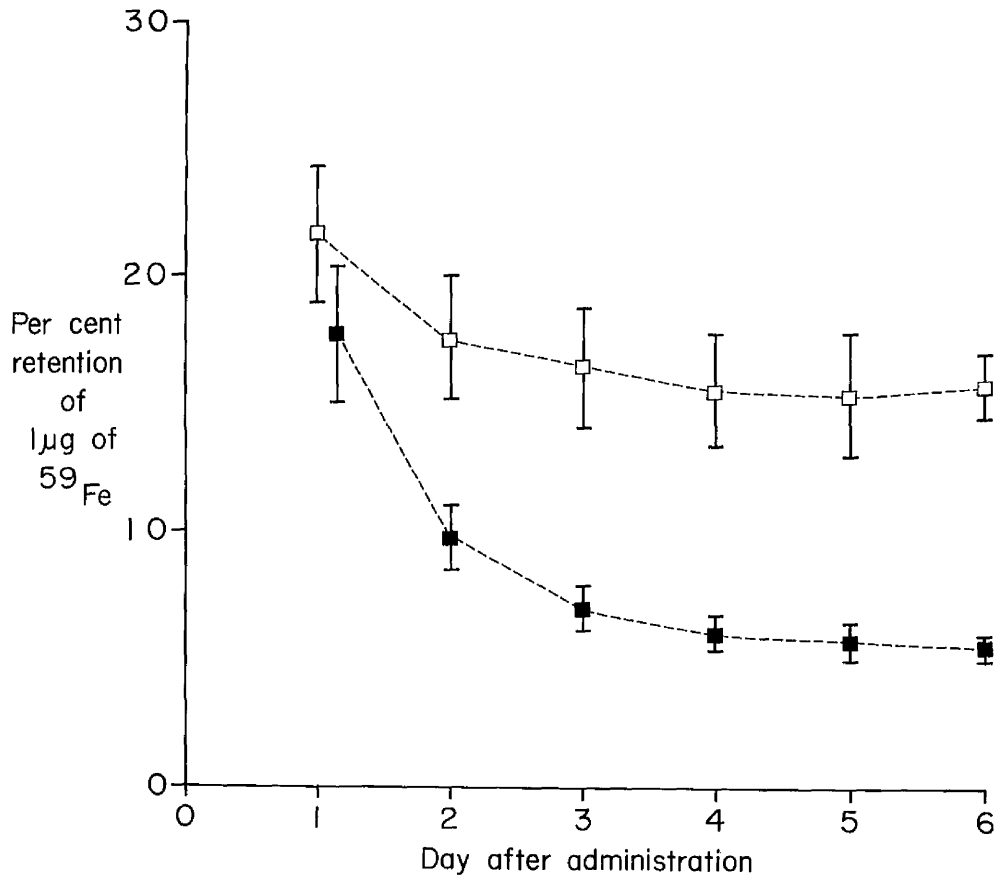


Fig. 7.6: The retention of ^{59}iron-tagged ferrous sulphate in oral doses containing 1.0 µg of elemental iron, on each of 6 days following administration. The open squares represent the means for normal (+/-) mice, and the closed squares, the means for anaemic (sla/-) mice; the vertical bars represent ±1 standard error of the mean. (See text.)

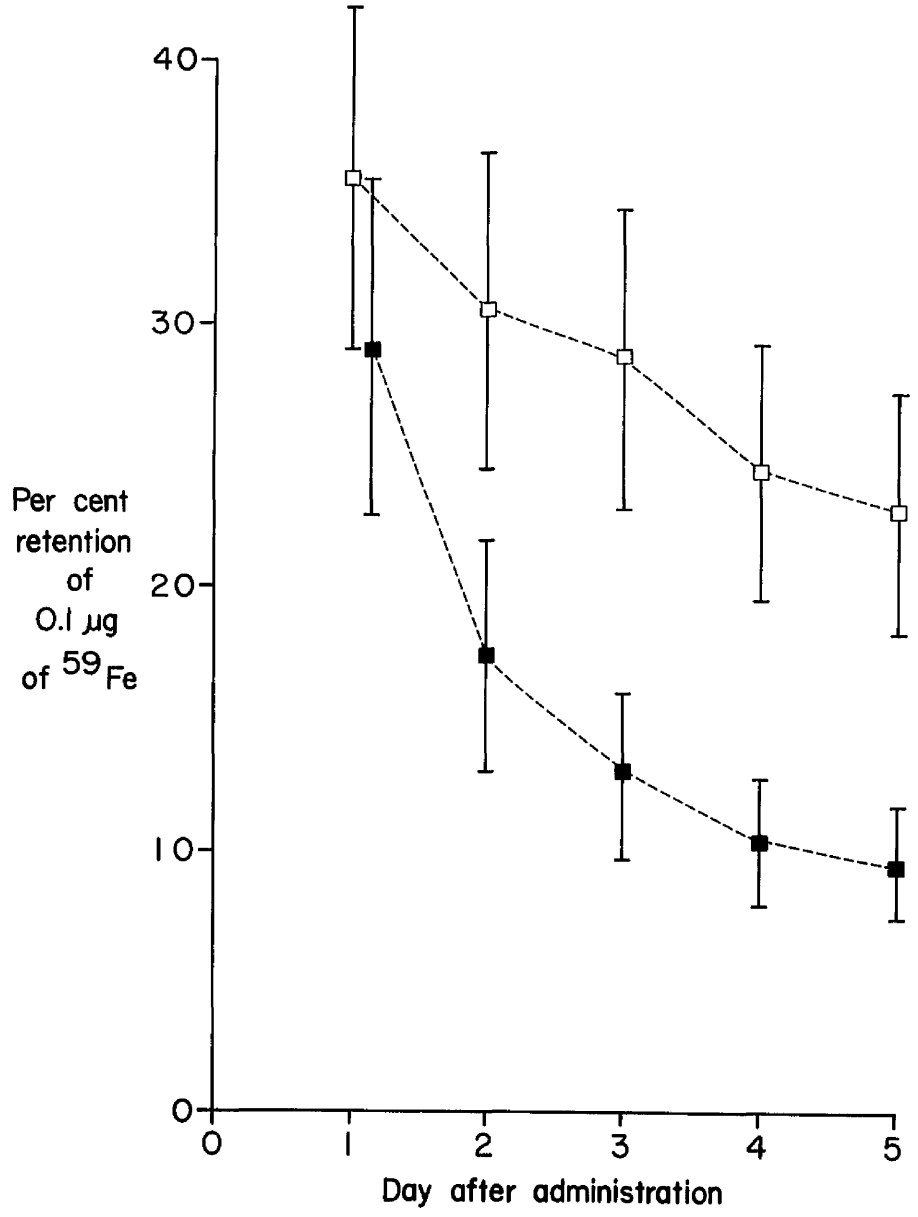


Fig. 7.7: The retention of ^{59}iron-tagged ferrous sulphate in oral doses containing 0.1 µg of elemental iron, on each of 5 days following administration. The open squares represent the means for normal (+/-) mice, and the closed squares the means for anaemic (sla/-) mice; the vertical bars represent ±1 standard error of the mean. (See text.)

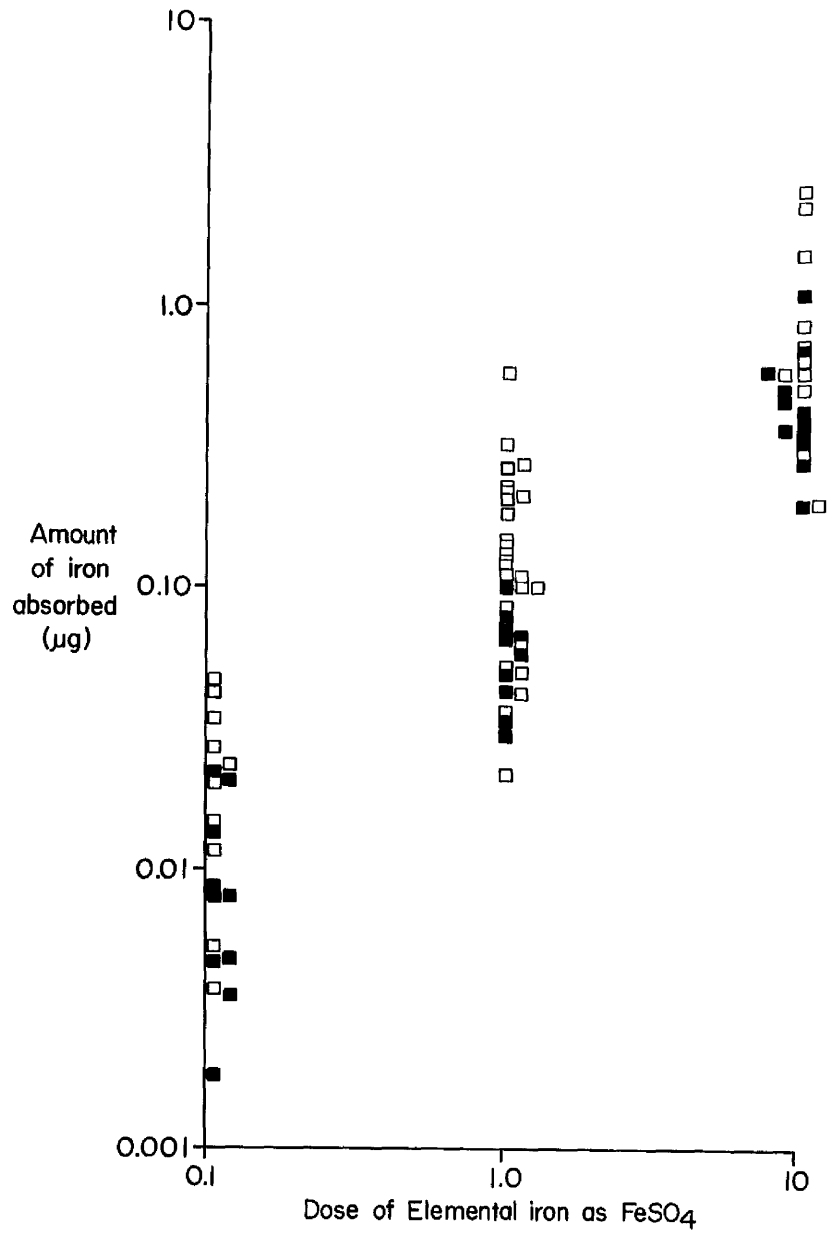


Fig. 7.8: The absorption of inorganic iron by normal (+/-) and anaemic (sla/-) mice, as ^{59}iron-tagged ferrous sulphate in doses containing 0.1, 1.0 and 10 µg of elemental iron, expressed as absolute amounts of iron absorbed, presented on a log-log scale. The open squares represent individual values for normal mice and the closed squares, individual values for anaemic mice. (See text, and Tables 7.9 and 7.10.)

The intestinal absorption of haemoglobin tagged with ^{59}iron, in a dose containing 10 µg of elemental iron, was estimated in normal and anaemic mice. A significant difference in absorption was not seen (Table 7.11). However, the low level of specific activity achieved in ^{59}iron-tagged haemoglobin, and an apparently low level of absorption combined to give very low whole body counting rates 5 days after dosing. The errors involved in counting at such low levels of activity may have influenced the results, which must therefore be regarded as inconclusive.

Full details of the data from which these tables and diagrams have been prepared are given in Appendix C.

The intestinal transit time in normal and anaemic mice has been compared using carmine red as a faecal marker. The detailed results of the experiment are given in Table 7.13 where it can be seen that the rate of faecal excretion of an oral dose of carmine red is similar in normal (+/-) and anaemic (sla/-) mice. In all 8 animals examined, carmine red was obviously present in the stools during the first 24 hours after administration; traces only were found in the faeces of 2 anaemic and 2 normal mice at 24-48 hours and none was seen on the third and fourth days.

The absorption of fat, zinc, cobalt and copper:

In view of the demonstration of defective iron

absorption, preliminary observations of the absorption of other substances have been made, using radio-isotopes. The absorption of 6.25 μg of radio-iodinated triolein, suspended in margarine, was similar in normal and anaemic mice (Figure 7.9 and Table 7.12). A dose of ^{65}zinc-tagged zinc chloride containing 3.5 μg of elemental zinc was given. Although normal mice tended to absorb more zinc than did anaemic mice, considerable overlap between the two groups was seen (Figure 7.9) and the differences are not statistically significant. Absorption of cobaltous chloride, tagged with ^{57}cobalt, was tested, using a dose of 0.0006 μg of elemental cobalt. Normal and anaemic mice absorbed approximately the same amount of this element (Figure 7.9 and Table 7.12).

Absorption of copper as ^{64}cuprous nitrate was tested 4 days after oral administration, the shorter period being necessitated by the rapid rate of decay of ^{64}copper (half-life, 12.8 hours; The Radio-Chemical Manual, 1966). A dose of 10 μg of elemental copper was required to give sufficient counts for adequate assessment of the retention of this element. As in the case of zinc, anaemic (sla/-) mice appeared to absorb rather less copper than normal mice, but again the overlap between the two groups was considerable (Figure 7.9) and the difference of the means was not statistically significant (Table 7.12).

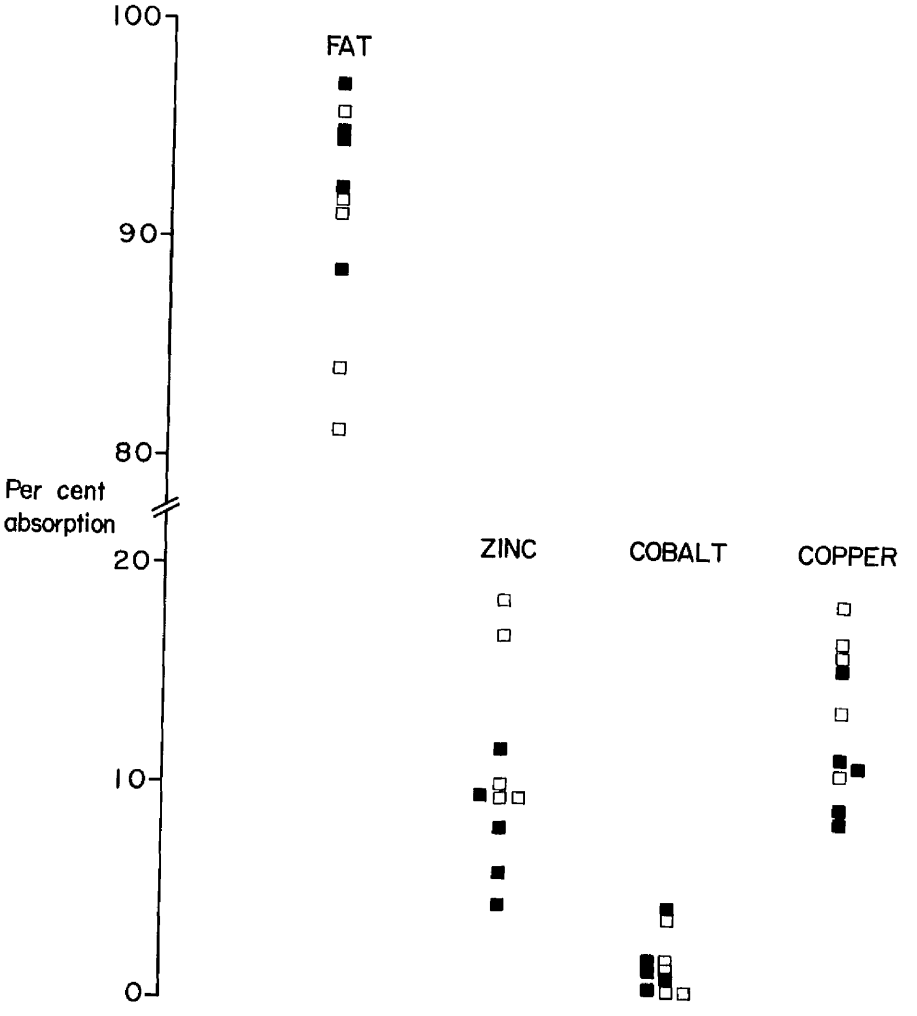


Fig. 7.9: The absorption of fat, zinc, cobalt and copper by normal (+/-) and anaemic (sla/-) mice. Individual values for normal and anaemic mice are indicated by open and closed squares, respectively.

SUMMARY:

The results of the experiments presented in this chapter have demonstrated that anaemic (sla/-) mice have a significantly increased utilization of tracer doses of ^{59}iron for erythropoiesis, and that the reappearance of the parenterally administered radio-iron in the peripheral blood is more rapid than normal. Further, the clearance of tracer doses of radio-iron from the plasma is also more rapid than normal. Clear evidence of excessive loss of iron by anaemic mice is lacking.

The intestinal absorption of iron at three dose levels is significantly reduced in anaemic mice. The absorption of fat and cobalt is apparently unaffected by the sla gene, while that of copper and zinc is reduced in the anaemic animals. However, the differences between normal and anaemic mice in respect of the absorption of copper and zinc are not statistically significant.

Anaemic (sla/-) mice have increased blood and plasma volumes, and decreased red cell mass.

CHAPTER 8

Miscellaneous Investigations

A variety of other investigations designed to throw light on the disorder of iron metabolism in X-linked mouse anaemia have been carried out, and the results of these are reported in this chapter. The serum iron, total iron binding capacity (T.I.B.C.), total body iron content, and free erythrocyte protoporphyrin (F.E.P.) have been estimated. Haemoglobin electrophoresis in starch gel has been performed in an attempt to detect qualitative differences between normal and anaemic mice, and electrophoresis of serum proteins has been carried out to demonstrate the presence of transferrin in anaemic mice, with and without prior labelling of the transferrin with ^{59}iron.

Haemopoietic cells from the bone marrow and spleen of anaemic mice have been transplanted to lethally irradiated host mice, and the haematological picture in these host mice assessed 116 days after transplantation, to determine whether sla/- haemopoietic cells, transplanted to a normal environment are capable of producing normal haemoglobin concentrations and normal erythrocytes.

The methods employed in these experiments are described in Chapter 4.

Serum iron concentration and total iron binding capacity:

The serum iron has been measured in 27 normal mice and 25 anaemic mice. The mean serum iron concentration in normal mice is 254 µg/100 ml; in anaemic mice the mean serum iron concentration is reduced to 142 µg/100 ml and the difference of the means is statistically significant (Table 8.1). Individual values are illustrated in Figure 8.1.

The results of estimations of the T.I.B.C. are also given in Figure 8.1 and in Table 8.1. The mean T.I.B.C. is 420 µg/100 ml in normal mice and 612 µg/100 ml in anaemic (sla/-) animals; the difference is statistically significant.

The mean percentage saturation of the T.I.B.C. is 60.5 and 23.2 in normal and anaemic mice respectively.

Detailed results on the animals studied are given in the Appendix D.

Total body iron content:

The total body iron content, in mg/100 G of body weight, of mice at different ages is given in Table 8.2 and Figure 8.2. At all ages the total body iron content of anaemic mice is reduced significantly when compared with normal mice, and this difference is most noticeable over 300 days of age, in spite of the fact that 6 of the 8 mice of anaemic genotype in this age range had normal haemoglobin concentrations. As normal mice age there

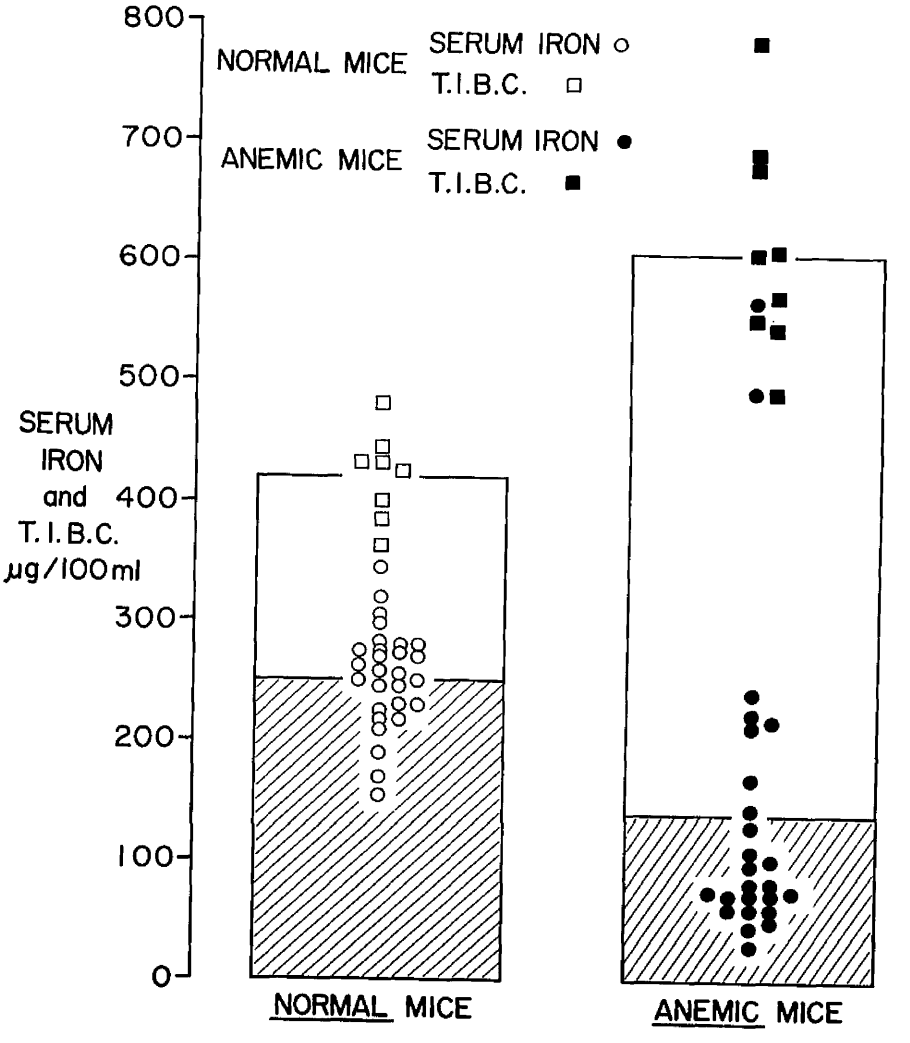


Fig. 8.1: Serum iron concentration and total iron binding capacity in normal (+/-) mice (left) and anaemic (sla/-) mice (right). The crosshatched columns represent the mean serum iron concentration, and the total height of the columns, the total iron binding capacity. The points indicate individual estimations of serum iron concentration and total iron binding capacity, according to the key at the top of the diagram.

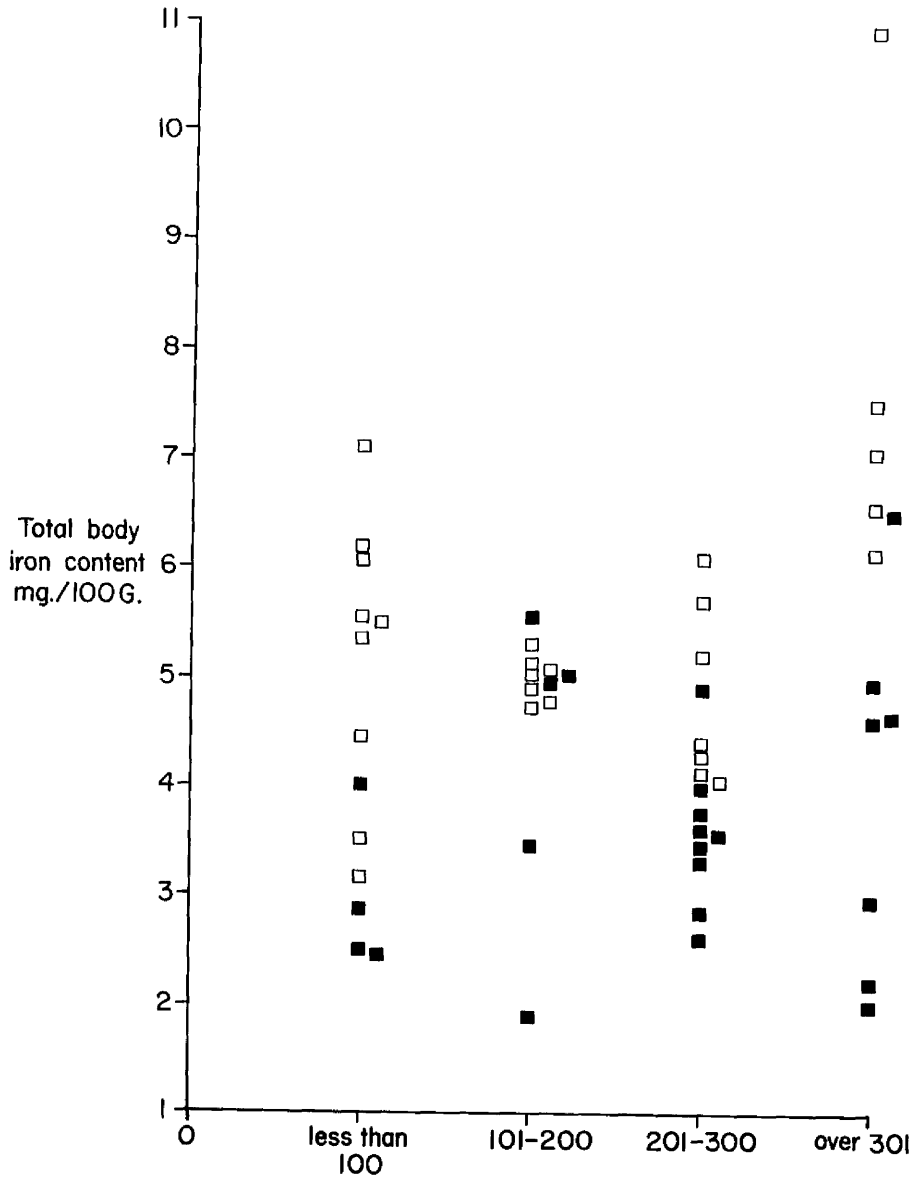


Fig. 8.2: The total body iron content of normal and anaemic mice, represented respectively by open and closed squares. Individual values are given for each of 53 animals, grouped according to age in days (abscissa).

is an increase in the total body content, and, while an increase is also seen in anaemic mice, it is comparatively slight. Further details of the animals used for this investigation are available in Appendix D.

Free erythrocyte protoporphyrin

Eleven estimations of the free erythrocyte protoporphyrin (F.E.P.) have been made on normal mice, and 8 on anaemic (sla/-) animals. The results are presented in Figure 8.3 and details of the analysis of these results are given in Table 8.3. There is a highly significant elevation of the F.E.P. concentration in the anaemic group. Individual estimations and details of the animals used are presented in Appendix D. The values obtained for normal mice are slightly higher than those reported by Nakao, Wada, Takaku, Sassa, Yano and Urata (1967).

Haemoglobin electrophoresis:

The haemoglobin pattern in 8 anaemic (sla/- and sla/sla), 5 heterozygous carrier (sla/+) and 7 normal male (+/-) mice has been examined by starch gel electrophoresis. Every animal, whether of the original mixed stock or a hybrid of the mixed stock and the C57Bl/6 Jax strain, showed the same "single" band pattern (see Figure 8.4).

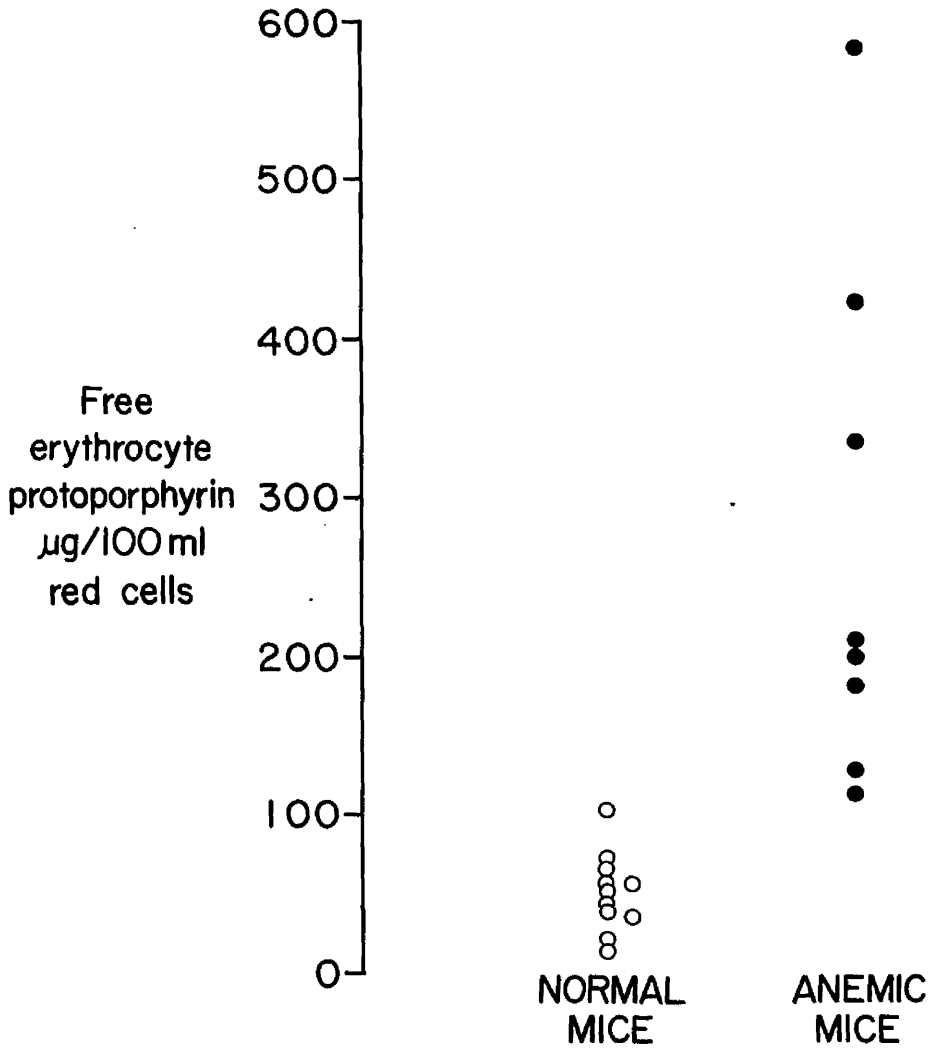


Fig. 8.3: Results of free erythrocyte protoporphyrin estimations in normal mice and anaemic mice. The open circles represent normal animals, and the closed circles, anaemic mice.

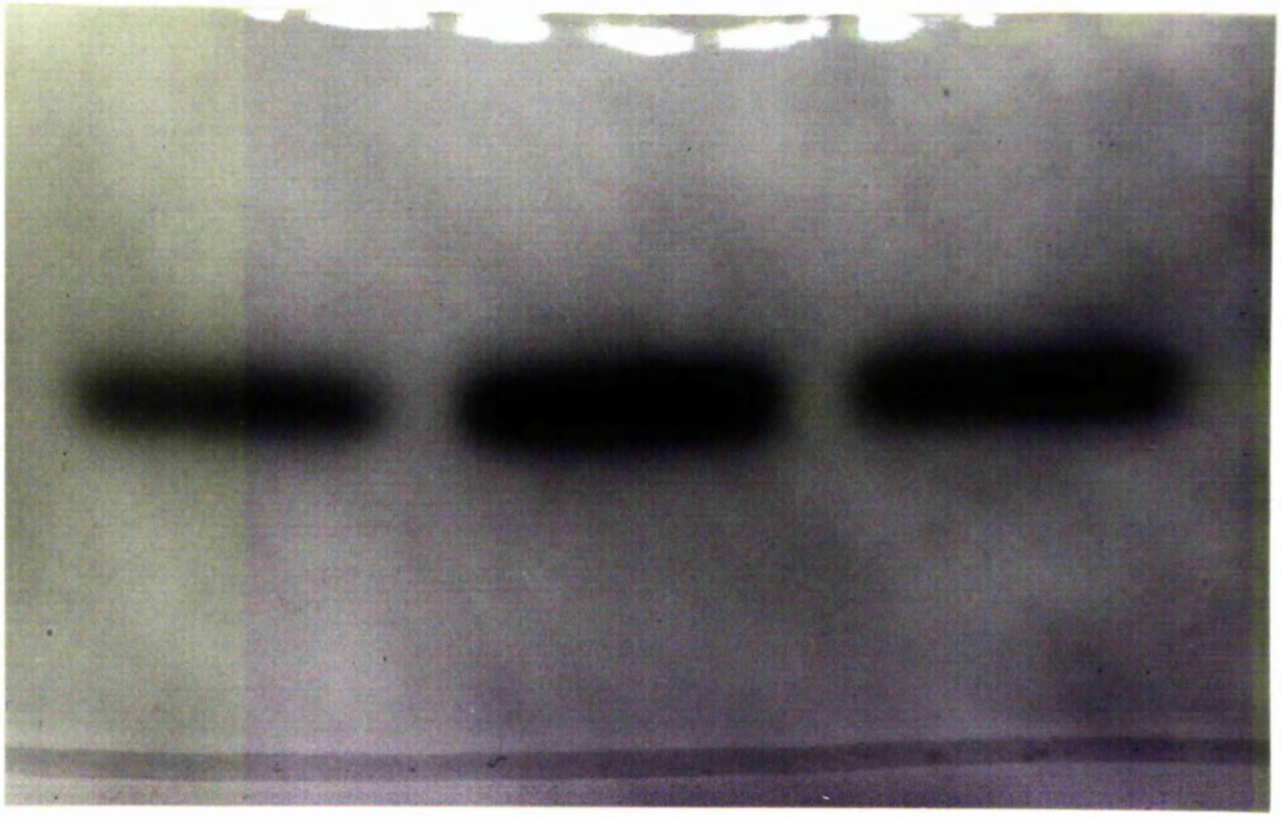


Fig. 8.4: Haemoglobin electrophoresis in starch gel at pH 8.8 (see Chapter 4). The origin is at the bottom of the photograph, and the bands, from left to right, are from a C57Bl/6 J mouse, a hybrid (F_4) normal (+/-) mouse and its anaemic littermate brother. The gel has not been stained.

Serum protein electrophoresis:

Serum protein electrophoresis was carried out, without in vitro labelling of the transferrin with ^{59}iron, in 13 normal (+/-), 4 heterozygous carrier (sla/+) and 14 anaemic (sla/- and sla/sla) mice. All except one anaemic (sla/-) and one heterozygous (sla/+) mouse showed a single transferrin band in the position of transferrin b (Cohen and Shreffler, 1961). The two exceptions were heterozygotes for transferrins a and b.

The serum proteins from 5 anaemic (sla/- and sla/sla), 3 heterozygous carrier (sla/+) and 4 normal mice were electrophoresed in starch gel after prior incubation at 20°C with ^{59}iron. After electrophoresis, the starch gel was stained with amido-black and the strip cut into sections for radioactive counting. In each instance most of the radioactivity in the starch gel strip was located in the transferrin band, which was identified from a normal control specimen from a C57Bl/6 Jax mouse, unlabelled and run concurrently. Figure 8.5 illustrates the patterns of radioactivity found in the electrophoretic strips from 2 normal and 2 anaemic mice. Details of the counts obtained in all animals examined are given in Appendix D.

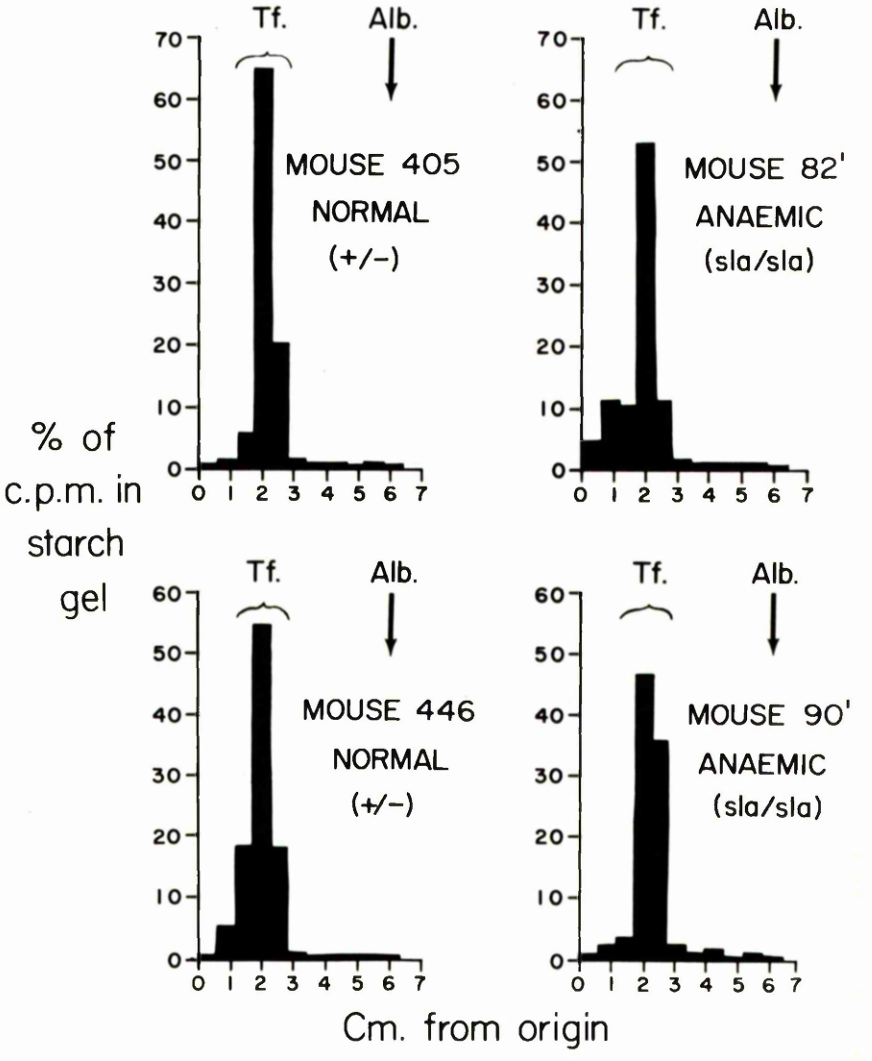


Fig. 8.5: The radioactivity associated with the serum proteins after starch gel electrophoresis following labelling with ^{59}iron in two normal and two anaemic mice. There is a distinct peak of radioactivity in the region of the transferrin band in all four animals.

The ability of transplanted sla/- haemopoietic tissue to form normal red cells and maintain a normal haemoglobin concentration in lethally irradiated hosts:

Suspensions of spleen and bone marrow cells from anaemic and normal male littermate mice of the fourth backcross generation of the original mixed stock and the C57Bl/6 Jax strain were injected into male mice of 10-12 weeks of age, of the C57Bl/10 Jax strain, in which endogenous bone marrow had been ablated by X-irradiation; preliminary experiments had shown these animals to accept such transplants. Fuller details of the methods employed are given in Chapter 4. Haematological data on the donor animals are given in Table 8.4. Groups of 12 mice received either normal or anaemic bone marrow, or normal or anaemic spleen, cell suspensions. Five mice receiving normal bone marrow, 10 receiving sla/- bone marrow and 2 receiving sla/- spleen cells were still surviving 116 days after injection and details of their haematological assessment at that time are given in Table 8.5. The progeny of injected bone marrow cells of normal and anaemic mice, and of spleen cells of anaemic mice are capable of maintaining normal haemoglobin concentrations and/or haematocrits in the lethally irradiated hosts. The M.C.H.C. in all instances was over 30 per cent and the morphological appearance of the red cells produced in all hosts, irrespective of

the source of grafted haemopoietic cells, was normal (Figures 8.6 and 8.7).

Summary

Chemical estimates of the serum iron concentration show reduced levels in anaemic mice, whereas the total iron binding capacity is elevated. The total body iron content is reduced in X-linked anaemia, in keeping the histochemical evidence of decreased body iron stores reported in Chapter 6.

Elevated levels of free erythrocyte protoporphyrin are seen in anaemic mice.

Qualitative differences in haemoglobin and transferrin have not been demonstrated in normal and anaemic mice, by starch gel electrophoresis, and the transferrin of anaemic mice binds ^{59}iron in vitro in a manner similar to normal animals.

Transplanted bone marrow and spleen cells from an anaemic (sla/-) mouse, injected into hosts whose own haemopoietic tissue has been ablated by X-irradiation, are capable of producing in these hosts, morphologically normal erythrocytes, and of maintaining normal haemoglobin concentrations and haematocrit levels.

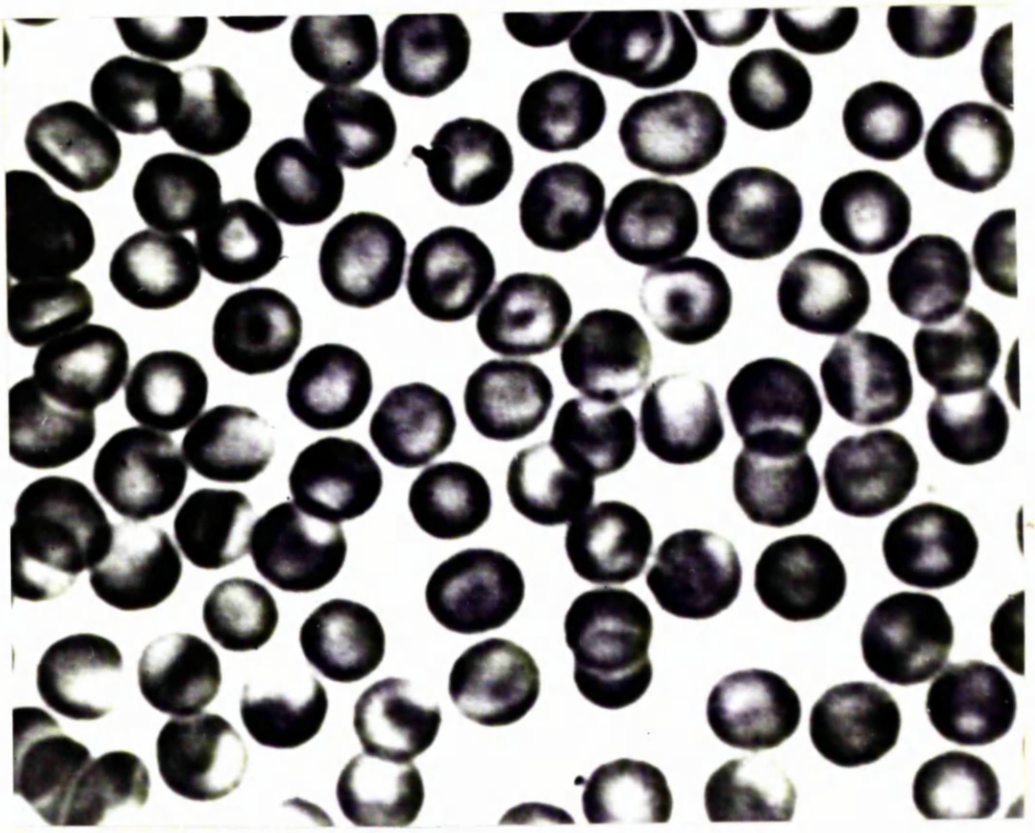


Fig. 8.6: Blood film of irradiated host, 116 days after grafting with normal (+/-) bone marrow cells. The red cells appear normal. Wright's stain, x 1,800.

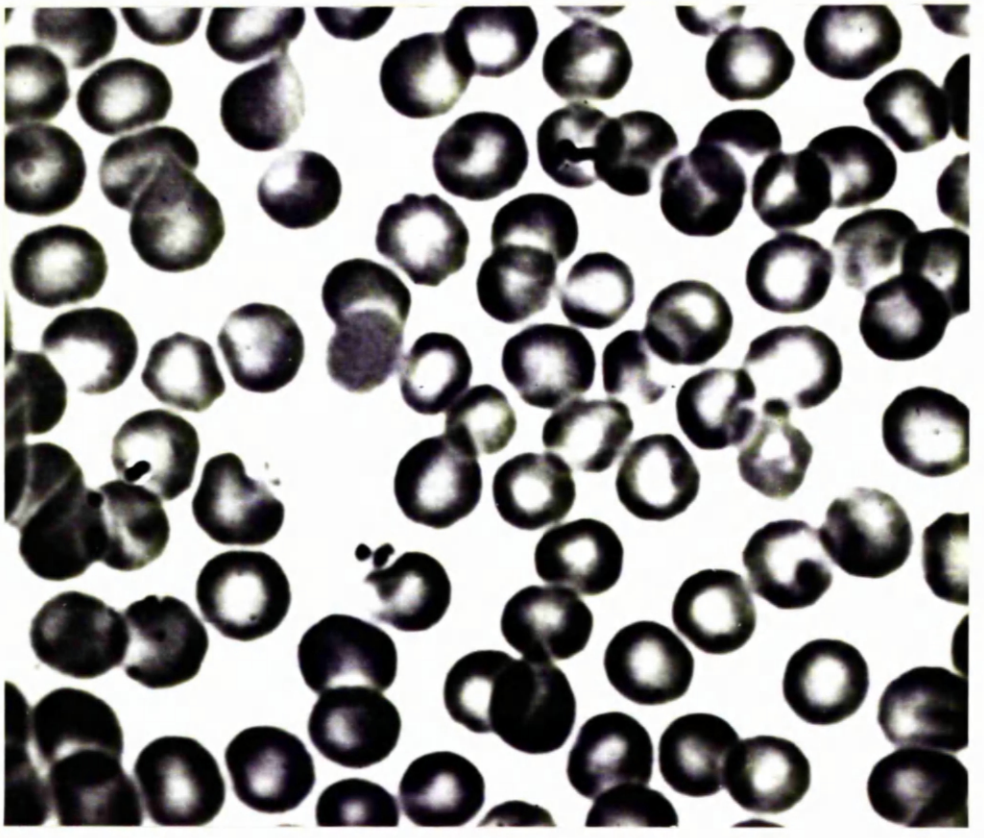


Fig. 8.7: Blood film of irradiated host 116 days after grafting with bone marrow cells from an anaemic (sla/-) mouse. The red cells appear normal. Wright's stain, x 1,800.

CHAPTER 9

The Effect of Treatment on X-Linked Mouse Anaemia

As part of the investigation of the nature of X-linked anaemia in mice, the effect of various therapeutic agents on the course of the anaemia has been studied. Details of the results of these investigations are given in the Appendix E.

Iron:

Iron dextran ("Imferon"), in doses containing 0.5 mg of elemental iron, was given intraperitoneally to 11 anaemic male (sla/-) and 6 anaemic female (sla/sla) mice. Of these 17 animals, 9 showed a rapid rise (within 7 days) in haematocrit to normal levels, which was sustained over periods varying from 15 to 57 weeks in 6 mice; in the remaining 3 a fall in haemotocrit was observed to occur between 7 and 32 weeks after treatment. Eight mice showed a response to this treatment which was delayed, or did not reach normal levels. The response of anaemic mice to 0.5 mg doses of iron is illustrated in Figure 9.1. The appearances of the blood film of a young anaemic mouse 3 weeks after treatment are shown in Figure 9.2. The red cells are uniform in size and shape, and show good haemoglobinization (c.f. Figs. 5.4, 5.6, 5.7 and 5.8).

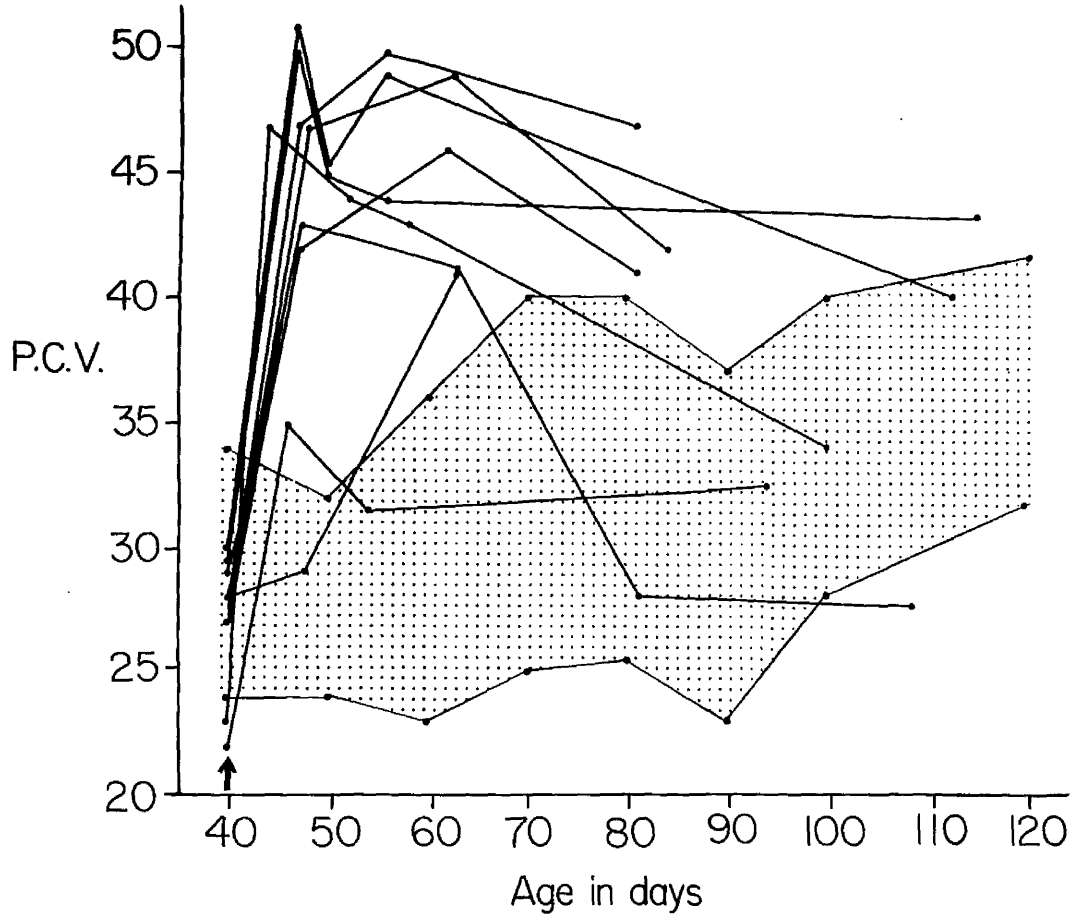


Fig. 9.1: The effect of treatment with 0.5 mg doses of iron, as "Imferon" parenterally, on X-linked mouse anaemia. Changes in packed cell volume (P.C.V.) are shown over a period of 80 days after treatment in 9 young anaemic mice. The stippled area represents the range of packed cell volume for untreated anaemic mice between the ages of 40 and 120 days.

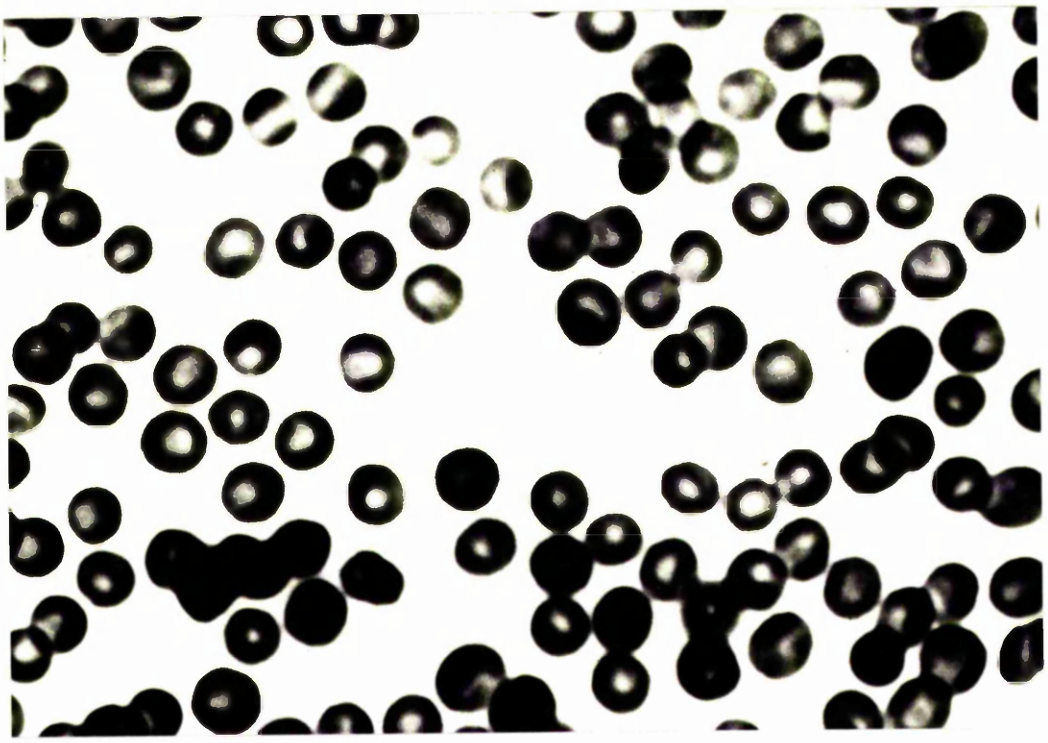


Fig. 9.2: The appearances of the blood film of an anaemic female (sla/sla) mouse 3 weeks after receiving 0.5 mg of elemental iron as "Imferon" intraperitoneally. The blood film is indistinguishable from normal (see Fig. 5.4) and the packed cell volume was 48%. Wright's stain x 1400.

Eight male anaemic mice were given 5 mg of elemental iron, as iron dextran ("Imferon"), intraperitoneally. In all, the haematocrit rose to normal within 7 days, and in all the response was sustained for 12 weeks at which time the animals were killed for histological examination.

Tissues were available for histological examination from 3 animals which received 0.5 mg of iron parenterally. In one the haematocrit had fallen from 44.5% at 16 weeks after treatment to 29% at 53 weeks after treatment, the initial response being good. Stainable iron was not present in the spleen, heart or lungs. In another, the rise in haematocrit was well sustained for 3 weeks after treatment, at which time the animal died of an unknown cause; the spleen, liver and heart were examined histologically and showed a complete absence of stainable iron. The third mouse died 3 days after treatment, before any haematological investigations were carried out; stainable iron was not present in the spleen or liver.

All 8 animals which received 5 mg of iron were sacrificed 12 weeks after treatment, when they were aged between 215 and 251 days. In all, the spleen iron stores were abundant (grade 4), and the spleen itself was of normal size with a normal proportion of red pulp. The liver parenchymal cells and Kupffer cells contained plentiful stainable iron, and the sternal bone marrow

(examined in 6 of the animals) showed an absence of stainable iron. The duodenal mucosal epithelium contained quantities of iron similar to those detected in the untreated mice (grade 2-4).

Pyridoxine hydrochloride:

Four anaemic male mice received a single parenteral dose of 0.1 mg of this agent, without a significant improvement in haematocrit. The mean haematocrit before treatment was 27% and 1 week after treatment, 28% ($p > 0.1$). Four anaemic male mice also received 5 mg of pyridoxine without significant improvement in the anaemia. In this group the mean haematocrit before treatment was 28% and 1 week after treatment, 30% ($p > 0.1$).

Vitamin B_{12} and folic acid:

Six anaemic male mice received 100 µg of vitamin B_{12} in a single parenteral dose. Consistent improvement in the anaemia was not seen and the mean haematocrit 7 days after treatment (28%) did not differ significantly from that before treatment (29%) ($p > 0.1$).

Folic acid in a single dose of 0.5 mg was given parenterally to 6 anaemic male mice; again consistent improvement in the anaemia was not seen, and the mean haematocrit 7 days after treatment (27%) did not differ significantly from that before treatment (26%) ($p > 0.1$).

Male hormone:

Six anaemic male mice received 20 mg of testosterone enanthate ("Delatestryl", Squibb) intraperitoneally, without significant improvement in the haematocrit. The mean haematocrit both before and after treatment was 28%.

Summary:

The anaemia has been treated with parenteral iron (0.5 mg and 5 mg), vitamin B_{12}, folic acid, pyridoxine hydrochloride and male hormone. None produced improvement except iron. With smaller doses the response was generally good, although sometimes less than maximal or poorly sustained. With the larger dose the improvement in the anaemia was uniformly good, the haematocrit rising to the normal range, where it remained for 3 months in all 8 animals so treated. Stainable iron was demonstrable in the tissues of these animals, but not in the tissues of 3 animals which had received the smaller (0.5 mg) dose.

CHAPTER 10

Discussion

Numerous facets of the problem of X-linked mouse anaemia require discussion, and these have been gathered under five main headings. The nature and causation of the anaemia are considered and its relevance to the Lyon hypothesis of X-chromosome inactivation is examined. The value of *sla* as a model for human disease is assessed. Intestinal iron absorption, and, in particular, the role of mucosal epithelial cell in iron absorption, is reviewed, and a model proposed for the regulation of intestinal iron absorption; within this model, possible sites of action of the *sla* gene are proposed. The chapter ends with a brief comment on the usefulness of the *sla* gene in experimental haematology.

The nature of X-linked anaemia of mice

The X-linked anaemia of mice is hypochromic and microcytic with poikilocytosis, and target cell formation, as is evident from blood films. The red cell indices, which show reduction in the M.C.H.C. and the M.C.V. confirm the hypochromic microcytic nature of the anaemia, and the red cell diameter estimations and red cell size scans obtained with the Coulter Model J particle size analyser also indicate microcytosis. The degree of anaemia reported here is similar to that found by Grewal (1962) but the severity of the changes in the red cells is greater in the Buffalo colony. This may be due to differences in genetic background, since the mice examined here in the assessment of the haematological picture were partially inbred from the original mixed stock supplied by Dr. Falconer, whereas Grewal's mice were descended from a single anaemic male of the original mixed stock, outcrossed with CBA/Gr x C57Bl/Gr hybrid females. Dietary differences may also have had an effect, but, since details of the diet used by Grewal are not available, assessment of this factor is not possible.

Red cell osmotic fragility is reduced in X-linked mouse anaemia (sla), and this may also be seen in hypochromic anaemias in man (Cassells, 1938; Valentine and Neel, 1944; Mooney, 1952; Dacie, 1960; Buchanan, Kinloch, Hutchison, Pinkerton and Cassidy, 1963). Grewal

(1962) found the osmotic fragility in his mice with sla to be normal; the reasons for this difference from my findings are not clear, but it may reflect the differences between the severity of changes in the red cells in Grewal's and in the Buffalo colony, and have the same possible explanations. Differences in technique could also be responsible.

Elevated reticulocyte counts in some anaemic mice, and the accompanying polychromatophilia, indicate increased activity on the part of erythropoietic system. The possibility that the reticulocytosis is a consequence of increased haemolysis has not been explored, but there is good evidence for decreased red cell survival in human hypochromic anaemia, including thalassaemia (Dacie, 1960; Bannerman, 1961; Weatherall, 1965) and chronic iron deficiency anaemia due to hookworm infestation (Loria, Sanchez-Medal, Lisker, de Rodriguez, and Labardini, 1967); decreased red cell survival has also been observed in baboons with chronic iron deficiency anaemia (Huser, Rieber and Berman, 1966).

The total white cell count, the white cell differential proportions and the platelet count are within normal limits in anaemic mice, and these findings together with the reticulocytosis, suggest that the proliferative and differentiating capacity of the haemopoietic system in mice with sla is unimpaired.

This suggestion is supported by the transplantation studies of Bennett, Pinkerton, Cudkowicz and Bannerman (in preparation) who have shown that the haemopoietic progenitor cells of mice with sla are as capable as their normal counterparts of repopulating the haemopoietic system of normal, lethally irradiated, host animals and of producing normal red cells in these hosts. It may also be noted here that the anaemias which result from disorders of differentiation and proliferation are macrocytic (see Chapter 2), whereas sla is hypochromic and microcytic.

Hypochromic anaemias are a consequence of inadequate haemoglobin formation within the red cell. Failure either of globin synthesis or of haem synthesis may be involved; the latter process may be defective either in the synthetic pathway for the formation of protoporphyrin IX, or in the supply of iron, or in the union of these two moieties in forming haem under the influence of haem synthetase (Goldberg, Ashenbruker, Cartwright and Wintrobe, 1956; Schwartz, Cartwright, Smith and Wintrobe, 1959). Further investigation of the mechanism of sla was therefore turned towards aspects of haemoglobin formation. The structure of the globin moiety of mouse haemoglobin is by autosomal genes (Popp and St. Amand, 1960; Popp, 1962b) whereas the anaemia described here is X-linked. In man, genes affecting quantitative globin chain synthesis seem to be closely linked to the structural genes; for example, the gene for the

hereditary persistence of haemoglobin F is closely linked to the structural genes for β- and δ- chain synthesis (Weatherall, 1965), and the thalassaemia genes, α and β, which disturb the rates of α- and β-chain manufacture, are also closely linked to, or are allelic with, the structural genes for α - and β-chain synthesis respectively (Weatherall, 1965). While the conclusions of human genetics cannot be directly applied to mice, it seems unlikely that a gene on the X-chromosome, (and thus remote from autosomal structural gene or genes) would directly influence the quantitative synthesis of globin in the mouse.

The qualitative characters of the haemoglobin of mice with X-linked anaemia was found not to differ from that of normal or heterozygous animals on starch gel electrophoresis, all animals examined showing the single band pattern (Ranney and Gluecksohn-Waelsch, 1955).

Estimations of the free erythrocyte protoporphyrin concentration in anaemic and normal mice revealed considerable elevation in the former. It was therefore considered improbable that X-linked mouse anaemia was a consequence of a defective step in the haem synthetic pathway up to the stage of protoporphyrin IX. Furthermore, closer examination of the possibility of iron deprivation of the erythron seemed likely to be rewarding, since raised free erythrocyte protoporphyrin concentrations are found in iron deficiency states in man (Pagliardi, Prato,

Giangrandi and Fiorina, 1959; Dagg, Goldberg and Lochhead, 1966). The evidence accumulated by histochemical and chemical studies of iron, and by investigations with radio-iron, in X-linked mouse anaemia support the hypothesis that iron deficiency is the cause of the anaemia. This evidence will now be summarized.

(i) Histochemical Studies. Cells containing haemosiderin granules were found in all the tissues of the normal mouse which were examined, except the testes and the pancreas. The principal iron storage site in the normal mouse is the spleen (Dunn, 1954) and the present study bears this out. The liver shows variable desposits of stainable iron in normal mice. Other organs have been found to show only scanty haemosiderin containing cells, in agreement with the observations of others (Morris et al, 1954). In sla, the tissue deposits of stainable iron (other than those in the duodenal mucosal epithelium, which will be considered more fully below) are virtually absent. Of 35 anaemic mice, only 8 showed any stainable iron whatever in the spleen and this amounted only to the merest traces; by contrast, all 25 normal mice examined had abundant haemosiderin in the spleen. Stainable iron was also found in some normal mice in the liver, bone marrow, kidney and heart, and in the mesenchymal tissue of the lungs, stomach and small bowel; such traces of stainable iron were not found in any anaemic mouse. Thus, the histochemically-demonstrable stores of iron are

clearly depleted in X-linked mouse anaemia.

Haemosiderin-containing erythroblasts were sought in the bone marrow smears of normal and anaemic mice; such cells (sideroblasts) were not found. The absence of sideroblasts in mice with sla is regarded as indirect support for the hypothesis that haem synthesis is not primarily affected, since disorders of the haem synthetic pathway characteristically give rise to a sideroblastic type of anaemia in man (Goldberg, 1965; Morrow and Goldberg, 1965) and experimental animals (Harriss, McGibbon and Mollin, 1965). The absence of sideroblasts in anaemic mice is consistent with iron deprivation but cannot be interpreted as direct support for a diagnosis of iron deficiency, since sideroblasts are also absent in normal mice with abundant stores of haemosiderin elsewhere.

The presence of haemosiderin-containing macrophages in the pulmonary alveoli and bronchioles of some of the anaemic mice would not invalidate the suggestion of iron deprivation of the erythron and other tissues since this iron is probably not available for haemopoiesis (Bothwell and Finch, 1962).

(ii) Estimates of total body iron content. Chemical estimates of total body iron content give low results in sla, providing further support the contention that mice with X-linked anaemia are iron-depleted.

(iii) Serum iron concentration and total iron binding capacity. Lowering of the serum iron concentration

with elevation of the total serum iron binding capacity, which is also present in sla, is characteristic of iron deficiency in man (Bothwell and Finch, 1962). In spite of the fact that the serum iron concentration is generally low in mice with X-linked anaemia, whether fasting or not, occasional very high values have been obtained. These are not due to the presence of haemoglobin iron in the serum upon which estimations of serum iron were made, since samples showing any trace of haemolysis were rejected. Contamination with extraneous iron cannot be excluded.

(iv) Radio-isotope studies. The clearance of iron from the plasma of anaemic mice is significantly faster than that in normal animals, and its reappearance in the circulation of anaemic mice is both more complete and more rapid than normal. These results are fully consistent with iron deficiency (Bothwell and Finch, 1962), although increased haemolysis can produce similar changes; and, indeed, an element of increased red cell destruction has not been excluded in sla.

In previous reports of preliminary studies of X-linked anaemia, Bannerman and Cooper (1964, 1966), and Pinkerton and Bannerman (1966) concluded that the utilization of ^{59}iron for haemopoiesis was similar in normal and anaemic mice. The calculations upon which this conclusion was based involved the assumption of a blood volume of 7 ml/100G

mouse (Bannerman, 1965a). It was considered important to ascertain if the blood volumes of normal and anaemic mice were indeed the same, on a volume/weight basis, in order that the calculations of utilization of iron for haemopoiesis might be as accurate as possible. The need for an assessment of blood volume in normal and anaemic mice was further increased by the knowledge that splenomegaly in man (also a feature of X-linked mouse anaemia), is associated with considerable increases in blood and plasma volume (McFadzean, Todd and Tsang, 1958; Bowdler, 1967; Richmond, Donaldson, Williams, Hamilton and Hutt, 1967); and by an impression gained during the taking of blood samples by cardiac puncture for serum iron and other estimations, that the quantities of blood obtained from anaemic mice were as large as these from normal mice, in spite of the smaller size of anaemic mice (see Chapter 5). As it transpired, anaemic mice proved to have a significantly increased blood volume, as compared with my estimations, and the estimations of others (see Chapter 1), of the blood volume of normal mice.

When the values for the total blood volume, obtained by the ^{51}Chromium red cell dilution technique, were applied to the calculations of the utilization of iron for erythropoiesis, the clear difference between normal and anaemic (sla/-) mice was obtained. It is obvious

from the blood volume, red cell mass and plasma volume data presented in Chapter 7 that the anaemia produced by the sla/- gene is in part a haemodilution effect.

(v) Effect of treatment. The favourable response of anaemic mice to intraperitoneal iron therapy, previously reported (Bannerman and Cooper, 1966) and confirmed here, suggests iron deficiency. Five mg of elemental iron produced an immediate rise in haematocrit which was well sustained for 3 months; the treated animals were then sacrificed for histochemical studies of the stores of iron, which proved to be abundant in the spleen and liver. It is of interest in this connection that adequate treatment with iron not only cures the anaemia and fills the iron stores, but also restores to normal, increases in proliferative activity in the erythropoietic tissues of sla/- mice, (Bennett et al, in preparation). The anaemia has also been found to respond favourably to smaller (0.5 mg) parenteral doses of iron. However, the response is sometimes incomplete or ill-sustained and, at autopsy, no iron is found in the stores, histochemically. It therefore seems likely that a dose of 0.5 mg of iron is inadequate to restore and maintain the haemoglobin concentration within normal limits, and to restock the iron stores; and that, in those animals where the improvement in haematocrit was poorly sustained, continuing negative iron balance was

responsible for the reappearance of the anaemia. In those animals in which the response to parenteral iron was very poor, no explanation is fully satisfactory. The possibility that the "intraperitoneal" injection of iron was, in fact, made into the bowel or bladder with subsequent excretion of some or all of the dose cannot be discounted.

Treatment with a variety of therapeutic agents (pyridoxine, vitamin B_{12}, folic acid, and testosterone derivatives) has been tried without benefit. Pyridoxine was given since X-linked human hypochromic anaemia may sometimes respond to that agent (Bishop and Bethell, 1959).

Intestinal absorption in sla.

The first direct evidence of a fault in iron absorption came to light during studies of the histochemical deposits of iron in the tissues. It was observed that the anaemic animals (with one exception) showed the presence of iron deposition in the mucosal epithelium of the duodenum, even after 6 hours fasting, whereas the majority of normal animals did not have any iron in the duodenal mucosal epithelium, and when present, only small amounts were seen; fasting normal mice showed total absence of such iron deposition. These observations are in sharp contrast to the presence or absence of stainable iron

in other tissues in normal and anaemic mice, respectively.

Studies of the intestinal absorption of iron using ^{59}iron-tagged ferrous sulphate in doses containing 0.1 µg, 1 µg and 10 µg of elemental iron revealed reduction of iron absorption by anaemic mice at all three dose levels, compared with normal animals. The differences between normal and anaemic mice were significant in spite of the wide range of variation in iron absorption in the mouse. The reduction in absorption of ^{59}iron-tagged ferrous sulphate is all the more striking when it is considered that iron deficient mammals, including man, tend to absorb more iron than normal (Bothwell, Pirzio-Biroli and Finch, 1958; Bannerman, O'Brien and Witts, 1962; Conrad and Crosby, 1963; Hallberg and Solvell, 1967).

No significant difference in iron absorption, between normal and anaemic mice, was found when 10 µg doses of iron, as ^{59}iron-tagged haemoglobin were given. However, the specific activity of the labelled haemoglobin was very low, a small dose was given and only a very small fraction of it was absorbed; thus, the residual levels of radio-activity retained by the animals 5 days after dosing was extremely low, scarcely above background, and errors in counting could well have obscured small differences between normal and anaemic mice. The results of this experiment are therefore regarded as inconclusive.

The X-linked anaemia of mice exhibits many of the

characteristics of iron deficiency in man, and seems to be a consequence of the failure adequately to absorb iron from the gastro-intestinal tract. The findings leading to this conclusion are summarised diagrammatically in Figure 10.1.

It is, of course, clear that the impairment of iron absorption in X-linked anaemia of mice must be less than complete, as total failure of iron absorption would be incompatible with life. The degree of impairment of iron absorption produced by the sla gene appears to vary, as there is negative correlation between the amount of iron deposited in the duodenal epithelial cells of anaemic mice, and the haematocrit. Animals with a less severe defect in iron absorption thus tend to show less iron deposition in the mucosal epithelium and less severe anaemia.

The possibility that the malabsorption of iron is not an isolated defect led to investigation of absorption of other substances - fat (in the form of ^{131}iodine-labelled triolein), ^{57}cobalt, ^{65}zinc and ^{64}copper. The absorption of fat was, if anything greater in anaemic mice, but the difference from normal animals was not significant, and the absorption of tracer doses of cobalt was similar in normal and anaemic mice. The absorption of both copper and zinc was rather less than normal in anaemic mice, but again the differences were not

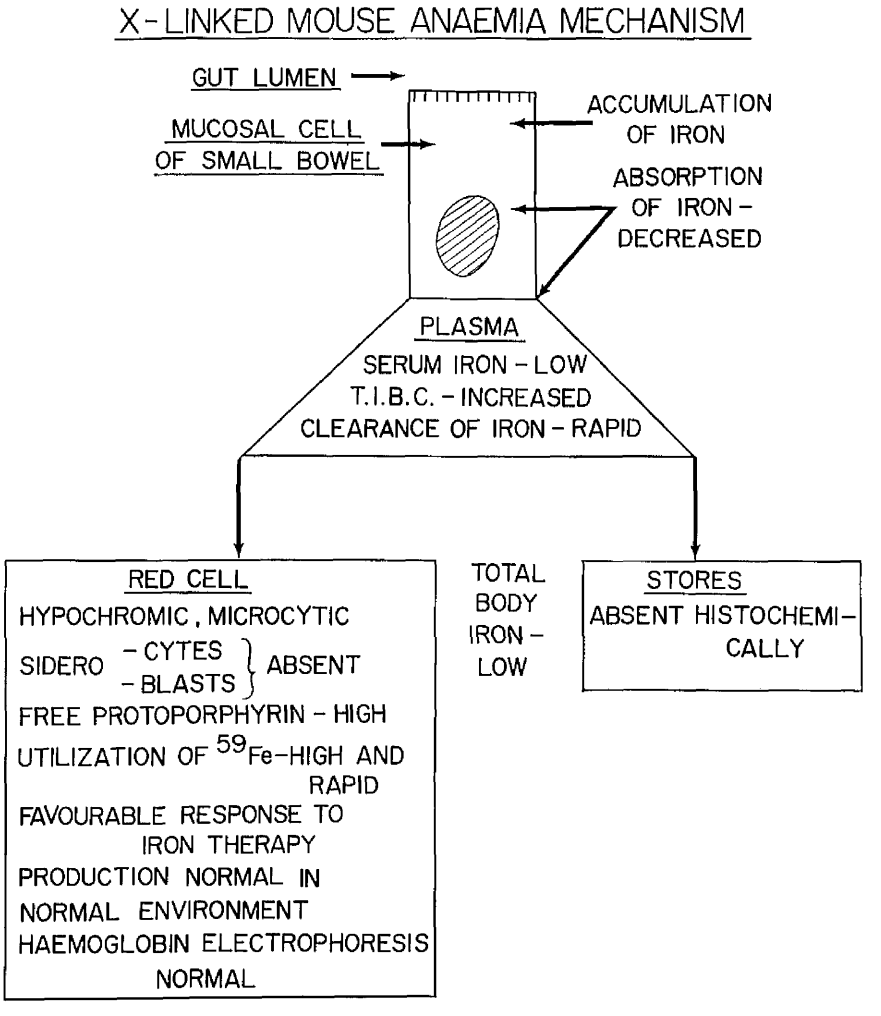


Fig. 10.1: Summary of the results of investigations leading to the conclusion that X-linked mouse anaemia is due to iron deficiency and intestinal malabsorption of iron.

statistically significant. The number of animals studied was small and the evidence that copper of zinc absorption is not abnormal in anaemic mice requires confirmation for certainty. Although copper deficiency may give rise to hypochromic anaemic, hypoferraemia, elevated total plasma iron-binding capacity (Lahey, Gubler, Chase, Cartwright and Wintrobe, 1952) and defective iron absorption (Gubler, Lahey, Chase, Cartwright and Wintrobe, 1952) in swine, the anaemia is not cured by the administration of iron (Cartwright, Gubler, Bush and Wintrobe, 1956) and the utilization of iron for haemopoiesis is defective (Gubler et al, 1952). Hence, it is unlikely that, even if copper were poorly absorbed in X-linked mouse anaemia, the anaemia is primarily a consequence of copper deficiency, since X-linked mouse anaemia responds to iron therapy and shows an increased utilization of tracer doses of iron for erythropoiesis.

Experimental zinc deficiency in rats leads to elevation of the haematocrit, testicular atrophy, sparse hair growth and splenic atrophy (Macapinlac, Pearson and Darby, 1966); none of these features is seen in mice with X-linked anaemia. In man, zinc deficiency has been seen in combination with other deficiencies, notably of iron, and the effect of zinc supplements in such persons has not included a rise in haematocrit, which only followed

treatment with iron (Prasad, 1966). Thus, it is unlikely that zinc deficiency alone caused the anaemia. A syndrome resembling hypopituitarism with delayed puberty is also seen in man in zinc deficiency (Sandstead, Prasad, Farid, Schulert, Miale, Bassilly and Darby, 1966); mice with X-linked anaemia show no evidence of delayed puberty, being as fecund as their normal counterparts.

It thus seems highly unlikely that zinc deficiency could be playing any significant role in sla.

The relationship of the sla gene to the process of intestinal iron absorption is discussed further in a subsequent section.

It should be emphasised that the results obtained here for the haematological assessment of normal control animals, the appearances noted in the normal mouse tissues, and the values for the serum iron concentration, total body iron content, plasma iron clearance rate, iron utilization for haemopoiesis, blood volume (by the ^{51}chromium and RISA techniques) and iron absorption in normal mice, agree well with previous reports in the literature, summarised in Chapter 1.

A number of miscellaneous findings, not directly related to the nature of X-linked mouse anaemia, merit discussion. Splenomegaly was frequently noted in anaemic mice and was generally related in degree to the severity of the anaemia; the increase in size was due to hyperplasia of the red pulp, and splenomegaly could

be attributed to increased haemopoietic activity, evidence of which was also seen in the abnormal persistence of haemopoietic foci in the livers of anaemic mice. The presence of increased erythropoietic activity in the spleen, as measured by the relatively greater numbers of erythroid precursors in anaemic mice, is consistent with the finding of increased numbers of erythropoietic ancestor cells in the spleens of anaemic mice by transplantation techniques (Bennett et al, in preparation). By contrast, there is no evidence to suggest hyperplasia of bone marrow in anaemic mice; significant alteration from normal in the erythroblast/granulocyte ratio in anaemic mouse bone marrow was not found and increase in the proportion of haemopoietic tissue in the medullary cavity at the expense of the vascular channels was also not seen in anaemic mice. Furthermore, there was no absolute increase in erythroblastic activity in the bone marrow of anaemic mice when bone marrow cells were transplanted into lethally irradiated hosts (Bennett et al, in preparation).

Part of the explanation for the presence of obvious erythroid hyperplasia in the spleen of anaemic mice, and the apparent absence of such hyperplasia from the bone marrow, may lie in recently demonstrated differences in the haemopoietic processes in the spleen and bone marrow of the mouse. When haemopoietic precursor cells from

mouse bone marrow are transplanted to lethally irradiated hosts and form colonies of haemic cells in the bone marrow and spleen of those hosts, the colonies in the bone marrow are predominantly granulopoietic and those in the spleen mainly erythropoietic (Curry and Trentin, 1967; Curry, Trentin and Wolf, 1967; Wolf and Trentin, 1968). Situations leading to erythroid hyperplasia, such as hypoxia, are known to affect the spleen of the mouse more dramatically than the bone marrow (Bruce and McCulloch, 1964) and a variant of Friend murine leukaemia virus, which causes a disease resembling polycythaemia vera, appears to affect predominantly the spleen (Mirand, 1967; Pinkerton, unpublished). Thus, it may be that in the mouse, the "soil" of the spleen is particularly conducive to erythroid proliferation, whereas that of marrow is not, and that, as a result, all or most of the hyperplasia of the erythroid series in mice with X-linked anaemia occurs in the spleen, rather than in bone marrow.

The blood volume determinations seem worthy of comment.

Mice with X-linked anaemia, in spite of reduction in the red cell mass, show an increase in plasma volume. In seeking an explanation for the increased blood volume in X-linked anaemia, the influence of splenomegaly requires consideration. Excessive and extremely rapid sequestration of the ^{51}Cr-labelled red cells in the enlarged spleens of anaemic mice could explain the high result with this

technique, but could not be the explanation for the difference using radio-iodinated serum albumin (RISA). The fact that two different labelling techniques gave very similar differences between normal and X-linked anaemic mice is interpreted as suggesting that there is indeed a true increase in blood volume in the anaemic animals.

An increase in blood volume has been found in man with chronic anaemia and splenomegaly, due to expansion of the plasma compartment (Keith, 1923; Berlin, Lawrence & Gartland, 1950; McFadzean et al, 1958; Bowdler, 1967; Richmond et al, 1967; Pryor, 1967). It has been suggested (Bowdler, 1967) that the increased blood and plasma volume is due to an expansion of the vascular space as a consequence of splenomegaly, but the study of Richmond et al (1967) indicates that the increase in plasma volume is too great to be accounted for merely by the plasma in the spleen pool. The mean increase in total blood volume in mice with sla is 3.08 ml/100G by the ^{51}chromium red cell method, and 2.53 ml/100G by the RISA method, while the mean increase in spleen size is only 0.59 G/100G body weight, and the greatest increase is less than 2 G/100G. The increase in spleen size alone is thus insufficient to accommodate the expansion of the blood volume. Both man and mouse with splenomegaly have an increase in blood volume which cannot be attributed merely to the increased size of the vascular pool. Further

detailed investigation of this aspect of the X-linked anaemia of mice may throw light on the mechanism by which enlargement of the spleen influences the plasma volume and causes a "dilution" type of anaemia. The effect of splenectomy on the plasma and blood volume in sla has not been studied, although, in man with splenomegaly the blood and plasma volumes are reduced by removal of the spleen (Richmond et al, 1967).

One of the early observations on X-linked mouse anaemia was its spontaneous improvement with age (Bannerman and Cooper, 1964, 1966) and the present study has confirmed this. Not only does the haemoglobin concentration rise, but the degree of morphological abnormality in blood films lessens, and the M.C.H.C., mean cell volume, and red cell volume distribution curves, tend to become normal. However, in spite of restoration of the haemoglobin concentration to normal, the total body iron stores, as measured both chemically and histochemically, remain deficient, and histochemical evidence of iron deposition in the duodenal mucosal epithelium persists. Thus, the histochemical evidence indicates that the fundamental metabolic defect in sla persists as the animal ages, in spite of the apparent improvement as reflected by the peripheral blood findings. The improvement in the anaemia with age is probably a manifestation of more favourable iron balance resulting from decreased iron requirements as the growth

rate slackens; thus, the defective iron absorption mechanism becomes relatively better able to meet the demands for iron erythropoiesis.

X-linked mouse anaemia and the hypothesis of X-chromosome inactivation

The hypothesis concerning inactivation of the X-chromosome in mammals was put forward by Lyon (1961) and, independently, by Russell (1961), on the basis of observations on mice. The normal phenotype of female mice of chromosome constitution XO implied that only one X-chromosome is necessary for normal development in the mouse. Also, female mice heterozygous for various X-linked genes affecting coat colour (e.g. Mottled, Mo, and Tabby, Ta) show patches of normal and mutant colour. To explain these observations, the hypothesis of X-chromosome inactivation (known as the "Lyon hypothesis") was drawn up. Briefly, the hypothesis states that, in each somatic cell of the female, only one of the two X-chromosomes is functioning and that inactivation of one of the two X-chromosomes occurs early in embryological development. Whether the paternal or maternal X-chromosome becomes inactive in a matter of chance, and once an X-chromosome is inactivated in any developing cell, all the progeny of that cell maintain the same X-chromosome inactive,

whether it is paternal or maternal. Thus, approximately half the cells of the female have the paternal X-chromosome active, and in the other half the maternal X-chromosome is functioning; and the female heterozygote for any two allelic genes at the same locus on the X-chromosome must, by implication, appear as a "mosaic" of cells (half of which exhibit the paternal, and half the maternal, gene effect) in the tissues in which the allelic genes are capable of expression.

The "Lyon hypothesis" has been extensively and frequently reviewed (Lancet, 1963; British Medical Journal, 1963; McKusick, 1964; Davidson, 1964; Teplitz and Beutler, 1966; Lyon, 1966) and appears to have met with general acceptance, although Grüneberg (1966, 1967 a and b) remains highly critical. A detailed discussion of the rapidly accumulating evidence favouring the validity of the Lyon hypothesis in various species would not be appropriate here, but a few examples of the phenomenon as observed in the human female heterozygous for X-linked mutations may be cited. In women heterozygous for glucose-6-phosphate dehydrogenase (G-6-PD) deficiency, clones of cultured fibroblasts are divisible into two distinct populations, one with normal G-6-PD activity and one with much reduced G-6-PD activity. None of the clones examined showed intermediate activity (Davidson, Nitowsky and Childs, 1963). Furthermore, clones of fibroblasts grown from negro women, heterozygous for the A and B electrophoretic

variants of G-6-PD, showed either the A (fast) or B (slow) pattern, but in no clone was a double (A plus B) pattern found. A mosaic pattern of erythrocytes in heterozygotes for G-6-PD deficiency has also been demonstrated - one population normal, the other with reduced enzyme activity (Beutler and Baluda, 1964).

In X-linked hypochromic anaemia of man a duality of red cell population may sometimes be seen in blood smears from female "carriers", with approximately half the cells hypochromic and microcytic and half normocytic and normochromic (Pinkerton, 1967). Recently, evidence tending to confirm the duality of red cell population in this disease has been provided. A woman and two daughters exhibited a blood picture with striking duality of red cell morphology, similar to that described above. Xg^a blood groups were determined in all family members. The mother, the two affected daughters and two normal daughters were Xg^a positive and the father, Xg^a negative. In the mother and one affected daughter, the two red cell populations were separated by differential centrifugation, and Xg^a blood groups determined; in the mother both populations were Xg^a positive; in the daughter the microcytic population of red cells was Xg^a positive (paternal X-chromosome inactive) and the normal-looking cells were Xg^a negative (maternal X-chromosome inactive) (MacDiarmid, Lee, Cartwright and Wintrobe, 1967).

The question of X-chromosome inactivation in carriers of the sla gene will now be considered. It was soon observed that heterozygotes (sla/+) showed little or no haematological abnormality (Falconer and Isaacson, 1962; Grewal, 1962) and the latter considered them sufficiently normal to employ as normal controls for comparison purposes. In general, the results obtained in the Buffalo colony are in agreement with Grewal's observations. However, in young heterozygous (sla/+) mice, the haemoglobin and haematocrit estimations occasionally gave rather low results and minor abnormalities have occasionally been seen in blood films (see Chapter 5). However, attempts to distinguish heterozygotes on the grounds of red cell morphology failed, as most could not be differentiated from the normal, and those with minor abnormalities could not be told apart from older anaemic mice in which spontaneous recovery was nearly complete. Certainly, no heterozygote demonstrated a duality of red cell population.

Furthermore, evidence of a dual population of red cells was not detected in red cell diameter or volume distribution curves, nor was a "tail" of abnormally resistant cells found during osmotic fragility determinations. Thus, if the provisions of the Lyon hypothesis hold true, the primary gene effect of the sla mutant, and therefore of its normal allele, was unlikely to be one

controlling a function within the erythron, but could only be affecting the red cells indirectly. These observations are consistent with absence of interference with protoporphyrin and globin synthesis, which have been discussed earlier, and they are also in keeping with the ability of haemopoietic cells of anaemic male mice to produce morphologically normal red cells when transplanted into a host environment normal at the sla locus. They are further consistent with the suggestion put forward above that X-linked anaemia is due to iron deprivation of the haemopoietic system.

The histochemical stores of iron in the heterozygous females showed a distribution similar to that seen in the normal mouse, but in some the quantity of stainable iron in the spleen was less than normal. There was no evidence of haemopoietic hyperplasia. Deposits of stainable iron were present in the duodenal epithelium of all heterozygotes examined, even after 6 hours fasting. Iron deposition was intermediate between the normal and anaemic mice, and tended to be patchy in distribution. This finding may be considered in the light of Grüneberg's (1967a) criteria for acceptance of an X-linked recessive trait in a human heterozygote as fitting the Lyon hypothesis. The following criteria are relevant to X-linked mouse anaemia:

1) Both hemizgous phenotypes must be known;
2) The contrasted patches of heterozygote must be arranged at random;

3) The patches of the heterozygote must correspond to the phenotypes of the hemizygotes;

4) Large areas of uniformly intermediate phenotype must not be present;

5) The contrasted patches should, on average, cover equal areas in the heterozygote.

Criteria 1, 2 and 4 are satisfied. In respect of criterion 3, there is overlap in the appearances between normal and heterozygous mice on the one hand, and between heterozygous and anaemic mice on the other, especially in the non-fasting group. Also it has not been possible to assess accurately in histological sections whether the contrasted patches occupy equal areas. Clearly, detailed examination of this problem by more refined techniques is required before an accurate assessment can be made of the relationship of the haemosiderin deposits in the small bowel epithelial cells of the heterozygotes for the sla gene to the Lyon hypothesis. It can safely be stated, however, that none of the findings in the small bowel, so far, are inconsistent with the X-chromosome inactivation theory.

X-linked mouse anaemia and the inherited hypochromic anaemias of man

It is part of the purpose of this thesis to assess the value of X-linked mouse anaemia as a model for human

disease, and other authors have already suggested a resemblance to β-thalassaemia (Cooley's anaemia) and to the X-linked hypochromic anaemia also described by Cooley (1945), and by Rundles and Falls (1946).

Grewal (1962) first pointed out a resemblance to the thalassaemias in man, which are disturbances in the quantitative synthesis of the constituent globin chains of the haemoglobin molecule (Weatherall, 1967). Unlike thalassaemia, the mouse disorder responds to iron therapy and improves spontaneously with age. In addition, on genetic grounds discussed previously in this chapter, the analogy is unlikely to be valid. Further evidence against any close resemblance between sla and thalassaemia is supplied by comparison of the iron status in the two disorders. In human β-thalassaemia, the serum iron is elevated with a high level of saturation of the iron binding capacity of the plasma (Smith, Sisson, Floyd and Siegal, 1950; Bannerman, 1961), and the histochemically demonstrable iron stores are almost always increased (Whipple and Bradford, 1932, 1936; Ellis, Schulman and Smith, 1954; Dacie, 1960; Bannerman, 1961). No evidence of a qualitative defect in haemoglobin synthesis has been discovered in sla, whereas abnormal persistence of haemoglobin F is a well recognized and characteristic feature of human β-thalassaemia (Dacie, 1960; Huehns and Shooter, 1965; Lehmann and Huntsman, 1966), and the

presence of the variant haemoglobin H (β_4) a frequent finding in human α-thalassaemia (Huehns and Shooter, 1965; Weatherall, 1965). Thus, the evidence presented here excludes a fundamental similarity between the human thalassaemias and X-linked mouse anaemia.

A closer analogy to the X-linked hypochromic anaemia of man has been postulated (Bannerman and Cooper, 1964; McKusick, 1964; Ohno, 1967), and the attractiveness of this suggestion is increased by the knowledge that certain analagous genes in divers species are known all to be X-linked, e.g., those for anti-haemophilic globin man, the dog (Graham, Buckwalter, Hartley and Brinkhous, 1949) and, probably, the horse (Nossel, Archer and Macfarlane, 1962), factor IX in man and the dog (Mustard, Rowsell, Robinson, Hocksema and Downie, 1960), and glucose-6-phosphate dehydrogenase in man, the horse and the donkey (Trijillo, Walden, O'Neil and Anstrall, 1965; Mathai, Ohno and Beutler, 1966).

The X-linked hypochromic anaemia of man (Cooley, 1945; Rundles and Falls, 1946) characteristically shows the presence of iron-containing erythroblasts in the bone marrow (Lukl, Wiederman and Barovik, 1958; Bourne, Elves and Israels, 1965; Losowsky and Hall, 1965; Hall and Losowsky, 1966). These cells ("sideroblasts") are not seen in sla, and the reduction in iron stores and serum iron concentration in these animals is in sharp contra-distinction to the increase usually seen in the human disorder

(Gelpi and Ende, 1958; Dacie, 1960; Byrd and Cooper, 1961; Morrow and Goldberg, 1965). A response to large doses of pyridoxine is sometimes seen in X-linked anaemia of man (Bishop and Bethell, 1959) but the murine anaemia is unaffected by this agent.

Thus it seems that the suggestion of an analogy between the X-linked hypochromic anaemia of man and the mouse is merely superficial.

Both thalassaemia and X-linked hypochromic (sideroblastic) anaemia of man are consequences of intrinsic defects in the red cell (Weatherall, 196?; Morrow and Goldberg, 1965). As discussed earlier, X-linked anaemia appears to have an extra-erythrocytic mechanism and thus could not represent a model for thalassaemia or for X-linked sideroblastic anaemia of man.

Atransferrinaemia in the human, an autosomally transmitted disorder, produces severe hypochromic anaemia with iron overload in the tissues (Heilmeyer, Keller, Vivell, Keiderling, Betke, Woehler and Schultz, 1961). This disorder has been excluded by the demonstration, both here and previously (Cohen, 1962), that transferrin is present in X-linked mouse anaemia and I have also shown that the transferrin of mice with X-linked anaemia is capable of binding ^{59}iron in vitro in a manner comparable to that of normal animals. A resemblance to the syndrome of congenital hypochromic anaemia with iron

overload described by Shahidi, Nathan and Diamond (1964) might also be suggested, but sla clearly differs from this in responding to iron therapy and in the absence of increased deposition of iron in the tissues.

Two other human disorders, of doubtful aetiology, bear some similarity to sla. Idiopathic pulmonary haemosiderosis in man is characterized by deposition of large quantities of iron in the lungs, and anaemia due to iron deficiency (Bothwell and Finch, 1962). While X-linked mouse anaemia shows many of the characteristics of human iron deficiency, the lungs of animals with sla do not contain large deposits of stainable iron. A few anaemic animals showed the presence of haemosiderin-containing macrophages in the alveoli, and so, too, did some of the normal mice; the appearances did not, however, resemble the massive deposition of iron seen in the lungs in human idiopathic pulmonary haemosiderosis (Bothwell and Finch, 1962; Cappell, 1964). The presence of malabsorption of iron indicated the necessity to exclude a form of "idiopathic steatorrhoea" in mice with X-linked anaemia. The appearance of the small bowel mucosa of anaemic mice did not show any alteration from the normal villous pattern, and conformed to the descriptions given by Malewitz (1965) and Hummell et al (1966); the only abnormality detected has been the presence of excessive iron in the mucosal epithelium.

In addition, there is no clear evidence of malabsorption of fat, cobalt, copper or zinc in anaemic mice. X-linked anaemia of mice cannot therefore be regarded as analagous to human coeliac disease or idiopathic steatorrhea, which, typically, exhibit stunting or flattening of the villi of the small intestinal mucosa (Paulley, 1954; Badenoch, 1960; Jeffries, Weser and Sleisenger, 1964; Lancet, 1967) and increased faecal excretion of fat.

Thus, a clear resemblance between X-linked mouse anaemia and an inherited human anaemia has not emerged during this study; however, it is quite possible that an analagous human disease will, in due course, come under observation.

X-linked mouse anaemia and iron deficiency in man

X-linked anaemia of mice appears to resemble acquired human iron deficiency anaemia more closely than any of the heritable hypochromic anaemias of man, and provides a model for study of the effects of iron deficiency on growth and metabolism in general, and on iron containing enzymes and other proteins in particular. Preliminary studies of the cytochrome enzymes in the tissues of mice with sla have already been carried out and reduction of their activity in some organs has been demonstrated (Doeblin, 1967).

X-linked mouse anaemia and iron absorption

Before discussing the relationship of X-linked mouse anaemia to iron absorption, the literature concerning this process will be reviewed. Since McCance and Widdowson (1937), in their classic review of iron balance which preceded the use of radio-isotopes, concluded that the control of the iron stores depended on iron absorption and not on regulated iron excretion (as had been widely held up until that time) much work, ingenuity and thought have been devoted to elucidating mechanisms whereby iron absorption can be influenced. While many aspects have been clarified mainly by the use of radioisotopes, we are still far from a full understanding of iron absorption.

A wide range of factors influence iron absorption and these have been summarized in Tables 10.1 and 10.2; some factors which, apparently, do not influence iron absorption, although they might reasonably have been expected to do so, are shown in Table 10.3. Iron absorption can occur throughout the length of the small bowel, but is maximal in the upper small intestine (Beutler, Fairbanks and Fahey, 1963; Brown, 1963; Wheby, Jones and Crosby, 1964) and the form in which iron is presented to the intestinal tract is of some importance. Iron is apparently more easily absorbed when given in the ferrous than in the ferric form (Furth and Scholl, 1936; Moore, Dubach, Minnich and Roberts, 1944; Hahn, Jones, Lowe, Meneely

and Peacock, 1945; Brise and Hallberg, 1962) in man, and rabbits, whereas little difference in absorption is seen when ferrous and ferric iron are compared in dogs, rats and mice (Underwood, 1938; Austoni and Greenberg, 1940; Moore et al, 1944); species differences may be important. It has been suggested that iron can only be taken up by the small bowel mucosa in the ferrous form (Bergeim and Kirch, 1949; Ventkatachalam, Brading, George and Walsh, 1956) and that ferric is converted to ferrous iron in the lumen of the small bowel; this is in keeping with the well known enhancement of iron absorption produced by the addition of reducing agents such as ascorbic acid (Moore and Dubach, 1951; Williams, 1959; Sorensen, 1965; Hallberg and Solvell, 1967). In contrast, the addition of certain substances, forming inscluble complexes with inorganic iron, inhibits absorption. Phytate decreases the absorption of inorganic iron (McCance, Edgecombe and Widdowson, 1943; Sharpe, Peacock, Cooke and Harris, 1950; Hallberg and Solvell, 1967) as does the presence of phosphate (Hegsted, Finch and Kinney, 1949). However, phytate does not reduce, nor does ascorbic acid clearly enhance, the absorption of haemoglobin iron (Hallberg and Solvell, 1967). Alcohol enhances the absorption of ferric iron (Charlton, Jacobs, Seftel and Bothwell, 1964), probably by stimulating the secretion of gastric acid. The administration of chelating agents (E.D.T.A. and desferrioxamine)

with doses of oral iron decreases the absorption of inorganic iron (Larsen, Bidwell and Hawkins, 1960; Hwang and Brown, 1963, 1965; Bannerman and Malpas, 1965). A wide variety of organic substances including fructose, lactate, pyruvate, cysteine (Pollack, Kaufman and Crosby, 1964b), and other organic acids (Groen, van den Broek and Veldman, 1947) have been shown to increase iron absorption, and it has recently been suggested by Saltman and his co-workers that iron is taken up by the mucosal epithelium as a chelate, in combination with low molecular weight organic compounds such as fructose and citrate (Saltman, 1965; Saltman and Helbock, 1965). There is, however, no clear evidence at present as to whether iron is required to be so chelated for uptake into the mucosal epithelium of the small bowel.

The iron derived from iron salts is more readily absorbed than is food iron or iron in the form of haemoglobin (Chodos, Ross, Apt, Pollycove and Halkett, 1957; Callender, Mallett and Smith, 1957; Schulz and Smith, 1958a, 1958b; Bannerman, 1965b; Hallberg and Solvell, 1967), and species differ in their relative ability to absorb organic and inorganic iron (Bannerman, 1965b). The process of absorption of haemoglobin iron is probably different from that of inorganic iron absorption, since the uptake of iron from haemoglobin is not influenced by desferrioxamine (Hwang and Brown,

1963, 1965; Bannerman and Malpas, 1965), or phytate Hallberg and Solvell, 1967) and is little affected by ascorbic acid (Halberg and Solvell, 1967). The rate of absorption of haemoglobin iron is slower than that of iron from inorganic sources (Callender, et al, 1957; Hallberg and Solvell, 1967). Haemoglobin iron is probably taken up by the mucosal epithelium as haem (Callender et al, 1957; Conrad, Weintraub, Sears and Crosby, 1966), which may explain the absence of an effect of desferrioxamine, which requires iron to be ionized for binding.

The importance of the stomach and its secretions have not yet been fully clarified in spite of extensive investigation. Partial gastrectomy in man has been shown to impair the absorption of both organic and inorganic iron (Stevens, Pirzio-Biroli, Harkins, Nyhus and Finch, 1959; Turnberg, 1966) although other workers have found no differences in iron absorption before and after partial gastrectomy (Smith and Mallett, 1957). In rats, total gastrectomy abolishes the ability of the animal to increase its iron absorption in response to iron deficiency, or blood loss (Whitehead and Bannerman, 1964; Murray and Stein, 1967b) and in man after partial gastrectomy, the administration of whole hog stomach enhances uptake from inorganic sources (Turnberg, 1966); absorption is impaired in achlorhydric iron-deficient subjects, when inorganic iron salts are used (Goldberg,

Lochhead and Dagg, 1963; Jacobs, Rhodes, Peters, Campbell and Eakins, 1966) but gastric acidity does not seen to affect the absorption of haemoglobin iron (Callender et al, 1957; Biggs, Bannerman and Callender, 1961). Jacobs, Rhodes and Eakins (1967) found that the addition of gastric juice from an anaemic, iron-deficient, patient increased iron absorption in achlorhydric individuals. However, hydrochloric acid alone produced a similar enhancement of iron absorption and thus it was concluded that gastric juice from an anaemic individual with iron deficiency exhibited an enhancing effect that could be ascribed simply to hydrochloric acid. This is at variance with the findings of Koepke and Stewart (1964), where iron absorption in normal dogs was found to be increased when an oral dose of iron was given with gastric juice from an iron deficient animal. A further claim has been put forward that a constituent of gastric juice other than hydrochoric acid influences iron absorption. Waxman, Pratt, Cuttner and Herbert (1966) found that hog intrinsic factor enhances iron absorption in both organic and inorganic forms; the previous addition of vitamin B_{12} did not inhibit this effect but exposure to anti-intrinsic factor antibody prevented enhancement of iron absorption.

Deller and his colleagues (Luke, Davis and Deller, 1967) have suggested that gastric juice may contain a substance, named by them "gastroferrin", which, hypothetically

binds iron and prevents its absorption. This substance is said to be present in reduced quantity in the gastric juice of iron deficient subjects, presumably thereby permitting a greater level of absorption. This hypothesis remains to be confirmed and a serious drawback is the absence of enhancement of iron absorption by removal of the stomach, and therefore of the postulated source of gastroferrin.

It is clear that the stomach and its secretions have an influence on iron absorption, especially where inorganic iron is concerned, but the precise mechanism or mechanisms by which this influence is mediated remain to be fully elucidated.

In 1935, it was noticed that ligation of the pancreatic duct led to siderosis in cats (Taylor, Stiven and Reid, 1935) and shortly afterwards Andersen (1938) observed a high incidence of siderosis among children with cystic fibrosis of the pancreas. Experimental pancreatic damage in animals, whether induced by ethionine or by a low protein diet, leads to excessive iron absorption (Kinney, Kaufman and Klavins, 1955; Kaufman, Klavins and Kinney, 1958). This has again been demonstrated by Brozovic, Popovic, Obradovic and Pendic (1966) who also showed that pancreatin would decrease iron absorption to near normal levels in animals previously treated with ethionine. Iron absorption in humans with

chronic pancreatitis is increased in a substantial proportion of cases (Davis and Badenoch, 1962) and iron absorption can be inhibited by the administration of pancreatin and pancreatic extract (Biggs and Davis, 1963; Smith, 1964; Davis and Biggs, 1967). In children with cystic fibrosis, an increase in iron absorption was detected when inorganic iron absorption was tested, but not when an organic tracer was used (Tönz, Weiss, Strahm and Rossi, 1965). Balcerzak and colleagues, on the other hand, found no increase in iron absorption, which could not be ascribed to iron deficiency, in 11 patients with chronic pancreatitis. Balcerzak, Peternel and Heinle, (1967), and Murray and Stein (1967a) did not obtain a reduction in iron absorption with crude powdered pancreas.

Davis and Biggs (1967) feel that it is unlikely that the mere lowering of pH in the duodenal contents by the absence of alkaline pancreatic secretion is enough to account for the increased iron absorption of pancreatic disease. However, the absorption of haemoglobin iron (as metalloporphyrin) is much inhibited by raising the pH, which has a polymerizing effect on haem, and hence, pancreatic secretions may be important in the regulation of the absorption of iron in organic form (Conrad, Cortell, Williams and Foy, 1966). A constituent of pancreatic extract, probably a polypeptide, and duodenal secretions from normal persons, can inhibit iron uptake by rat jejunum,

(Davis and Biggs, 1967). The mechanism whereby the pancreas influences iron absorption requires further clarification, and multiple factors may prove to be involved.

Iron absorption is also increased in cirrhosis of the liver (Callender and Malpas, 1963; Friedman, Schaefer and Schiff, 1966; Williams, Williams, Scheuer, Pitcher, Loiseau and Sherlock, 1967) and this enhancement is seen with both organic and inorganic iron tracers. Reduction in the level of iron absorption in these patients is produced by crude hog pancreas (Callender and Malpas, 1963). The cause of the increase in iron absorption in cirrhosis of the liver is not clear, but it is not due to iron deficiency (Williams et al, 1967). Since pancreatic damage is frequently associated with cirrhosis of the liver, especially that of alcoholic etiology (Sobel and Waye, 1963), it may be that increased iron absorption in hepatic cirrhosis is a consequence of concomitant pancreatic damage, rather than of cirrhosis per se.

Decreased iron absorption is seen in idiopathic steatorrhoea (Badenoch and Callender, 1954; Bonnet, Hagedorn and Owen, 1960) presumably as a result of the loss of absorptive surface area. An experimental syndrome in which various substances, including iron, are poorly absorbed from the intestine has also been described with the use of neomycin (Jacobson, Chodos and Faloon, 1960).

In the next few pages, various aspects of iron absorption in relation to the mucosal epithelial cell will be considered. Following the realisation that iron absorption is a major controlling factor in maintaining normal iron balance, attention focussed on the role of the small bowel, and Hahn and his collaborators (Hahn, Bale, Ross, Balfour and Whipple, 1943) and Granick (1946a, 1946b) put forward their classical theory of "mucosa block", in which the epithelial cell of the small intestinal mucosa played a key part. Since that time there has been considerable controversy over the "mucosa block" hypothesis; it seems now to be generally accepted that the mucosal cell of the small bowel does exert some control over the absorption of iron, although in a rather more complicated manner than was originally suggested by Granick (1946a, 1946b). This subject has been reviewed several times in recent years (Callender, 1959, 1967; Brown, 1963; Crosby, 1964, 1966; Wheby, 1966).

Hahn et al (1943) observed that the anaemia of chronic blood loss in dogs was associated with a 5 to 15 fold increase in iron absorption. Acute blood loss, on the other hand, did not produce an increase in iron absorption until several days had elapsed. Furthermore, if a large dose of iron was given 1 to 6 hours prior to testing with radioiron, absorption of tagged iron was found to be suppressed. On the basis of these findings,

the idea emerged of a "mucosa block" to iron absorption, which depended on alternate saturation and desaturation of an acceptor system in the mucosal cell. Desaturation was a slow process occurring over a period of days, whereas saturation could occur rapidly, within hours. Granick (1946a, 1946b) postulated a more specific mechanism for controlling iron uptake from the gut. He demonstrated a great increase in the amount of ferritin in the guinea-pig small bowel mucosa after oral administration of iron. The ferritin content of the mucosa was maximal 7 hours after administration and gradually decreased over 3-6 days. Granick suggested that, after iron is taken up by the epithelial cell, it is bound to an acceptor protein (apoferritin), forming ferritin, in which form the iron remains in the mucosal cell until required by the body, when the ferritin dissociates into iron (which is then transferred to the plasma) and apoferritin in the mucosal cells, and suggested that apoferritin formation was stimulated by the presence of iron in an unbound form (Granick, 1949, 1954).

It subsequently became clear that any such "mucosa block" was far from perfect. The absorptive mechanism apparently cannot be saturated with iron. Smith and Panacciuli (1958) demonstrated in man that, by increasing the dose of iron given orally, the absolute amount of iron absorbed can also be increased, although the proportion of the dose absorbed falls. Similar findings have

been reported in mice (Beutler, Kelly and Beutler, 1962; Gitlin and Cruchaud, 1962) and rats, in respect both of inorganic iron (Bannerman, O'Brien and Witts, 1962) and haemoglobin iron (Bannerman, 1965b). These findings do not negate the hypothesis of a mucosal block, but they clearly demonstrate that such a block is incomplete at any dose level of iron. Some of the observations in these experiments support the theory that iron absorption is influenced by the body's requirements. Iron-deficient persons, at all dose levels, absorb more iron than iron-replete individuals (Smith and Pannacciuli, 1958) and the same is true in rats (Bannerman, O'Brien and Witts, 1962).

Both Gitlin and Cruchaud (1962) and Bannerman, O'Brien and Witts (1962) found a curvilinear relationship between the size of the dose of iron given, and the amount absorbed. In higher dose ranges the relationship is linear, and, in the lower, it is curvilinear. These results are interpreted as indicating two absorptive mechanisms, one in which an enzymic, or "carrier", process is involved, and which is limited in capacity, and the other a process with first-order kinetics where the concentration of iron in the gut lumen is the limiting factor, and the maximum amount that can be absorbed depends on the size of the lethal dose. Both mechanisms appear to be facilitated by iron deficiency and inhibited, regardless of the size of the dose, by iron-loading

(Gitlin and Cruchaud, 1962). Thus, although "mucosa block" as postulated by Hahn et al, (1943) and Granick (1946a and b) is far from perfect, there is still some control of iron absorption even when near lethal doses of iron are given.

The experiments of Gitlin and Cruchaud (1962) and Bannerman, O'Brien and Witts (1962) suggest that a specific, controlling mechanism exists for iron absorption at the lower, more physiological, range of iron dosage, and that it is quite precise, adjusting rapidly as requirements change. The latter authors tested iron absorption in iron deficient rats on a low iron diet, then changed the diet to an iron-supplemented one and re-examined iron absorption. A rapid fall in iron absorption, to normal levels, was observed before the haemoglobin concentration had risen to normal. In the reverse experiment, rats changed from an iron-supplemented, to an iron-poor diet exhibited a rise in iron absorption before any effect was seen on the haemoglobin concentration. It was concluded that local factors, such as the amount of iron in the intestinal mucosa, were of greater importance than the haemoglobin concentration or the body stores of iron. Similar observations have been made by Pollack, Kaufman, and Crosby (1964a) and Charlton, Jacobs, Torrance and Bothwell (1965).

Autoradiographic studies of the small bowel

epithelium of rats have been carried out following oral and parenteral administration of radioiron (Conrad and Crosby, 1963). In normal rats, sections were made from the duodenum and jejunum at intervals up to 48 hours after oral dosing with iron. Two to 8 hours after the test dose, most of the villous epithelium showed radioactivity. At 12-24 hours, labelling was confined to the epithelium of the distal half of the villus, and at 36-40 hours, only the tips of the villi showed activity. Thus, iron from the bowel lumen taken up by the epithelial cell in the normally iron replete state is held there, at least in part, until the cell is shed. Sections from iron-deficient and iron-loaded rats showed little activity at any of these times. Normal and iron-loaded rats were killed 16 hours after parenteral injection of ^{59}iron and autoradiographs made of the upper small bowel. Radioactivity was principally located in the epithelial cells of the proximal half of the villi. Iron deficient rats showed little activity in the epithelium. These findings suggest that iron is incorporated into the mucosal epithelium as the cell is formed and remains there until the cell is shed, unless it is required for other purposes, such as in iron deficiency.

On the basis of these experiments, a hypothesis was elaborated for the regulation of iron absorption through the epithelium of the small bowel (Conrad and Crosby, 1963). It was suggested that, in the normally iron replete state, the quantity of iron in the body

stores regulates, to some extent, the acceptance of iron from the intestinal lumen, by the mucosal cell, through the amount of iron laid down in the cell at the time of its formation - the more iron laid down, the less can be "accepted". After entering the mucosal cell, dietary iron can be passed to the plasma, or lost when the cell is shed. (Fig. 10.2, left). In iron deficiency, the amount of iron incorporated in the cell is reduced and the acceptance of iron facilitated. More iron is absorbed than normal and little is lost in the exfoliated mucosal cell. (Fig. 10.2, centre). In the iron-loaded state, the quantity of iron laid down in the epithelial cell during its formation is excessive, and iron uptake from the intestinal lumen is obstructed; increased amounts of iron are lost as the mucosal cells are shed (Fig. 10.2, right).

Further experiments by these, and other workers, outlined below, are in keeping with this hypothesis. Conrad, Weintraub and Crosby (1964) observed that the maximal concentration of intravenously administered iron in the mucosal epithelium of rats occurs 16 to 24 hours after injection; suppression of iron absorption from an oral dose of tagged iron clearly followed 24 hours after a parenteral injection of 0.25 mg of iron, at which time there was a significant excess of iron in the mucosa, as measured chemically. Charlton et al (1965) noted depression in iron absorption which was detected as early as 6 hours after parenteral injection of iron and which

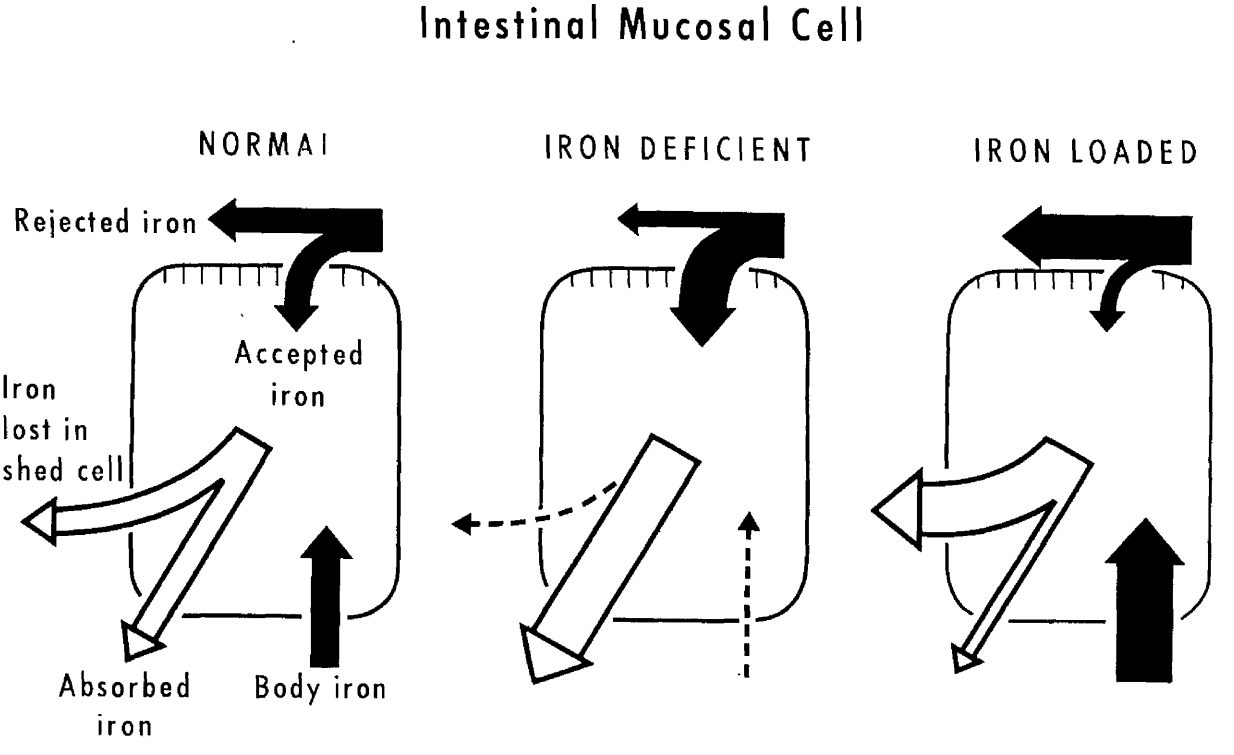


Fig. 10.2: A hypothesis for the regulation of iron absorption by the intestinal mucosal epithelium, under the influence of the body iron stores. After Conrad and Crosby (1963). See text.

reached a maximum at 18 hours. By 48 hours absorption of iron had risen again to near normal levels.

The possibility that iron is "held" in the small bowel epithelium in the normal individual until the mucosal cell is shed, and passed through the cell rapidly in the iron-deficient subject, has received indirect support from experiments in man using radio-iron and a faecal marker. Conrad and Crosby (1963) gave radio-iron and carmine red to normal and iron deficient subjects. In both groups, the carmine red was entirely evacuated within 5 days; faecal excretion of iron was complete at 3 to 7 days in iron deficient subjects, and 6 to 15 days in normal persons. Similar results have been obtained by Boender, Mulder, Ploem, de Wael and Verloop (1967) using ^{131}barium sulphate as the faecal marker. The lapse of time in completion of the faecal elimination of radio-iron in normal as compared with iron-deficient individuals is similar in length to the turnover time of duodenal epithelial cells in man, i.e. 5 to 6 days (MacDonald, Trier and Everett, 1964; Creamer, 1967). The fact that delay in intestinal transit may occur in iron-deficient rats (Copp and Greenberg, 1946; Bannerman, O'Brien and Witts, 1962) and dogs (Austoni and Greenberg, 1940) does not necessarily negate the conclusions of Conrad and Crosby (1963) and Boender et al (1967) based on investigations in man.

A negative correlation between the iron content of the bowel and iron absorption has been found in rats by Chirasiri and Izak (1966) and Pearson, Reich, Frank and Salamat (1967). Allgood and Brown (1967) estimated the non-haem iron concentration in duodenal biopsies in normal, iron-deficient and iron-overloaded human subjects and found no significant correlation of iron concentration with iron absorption. No obvious explanation emerged for the discrepancy between this and the studies in animals. Difficulty in ascertaining whether the iron measured was epithelial or non-epithelial, differences between the human and the experimental situation, and species and technical differences may all have played a part. More detailed investigations in man are clearly called for.

Extensive studies of the transport of iron by the small bowel mucosa of the rat, using everted gut sacs or loops of small bowel, have been carried out by Schachter and his colleagues (Dowdle, Schachter and Schenker, 1960; Manis and Schachter, 1962a, 1962b, 1964, 1965), Brown and co-workers (Brown and Justus, 1958; Brown and Rother, 1963) and by Jacobs, Bothwell and Charlton (1966). While caution is required in interpreting data obtained by such in vitro studies, where the conditions are quite unphysiological, the evidence which has emerged from these experiments is of considerable interest.

Brown and Justus (1958) found that iron transfer from lumen to serosa was similar in segments of small bowel

from all levels and suggested that the maximum absorption found in the duodenum *in vivo* was a consequence of its anatomical position. Transfer of iron in their experimental system was apparently a passive process, unaffected by changes in pH or the presence of metabolic inhibitors. However, under somewhat different experimental conditions, Dowdle et al (1960) demonstrated that iron could be transported from the mucosa to the serosa against a concentration gradient by a system of limited capacity. The transport mechanism apparently depended upon oxidative metabolism and the generation of phosphate bond energy. These findings were confined to the duodenum and were not demonstrable lower down the intestinal tract. Further investigations in the mouse, rat and golden hamster confirmed the observations of Dowdle et al (1960), and showed that mucosal uptake was similar throughout the length of the small bowel, whereas the capacity for active transfer from mucosa to serosa was sharply localized to the duodenum (Manis and Schachter, 1962a). Mucosal uptake of iron is faster than serosal transfer and the latter is the rate limiting step in the movement of iron from the lumen to the serosal surface in everted gut sacs; mucosal uptake increases in proportion to the concentration of iron in the ambient medium, in a range of concentrations where serosal transfer remains constant. Oxygen and hexose are required for serosal transfer, which is more liable than mucosal

uptake to inhibition by metabolic poisons such as cyanide. An oral dose of iron given to an experimental animal prior to construction of an everted gut sac inhibits both the mucosal uptake and the serosal transfer of iron (Manis and Schachter, 1962b) and a similar effect of high dietary iron concentration is also seen. Pregnancy increases the transfer of iron from the luminal to the serosal side of an everted gut sac. Manis and Schachter (1964) further demonstrated that iron was taken up by the mucosa as ferrous iron, entering a rapidly turning-over pool, whence it passed either to the serosal surface, or to a slowly turning-over protein-bound ferric iron pool, of which ferritin apparently formed a very small proportion, about 8%. Prior treatment of experimental animals with oral iron enlarged both the rapidly and the slowly turning-over pools, decreased the net uptake of iron by the mucosal epithelium and decreased the net transfer to the serosa. The decrease in serosal transfer from the mucosal cell is apparently a primary effect, since the mucosal ferrous iron pool is increased in size. Reduced iron transport from the mucosal cell to the serosa results in a secondary increase in size of the ferric iron pool. Manis and Schachter (1964) conclude that the inhibition of the transport mechanism for transfer of iron from the mucosa to the serosal surface occurs through an unknown mechanism which can be inhibited by iron itself.

The work of Schachter and his colleagues, demonstrating a rapid and a slow transit mechanism for iron uptake from the duodenal mucosa is in agreement with other observations. Ferrokinetic studies in man have shown two phases of iron absorption, one rapid, commencing about 10 minutes after oral dosing and continuing for 3 to 4 hours, and the other slow, probably from iron stored in the mucosa (Hallberg and Solvell, 1960). Wakisaka, Tomita, Kariyone, Takahashi, Yamaba, and Takagi (1965) have confirmed these findings and also noted that the increase in iron absorption associated with iron deficiency occurs mainly in the rapid phase; significant correlation with the serum iron level and the plasma iron disappearance rate was found. Similar conclusions have been reached by Stewart and Gambino (1961), employing dogs.

Using a loop of small bowel with an artificial circulation, Jacobs, Bothwell and Charlton (1966) found evidence to support Manis and Schachter's (1962a) suggestion of an active metabolic process involved in iron transfer across the small bowel mucosa. This process is inhibited by anoxia, azide, cyanide and iodacetamide, and enhanced by α-keto-glutaric acid, citrate and methylene blue, previous iron deprivation, venesection and phenylhydrazine-induced haemolysis.

Wheby and Crosby (1963), and Wheby, Jones and Crosby (1964) studied the differential proportions of an orally

administered dose of radio-iron in the gut of rats, and in the remainder of the carcass at various times after dosing. Their results indicate that iron is absorbed in two stages, the first from the gut lumen to the mucosa and the second from the mucosal cell to the other tissues; the former step occurs more rapidly, implying a storage mechanism for iron in the mucosal cell. Both steps vary quantitatively with the state of the iron stores, more iron being transferred, the smaller the iron stores; the transfer of iron from the mucosal cell to other tissues was more affected by the state of the iron stores and the prior oral administration of iron, than was the uptake of iron by the mucosal cells. Sixty to 80 per cent of the total absorption of iron occurred within two hours and the rest more slowly over 12 to 20 hours. The kinetics of the early phase of absorption were consistent with an enzyme dependent system, when the dose of iron was 50 µg or less in normal rats or 500 µg or less in iron deficient animals. The larger the oral dose of iron given the more iron, relatively, is put into the stable, slowly absorbed form, and the less, relatively, is passed through the rapid transport mechanism. Conversely, the smaller the dose of iron, the greater the proportion absorbed via the rapid transport mechanisms and the smaller the proportion passing to the stable form.

The nature of the iron as it passes through these

two phases in the mucosal cell is not clear. The stable form is protein bound and Charlton et al (1965), identified this iron-protein complex as ferritin. Furthermore, Hartman, Conrad, Hartman, Joy and Crosby (1963) have demonstrated ferritin "tetrads" by electron microscopy near the apex of duodenal epithelial cells, and these are less frequent in iron deficiency (Crosby, 1963, 1964). However, Brown and Rother (1963) were unable to show that the iron-protein complexes in their preparations of rat small bowel were ferritin by immuno-chemical and electrophoretic techniques, and Manis and Schachter (1964) could only show about 8 per cent of the protein-bound iron to be in the form of ferritin. The nature of the slowly turning-over protein-bound pool of iron in the small bowel mucosa requires further investigation to explain these discrepancies.

The nature of the iron in the rapid transit pool is also unclear. It is apparently in the ferrous state (Manis and Schachter, 1964). Brown and Rother (1963) claimed that this iron was associated with glycine and serine; however, this conclusion may be erroneous, based on an experimental artefact resulting from the inclusion of versene in solutions used for washing the small bowel tissue (Charlton et al, 1965; Manis and Schachter, 1965).

It was indicated above that haemoglobin iron was apparently absorbed in a different fashion from inorganic iron. Both are primarily absorbed in the duodenum. Haemoglobin is split enzymatically into metalloporphyrin and globin

degradation products in the bowel lumen; the haem is taken up by the mucosal epithelium where it can be demonstrated histochemically. Some of the iron is then released from haem in an inorganic form, but, in the guinea-pig, this quantity is too small to account for all the iron absorbed from haemoglobin, and most of the iron which passes through the mucosal cell to the plasma is transported thence as haem (Conrad, Weintraub, Sears and Crosby, 1966). In man, on the other hand, the passage of haem from the gut to the plasma has not been detected (Turnbull, Cleton and Finch, 1962). This may be due to species differences, such as more rapid breakdown of haem passed to the plasma in man, or more complete reduction of metalloporphyrin to inorganic iron in the gut lumen or mucosal epithelial cell of man.

The iron of purified haem is less well absorbed than that of haemoglobin and it appears that the globin degradation products may "protect" the haemoglobin-derived haem from the polymerizing effects of the alkaline secretions in the duodenal lumen (Conrad, Cortell, Williams and Foy, 1966).

The studies of Halberg and Solvell (1967) in man, using inorganic and haemoglobin iron differentially labelled with two isotopes of iron - ^{59}iron and ^{55}iron - demonstrated that inorganic iron is more rapidly absorbed than haemoglobin iron, consistent with delay either in freeing iron from the metalloporphyrin or in transfer of

haemoglobin iron into the mucosal cell, or both. A dose of unlabelled ferrous iron given with ^{59}iron-tagged haemoglobin decreased the absorption of haemoglobin iron; organic iron and inorganic iron containing the same dose of elemental iron given prior to a tagged oral dose of inorganic iron inhibited the absorption of the radio-iron to a similar extent.

Mutual interference with absorption of iron in the forms of haemoglobin and ferrous sulphate has also been demonstrated in the guinea pig (Conrad, Weintraub, Sears and Crosby, 1966). Unlabelled ferrous sulphate decreased the absorption of haemoglobin ^{59}iron significantly, whereas the decrease in ferrous ^{59}iron absorption produced by unlabelled haemoglobin iron was not statistically significant, and it was suggested that the slower rate of absorption of haemoglobin iron decreases its capacity for inhibiting the absorption of inorganic iron. The effects of unlabelled haemoglobin iron on inorganic ^{59}iron absorption, and of unlabelled inorganic iron on haemoglobin ^{59}iron absorption, are less in the guinea pig than the effects of unlabelled inorganic iron on inorganic ^{59}iron absorption and of unlabelled haemoglobin iron on haemaglobin ^{59}iron absorption, when equivalent doses are used. Conrad and his associates also provided evidence to indicate that ferrous sulphate inhibits haemoglobin iron absorption by decreasing transfer into the plasma of

intracellular iron released from haem rather than by affecting uptake from the lumen of the cell.

It may be that man is more capable of splitting the iron from haem in the mucosal epithelial cell than is the guinea pig, and that man utilizes a common pathway for most of his iron absorption from both inorganic and haem sources, whereas the guinea pig appears to have two separate pathways for iron absorption, one for inorganic iron, the other for haem, and perhaps other forms of organically bound, iron.

A wide variety of factors active on the systemic side, as opposed to the luminal side, of the mucosa also affect iron absorption. An increase in iron absorption is produced by blood loss, increased erythropoiesis, administration of erythropoietic hormone, hypoxia, iron deficiency and pregnancy (see Table 10.1), and a decrease is seen with bacterial infection, bone marrow suppression, copper deficiency, iron overload, blood transfusion and sterile abscess (see Table 10.2).

The mechanism of increased iron absorption in association with most of the conditions listed above may well be mediated through a high rate of removal of iron from the plasma (Bothwell and Finch, 1962). It has been shown that iron absorption is inversely related to the degree of transferrin saturation (Taylor and Gatenby, 1967) and transferrin given intravenously enhances iron absorption

(Solvell, 1960). Weintraub, Conrad and Crosby (1964) demonstrated in rats that, following blood loss, a 4 to 5 day period elapsed before the serum iron fell and the plasma iron clearance rate increased. Associated with these changes, the absorption of iron from the intestinal tract increased and the amount of iron in the small bowel decreased. They postulated that the iron content of the bowel, controlling (see Fig. 10.2) iron absorption, was in turn controlled by the rate of plasma iron turnover. Further experiments in which the rate of erythropoiesis was varied by altering the environmental oxygen tension tended to confirm this suggestion (Weintraub, Conrad and Crosby, 1965).

Beutler and Buttenweiser (1960) attempted to detect a humoral regulator of iron absorption. They injected plasma and crude tissue extracts from normal and iron-deficient rats or mice into animals of the same species, without producing any alteration in iron absorption. Fischer and Price (1963), on the other hand, were able to enhance iron absorption in rats by the transfusion of iron-deficient plasma; the possibility that this was an effect of transfusing unsaturated transferrin cannot be excluded. There is, at present, insufficient evidence for adequate assessment of the possible existence of a specific humoral factor affecting iron absorption.

The assumption that iron in the plasma and the

mucosal epithelium does not exchange with that in the lumen of the bowel has been confirmed by Crosby and his associates (Pollack, Kaufman and Crosby, 1964c).

In summary, iron can be absorbed from the small intestine either as inorganic iron, probably in the ferrous state, or in combination with organic compounds, such as in haem. The gastric and pancreatic secretions can affect iron absorption in ways which are not yet fully understood, and various substances given orally with iron may also influence its intestinal absorption. The mucosa of the proximal small intestine exerts some control over the uptake of iron from the luminal contents, through the interplay of several factors; firstly, the iron content of the mucosal epithelial cell at the time of its formation, and this is dependent on the body iron stores; secondly, the amount of iron taken up and retained by the mucosal cell from the food; and thirdly, the requirement of the body for iron as reflected by the rate of removal of iron from the plasma.

Iron taken into the mucosal cell from the gut lumen apparently enters one of two pools of iron, a divalent, non-protein bound rapidly turning-over pool, or a slowly turning-over protein bound iron pool, with iron in the trivalent form.

Iron entering the rapidly turning-over pool in the mucosal epithelial cell is quickly transferred from the cell to the plasma, and constitutes most of the iron

absorbed, whereas that entering the protein-bound pool can be absorbed only very slowly, and in small amount, with most of the iron in this form being shed as the epithelial cell exfoliates. Whether the protein-bound iron is in the form of ferritin is not at present clear, and the conflicting findings of various workers have been mentioned above. It seems likely that iron is transferred from the mucosal cell to the plasma by a mechanism which is capable of working against a concentration gradient, which involves an energy-consuming enzyme-mediated step, and which is in some way limited by the amount of iron in the mucosal cell, and the amount of iron required to be uplifted by the plasma to meet demands elsewhere.

While it is generally agreed that the intestinal mucosa exerts some control over iron absorption, it is equally agreed that this control is far from perfect and the mechanism cannot fully regulate the passage of iron through the intestinal mucosa when the concentration of iron in the intestinal lumen is high.

The situation in the X-linked anaemia of mice, where there is decreased iron absorption, and iron deficiency is in sharp contrast to that in iron deficient animals and humans, where it is usual to find increased iron absorption (Hahn et al, 1943; Bothwell et al, 1958; Bannerman, O'Brien and Witts, 1962; Conrad and Crosby, 1963; and others).

Dietary factors cannot be involved, since both normal and anaemic mice received identical food and water. It is unlikely that gastric or pancreatic factors are involved although the possibility cannot be entirely excluded. The stomach appears histologically normal and acid-secreting cells are present in similar numbers in normal and anaemic mice. A disorder involving the hypothetical gastric factor, "gastroferrin", (Luke et al, 1967) and leading to iron malabsorption, would necessitate the oversecretion of "gastroferrin" by the mutant, and this is an improbable event on genetic grounds. A pancreatic disorder producing malabsorption of iron would also require a postulate of excessive secretion. The pancreas, in anaemic mice, also appears histologically normal. The influence of factors operating in the lumen of the small bowel (i.e., dietary factors, and exocrine secretions) is apparently to facilitate, or inhibit, uptake of iron by the epithelial cells of the small bowel. It therefore seems unlikely that a factor or factors, operating within the bowel lumen alone, would inhibit iron absorption and yet permit excessive deposition of iron within the mucosal epithelium. Yet it seems inescapable that the iron deposited in the small bowel epithelium in mice with sla is derived from the diet. The alternative source, the body iron stores, is improbable in view of the lack of evidence of excessively rapid iron loss in anaemic animals, and the high rate and level of iron utilization

for haemopoiesis implies that comparitively little would be left for incorporation as "unwanted" iron in the small bowel. The conditions on the serosal, or systemic, side of the small bowel epithelium are highly conducive to iron transfer from the mucosa to the plasma, and the metabolic defect leading to X-linked mouse anaemia appears to be inherent in the epithelial cells of the small bowel.

The proportions of an oral dose of ^{59}iron retained by normal and anaemic mice at 24 hours after administration are similar*, and the clear-cut differences only emerge from the second day after dosing onwards. Of the iron retained by the normal mouse from a 1 µg oral dose on the first day after administration, 64 per cent remains on the 5th day, when, as discussed earlier, faecal elimination and mucosal exfoliation of ^{59}iron are complete; it is probable that some of the iron remaining in the body at 24 hours after dosing and which is eliminated over the next day or two, is present in the mucosal epithelial cells (Conrad and Crosby, 1963). In anaemic mice, by contrast, only 32 per cent of the iron retained on the first day after dosing still remains on the 5th day. If rejection of dietary iron by the mucosal epithelial cell were the mechanism of malabsorption of iron in anaemic mice, in the presence

---

* See Table 7.9 and Figs. 7.6 and 7.7. No significant difference is seen in the proportions of iron retained from 0.1 µg and 1.0 µg doses of ^{59}iron in anaemic and normal mice ($p > 0.1$), 24 hours after administration.

of a normal rate of faecal elimination, then the relative proportions of iron retained on the first and fifth days after dosing in anaemic mice would necessarily be more similar to the normal, and significant differences in iron retention between normal and anaemic mice would probably have emerged on the first day after dosing. It seems more likely that iron can be taken up in more or less normal quantities by the small bowel epithelium of anaemic mice, but that the mucosal cells are incapable of transferring the iron further, to the plasma and tissues, in adequate amounts; the untransferred iron would then be shed from the villous tip in the mucosal epithelial cells, with the resultant delay in the appearance of significant differences in the retention of iron in normal and anaemic mice. The rapid fall in retained radioactivity in anaemic mice during the second day, with a slower fall off during the third day, is in keeping with the known turnover time of 2 days for duodenal epithelial cells in the mouse (Creamer, 1967).

Since iron can apparently enter the small bowel epithelial cells of mice with X-linked anaemia, and the available evidence indicates that the source of such iron is the diet, it must be supposed that the obstruction to iron absorption lies in the transfer of iron from the mucosal cell to the plasma and tissues - a defect at the "back-door" of the mucosal cell, so to speak.

In assessing the role of the sla gene in the mucosal epithelial cell, only the process of inorganic iron absorption can be considered in detail, since the ability of mice with sla to absorb haemoglobin iron has not yet been adequately investigated; furthermore, it is not clear whether the same or different pathways of iron transport are involved in the absorption of haem and inorganic iron in the mouse, and species differences are probably important (Conrad, Weintraub, Sears and Crosby, 1966).

Figures 10.3 and 10.4 present a hypothetical scheme for iron transfer by the mucosal epithelial cell to the plasma and the control of iron absorption, and within this scheme possible sites of action of the sla gene will be indicated.

The movement of iron from the lumen of the bowel to the epithelial cell will be referred to as "uptake", and the movement of the iron through the mucosal cell to the plasma as "transfer"; the sum of these is iron "absorption".

The absorption of inorganic iron from low ("physiological") concentrations in the lumen of the small intestine will be considered first. It is uncertain in what form or forms inorganic iron can be taken up by the mucosal cell (step a, Fig. 10.3). However, once iron has entered the cell (step b), it either passes rapidly through the cell(pathway c and e-g) or is converted into a trivalent form, entering a protein-bound, slowly turning over pool

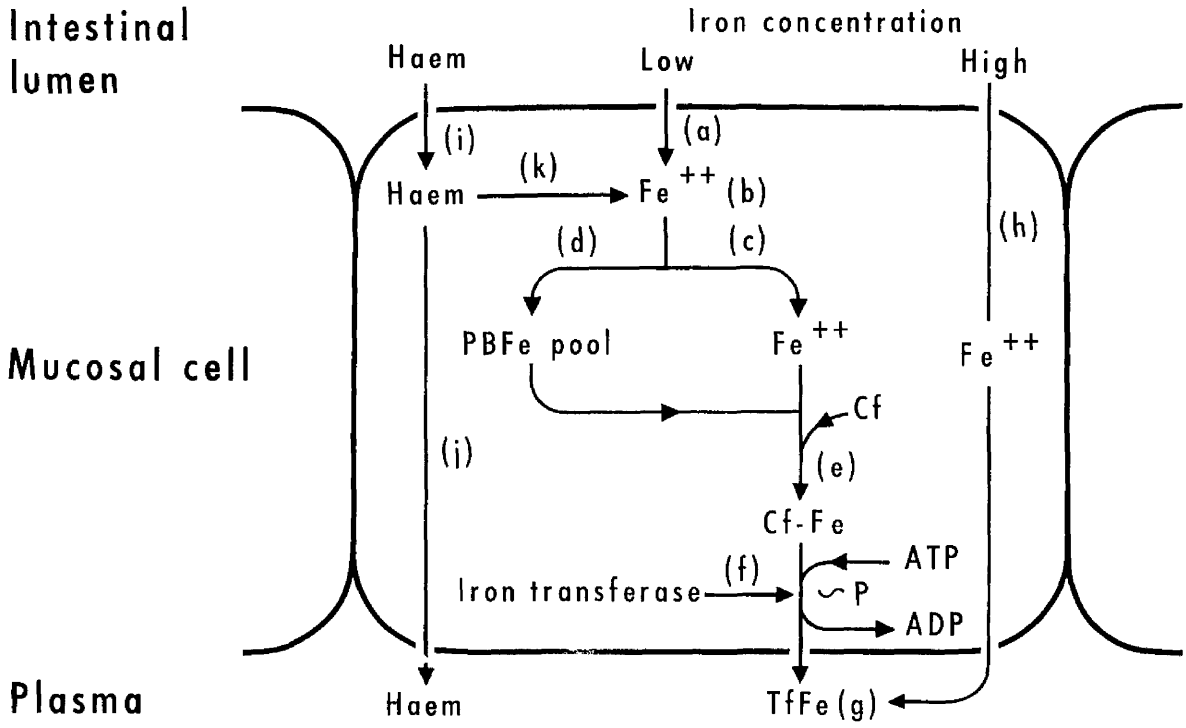


Fig. 10.3: A hypothesis for the intestinal absorption of iron and its control by the intestinal mucosal epithelium. Fe^{++}, ferrous iron; Cf, "cytoferrin"; ATP, adenosine triphosphate; ADP, adenosine diphosphate; ~P, energy-rich phosphate; Tf, transferrin; PBFe, protein-bound iron; Cf-Fe, "cytoferrin"-bound iron; Tf-Fe, transferrin-bound iron. See text.

(step d) (Manis and Schachter, 1964) from which little iron is subsequently transferred to the plasma and most of which is lost with the exfoliated mucosal epithelial cell (Conrad and Crosby, 1963). As discussed previously, the nature of this protein-bound iron is uncertain; however, the iron seen in the epithelial cells of the duodenum of sla and normal mice appears as haemosiderin, which is believed contain ferritin (Richter, 1957a, 1957b). It is probable that such transfer of iron to the plasma from the protein-bound pool as does occur, involves the passage of iron back through the divalent iron pool (Manis and Schachter, 1962a, 1964).

The details of the mechanism whereby iron transfer to the plasma is inhibited by adequate body iron stores, or previous exposure to iron either from the intestinal lumen or from the systemic side of the mucosal epithelium are not at present known. Manis and Schachter (1964) have suggested that the inhibition may be a primary effect of iron itself, active at the stage of transfer of iron from the mucosal cell to the plasma. It has also been demonstrated by in vitro experiments that iron transfer is enzyme-mediated and consumes phosphate bond energy (Dowdle et al, 1960; Manis and Schachter, 1962a; Jacobs et al, 1965). In Figure 10.3, steps (e) and (f) incorporate these observations. "Cytoferrin" (Cf) is a hypothetical iron-binding substance, formed within the mucosal epithelial cell; it is

suggested that the amount is limited in the individual cell and that its presence is required for the transfer of iron through the cell membrane to the plasma. The regulation of the cellular content of "cytoferrin" could be mediated through the quantity of iron laid down in the mucosal cell at the time of its formation and this is considered further below. Once "cytoferrin" has been formed and consumed in taking iron across the cell membrane, it would no longer be available to transfer iron by this pathway.

"Cytoferrin"-bound iron is, according to the hypothesis, transferred to the plasma via the energy-consuming step, indicated by (f). The enzyme involved, for which the evidence has been discussed above, is tentatively described as "iron transferase". It is suggested that "iron transferase" expedites the movement of "cytoferrin"-bound iron to the plasma where the iron is taken up by transferrin (step g) for delivery to the various cells synthesizing iron compounds. Two other possibilities could be put forward - firstly, that "cytoferrin" and "iron-transferase" are identical, which would require "iron-transferase" to be a self-consuming enzyme; and secondly, that "iron transferase" unites "cytoferrin" and iron, making a compound capable of transferring iron from the mucosal cell to the plasma.

The defect in intestinal iron transport induced by the inhibition of protein synthesis by cycloheximide

(Greenberger and Ruppert, 1966) and tetracycline (Greenberger, Ruppert and Cuppage, 1967) could be explained by defective manufacture either of "cytoferrin" or of "iron transferase". Indeed, Greenberger and Ruppert (1966) suggest that "the interference in iron transport" (induced by cycloheximide) "may be due to a deficiency of an unidentified carrier substance". They also suggest that such a carrier might be involved in the uptake of iron by the mucosal epithelial cell, since decreased mucosal iron uptake was found in cycloheximide treated animals. Their findings could equally well be explained by a "backing-up" of iron in the mucosal cell consequent upon "cytoferrin" or "iron-transferase" deficiency, with an alteration in the luminal-mucosal cell iron concentration gradient, inhibiting entry of iron into the epithelial cell from the intestinal contents.

In Figure 10.4, a hypothetical model is laid out, whereby the state of the body iron stores could determine the capability of the intestinal epithelial cell for iron absorption, through the "cytoferrin" mechanism. In the normal individual, iron is incorporated into the cell in excess of "essential" requirements; the excess, it is postulated, is taken up by "cytoferrin" manufactured in a set quantity within the developing epithelial cell; the iron so taken up would then tend to be resorbed, leaving some "cytoferrin" behind to participate in the subsequent absorption of iron from the intestinal lumen (Fig. 10.4, left). In iron deficient states, the quantity of iron laid

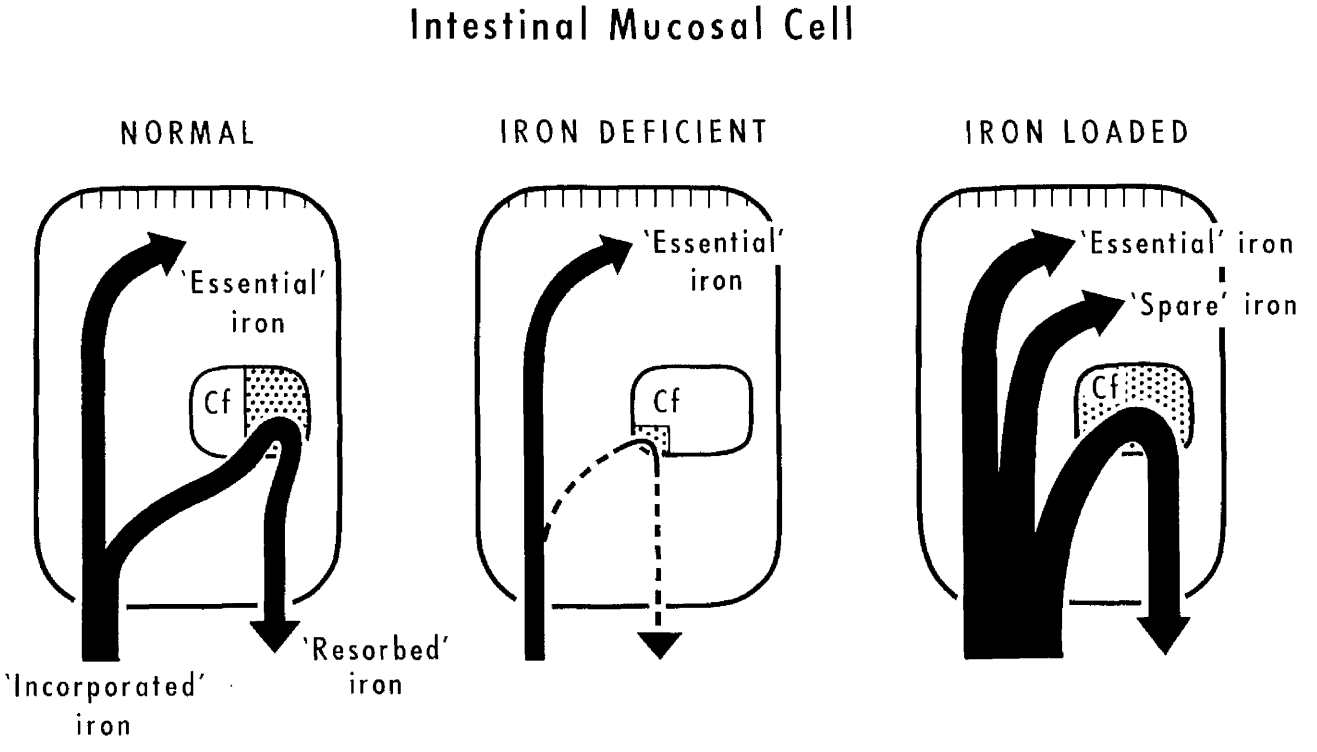


Fig. 10.4: The regulation of intestinal iron absorption through the interaction of the quantity of iron incorporated into the mucosal cell at the time of its formation, and the "cytoferrin" (Cf) mechanism. (See text.)

down in the developing cell is barely sufficient to meet essential requirements, and little or none is left to attach to "cytoferrin" and be resorbed; thus abundant "cytoferrin" is available to facilitate the transfer of iron from cell to plasma (Fig. 10.4, centre). In the case of iron overload large quantities of iron are incorporated into the epithelial cell during its formation; "cytoferrin" would then be completely bound and consumed, and the remaining excess of iron over essential requirements ("spare" iron) would then remain to be lost as the mucosal cell is exfoliated (Fig. 10.4, right). The consumption of "cytoferrin" and the presence of "spare" iron would serve to inhibit the mucosal uptake of iron, by providing a gradient in iron concentration which is hard to overcome. These suggestions represent a modification and extension of the hypothesis of Conrad and Crosby (1963) outlined earlier in this chapter.

Exposure of the small bowel mucosa to high, unphysiological, doses of iron increases the absorption of iron, by a process with kinetic characteristics which do not suggest an enzyme-mediated process (step h, Fig. 10.3), and it is postulated that the "cytoferrin"-"iron transferase" mechanism would only be relevant to low and physiological doses of iron.

Since haem iron is apparently handled differently by the small bowel mucosa of guinea-pigs and man (Turnbull, Cleton and Finch, 1962; Conrad, Weintraub,

Sears and Crosby, 1966) with, apparently, the former showing the presence of haem in the plasma and the latter not, it seems reasonable to suppose that two pathways of absorption from the intestinal lumen may be involved. It is suggested that haem is taken up by the mucosal cell (step i, Fig. 10.3) and either transferred as such to the plasma (step j) or split into iron and tetrapyrrole (or the products of its catabolism), with the iron passing into the ferrous iron pool (step k). Species variation might well give rise to a difference in the relative importance of the two pathways involved with, for example, step (j) predominating in the guinea pig and step (k) predominating in man. The relative delay in absorption of haem iron as compared with inorganic iron in man (Hallberg and Solvell, 1967) may be due in part, at least, to the extra step (k) required to convert haem to inorganic iron during transfer.

The malabsorption of iron in X-linked mouse anaemia could equally be a consequence of genetically controlled deficiency of "cytoferrin" or of "iron transferase". A shortage of "cytoferrin" would produce an inadequate supply of bound iron suitable for passage through the "iron transferase" controlled pathway, and would obstruct the movement of iron from the ferrous pool to the plasma. Iron would then be diverted to the protein-bound pool where it could be represented by the haemosiderin seen in the anaemic animals. Alternatively, obstruction of the pathway by

deficiency of "iron transferase" would prevent "cytoferrin"-bound iron being transferred to the plasma, with resultant build-up of iron in the ferrous and protein-bound pathways. Comment cannot at present be made on the relationship of the sla gene to the proposed scheme of haem iron absorption shown in Fig. 10.3.

The future of X-linked anaemia of mice as a tool in experimental haematology

The field of study to which the sla mutant is most applicable is obviously that of iron absorption. It is extremely likely, as discussed earlier, that the inherited defect operates within the mucosal epithelial cell of the small bowel. However, it is still necessary completely to exclude any possibility that gastric or pancreatic factors, affecting the form of iron taken up by the mucosal cell and its consequent handling by that cell, are involved; this could well be done by the use of in vitro preparations of loops of small bowel, which would permit experimental variation in the form in which iron is presented to the mucosa of the small bowel. Such loops could also be used to study the effect of varying the medium surrounding the serosa and thus exclude a possible defect acting outside the epithelial cell, but influencing the transfer of iron from the mucosa to the unsaturated transferrin.

Further studies of the excretion of iron by, and of

the transfer of iron from body stores to, the mucosal epithelium are required to confirm the results of the preliminary experiments on iron excretion reported here, which indicate that the source of mucosal iron is the diet.

These steps are necessary to establish further that the metabolic defect induced by the sla mutant is intrinsic to the intestinal epithelial cell and involves the process of transfer of iron from the mucosal cell to the plasma.

Attention could then be turned to the mucosal cell itself, perhaps with the models outlined in Fig. 10.3 and 10.4 as a working hypothesis. The precise nature of iron transfer from mucosal cell to plasma has remained obscure in spite of study by many techniques. However, a specific defect in a single step in the metabolic pathway of iron absorption has not hitherto been discovered, and previous experiments have involved the use of normal tissues exposed to a wide variety of experimental conditions. These conditions can now be applied to the normal (+/-) and abnormal (sla/-) small bowel mucosa in parallel.

Autoradiographic studies using oral and parenteral radioiron can reveal movement of iron into and out of the mucosal cells at various intervals following isotope administration and use of the electron microscope may indicate the intra-cellular organelles in which iron accumulation is occurring. Inferences about sites of hold-up and delay in iron transfer in anaemic and normal mice may then be possible.

The influence of previous exposure of duodenal mucosa to iron orally and parenterally in various doses can be studied, and the effect of curing the anaemia on iron absorption could also be recorded.

A search for the hypothetical "cytoferrin" might be attempted by homogenation and fractionation techniques, and studies with gut loops similar to those of Dowdle et al, (1960), and Manis and Schachter (1962a, 1964) on the kinetics of the rapidly and slowly turning-over pools of iron in the mucosal epithelial cell should also be undertaken. At the present time it is not clear how the fundamental metabolic abnormality in X-linked mouse anaemia can be best identified. However, attempts to clarify further its nature should also throw light on the mysteries of intestinal iron absorption.

X-linked mouse anaemia should prove a useful tool in the investigation of the tissue effects of iron deficiency. The anaemia can be induced in sla mutants while they are receiving the same diet as normal "control" animals, and thus the environmental conditions of experiments involving normal and anaemic mice can be made identical. Furthermore, animal management is easier if all mice are on the same diet, rather than on two different diets, when mistakes may easily arise to invalidate experiments.

Deficiency of cytochrome enzymes has already been detected (Doeblin, 1967) and the effect of prolonged iron

deficiency on haemopoietic stem cell behaviour is at present under investigation (Bennett et al, in preparation). The unbalancing effect of chronic iron deficiency on haem and globin synthesis could also be studied in X-linked mouse anaemia.

CHAPTER 11

Summary

The X-linked anaemia of mice (gene symbol, sla) is hypochromic and microcytic in character. Typically, the mean corpuscular haemoglobin concentration, the mean cell volume, and the mean cell diameter are reduced. Anaemia is moderately severe in young hemizygous male and homozygous female mice, and regresses spontaneously with age. Heterozygous female animals show little or no haematological abnormality, and duality of the red cell population is not seen.

The iron stores of anaemic mice, as determined both chemically, and histochemically, are depleted at all ages. The clearance of iron from the plasma is rapid and the appearance of radio-iron in the red cells, after parenteral injection, is both greater and more rapid than normal. The serum iron concentration is low and the total serum iron binding capacity is elevated, as is the free erythrocyte protoporphyrin concentration. The anaemia responds well to parenteral iron dextran. Together, these findings are characteristic of iron deficiency.

In contrast to the usual situation in iron deficiency in man and other mammals, intestinal iron absorption is not increased in X-linked anaemia of mice;

indeed a significantly low level of absorption of inorganic iron at three dose levels (0.1, 1.0 and 10 µg) has been found. Histochemical studies reveal abnormal accumulation of stainable iron in the mucosal epithelium of the small bowel of anaemic and heterozygous mice.

These findings imply that the deficiency of iron is a consequence of malabsorption of iron by the small bowel, probably as a result of a primary, genetically controlled, fault in the metabolic processes of iron transfer from the mucosal cell to the plasma. The histochemical findings in the mucosal epithelium of heterozygous carriers are consistent with, although they do not constitute proof of, the provisions of the Lyon hypothesis of X-chromosome inactivation.

X-linked mouse anaemia does not, at present, seem to be a model for any known form of heritable human anaemia, but is potentially a useful tool in the investigation of the still mysterious processes whereby iron is transferred from the mucosal epithelium to the plasma. A hypothetical model explaining known features of iron absorption, and incorporating possible sites of action of the sla gene, is presented and discussed. It is postulated that the sla gene controls the production either of an enzyme ("iron transferase") necessary for the transfer of iron to the plasma from the mucosal epithelial cell of the small bowel, or of a carrier substance ("cytoferrin") to which iron must be bound for this transfer to be achieved.

REFERENCES

Aarons, I. (1964). Renal immunofluorescence in NZB/NZW mice. Nature (Lond), 203,1080.

Albritton, E. C. (1952). Standard values in blood. W. B. Saunders, Philadelphia.

Allen, R. C., H. Meier and W. G. Hoag. (1962). Distribution of coagulation proteins in normal mouse plasma. Science, 135,103.

Allgood, J. W. and E. B. Brown. (1967). The relationship between duodenal mucosal iron concentration and iron absorption in human subjects. Scand. J. Haemat., 4,217.

Altman, K. I. and E. S. Russell. (1964). Heme synthesis in normal and genetically anaemic mice. J. cell. comp. Physiol., 64,293.

Altman, K. I., E. S. Russell, K. Saloman and J. K. Scott. (1953). Chemopathology of hemoglobin synthesis in mice with a hereditary anemia. Fed. Proc., 12,168.

Andersen, D. H. (1938). Cystic fibrosis of the pancreas and its relation to celiac disease. Amer. J. Dis. Childh., 56,344.

Anderson, R., R. R. Huestis and A. G. Motulsky. (1960). Hereditary spherocytosis in the deer mouse. Its similarity to human disease. Blood., 15,491.

Anderson, R. and A. G. Motulsky. (1966). Adverse effect of raised environmental temperature on the expression of hereditary spherocytosis in deer mice. Blood, 28,365.

Andrew W. (1965). Comparative Hematology. Grune & Stratton, New York.

Attfield, M. (1951). Inherited macrocytic anaemias in the house mouse. III. Red blood cell diameters. J. Genet., 50,250.

Austoni, M. E. and D. M. Greenberg. (1940). Studies on iron metabolism with the aid of its artificial radioactive isotope. J. biol. Chem., 134,27.

Badenoch, J. (1960). Steatorrhoea in the adult. Brit. med. J., 2, 879 and 963.

Badenoch, J. and S. T. Callender. (1954). Iron metabolism in steatorrhoea. The use of radioactive iron in studies of absorption and utilization. Blood, 9,123.

Baker, P. J. and J. B. Wilson. (1965). Hypoferremia in mice and its application to the bioassay of endotoxin. J. Bact., 90,903

Balcerzak, S. P., W. W. Peternel and E. W. Heinle. (1967). Iron absorption in chronic pancreatitis. Gastroenterology, 53,257.

Balfour, W. M., P. F. Hahn, W. F. Bale, W. T. Pommerenke, G. H. Whipple. (1942). Radioactive iron absorption in clinical conditions. Normal, pregnancy, anemia and hemochromatosis. J. exper. Med., 76,15.

Ballantyne, J., F. G. Beck, L. C. Strong and W. C. Quevedo. (1962). Another allele at the W locus of the mouse. J. Hered., 52,200.

Bannerman, R. M. (1961). Thalassemia. A survey of some aspects. Greene and Stratton, New York and London.

Bannerman, R. M. (1965a). Personal communication.

Bannerman, R. M. (1965b). Quantitative aspects of hemoglobin iron absorption. J. Lab. clin. Med., 65,944.

Bannerman, R. M., S. T. Callender and D. L. Williams. (1962). Effect of desferrioxamine and D.T.P.A. in iron overload. Brit. med. J., 2,1573.

Bannerman, R. M. and R. G. Cooper. (1964). Sex-linked anemia; a hypochromic anemia of mice. Xth Congr. Int. Soc. Hemat., abstr. E22.

Bannerman, R. M. and R. G. Cooper. (1966). Sex-linked anemia: a hypochromic anemia of mice. Science., 151,581.

Bannerman, R. M. and J. S. Malpas. (1965). Studies on desferroxamine in relation to the absorption of iron. Brit. J. Haemat., 11,15.

Bannerman, R. M., J. R. P. O'Brien and L. J. Witts. (1962). Studies in iron metabolism IV. Iron absorption in experimental iron deficiency. Blood, 20,532.

Bannerman, R. M. and P. H. Pinkerton. (1967). X-linked hypochromic anaemia of mice. Brit. J. Haemat., 13,1000.

Barnes, R. D. S. and M. Tuffrey. (1966). Sites of red cell destruction in the haemolytic anaemia of adoptively immunized NZB mice. Brit. J. Haemat., 12,717.

Barrowman, J. and M. Craig. (1961). Haemoglobins of foetal C57B1/6 mice. Nature (Lond.), 190,818.

Barrowman, J. and K. B. Roberts. (1961). Haemoglobins of foetal CBA mice. Nature (Lond.), 189,409.

Benjamin, B. I., S. Cortell and M.E. Conrad. (1967). Bicarbonate-induced iron complexes and iron absorption: one effect of pancreatic secretions. Gastroenterology, 53,389.

Bennett, D. (1956). Developmental analysis of a mutation with pleiotropic effects in the mouse. J. Morph., 98,199.

Bergeim, O. and E. R. Kirch. (1949). Reduction of iron in the human stomach. J. biol. Chem., 177,591.

Berlin, N. I., J. H. Lawrence and J. Gartland. (1950). The blood volume in chronic leukemia as determined by P^{32}-labelled red blood cells. J. Lab. clin. Med., 36,435.

Bernstein, S. E. (1962). Acute radiosensitivity in mice of differing W genotype. Science, 137,428.

Bernstein, S. E. (1963a). Modification of radiosensitivity of genetically anemic mice by implantation of blood forming tissue. Rad. Res., 20,695.

Bernstein, S. E. (1963b). Analysis of gene action and characterization of a new hematological abnormality, hemolytic anemia. Proc. IXth. Int. Congr. Genet., 1,186.

Bernstein, S. E. (1966). Cited by Russell, E. S. and S. E. Bernstein, (1966).

Bernstein, S. E. and Newburger. (1966). Cited by Russell, E. S. and S. E. Bernstein, (1966).

Bernstein, S. E. and E. S. Russell. (1959). Implantation of normal blood forming tissue in genetically anemic mice, without X-irradiation of host. Proc. Soc. exper. Biol. Med., 101,769.

Bertles, J. F. (1957). Sodium transport across the surface membrand of red blood cells in hereditary spherocytosis. J. clin. Invest., 36,816.

Beutler, E. and M. C. Baluda. (1964). The separation of glucose-6-phosphate dehydrogenase deficient erythrocytes from the blood of heterozygotes for glucose-6-phosphate dehydrogenase deficiency. Lancet, 1,189.

Beutler, E. and E. Buttenweiser. (1960). The regulation of iron absorption. I. A search for humoral factors. J. Lab. clin. Med., 55,274.

Beutler, E., V. F. Fairbanks and J. L. Fahey. (1963). Clinical disorders of iron metabolism. Grune and Stratton. New York.

Beutler, E., B. M. Kelly and F. Beutler. (1962). The regulation of iron absorption. II. Relationship between iron dosage and iron absorption. Amer. J. clin. Nutr., 11,559.

Bianchi, A. (1951). Primi tenativi de correzione di anemia macrocitica ereditaria nel topo. Arch. Sci. Biol., 35,147.

Bielschowsky, M. and F. Bielschowsky. (1962). Reaction of the reticular tissue of mice with auto-immune haemolytic anaemia to 2-aminofluorene. Nature (Lond.), 194,692.

Bielschowsky, M. and F. Bielschowsky. (1964). Observations on NZB/Bl mice. Differential fertility in reciprocal crosses and the transmission of the auto-immune haemolytic anaemia to NZB/Bl x NZC/Bl hybrids. Aust. J. exp. Biol. med. Sci., 42,561.

Bielschowsky, M., B. J. Helyer and J. B. Howie. (1959). Spontaneous haemolytic anaemia in mice of the NZB/Bl strain. Proc. Univ. Otago med. Sch., 37,9.

Biggs, J. C., R. M. Bannerman and S. T. Callender. (1961). Iron absorption in achlorhydria. Proc. VIIIth Congr. Europ. Soc. Haematol., Vienna, 1, Abst. 236.

Biggs, J. C. and A. E. Davis. (1963). Relationship of diminished pancreatic secretion to haemochromatosis. Lancet, 2,814.

Bishop, C. W. (1965). Personal communication.

Bishop, R. C. and F. H. Bethell. (1959). Hereditary hypochromic anemia with transfusion hemosiderosis, treated with pyridoxine. New Engl. J. Med., 261,486.

Bishop, R. C. and F. H. Bethell. (1960). Hereditary hypochromic anaemia with transfusion siderosis treated with pyridoxine. Proc. 7th Congr. int. Soc. Haemat., Rome, 1958, 2,382.

Boender, C. A., E. Mulder, J. E. Ploem, J. de Wael and M. C. Verloop. (1967). Iron absorption and retention in man. Nature (Lond.), 213,1236.

Bonnet, J. A., A. B. Hagedorn and C. A. Owen. (1960). A quantitative method for measuring the gastrointestinal absorption of iron. Blood, 15,36.

Borghese, E. (1951). Osservazioni sul'emopoiesi epatica nei topi anemici WW. Monit. zool. ital., 59,1.

Borghese, E. (1952a). Ricerche embriologiche ed istologiche sui mutanti del locus W nei topi. II. L'empoiesi midollare nei topi anemici W/W. Symp. genet. Pavia, 3,107.

Borghese, E. (1952b). Nuove osservazzioni sul'emopoiesi nei topi anemici WW. Atti. d. Soc. ital. di Anat. XIII Convegno Sociale., p. 1.

Borghese, E. (1952c). Ricerche embriologiche ed istologiche sui mutanti del locus W nei topi. I- L'emopoiesi epatica nei topi anemici W/W. Symp. genet. Pavia, 3,86.

Borghese, E. (1952d). Foyers d'hématopoiese dans la glande surrénale foetale de Mus musculus. Acta Anat., 16,54.

Borghese, E. (1954). Recherches embryologiques sur les souris anémiques W/W. Gaz. med. Portug., 7,262.

Borghese, E. (1955). Nouvelles recherches sur les souris anémiques W/W. C.R. Ass. Anat., 42,339.

Borghese, E. (1956). Gonads of W mice cultured in vitro. Anat. Rec., 124,481.

Borghese, E. (1957). Lo sviluppo in vitro delle gonadi embrionali dei topi anemici W/W. Symp. genet. Pavia, 5,84.

Borghese, E. (1959a). The present state of research on WW mice. Acta anat. (Basel), 36,185.

Borghese, E. (1959b). L'empoiesis splenica nei topi anemici W/W. Monit. zool. ital., 67,1.

Bothwell, T. H. and C. A. Finch. (1962). Iron Metabolism. Little, Brown & Co., Boston.

Bothwell, T. H., G. Pirzio-Biroli and C. A. Finch. (1958). Iron absorption. I. Factors influencing absorption. J. Lab. clin. Med., 51, 24.

Bothwell, T. H., H. van W. van Doorn-Wittkampf, M. L. du Preez and T. Alper. (1953). The absorption of iron. Radioiron studies in idiopathic hemochromatosis, malnutritional cytosiderosis, and transfusional hemosiderosis. J. Lab. clin. Med., 41,836.

Bourne, M. S., M. W. Elves and M. C. G. Israels. (1965). Familial pyridoxine-responsive anaemia. Brit. J. Haemat., 11,1.

Bowdler, A. J. (1967). Dilution anaemia associated with enlargement of the spleen. Proc. roy. Soc. Med., 60,44.

Brise, H. and L. Hallberg. (1962). A method for comparitive studies on iron absorption in man using two radio-iron isotopes. Acta. med. scandinav., 171, Suppl. 376,7.

British Medical Journal. (1963). Leading article. The Lyon Hypothesis. 2, 1215.

Brodsky, I., L. H. Dennis, S. B. Kahn and L. W. Brady. (1966). Normal mouse erythropoiesis. I. The role of the spleen in mouse erythropoiesis. Cancer Res., 26,198.

Brown, E. B. (1963). The absorption of iron. Amer. J. clin. Nutr., 12,205.

Brown, E. B. and B. W. Justus. (1958). In vitro absorption of radio-iron by everted pouches of rat intestine. Amer. J. Physiol., 194,319.

Brown, E. B. and M. L. Rother. (1963). Studies of the mechanism of iron absorption. I. Iron uptake by the normal rat. J. Lab. clin. Med., 62,357.

Brown, H. E. and T. F. Dougherty. (1956). The diurnal variation of blood leucocytes in normal and adrenalectomized mice. Endocrinology, 58,365.

Brozovic, B., O. Popovic, D. Obradovic and S. Pendic. (1966). Iron absorption in normal and d, l-ethionine treated rats before and after the administration of pancreatin. Gut, 7,531.

Bruce, W. R. and E. A. McCulloch. (1964). The effect of erythropoietic stimulation on the hemopoietic colony forming cells of mice. Blood, 23,216.

Buchanan, K. D., J. D. Kinloch, H. E. Hutchison, P. H. Pinkerton and P. Cassidy. (1963). Thalassaemia in Scots. J. clin. Path., 16,596.

Bunting, C. H. (1922). The leukocytes. Physiol. Rev., 2,505.

Burnet, F. M. and M. C. Holmes. (1965). Genetic investigations of autoimmune disease in mice. Nature (Lond.), 207,368.

Burwell, E. L., B. A. Brickley and C. A. Finch. (1953). Erythrocyte life span in small animals. Comparison of two methods of employing radioiron. Amer. J. Physiol., 172,718.

Byrd, R. B. and T. Cooper. (1961). Hereditary iron loading anemia with secondary hemochromatosis. Ann. intern. Med., 55,103.

Callender, S. T. (1959). Iron absorption. Brit. Med. Bull., 15,5.

Callender, S. T. (1967). The intestinal mucosa and iron absorption. Brit. med. Bull, 23,263.

Callender, S. T., B. Mallett, B. J. and M. D. Smith. (1957). Absorption of haemoglobin iron. Brit. J. Haemat., 3,186.

Callender, S. T. and J. S. Malpas. (1963). Absorption of iron in cirrhosis of the liver. Brit. med. J., 2,1516.

Cappell, D. F. (1930). The late results of intravenous injection of colloidal iron. J. Path. Bact., 33,175.

Cappell, D. F. (1964). Muir's Textbook of Pathology, 8th Ed., Arnold, London.

Caraway, W. T. (1963). Macro and micro methods for the determination of serum iron and iron binding capacity. Clin. Chem., 9,188.

Carter, T. C. (1951). The genetics of luxate mice. I. Morphological abnormalities of the heterozygotes and homozygotes. J. Genet., 50,277.

Cartwright, G. E., C. J. Gubler, J. A. Bush and M. M. Wintrobe. (1956). Studies on copper metabolism. XVII. Further observations on the anaemia of copper deficiency in swine. Blood, 11,143.

Casey, T. P. and J. B. Howie. (1965). Autoimmune hemolytic anemia in NZB/Bl mice treated with the corticosteroid drug betamethasone. Blood, 25,423.

Cassells, D. A. K. (1938). Red cell fragility in various blood conditions. J. Path. Bact., 47,603.

Channing, A. A., T. Kasuga, R. E. Horowitz, E. L. Dubois and H. B. Demopoulos. (1965). An ultrastructural study of spontaneous lupus nephritis in the NZB/Bl - NZW mouse. Amer. J. Path., 47,677.

Chappelle, E., B. W. Gabrio, A. R. Stevens and C. A. Finch. (1955). Regulation of body iron content through excretion in the mouse. Amer. J. Physiol. 182,390.

Charlton, R. W., P. Jacobs, H. Seftel and T. H. Bothwell. (1964). Effect of alcohol on iron absorption. Brit. med. J., 2,1427.

Charlton, R. W., P. Jacobs, J. D. Torrance and T. H. Bothwell. (1965). The role of the intestinal mucosa in iron absorption. J. clin. Invest., 44,543.

Chase, M. S., C. J. Gubler, G. E Cartwright and M. M. Wintrobe. (1952). Studies on copper metabolism. IV. The influence of copper on the absorption of iron. J. biol. Chem., 199,757.

Cheney, B. and C. A. Finch. (1960). Effect of inosine on iron absorption in rats. Proc. Soc. exper. Biol. Med., 103,37.

Chirasiri, L., and G. Izak. (1966). The effect of acute haemorrhage and acute haemolysis on the intestinal iron absorption in the rat. Brit. J. Haemat., 12,611.

Chodos, R B. and J. F. Ross. (1953). Absorption of radioactive iron in normal, anemic and hemochromatotic subjects. Amer. J. Med., 14,499.

Chodos, R. B., J. F. Ross, L. Apt, M. Pollycove and J. A. E. Halkett. (1957). The absorption of radioiron labelled foods and iron salts in normal and iron deficient subjects, and in idiopathic hemochromatosis. J. clin. Invest., 36,314.

Clark, F. H. (1934). The inheritance and linkage relations of a new recessive spotting in the house mouse. Genetics, 19,365.

Cohen, B. L. (1960). Genetics of plasma transferrins in the mouse. Genet. Res., Camb., 1,431.

Cohen, B. L. (1962). Cited by Grewal, M. S. (1962).

Cohen, B. L. and D. C. Shreffler. (1961). A revised nomenclature for the mouse transferrin locus. Genet. Res. Camb., 2,306.

Conrad, M. E., B. I. Benjamin, H. L. Williams and A. L. Foy. (1967). Human absorption of hemoglobin iron. Gastroenterology, 53,5.

Conrad, M. E., S. Cortell, H. L Williams and A. L. Foy. (1966). Polymerization and intra-luminal factors in the absorption of hemoglobin iron. J. Lab. clin. Med., 68,659.

Conrad, M. E. and W. H. Crosby. (1963). Intestinal mucosal mechanisms controlling iron absorption. Blood, 22,406.

Conrad, M. E., L. R. Weintraub and W H. Crosby. (1964). The role of the intestine in iron kinetics. J. clin. Invest., 43,963.

Conrad, M. E., L. R. Weintraub, D. A. Sears and W. H. Crosby. (1966). Absorption of hemoglobin iron. Amer. J. Physiol., 211,1123.

Cooley, T. B. (1945). A severe type of hereditary anaemia with elliptocytosis. Interesting sequence of splenectomy. Amer. J. med. Sci., 209,561.

Copley, A. L. and T. P. Robb. (1942a). Studies on platelets. I. The method of Vilarino and Pimental and a new direct method of counting blood platelets. Amer. J. clin. Path., 12,362.

Copley, A. L. and T. P. Robb. (1942b). Studies on platelets. III. The effect of heparin in vivo on the platelet count in mice and dogs. Amer. J. clin. Path., 12,563.

Copp, D. H. and D. M. Greenberg. (1946). A tracer study of iron metabolism with radioactive iron. 1. Methods: absorption and excretion of iron. J. biol. Chem., 164,377.

Coulombre, J. L. and E. S Russell. (1954). Analysis of the pleiotropism at the W - locus in the mouse. J. exper. Zool., 126,277.

Cowan, J. W., M. Esfahani, J. P. Salji and S. A. Azzam. (1966). Effect of phytate on iron absorption in the rat. J. Nutr., 90,423.

Craig, M. L. and E. S. Russell. (1963). Electrophoretic patterns of hemoglobin from fetal mice of different inbred strains. Science, 142,398.

Craig, M. L. and E. S. Russell. (1964). A developmental change in hemoglobins correlated with an embryonic red cell population in the mouse. Dev. Biol., 10,191.

Creamer, B. (1967). The turnover of the epithelium of the small intestine. Brit. med. Bull., 23,226.

Crosby, W. H. (1963). The control of iron balance by the intestinal mucosa. Blood, 22,441.

Crosby, W. H. (1964). The control of iron absorption. Series haematologica, 6,66.

Crosby, W. H. (1966). Mucosal block. An evaluation of concepts relating to control of iron absorption Seminars in Hematology, 3,299.

Cudkowicz, G. (1967). Personal communication.

Curry, J. L. and J. J. Trentin. (1967). Hemopoietic spleen colony studies. 1. Growth and differentiation. Develop. Biol., 15,395.

Curry, J. L., J. J. Trentin and N. Wolf. (1967). Hemopoietic spleen colony studies. II. Erythropoiesis. J. exper. Med., 125,703.

Dacie, J. V. (1960). The haemolytic anaemias. I. Congenital anaemias. 2nd Ed. Churchill, London.

Dacie, J. V. (1962). The haemolytic anaemias. II. The auto-immune haemolytic anaemias. 2nd Ed. Churchill, London.

Dacie, J. V. and S. M. Lewis. (1963). Practical Haematology. 3rd Ed., Churchill, London.

Dagg, J. H., A. Goldberg, and A. Lochhead. (1966). Value of erythrocyte protoporphyrin in the diagnosis of latent iron deficiency (sideropenia). Brit. J. Haemat., 12,326.

Danon, D. (1963). A rapid micro-method for recording red cell osmotic fragility by continuous decrease of salt concentration. J. clin. Path., 16,377.

Danon, D. (1967). The Fragiligraph and its application. Brit. J. Haemat., 13 (Suppl.), 61.

Davidson, R. G. (1964). The Lyon hypothesis. J. Pediat., 65,765.

Davidson, R. G., H. M. Nitowsky and B. Childs. (1963). Demonstration of two populations of cells in the human female heterozygous for glucose-6-phosphate dehydrogenase variants. Proc. nat. Acad. Sci. U.S., 50,481.

Davis, A. E. and J. Badenoch. (1962). Iron absorption in pancreatic disease. Lancet, 2,6.

Davis, A. E. and J. C. Biggs. (1965). The pancreas and iron absorption. Gut, 6,140.

Davis, A. E. and J. C. Biggs. (1967). The pancreas and iron absorption: current views. Amer. J. dig. Dis., 12,293.

Davis, P. S. and D. J Deller. (1966). Effect of a xanthine oxidase inhibitor (allopurinol) on radioiron absorption in man. Lancet, 2,470.

de Aberle, S. B. (1924). An hereditary intra-uterine developmental deficiency in the dominant white mouse. Anat. Rec., 27,177.

de Aberle, S. B. (1935). Hereditary anemia in mice and its relation to dominant spotting. Amer. Nat., 59,327.

de Aberle, S. B. (1927a). A study of the hereditary anemia of mice. Amer. J. Anat., 40,219.

de Aberle, S. B. (1927b). Reticulation and age of red blood corpuscles in normal and anemic mice. Anat. Rec., 35,30.

Deol, M. S. (1961). Genetical studies on the skeleton of the mouse. XXVIII. Tail short. Proc. roy. Soc. B., 155,78.

Detlefsen, J. A. (1923). A lethal type in mice, which may live for a few days after birth. Anat. Rec., 24,417.

Dickie, M. M. (1966). Keeping Records. In The Biology of the Laboratory Mouse, Chapt. 3. 2nd Ed. Ed. E. L. Green. McGraw-Hill Book Co.,New York.

Doeblin, T. D. (1967). Personal communication.

Dowdle, E. B., D. Schachter and H. Schenker. (1960). Active transport of Fe^{59} by everted segments of rat duodenum. Amer. J. Physiol., 198,609.

Dreyer, G. and W. Ray. (1910). The blood volume of mammals as determined by experiments upon rabbits, guinea-pigs and mice; and its relationship to the obdyweight and surface area expressed in a formula. Phil. Trans. roy. Soc. B., 201,133.

Dubois, E. L., R. E. Horowitz, H. B. Demopoulos and R. Teplitz. (1966). NZB/NZW mice as a model of SLE. J. amer. med. Ass., 195,285.

Dunn, T. B. (1954). Normal and pathologic anatomy of the reticular tissue in laboratory mice, with a classification and discussion of neoplasms. J. nat. Cancer. Inst., 14,1281.

Edwards, J. A. (1967). Personal communication.

Eickholt, T. H. and W. F. White. (1964). Determination of iron content in mice. J. pharm. Sci., 53,1418.

Ellis, J. T., I. Schulman and C. H. Smith. (1954). Generalized siderosis with fibrosis of liver and pancreas in Cooley's (Mediterranean) anemia. Amer. J. Path., 30,287.

Endicott, K. M. and H. Gump. (1947). Hemograms and myelograms of healthy female mice of C57 brown and CFW strains. Blood, Special Issue, 1,60.

Erlandson, M. E., B. Walden, G. Stern, M. W. Hilgartner, J. Wehman and C. H. Smith. (1962). Studies on congenital hemolytic syndromes. IV. Gastro-intestinal absorption of iron. Blood, 19,359.

Erslev, A. J. (1959). The effect of anemic anoxia on the cellular development of nucleated red cells. Blood, 14,386.

Ewing, K. L. and O. E. Tauber. (1964). Hematological changes in aging male C57Bl/6 Jax mice. J. Geront., 19,165.

Falconer, D. S. (1952). A totally sex-linked gene in the house mouse. Nature, (Lond.), 169,664.

Falconer, D. S. (1953). Total sex-linkage in the house mouse. Z. f. indukt. Abstamm. u. Vererbs., 85,210.

Falconer, D. S. (1965). Personal communication.

Falconer, D. S. and J. H. Isaacson. (1962). The genetics of sex-linked anaemia in the mouse. Genet. Res. Camb., 3,248.

Fantoni, A., A. de la Chapelle, R. A. Rifkind and P. A. Marks. (1967). Differentiation of erythroid cells in fetal mice. Proc. 10th Ann. Meeting, Amer. Soc. Hemat., Abst. 32.

Fekete, E. (1941). In The Biology of the Laboratory Mouse. Ed. G. D. Snell. The Blakison Co., Philadelphia, Pa.

Fekete, E., C. C. Little and A. M. Cloudman. (1941). Some effects of the gene W^V (dominant spotting) in mice. Proc. nat. Acad. Sci., 27,114.

Field, M., M. Seki, M. L. Mitchell and T. C. Chalmers. (1960). Studies in iron absorption. I. Determination in rats by measurement of total body radioactivity. J. Lab. clin. Med., 55,929.

Fischer, D. S. and D. C. Price. (1963). A possible humoral regulator of iron absorption. Proc. Soc. exper. Med., 112,228.

Forsthoefel, P. F. and A. M. Kuharcik. (1961). The problem of anemia in mice homozygous for Strong's luxoid gene. Genetics, 46,864.

Fortuyn, A.B.D. (1939). A mutation from agouti with recessive spotting to dominant spotting in Mus musculus. Genetica, 21,92.

Francis, L. D. and L. C. Strong. (1938). Hemoglobin studies on the blood of female mice of the CBA strain: effects of age, diet, strain and reproduction. Amer. J. Physiol., 124,511.

Fraser, A. S., S. Sobey and C. C. Spicer. (1953). Mottled, a sex modified lethal in the house mouse. J. Genet., 51,217.

Friedman, B. I., J. W. Schaefer and L. Schiff. (1966). Increased iron - 59 absorption in patients with hepatic cirrhosis. J. nucl. Med., 7,594.

Furth, J. and H. Sobel. (1946). Hypervolemia secondary to grafted granulosa cell tumor. J. nat. Cancer Inst., 7,103.

Furth, O. and R. Scholl. (1936). The absorption of ferrous and ferric compounds from the intestines of rabbits. J. Pharmacol. exper. Therap., 58,14.

Garber, E. D. (1952). "Bent-tail", a dominant sex-linked mutation in the mouse. Proc. nat. Acad. Sci., 38,876.

Gardner, M. V. (1947). The blood picture of normal laboratory animals. A review of the literature 1936-1946. J. Franklin Inst., 243,172.

Gelmetti, L. (1952). Ricerche embriologiche ed istologiche sui mutanti del locus W nei topi. III. L'emopoiesi nella milza. Symp. genet., Pavia., 3,119. Cited by Borghese, E. (1959a).

Gelpi, A. P. and N. Ende. (1958). An hereditary anemia with hemochromatosis. Studies of an unusual hemopathic syndrome resembling thalassemia. Amer. J. Med., 25,303.

Giltinan, P. J., M. C. Holmes and F. M. Burnet. (1965). Cortisone acetate treatment of haemolytic anaemia in NZB mice. Aust. J. exper. Biol. med. Sci., 43,523.

Gitlin, D. and A. Cruchaud. (1962). On the kinetics of iron absorption in mice. J. clin. Invest., 41,344.

Gluecksohn-Waelsch, S. (1960). The inheritance of hemoglobin types and other biochemical traits in mammals. J. cell. comp. Physiol., 56, Suppl. 1, 89.

Gluecksohn-Waelsch, S., H. M. Ranney and B. F. Sisken. (1957). The hereditary transmission of hemoglobin differences in mice. J. clin. Invest., 36,753.

Goldberg, A. (1965). Sideroblastic anaemia: a commentary. Brit. J. Haemat., 11,821.

Goldberg, A., N. Ashenbruker, G. E. Cartwright and M. M. Wintrobe. (1956). Studies in the biosynthesis of haem in vitro by avian erythrocytes. Blood, 11,821.

Goldberg, A., A. C. Lochhead and J. H. Dagg. (1963). Histamine - fast achlorydria and iron absorption. Lancet, 1,848.

Goodall, A. (1910). The numbers, proportions and characters of the red and white blood corpuscles in certain animals. J. Path. Bact., 14,195.

Goodman, J. W. and L. H. Smith. (1961). Erythrocyte life span in normal mice and in radiation bone marrow chimeras. Amer. J. Physiol., 200,764.

Gorer, P. A. (1936a). The detection of antigenic differences in mouse erythrocytes by the employment of immune sera. Brit. J. exper. Path., 17,42.

Gorer, P. A. (1936b). The detection of a hereditary antigenic difference in the blood of mice by means of human group A serum. J. Genet., 32,17.

Gorini, P., E. G. Rondanelli and A. Ferrata. (1957). Ricerche sulla genesi della macroeritrocitosi nell'anemia aplastica ereditaria dei tope W/W. Symp. genet., Pavia, 5,310.

Goulden, F. and F. L. Warren. (1944). The hemoglobin content of the blood of mice of the R111 and CBA strains. Cancer. Res., 4,421.

Gowen, J. W. and M. L. Calhoun. (1943). Factors affecting genetic resistance of mice to mouse typhoid. J. infect. Dis., 73,40.

Gowen, J. W. and E. H. Gay. (1932). Physiological factors necessary to alleviate genetic lethal anemia in mice. Amer. Nat., 66,289.

Graham, J. B., J. A. Buckwalter, L. J. Hartley and K. M. Brinkhous. (1949). Canine hemophilia. Observations on the course, the clotting anomaly and the effect of blood transfusions. J. exper. Med., 90,97.

Granick, S. (1946a). Protein apoferritin and ferritin in iron feeding and absorption. Science, 103,107.

Granick, S. (1946b). Ferritin. IX. Increase of the protein apoferritin in the gastrointestinal mucosa as a direct response to iron feeding. The function of ferritin in the regulation of iron absorption. J. biol. Chem., 164,737.

Granick, S. (1949). Iron metabolism and hemochromatosis. Bull. N.Y. Acad. Med., 25,403.

Granick, S. (1954). Iron metabolism. Bull. N. Y. Acad. Med., 30,81.

Granick, S. and R. D. Levere. (1964). Heme synthesis in erythroid cells. In Progress in Hematology, Vol. IV, Ed. C. V. Moore and E. B. Brown. Grune and Stratton, New York and London, p. 1.

Green, M. C. (1955). Luxoid - a new hereditary leg and foot abnormality in the house mouse. J. Hered., 46,91.

Green, M. C. (1966). Mutant genes and linkages. In The Biology of the Laboratory Mouse, Chapt. 8. 2nd Ed. Ed. E. L. Green. McGraw-Hill Book Co., New York.

Greenberg, S., H. Wong, S. A. Miller, R. W. Scarlata and T. C. Chalmers. (1960). Iron absorption and turnover in hypoxia. J. clin. Invest., 39,992.

Greenberger, N. J. and R. D. Ruppert. (1966). Inhibition of protein synthesis: a mechanism for the production of impaired iron absorption. Science, 153,315.

Greenberger, N. J., R. D. Ruppert and F. E. Cuppage. (1967). Inhibition of intestinal iron transport induced by tetracycline. Gastroenterology, 53,590.

Grewal, M. S. (1962). A sex-linked anaemia in the mouse. Genet. Res. Camb., 3,238.

Grinstein, M. and M. M. Wintrobe. (1948). Spectrophotometric micromethod for the quantitative determination of the free erythrocyte protoporphyrin. J. biol. Chem., 172,459.

Groen, J., W. A. van den Broek and H. Veldman. (1947). Absorption of iron compounds from the small intestine in the rat. Biochem. Biophys. Acta., 1,315.

Grüneberg, H. (1939). Inherited macrocytic anaemias in the house mouse. Genetics, 24,777.

Grüneberg, H. (1941). The growth of the blood of the sucking mouse. J. Path. Bact., 52,323.

Grüneberg, H. (1942a). The anaemia of the flex-tailed mice (Mus musculus L.) I. Static and dynamic haematology, J. Genet., 4345.

Grüneberg, H. (1942b). Inherited macrocytic anaemias in the house mouse. II. Dominance relationships. J. Genet., 43,285.

Grüneberg, H. (1942c). The anaemia of flex-tailed mice (Mus musculus L.). II. Siderocytes. J. Genet., 44,246.

Grüneberg, H. (1952). The Genetics of the Mouse. 2nd. Ed. Martinus Nijhoff, The Hague.

Grüneberg, H. (1963). The Pathology of Development. A study of inherited skeletal disorders in animals. John Wiley and Sons, Inc., N. Y.

Grüneberg, H. (1966). More about the tabby mouse and about the Lyon hypothesis. J. Embroyol. exp. Morph., 16,569.

Grüneberg, H. (1967a). Sex-linked genes in man and the Lyon hypothesis. Ann. hum. Genet., Lond., 30,239.

Grüneberg, H. (1967b). Gene action in the mammalian X-chromosome. Genet. Res., (Camb.), 9,343.

Gubler, C. J., G. E. Cartwright and M. M. Wintrobe. (1950). The anemia of infection. X. The effect of infection on the absorption and storage of iron by the rat. J. biol. Chem., 184,563.

Gubler, C. J., M. E. Lahey, M. S. Chase, G. E. Cartwright, and M. M. Wintrobe. (1952). Studies on copper metabolism. III. Metabolism of iron in copper deficient swine. Blood, 7,1075.

Gurney, C. W., L. G. Lajtha and R. Oliver. (1962). A method for the investigation of stem cell kinetics. Brit. J. Haemat., 8,461.

Guzman, T. G. and A. Briones. (1946). Algunos datos eritrocitometricos de la sangre del raton recien nacido de la ciudad de Mexico. Biol. Abstr., 20,1139.

Hahn, P. F., W. F. Bale, J. F. Ross, W. M. Balfour and G. H. Whipple. (1943). Radioactive iron absorption by gastrointestinal tract. Influence of anemia, anoxia, and antecedent feeding. Distribution in growing dogs. J. exper. Med., 78,169.

Hahn, P. F., W. F. Bale and G. H. Whipple. (1946). Effects of inflammation (turpentine abscess) on iron absorption. Proc. Soc. exper. Biol. Med., 61,405.

Hahn, P. F., E. L. Carothers, W. J. Darby, M. Martin, C. W. Sheppard, R. O. Cannon, A. S. Beam, P. M. Deusen, J. C. Peterson and G. S. McClellan. (1951). Iron metabolism in human pregnancy as studied with the radio-isotope, ^{59}Fe. Amer. J. Obstet. Gynec., 61,477.

Hahn, P. F., E. Jones, R. C. Lowe, G. R. Meneely and W. Peacock. (1945). The relative absorption and utilization of ferrous and ferric iron in anaemia as determined with the radioactive isotope. Amer. J. Physiol., 143,191.

Halberg, F., M. B. Visscher and J. J Bittner. (1953). Eosinophil rhythm in mice: range of occurrence; effects of illumination, feeding and adrenalectomy. Amer. J. Physiol., 174,109.

Hall, R. and M. S. Losowsky. (1966). The distribution of erythroblast iron in sideroblastic anaemias. Brit. J. Haemat., 12,334.

Hallberg, L. and L. Solvell. (1960). Absorption of a single dose of iron in man. Acta. med. scandinav., 168, Suppl. 358,19.

Hallberg, L. and L. Solvell. (1967). Absorption of haemoglobin iron in man. Acta. med. scandinav., 181,335.

Harriss, E. B. (1962). Iron metabolism in experimental pyridoxine deficiency. In Radio-isotope in der Hamatologie: Internationales Symposion, Freiburg. Friedrich-Karl Schattauer-Verlag, Sluttgart.

Harriss, E. B., B. H. MacGibbon and D. L. Mollin. (1965). Experimental sideroblastic anaemia. Brit. J. Haemat., 11,99.

Hartman, R. S., M. E. Conrad, R. E. Hartman, R. J. T. Joy and W. H. Crosby. (1963). Ferritin containing bodies in human small intestinal epithelium. Blood, 22,397.

Hegsted, D. M., C. A. Finch and T. D. Kinney. (1949). The influence of diet on iron absorption. II. The interrelation of iron and phosphorus. J. exper. Med., 90,147.

Heilmeyer, L., W. Keller, O. Vivell, W. Keiderling, K. Betke, F. Woehler and H. E. Schultz. (1961). Congenital transferrin deficiency in a 7 year old girl. Germ. med. Mth., 6,385.

Heinecke, H. and M. Wagner. (1964). Haemoglobin types of the wild house mouse (Mus musculus domesticus Rutty 1772). Nature (Lond.), 204,1099.

Helyer, B. J. and J. B. Howie. (1961). Positive lupus erythematosus tests in a crossbred strain of mice NZB/Bl - NZY/BL. Proc. Univ. Otago. med. Sch., 39,17.

Helyer, B. J. and J. B. Howie. (1963a). Spontaneous autoimmune disease in NZB/BL mice. Brit. J. Haemat., 9,119.

Helyer, B. J. and J. B. Howie. (1936b). Renal disease associated with positive lupus erythematosus tests in a crossbred strain of mice. Nature (Lond.), 197,197.

Hertwig, P. (1942a). Neue Mutationen und Koppelungsgruppen bei der Hausmaus. Z. indukt. Abstamm. -u. Vererb.-Lehre., 80,220.

Hertwig, P. (1942b). Sechs neue Mutationen bei der Hausmaus in ihrer Bedeutung fur allgemeine Vererbungsfragen. Z. Menschl. Vererb.-u. Konstit.-Lehre.,26,1.

Hertwig, P. (1956). Erbliche Anamien bei Mausen. Zool. Anzeig., Suppl. 20,185.

Hill, A. B. (1966). Principles of Medical Statistics. 8th Ed. Oxford Univ. Press, New York.

Hochhaus, H. and H. Quincke. (1896). Ueber Eisen-Resorption und Ausscheidung im Darmkanal. Naunyn-Schmeideberg's Arch. exp. Path. Pharmak., 37,159.

Holborrow, E. J., R. D. S. Barnes and M. Tuffrey. (1965). A new red-cell autoantibody in NZB mice. Nature (Lond.), 207,601.

Holmes, M. C (1965). Coombs test conversion in young NZB mice induced by transfer of lymphoid cells from Coombs positive donors. Aust. J. exper. Biol. med. Sci., 43,399.

Holmes, M C. and F. M. Burnet. (1963a). The influence of splenectomy in NZB mice. Aust. J. exper. Biol. med. Sci., 41,449.

Holmes, M. C. and F. M. Burnet. (1936b). A natural history of autoimmune disease in NZB mice. A comparison with the pattern of human autoimmune manifestations. Ann. intern. Med., 59,265.

Holmes, M. C. and F. M. Burnet. (1964). The inheritance of autoimmune disease in mice: a study of hybrids of the strains NZB and C_3H. Heredity, 19,419.

Holmes, M. C., J. Gorrie and F. M. Burnet. (1961). Transmission by splenic cells of an auto-immune disease occurring spontaneously in mice. Lancet, 11,638.

Horowitz, R. E., E. L. Bubois and A. A. Channing. (1965). Pathology of systemic lupus erythrematosus in the mouse. Fed. Proc., 24,683.

Huehns, E. R. and E. M. Shooter. (1965). Human haemoglobins. J. med. Genet., 2,48.

Huestis, R. R. and R. Anderson. (1954). Inherited Jaundice in Peromyscus. Science, 120,852.

Huestis, R. R., R. S. Anderson and A. G. Motulsky. (1956). Hereditary spherocytosis in Peromyscus 1. Genetic studies. J. Hered., 47,225.

Hummell, K. P., F. L. Richardson and E. Fekete, (1966). Anatomy. Chapt. 13 of The Biology of the Laboratory Mouse, 2nd Ed., Ed. E. L. Green; McGraw-Hill Book Co., New York.

Hunt, H. R., R. Mixter and D. Permar. (1933). Flexed-tail in the mouse, Mus musculus. Genetics, 18,335.

Hunt, H. R. and D. Permar. (1928). Flexed-tail, a mutation in the house mouse. Anat. Rec., 41,117.

Huser, H. J., E. E. Rieber and A. R. Berman. (1966). Experimental evidence of excessive hemolysis in chronic iron deficiency anemia. Blood, 28,1004.

Hutchison, H. E., P. H. Pinkerton, M. Aiton and P. Cassidy. (1963). A method for the estimation of the concentration of haemoglobin variants with particular reference to haemoglobins A_2 and S and the recognition of thalassaemia minor. Scot. med. J., 8,149.

Hutton, J. J., J. Bishop, R. Schweet and E. S. Russell. (1962 a). Hemoglobin inheritance in inbred mouse strains, II. Genetic studies. Proc. nat. Acad. Sci., 48,1718.

Hutton J. J., J. Bishop, R. Schweet and E. S. Russell. (1962b). Hemoglobin inheritance in inbred mouse strains 1. Structural differences. Proc. nat. Acad. Sci., 48,1505.

Hutton, J. J., R. S. Schweet, H. G. Wolfe, and E. S. Russell. (1964). Hemoglobin solubility and α-chain structure in crosses between two inbred mouse strains. Science, 143,252.

Hwang, Y. F. and E. B. Brown. (1963). Studies on the effects of desferroxamine on human iron absorption and excretion. J. Lab. clin. Med., 62,885.

Hwang, Y. F. and E. B. Brown. (1965). Effect of desferroxamine on iron absorption. Lancet, 1,135.

Ilan, J., K. Guggenheim and M. Ickowitz. (1963). Characterization of the "meat anaemia" in mice and its prevention and cure by copper. Brit. J. Haemat., 9,25.

Ingram, V. M. (1963). The hemoglobins in genetics and evolution. Columbia University Press, New York and London.

Ingram, V. M. and A. O. W. Stretton. (1961). Human hemoglobin A_2: chemistry, genetics and evolution. Nature (Lond.), 190,1079.

Jacobs, A., J. Rhodes and J. D. Eakins. (1967). Gastric factors influencing iron absorption in anaemic patients. Scand. J. Haemat., 4,105.

Jacobs, A., J. Rhodes, D. K. Peters, H. Campbell and J. Eakins. (1966). Gastric acidity and iron absorption. Brit. J. Haemat., 12,728.

Jacobs, P., T. H. Bothwell and R. W. Charlton. (1966). Intestinal iron transport: studies using a loop of gut with an artificial circulation. Amer. J. Physiol., 210,694.

Jacobson, E. D. R. B. Chodos and W. W. Faloon. (1960). An experimental malabsorption syndrome induced by neomycin. Amer. J. Med., 28,524.

Jacobson, L. O. (1944). The effect of estrogens on the peripheral blood and bone marrow of mice. Endocrinology, 34, 240.

Jeffries, G. H., E. Weser and M. H. Sleisenger. (1964). Progress in gastroenterology. Malabsorption. Gastroenterology, 46,434.

Joe, M., J. M Teasdale and J. R. Miller. (1962). A new mutation (sph) causing neonatal jaundice in the house mouse. Can. J. Genet. Cytol., 4,219.

Kales, A. N., W. Fried and C. W. Gurney. (1966). Mechanism of the hereditary anemia of Sl^m mice. Blood, 28,387.

Kaliss, N. and D. Pressman. (1950). Plasma and blood volumes of mouse organs, as determined with radioactive isotopes. Proc. Soc. exper. Biol. Med., 75,16.

Kamenoff, R. J. (1935). Effects of the flexed-tail gene on the development of the house mouse. J. Morph., 58,117.

Kamenoff, R. J. (1937). Erythrocyte count in 4 inbred strains of mice. Proc. Soc. exper. Biol. Med., 36,411.

Kato, K. (1941). A simple and accurate microfragility test for measuring erythrocyte resistance. J. Lab. clin. Med., 26,703.

Kaufman, N., J. V. Klavins and T. D. Kinney. (1958). Excessive iron absorption in rats fed low protein, high fat diets. Lab. Invest., 7,369.

Keeler, C. E. (1931). A new mutation to "dominant spotting" (W) in the house mouse. J. Hered., 22,273.

Keighley, G. H. (1966). Cited by Russell, E. S. and S. E. Bernstein. (1966).

Keighley, G. H., P. Lowy, E. S. Russell and M. W. Thompson. (1966). Analysis of erythroid homeostatic mechanisms in normal and genetically anaemic mice. Brit. J. Haemat., 12,461.

Keighley, G., E. S. Russell and P. H. Lowy. (1962). Response of normal and genetically anaemic mice to erythropoietic stimuli. Brit. J. Haemat., 8,429.

Keith, N. M. (1923). The total circulating volume of blood and plasma in cases of chronic anemia and leukemia. Amer. J. med. Sci., 165,174.

Kienle, M. and L. C. Strong. (1959). Haematological studies of anaemia in luxoid mice of a polydactylous descent. Blut, 5,335.

Kinney, T. D., D. M. Hegsted and C. A. Finch. (1949). The influence of diet on iron absorption. I. The pathology of iron excess. J. exper. Med., 90,137.

Kinney, T. D., N. Kaufman and J. Klavins. (1955). Effect of ethionine-induced pancreatic damage on iron absorption. J. exper. Med., 102,151.

Koepke, J. A. and W. B. Stewart. (1964). Role of gastric secretion in iron absorption. Proc. Soc. exper. Biol. Med., 115,927.

Konitzer, K. and K. Michalke. (1965). Der Eisenstoffwechsel der weissen Maus. Acta biol. med. german., 14,489.

Kovach, J. S., E. S. Russell and P. A. Marks. (1966). Erythroid cell development in fetal mice: biochemical and ultrastructural analysis. Clin. Res., 14,319.

Kozma, C., R. A. Salvador and G. B. Elion. (1967). Allopurinol and iron storage. Lancet, 2,1040.

Krantz, S., E. Goldwasser and L. O. Jacobson. (1959). Studies on erythropoiesis. XIV. The relationship of humoral stimulation to iron absorption. Blood, 14,654.

Kroe, D. J., N. Kaufman, J. V. Klavins and T. D. Kinney. (1966). Interrelation of amino-acids and pH on intestinal iron absorption. Amer. J. Physiol., 211,414.

Kuharcik, A. M. and P. F. Forsthoefel. (1963). A study of anemia in Strong's luxoid mutant. J. Morph., 112,13.

Kunze, H-G. (1954). Die Erythropoiese bei einer erbilichen Anamie rontgenmutierter Mause. Folia haemat. (Lpz.), 72,391.

Lajtha, L. G. (1961). The use of isotopes in haematology, Blackwell, Oxford.

Lahey, M. E., C. J. Gubler, M. S. Chase, G. E. Cartwright and M. M. Wintrobe. (1952). Studies on copper metabolism. II. Hematologic manifestations of copper deficiency in swine. Blood, 7,1053.

Lancet. (1963). Leading article. Lyonisation of the X-chromosome, 2,769.

Lancet. (1967). Leading article. Structure and function in idiopathic steatorrhoea, 2,873.

Lange, R. D. and V. Pavlovic-Kentera. (1964). Erythropoietin. In Progress in Hematology Vol. IV. Ed. C. V. Moore and E. B. Brown. Grune and Stratton, New York and London p. 72.

Larsen, B. A., R. G. S. Bidwell and W. W. Hawkins. (1960). The effect of ingestion of di-sodium ethylene diamine tetra-acetate on the absorption and metabolism of radio-active iron by the rat. Canad. J. Biochem. Physiol., 38,51.

Lehmann, H. and R. G. Huntsman. (1966). Man's Haemoglobins. North-Holland Publishing Co. Amsterdam.

Lewis, J. P., L. F. O'Grady, S. E. Bernstein, E. S. Russell and F. E. Trobaugh. (1965). Genetic influence on repopulation and differentiation of transplanted marrow. Proc. 8th Ann. Meeting of Amer. Soc. Hemat. Blood, 26,870.

Lie-Injo Luan Eng. (1962). Alpha-chain thalassemia and hydrops fetalis in Malaya. Report of five cases. Blood, 20,581.

Lindsey, E. S., G. W. K. Donaldson and M. F. A. Woodruff. (1966). Erythrocyte survival in normal mice and in mice with auto immune haemolytic anaemia. Clin. exper. Immunol., 1,85.

Little, C. C. (1915). The inheritance of black-eyed white spotting in mice. Amer. Nat., 49,727.

Little, C. C. (1917). The relation of yellow coat color to black-eyed white spotting of mice in heredity. Anat. Rec., 11,501.

Little, C. C. and A. M. Cloudman. (1937). The occurrence of a dominant spotting mutation in the house mouse. Proc. nat. Acad. Sci., 23,535.

Long, G., M C. Holmes and F. M. Burnet. (1963). Auto antibodies produced against mouse erythrocytes in NZB mice. Austr. J. exp. Biol. med. Sci., 44,315.

Loria, A., L. Sanchez-Medal, R. Lisker, E. de Rodriguez, J. Labardini. (1967). Red cell life-span in iron deficiency anaemia. Brit. J. Haemat., 13,294.

Losowsky, M. S. and R. Hall. (1965). Hereditary sideroblastic anaemia. Brit. J. Haemat., 11,70.

Luke, C. G., P. S. Davis and D. J. Deller. (1967). Change in gastric iron binding protein (gastroferrin) during iron deficiency anaemia. Lancet, 1,926.

Lukl, P., B. Wiedermann and M. Barborik. (1958). Hereditäre Leptocyten - Anämie bei Männeon mit Hämochromatose. Folia haemat. (Frankfurt), N.F., 3,17.

Lyon, M. F. (1961). Gene action in the X-chromosome of the mouse (Mus musculus L.). Nature (Lond.), 190,372.

Lyon, M. F. (1966). X-chromosome inactivation in mammals. In Advances in Teratology, Ed. D. H. M. Wollain, p. 25. Logos Press, London.

Macapinlac, M. P., W. N. Pearson and W. J. Darby. (1966). Some characteristics of zinc deficiency in the albino rat. In Zinc Metabolism, Ed. A. S. Prasad, P. 142. Chas. C. Thomas, Springfield, Illinois.

MacDiarmid, W D., G. R. Lee, G. E. Cartwright and M. M. Wintrobe. (1967). X-inactivation in an unusual X-linked anaemia and the Xg^a blood group. Clin. Res., 15,132.

MacDonald, W. C., J S. Trier and N. B. Everett. (1964). Cell proliferation and migration in the stomach duodenum and rectum of man: radioautographic studies. Gastroenterology, 46,405.

Malewitz, T. D. (1965). Normal histology of the digestive tract of the mouse. Okajimas Fol. anat. jap., 41,21.

Manis, J. G. and D. Schachter. (1962a). Active transport of iron by intestine: features of the two-step mechanism. Amer. J. Physiol, 203,73.

Manis, J. G. and D. Schachter. (1962b). Active transport of iron by intestine: effects of oral iron and pregnancy. Amer. J. Physiol., 203,81.

Manis, J. G. and D. Schachter. (1964). Active transport of iron by intestine: mucosal iron pools. Amer. J. Physiol., 207,893.

Manis, J. G. and D. Schachter. (1965). Fe^{59}-amino-acid complexes: are they intermediates in Fe^{59} absorption across the intestinal mucosa. (30409). Proc. Soc. exper. Biol. Med., 119,1185.

Margolis, F. L. (1965). Reduced δ-aminolevulinate dehydrase (ALD) activity in mutant mouse anemia. Fed. Proc., 24,469.

Margolis, F. L. and E. S. Russell. (1965). Delta-aminolevulinate dehydratase activity in mice with hereditary anemia. Science, 150,496.

Mathai, C. K., S. Ohno and E. Beutler. (1966). Sex-linkage of glucose-6-phosphate dehydrogenase gene in equidae. Nature, (Lond.), 210,115.

McCance, R. A., C. N. Edgecombe, and E. M. Widdowson. (1943). Phytic acid and iron absorption. Lancet, 2,126.

McCance, R. A. and E. M. Widdowson. (1937). Absorption and excretion of iron. Lancet, 2,680.

McCulloch, E. A., L. Siminovitch and J. E. Till. (1964). Spleen colony formation in anemic mice of genotype WW^v. Science, 144,844.

McCulloch, E. A., L. Siminovitch, J. E. Till, E. S. Russell and S. E. Bernstein. (1965). The cellular basis of the genetically determined hemopoietic defect in anemic mice of genotype Sl/Sl^{d}. Blood, 26,399.

McDowell, E. C. and J. E. Hubbard. (1922). On the absence of isoagglutinins in mice. Proc. Soc. exper. Biol. Med., 20,93.

McFadzean, A. J. S., D. Todd and K. C. Tsang. (1958). Observations on the anemia of cryptogenic splenomegaly. II. Expansion of the plasma volume. Blood, 13,524.

McKusick, V. A. (1964). On the X-chromosome of man. American Institute of Biological Sciences.

Meier, H., R. C. Allen and W. G. Hoag. (1961). Normal blood clotting of inbred mice. Amer. J. Physiol., 201,375.

Meier, H., R. C. Allen and W. G. Hoag. (1962). Spontaneous hemorrhagic diathesis in inbred mice due to single or multiple "prothrombin-complex" deficiencies. Blood, 19,501.

Meier, H., W. G. Hoag and R. C. Allen. (1961). Spontaneous hemorrhagic diathesis in inbred mice due to "prothrombin-complex" deficiencies. Fed. Proc., 20,54.

Mellors, R. C. (1965). Auto-immune disease in NZB/Bl mice. I. Pathology and pathogenesis of a model system of spontaneous glomerulonephritis. J. exper. Med., 122,25.

Mellors, R. C. (1966). Auto-immune disease in NZB/Bl mice. II. Autoimmunity and malignant lymphoma. Blood, 27,435.

Mellors, R. C., and C. Y. Huang. (1966). Immunopathology of NZB/Bl mice. V. Virus-like (filterable) agent separable from lymphoma cells and identifiable by electron microscopy. J. exper. Med., 124,1031.

Mendel, G. A. (1961). Studies on iron absorption. I. The relationship between the rate of erythropoiesis, hypoxia and iron absorption. Blood, 18,727.

Mendel, G. A., R. J. Weiler and A. Mangalik. (1963). Studies on iron absorption. II. The absorption of iron in experimental anemias of diverse etiology. Blood, 22,450.

Mintz, B. (1957). Embryological development of primordial germ-cells in the mouse: effect of a new mutation, W^J. J. Embryol. exp. Morph., 5,396.

Mintz, B. and E. S. Russell. (1955). Developmental modifications of primordial germ cells induced by the W-series of genes in the mouse embryo. Anat. Rec., 122,443.

Mintz, B. and E. S. Russell. (1951). Gene-induced embryological modifications of primordial germ cells in the mouse. J. exper. Zool., 134,207.

Mirand, E. A. (1966). Personal communication.

Mirand, E. A. (1967). Virus-induced erythropoiesis in hypertransfused polycythemic mice. Science, 156,832.

Mirand, E. A. and G. Gutman. (1963). The inability of genetically anemic mice to respond to various erythropoietin preparations. Amer. Zool., 3,551.

Mirand, E. A. and T. C. Prentice. (1964). The ability of genetically anemic mice (WW^V) to respond only to erythropoietin from WW^V mice. Amer. Zool., 4,326.

Mirsky, R. (1949). A histological study of the livers of W and W^V alleles. Jackson Lab., Summer student report. Cited by Mintz, B. (1957).

Mixter, R. and H. R. Hunt. (1933). Anemia in the flex-tailed mouse, Mus musculus. Genetics, 18,367.

Miyasato, F., J. R. Manaligod and V. E. Pollak. (1967). Auto-immune disease in NZB and NZB-NZW F_1 mice. Arch. Path., 83,20.

Moller, G. (1965). Survival of H-2 incompatible mouse erythrocytes in untreated and isoimmune recipients. Immunology, 8,360.

Mooney, F. S. (1952). The diagnosis of carriers of Cooley's anaemia. J. clin. Path., 5,154.

Moore, C. V. and R. Dubach. (1951). Observations on the absorption of iron from foods tagged with radioiron. Trans. Ass. amer Phys., 64,245.

Moore, C. V., R. Dubach, V. Minnich and H. K. Roberts. (1944). Absorption of ferrous and ferric radioactive iron by human subjects and by dogs. J. clin. Invest., 23,755.

Morgan, W. C. (1950). A new tail-short mutation in the mouse whose lethal effects are conditioned by the residual genotypes. J. Hered., 41,208.

Morris, H. P., T. B. Dunn and B. P. Wagner. (1954). Influence of gonadotroplin on pyridoxine-deficient and diet restricted female mice. J. nat. Cancer Inst., 14,493.

Morrow, J. J. and A. Goldberg. (1965). The sideroblastic anaemias. Postgrad. med. J., 41,740.

Motulsky, A. G., R. R. Huestis and R. Anderson. (1956). Hereditary spherocytosis in mouse and man. Acta. genet., 6,240.

Murray, M. J. and N. Stein. (1967a). Effect of pancreas extract on Fe^{59} absorption by anemic rats. J. Lab. clin. Med., 70,361.

Murray, M. J. and N. Stein. (1967b). The integrity of the stomach as a requirement for maximal iron absorption. J. Lab. clin. Med., 70,673.

Mustard, J. F., H. C. Rowsell, G. A. Robinson, T. D. Hoeksema and H. G. Downie. (1960). Canine haemophilia B (Christmas disease). Brit. J. Haemat., 6, 259.

Nairn, R. C., A. R. McGiven, P. N. J. Ironside and L. C. Norins. (1966). Plasma proteins in the glomerular lesions of NZB/NZW mice. Brit. J. exper. Path., 47,99.

Nakao, K., O. Wada, F. Takaku, S. Sassa, Y. Tano and G. Urata. (1967). The origin of the increased protoporphyrin of mice with experimentally induced porphyria. J. Lab. clin. Med., 70,923.

Nash, D. J., E. Kent, M. M. Dickie and E. S. Russell. (1964). The inheritance of "mick", a new anemia in the house mouse. Amer. Zool., 4,404.

Niece, R. L., E. C. McFarland and E. S. Russell. (1963). Erythroid homeostasis in normal and genetically anemic mice: reaction to induced polycythemia. Science, 142,1468.

Norins, L. C. and M. C. Holmes. (1964a). Globulins on NZB mouse erythrocytes. J. Immunol., 93,897.

Norins, L. C. and M. C. Holmes. (1964b). Antinuclear factor in mice. J. Immunol., 93,148.

Nossel, H. L., R. K. Archer and R. G. Macfarlane. (1962). Equine haemophilia: Report of a case and its response to multiple infusions of heterospecific A. H. G. Brit. J. Haemat., 8,335.

Oakley, C. L. and G. H. Warrack. (1940). The blood volume of the mouse. J. Path. Bact., 50,372.

Odell, T. T. and T. P. McDonald. (1960). Peripheral counts and survival of blood platelets of mice. Fed. Proc., 19,63.

Odell, T. T. and T. P. McDonald. (1961). Life span of mouse platelets. Proc. Soc. exper. Biol. Med., 106,107.

Ohno, S. (1967). Sex chromosomes and sex-linked genes. Springer-Verlag. Berlin, Heidelberg, New York.

Oliner, H., R. Schwartz and W. Dameshek. (1961). Studies in experimental autoimmune disorders. I. Clinical and laboratory features of autoimmunization (Runt Disease) in the mouse. Blood, 17,20.

Padowetz, W. (1965). Personal communication.

Pagliardi, E., V. Prato, E. Giangrandi and L. Fiorina. (1959). Behaviour of the free erythrocyte protoporphyrins and of the erythrocyte copper in iron deficiency anaemia. Brit. J. Haemat., 5,217.

Panzenhagen, H., and R. Speirs. (1953). Effect of horse serum adrenal hormones, and histamine on the number of eosinophils in the blood and peritoneal fluid of mice. Blood, 8,536.

Parsons, L. D. (1936). Blood changes in mice bearing experimental sarcomas: (A) Sarcomas induced by a derivation of 1:2:5:6 dibenzanthracene; (B) Sarcomas produced by cell-free filtrates of Mal. Sarcoma I. J. Path. Bact., 43,1.

Paulley, J. W. (1954). Observations on the aetiology of idiopathic steatorrhoea. Jejunal and lymph node biopsies. Brit. med. J., 2,1318.

Pearson, W. N., M. Reich, H. Frank, and L. Salamat. (1967). Effects of dietary iron level on gut iron levels and iron absorption in the rat. J. Nutr., 92,53.

Penny, R. H. C. (1967). The blood and marrow picture of the laboratory mouse. Brit. vet. J., 123,227.

Petri, S. (1933). Morphologie und Zahl der Blutkörperchen bei 7-ca. 30G Schweren Normalen weissen Laboratoriumsmäusen. Acta path. et microbiol. scandinav., 10,159.

Phillips, R. J. S. (1954). Jimpy, a new totally sex-linked gene in the house mouse. Z. f. indukt. Abstamm. u. Vererbs., 86,322.

Pinkerton, P. H. (1967). X-linked hypochromic anaemia. Lancet, 1,1106.

Pinkerton, P. H. (1968). Histological evidence of disordered iron transport in the X-linked hypochromic anaemia of mice. J. Path. Bact., in the press.

Pinkerton, P. H. and R. M. Bannerman. (1966). X-linked hypochromic anemia of mice - a disorder of iron metabolism. Blood, 28,987.

Pinkerton, P. H. and R. M. Bannerman. (1967). Hereditary defect in iron absorption in mice. Nature (Lond.), 216,482.

Pinkerton, P. H., and R. M. Bannerman. (1968). The heritable mouse anemias. Hemat. Rev., in the press.

Pinkerton, P. H., M. Kreimer-Birnbaum and R. M. Bannerman. (1966). Iron metabolism in the X-linked hypochromic anemia of mice. Fed. Proc., 25,235.

Pirzio-Biroli, G., T. H. Bothwell and C. A. Finch. (1958). Iron absorption. II. The absorption of radioiron administered with a standard meal in man. J. Lab. clin. Med., 51,37.

Pirzio-Biroli, G. and C. A. Finch. (1960). Iron absorption. III. The influence of iron stores on iron absorption in the normal subject. J. Lab. clin. Med., 55,216.

Platt, W. R. and O. A. Zeller. (1951). Possible effects of hypersplenic extracts on the haemopoietic organs of mice. Arch. Path., 51,38.

Pollack, S., R. M. Kauffman and W. H. Crosby. (1964a). Iron absorption: the effect of an iron-deficient diet. Science, 144,1015.

Pollack, S., R. M. Kauffman and W. H. Crosby. (1964b). Iron absorption: Effects of sugars and reducing agents. Blood, 24,577.

Pollack, S., R. M. Kauffman and W. H. Crosby. (1964c). An investigation of exchange of iron across the intestinal mucosa. J. Lab. clin. Med., 63,847.

Popp, R. A. (1962a). Studies on the mouse hemoglobin loci VI. A third allele, Sol^3, at Sol locus. J. Hered., 53,147.

Popp, R. A. (1962b). Studies on the mouse hemoglobin loci III. Heterogeneity of electrophoretically indistinguishable single type hemoglobins. J. Hered., 53,75.

Popp, R. A. (1962c). Studies on the mouse hemoglobin loci V. Differences among tryptic peptides of the β-chain governed by alleles at the Hb locus. J. Hered., 53,142.

Popp, R. A. (1962d). Studies on the mouse hemoglobin loci VII. Differences among tryptic peptides of the α-chain governed by alleles at the Sol locus. J. Hered., 53,148.

Popp, R. A. (1963). Hemoglobin loci: Mice classified for their Hb and Sol alleles. Science, 140,893.

Popp, R. A. (1965). Hemoglobin variants in mice. Fed. Proc., 24,1252.

Popp, R. A., D. M. Popp and B. C. Webb. (1963). Amino-acid analysis of tryptic peptides of the α-chain of mouse hemoglobin. Amer. Zool., 3,490.

Popp, R. A. and W. St. Amand. (1958). The mouse hemoglobin locus. Anat. Rec., 132,489.

Popp, R. A. and W. St. Amand. (1960). Studies on the mouse hemoglobin locus. I. Identification of hemoglobin type and linkage of hemoglobin with albinism. J. Hered., 51,141.

Prasad, A. S. (1966). Metabolism of zinc and its deficiency in human subjects. In Zinc Metabolism. Ed. A. S. Prasad. p. 250. Chas. C. Thomas, Springfield, Illinois.

Pryor, D. S. (1967). The mechanism of anaemia in tropical splenomegaly. Quart. J. Med., 143,337.

The Radiochemical Manual. (1966). 2nd Ed., Ed. B. J. Wilson, The Radiochemical Centre, Amersham.

Ranney, H. M. and S. Gluecksohn-Waelsch. (1955). Filter paper electrophoresis of mouse hemoglobin. Preliminary note. Ann. hum. Genet., 19,269.

Ranney, H. M., G. Marlow-Smith and S. Gluecksohn-Waelsch. (1960). Haemoglobin differences in inbred strains of mice. Nature (Lond.), 188,212.

Reynafarje, C., R. Lozano and J. Valdivieso. (1959). The polycythaemia of high altitudes: iron metabolism and related aspects. Blood, 14,433.

Richmond, J., G. W. K. Donaldson, R. Williams, P. J. S. Hamilton and M. S. R. Hutt. (1967). Haematological effects of the idiopathic splenomegaly seen in Uganda. Brit. J. Haemat., 13,348.

Richter, G. W. (1957a). Structure and deposition of hemosiderin in cells as disclosed by electron microscopy: Relationships of ferritin and hemosiderin. Amer. J. Path., 33,590.

Richter, G. W. (1957b). A study of hemosiderosis with the aid of electron microscopy. J. exper. Med., 106,203.

Rifkin, D., M. Rifkin and W. Konigsberg. (1965). Amino-acid composition of tryptic peptides of two strains of mouse hemoglobin. Fed. Proc., 24,532.

Riggs, A. (1965). Hemoglobin polymerization in mice. Science, 147,621.

Rosa, J., G. Schapira, L. C. Dreyfus, J. de Grouchy, G. Mathé and J Bernard. (1958). Different heterogeneities of mouse haemoglobin according to strains. Nature (Lond.), 182,947.

Rundles, R. W. and H. F. Falls. (1946). Hereditary (? sex-linked) anemia. Am. J. med. Sci., 211,641.

Rush, B., M. A. Figallo and E. B. Brown. (1966). Effect of a low iron diet on iron absorption. Amer. J. clin. Nutr., 19,132.

Russell, E. S. (1949). Analysis of pleiotropism at the W-locus in the mouse: relationship between the effects of W and W^V substitution on hair pigmentation and on erythrocytes. J. Genet., 34,708.

Russell, E. S. (1954). Review of the pleiotropic effects of the W-series genes on growth and differentiation, in Aspects of Synthesis and Order of Growth. p. 113. Ed. D. Rudnick. Princeton Univ. Press. 13th Symposium of the Society for the Study of Development and Growth.

Russell, E. S. (1963). Problems and potentialities in the study of genic action in the mouse, in Methodology in Mammalian Genetics. Ed., W. J. Burdette. Holden-Day, Inc., San Francisco.

Russell, E. S. (1966). Personal Communication.

Russell, E. S. and S. E. Bernstein. (1966). Blood and Blood Formation. Chapt. 17, in The Biology of the Laboratory Mouse, 2nd Ed., Ed. E. L. Green; McGraw-Hill Book Co., New York.

Russell, E. S., S. E. Bernstein, F. A. Lawson and L. J. Smith. (1959). Long continued function of normal blood forming tissue transplanted into genetically anemic hosts. J. nat. Cancer Inst., 23,557.

Russell, E S., S. E. Bernstein, E. C. McFarland and W. R. Modeen. (1963). The cellular basis of differential radiosensitivity of normal and genetically anemic mice. Rad. Res., 20,677.

Russell, E. S., J. L. Coulombre and E. Fekete. (1952). Contributions of studies of gonad development and function and of hair spotting pattern to analysis of the W-series pleiotropism. Genetics, 37,621.

Russell, E. S. and E. L. Fondal. (1951). Quantitative Analysis of the normal and four alternative degrees of an inherited macrocytic anemia in the house mouse. I. Number and size of erythrocytes. Blood, 6,892.

Russell, E. S. and P. S. Gerald. (1958). Inherited electrophoretic hemoglobin patterns among 20 inbred strains of mice. Science, 128,1569.

Russell, E. S., F. Lawson and G. Schabtach. (1957). Evidence for a new allele at the W-locus of the mouse. J. Hered., 48,119.

Russell, E. S., L. M. Murray, E. M. Small and W. K. Silvers. (1956). Development of embryonic mouse gonads transferred to the spleen: effects of transplantation combined with genotypic autonomy. J. Embryol. exp. Morph., 4,347.

Russell, E. S., E. F. Neufeld and C. T. Higgens. (1951). Comparison of normal blood picture of young adults from 18 inbred strains of mice. Proc. Soc. exper. Biol. Med., 78,761.

Russell, E. S., L. J. Smith and F. A. Lawson. (1956). Implantation of normal blood forming tissue in radiated genetically anemic hosts. Science, 124,1076.

Russell, E. S., C. M. Snow, L. M. Murray and J. P. Cornier. (1953). The bone marrow in inherited macrocytic anaemia in the house mouse. Acta haemat., 10,247.

Russell, L. B. (1961). Genetics of mammalian sex chromosomes. Science, 133,1795.

Russell, P. J., J. D. Hicks and F. M. Burnet. (1966). Cyclophosphamide treatment of kidney disease in (NZB x NZW) F_1 mice. Lancet, 1,1279.

Russell, R. L. and D. L. Coleman. (1963). Genetic control of hepatic δ-aminolevulinate dehydrase in mice. Genetics, 48,1033.

Russell, W. L. and E. S. Russell. (1948). Investigation of the sterility-producing action of the W^v gene in the mouse by means of ovarian transplantation. Genetics, 33,122.

Sagel, I., G. Treser, A. Ty, M. Wachstein and K. Lange. (1965). Auto-immune renal lesions in NZB mice. Fed. Proc., 24,243.

Saltman, P. (1965). The role of chelation in iron metabolism. J. chem. Educ., 42,682.

Saltman, P. and H. Helbock. (1965). The regulation and control of intestinal iron transport. In Radioisotopes in animal nutrition and physiology. p. 301. International Atomic Energy Agency, Vienna.

Sandstead, H. H., A. S. Prasad, Z. Farid, A. Schulert, A. Miale, S. Bassilly and W. J. Darby. (1966). Endocrine manifestations of human zinc deficiency. In Zinc Metabolism. Ed. A. S. Prasad. p. 304. Chas. C. Thomas, Springfield, Illinois.

Sarvella, P. A. and L. B. Russell. (1956). Steel - a new dominant gene in the house mouse with effects on coat, pigment and blood. J. Hered., 47,123.

Saylor, L. and C. A. Finch. (1953). Determination of iron absorption using two isotopes of iron Amer. J. Physiol., 172,372.

Scarborough, R. A. (1931). The blood picture of normal laboratory animals. Yale J. Biol. Med., 3,267.

Schade, S. G. and R. F. Schilling. (1967). Effect of pepsin on the absorption of food vitamin B_{12} and iron. Amer. J. clin. Nutr., 20,636.

Schulz, J. and N. J. Smith. (1958a). A qualitative study of the absorption of food iron in infants and children. Amer. J. Dis.Childh., 95,109.

Schulz, J. and N. J. Smith. (1958b). Quantitative study of absorption of iron salts in infants and children. Amer. J. Dis. Childh., 95,120.

Schwartz, H. C., G. E. Cartwright, E. L. Smith and M. M. Wintrobe. (1959). Studies on the biosynthesis of heme from iron and protoporphyrin. Blood, 14,486.

Searle, A. G. (1952). Inherited macrocytic anemias of the house mouse. IV. The alleviating effect of blood injections. J. Genet., 51,187.

Selwyn, J. G. and W. E. R. Hackett. (1949). Acquired haemolytic anaemia; survival of transfused erythrocytes in patients and normal recipients. J. clin. Path., 2,114.

Shahidi, D. G., D. G. Nathan and L. K. Diamond. (1964). Iron deficiency anaemia associated with an error in iron metabolism in two siblings. J. clin. Invest., 43,510.

Sharpe. L. M., W. C. Peacock, R. Cooke, and R. S. Harris. (1950). The effect of phytate and other food factors on iron absorption. J. Nutr., 41,433.

Shearer, G. M. and G. Cudkowicz. (1967). Deficient production of antibody-forming cells by genetically anemic $W^{V}W^{V}$ and $W^{V}+$ mice. Fed. Proc., 26,688.

Shreffler, D. C. (1960). Genetic control of serum transferrin type in mice. Proc. nat. Acad. Sci., 46,1378.

Shreffler, D. C. (1963). Linkage of the mouse transferrin locus. J. Hered., 54,127.

Simonds, J. P. (1925). The blood of normal mice. Anat. Rec., 30,99.

Singer, M. F., M. Foster, M. L. Petras, P. Towlin and R. W Sloane. (1964). A new case of blood group inheritance in the house mouse. Genetics, 50,285.

Smith, C. H., T. R. C. Sisson, J. W. H. Floyd and S. Siegal. (1950). Serum iron and iron binding capacity of the serum in children with Cooley's anemia. Pediatrics, 5,799.

Smith, M. D. and B. Mallett. (1957). Iron absorption before and after partial gastrectomy. Clin. Sci., 16,23.

Smith, M. D. and I. M. Pannacciulli. (1958). Absorption of inorganic iron from graded doses: its significance in relation to iron absorption tests and the mucosal "block" theory. Brit. J. Haemat., 4,428.

Smith, L. H. and J. Tohá. (1958). Survival of mouse-grown rat erythrocytes. Proc. Soc. exper. Biol. Med., 98,125.

Smith, R. S. (1964). Iron absorption in cystic fibrosis. Brit. med. J., 1,608.

Smithies, O. (1959). An improved procedure for starch-gel electrophoresis: further variations in the serum proteins of normal individuals. Biochem. J., 71,585.

Sobel, H. J. and J. D. Waye. (1963). Pancreatic changes in various types of cirrhosis in alcoholics. Gastroenterology, 45,341.

Solvell, L. (1960). Effect of iron and transferrin intravenously on iron absorption and turnover in man. Acta med. scandinav., 168, suppl. 358,17.

Sorensen, E. W. (1965). Studies on iron absorption. II. Experiments with iron deficient and non-iron deficient diets. Acta med. scandinav., 178,385.

Spray, C. M. and E. M. Widdowson. (1950). Effect of growth and development on the composition of mammals. Brit. J. Nutr., 4,332.

Staats, J. (1966). The Laboratory Mouse. Chapt. 1. In The Biology of the Laboratory Mouse, 2nd Ed. Ed. E. L. Green. McGraw-Hill Book Co., New York.

Stevens, A. R., G. Pirzio-Biroli, H. N. Harkins, L. M. Nyhus and C. A. Finch. (1959). Iron metabolism in patients after partial gastrectomy. Ann. Surg., 149,534.

Stevens, L. C. and J. A. Mackensen. (1958). The inheritance and expression of a mutation in the mouse affecting blood formation, the axial skeleton and body size. J. Hered., 49,153.

Stevens, L. C., J. A. Mackensen and S. E. Bernstein. (1959). A mutation causing neonatal jaundice in the house mouse. J. Hered., 50,35.

Stewart, W. B. and S. R. Gambino. (1961). Kinetics of iron absorption in normal dogs. Amer. J. Physiol., 201,67.

Stewart, W. B., P. S. Vassar and R. S. Stone. (1953). Iron absorption in dogs during anemia due to acetylphenylhydrazine. J. clin. Invest., 32,1225.

Stickel and M. F. A. Woodruff. (1966). Cited by Lindsey, E. S., Donaldson, G. W. K. and Woodruff, M. F. A. (1966).

Strong, L. C. and L. D. Francis. (1940). Differences in hemoglobin values in the blood of breeder female mice: a comparison between cancer susceptible and cancer resistant strains. Amer. J. Canc., 38,399.

Strong, L. C. and L. B. Hardy. (1956). A new "Luxoid" mutant in mice. J. Hered., 47,277.

Strong, L. C. and W. F. Hollander. (1953). Two non-allelic mutants resembling "W" in the house mouse. J. Hered., 44,41.

Taylor, A. (1945). Changes in hemoglobin concentration, total haemoglobin and blood volume associated with tumor growth. U. Texas Publ. No. 4507,95. Cited by Bernstein, S. E. The Biology of the Laboratory Mouse, Chapt. 16. 2nd Ed., Ed. E. L. Green; McGraw Hill Book Co., New York.

Taylor, J., D. Stiven and E. W. Reid. (1935). Experimental and idiopathic siderosis in cats. J. Path. Bact., 41,397.

Taylor, M. R. H. and P. B. B. Gatenby. (1966). Iron absorption in relation to transferrin saturation and other factors. Brit. J. Haemat., 12,747.

Tchakhotine, S. (1938). Hérédité du taux leucocytaire du Sang chez la Souris. C. R. Acad. Sci. (Paris), 206,533.

Teplitz, R. L and E. Beutler. (1966). Mosaicism, chimerism and sex-chromosome inactivation. Blood, 27,258.

Thoms, G. (1951). Das histologische verhalten von Milz, Leber und Knochenmark bei erblichen Anaemie röntgenmutierter Mäuse. Wiss. Z. der Martin-Luther Universitat., 4,13.

Thompson, M W., E. A. McCulloch, L. Siminovitch and J. E. Till. (1966a). The cellular basis for the defect in hemopoiesis in mice with Hertwig's anemia. Genetics, 54,366.

Thompson, M. W., E. A. McCulloch, L. Siminovitch and J. E. Till. (1966b). The cellular basis for the defect in haemopoiesis in flex-tailed mice. I. The nature and persistence of the defect. Brit. J. Haemat., 12,152.

Thompson, M. W., E. S. Russell and E. C. McFarland. (1963). Response of polycythemic WW^{v} anemic mice to erythropoietin. Proc. XIth Int. Congr. Genet., 1,185.

Tomsett, S. L. (1940). Factors influencing the absorption of iron and copper from the alimentary tract. Biochem. J., 34,961.

Tönz, O., S. Weiss, H. W. Strahm and E Rossi. (1965). Iron absorption in cystic fibrosis. Lancet, 2,1096.

Turnberg, L. A. (1966). The absorption of iron after partial gastrectomy. Quart. J. Med., 35,107.

Turnbull, A., F. Cleton and C. A. Finch. (1962). Iron absorption. IV. The absorption of hemoglobin iron. J. clin. Invest., 41,1897.

Trujillo, J. M., B. Walden, P. O'Neil and H. B. Anstall. (1965). Sex-linkage of glucose-6-phosphate dehydrogenase in the horse and donkey. Science, 148,1603.

Ultman, J. E. and C. S. Gordon. (1965). Life span and sites of sequestration of normal erythrocytes in normal and splenectomized mice and rats. Acta haemat. (Basel), 33,118.

Underwood, E. J. (1938). A comparison of ferrous and ferric iron in the nutrition of the rat. J. Nutr., 16,299.

Valentine, W. N. and J. V. Neel. (1944). Hematologic and genetic study of the transmission of thalassemia. Arch. intern. Med., 74,185.

Van Putten, L. M. (1958). The life span of red cells in the rat and the mouse as determined by labelling with DFP^{32} in vivo. Blood, 13,789.

Ventkatachalam, P. S., I. Brading, E. P. George and R. J. Walsh. (1956). An experiment in rats to determine whether iron is absorbed only in the ferrous state. Austral. J. exp. Biol., 34,389.

Von Ehrenstein, G. (1958). The life span of the erythrocytes of normal and of tumour-bearing mice as determined by glycine-2-^{14}C. Acta physiol. scandinav., 44,80.

Wack, J. P. and J P. Wyatt. (1959). Studies on ferrodynamics. I. Gastrointestinal absorption of ^{59}Fe in the rat under differing dietary states. Arch. Path., 67,237.

Wakisaka, G., S. Tomita, S. Kariyone, Y. Takahashi, M. Yamaba and Y. Takagi. (1965). Kinetics of iron absorption in iron deficiency anemia. Israel J. med. Sci., 1,736.

Waxman, S., P. Pratt, J. Cuttner and V. Herbert. (1966). Evidence suggesting facilitated absorption in man of organic (and inorganic) iron by a substance present in depepsinized, neutralized normal human gastric juice and in hog intrinsic factor concentrates. Blood, 28,1005.

Weatherall, D. J. (1965). The Thalassaemia Syndromes, Blackwell, Oxford.

Weatherall, D. J. (1967). The Thalassemias. In Progress in medical genetics, Vol V. Ed. A. G. Steinberg and A. G. Bearn, Grune & Stratton, New York & London.

Weintraub, L. R., M. E. Conrad and W. H. Crosby. (1964). The significance of iron turnover in the control of iron absorption. Blood, 24,19.

Weintraub, L. R., M. E Conrad and W. H. Crosby. (1965). Regulation of the intestinal absorption of iron by the rate of erythropoiesis. Brit. J. Haemat., 11,432.

Weir, J. A. and G. Schlager. (1962a). Selection for total leucocyte count in the house mouse. Genetics, 47,993.

Weir, J. A. and G. Schlager. (1962b). Selection for leucocyte count in the house mouse and some physiological effects. Genetics, 47,1199.

Wheby, M. S. (1966). Regulation of iron absorption. Gastroenterology, 50,888.

Wheby, M. S. and W. H. Crosby. (1963). The gastrointestinal tract and iron absorption. Blood, 22,416.

Wheby, M. S., L. G. Jones and W. H. Crosby. (1964). Studies on iron absorption. Intestinal regulatory mechanisms. J. clin. Invest., 43, 1433.

Whipple, G. H. and W. L. Bradford. (1932). Racial or familial anemia of children associated with fundamental disturbances of bone and pigment metabolism. (Cooley - von Jaksch.) Amer. J. Dis. Child., 44,336.

Whipple, G. H. and W. L. Bradford. (1936). Mediterranean disease - Thalassemia (Erythroblastic anemia of Cooley). Associated pigment abnormalities simulating hemochromatosis. J. Pediat. 9,279.

Whitehead, J. S. W. and R. M. Bannerman. (1964). Absorption of iron by gastrectomized rats. Gut, 5,38.

Wiadrowski, M. and D. Metcalf. (1963). Erythrocyte osmotic fragility in AKR mice with lymphoid leukaemia. Nature (Lond.), 198,1103.

Wigley, R. D. and K. G. Couchman. (1966). Polyarteritis Nodosa-like disease in outbred mice. Nature, (Lond.), 211,319.

Williams, J. (1959). The effect of ascorbic acid on iron absorption in post-gastrectomy anaemia achlorhydria. Clin. Sci., 18,521.

Williams, R., H. S. Williams, P. J. Scheuer, C. S. Pitcher, E Loiseau and S. Sherlock. (1967). Iron absorption and siderosis in chronic liver disease. Quart. J. Med., 36,151.

Wish, L., J. Furth, and R H. Storey. (1950). Direct determinations of plasma, cell and organ-blood volumes in normal and hypervolemic mice. Proc. Soc. exper. Biol. Med., 74,644.

Wolf, N. S. and J. J. Trentin. (1968). Hemopoietic colony studies. V. Effect of hemopoietic organ stroma on differentiation of pluripotent stem cells. J. exper. Med., 127, 205.

Young, L. E. (1955). Observations on inheritance and heterogeneity of chronic spherocytosis. Trans. Ass. Amer. Phycns., 68,141.

This form should be completed and sent in along with the Thesis submitted by each candidate.

University of Glasgow.

DEGREE OF M.D.

TITLE OF THESIS (*In Block Letters*)

X-LINKED ANAEMIA OF MICE: AN HEREDITARY DISORDER OF IRON ABSORPTION.

Full Name (*Surname first*) PINKERTON, PETER, HARVEY

Address 36 Goodyear Cres., Willowdale, Ontario, Canada.

Year of Graduation as M.B. of Glasgow: 1958

Other registrable qualifications M.R.C.P.E., M.C.Path.

Medical appointments held Director of Laboratory Haematology, Sunnybrook Hospital, Toronto; Assistant Professor of Pathology, University of Toronto. Formerly Fellow in Medicine at the State University of New York at Buffalo.

State whether work for Thesis was done in General Practice or in Hospital, Clinic, Laboratory or other Institution, giving place of general practice or name and situation of Institution:

The work for this Thesis was performed in the Laboratory of the Medical Genetics Unit of the Department of Medicine, State University of New York at Buffalo and the Buffalo General Hospital.

VOLUME 2

TABLES

Table 1.1

HAEMOGLOBIN, HAEMATOCRIT AND MCHC IN ADULT MICE

Reference	Strain	Age	Sex	Hb (G/100ml)	Haematocrit %	MCHC%
Petri (1933)	White	---	M	9.3-15.3	30-49	---
			F	8.8-13.9	32-48	---
Francis and Strong (1938)	CBA	40-480 days	F (virgin)	13.9-17.4	---	---
		120-840 days	F (breeder)	13.0-16.3	---	---
Goulden and Warren (1944)	CBA	250-900 days	M	7.7-18.8	---	---
	CBA	250-900 days	F	12.8-19.3	---	---
	R111	100-550 days	M	7.2-15.6	---	---
	R111	100-600 days	F	9.7-23.9	---	---
Endicott and Gump (1947)*	C57Br	2 mo.	F	18.1±0.55	54.4±2.8	---
	CFW	2 mo.	F	17±1.32	56±5.1	---

Table 1.1 continued

Reference	Strain	Age	Sex	Hb (G/100 ml)	Haematocrit %	MCH%
Russell et al (1951)	A/Jax	2-3 mo.	M and F	12.9±0.2	42.5±0.4	30
	A/He Jax			12.7±0.2	42.5±0.5	30
	AKR/Jax			13.9±0.2	45.6±1	30
	BALB/cAn Jax			14.5±0.2	46.5±0.8	31
	BALB/c Jax			15±0.2	48±0.7	31
	CBA/Jax			13.5±0.2	45±1.3	31
	C3H/Jax			12.2±0.4	39.5±0.7	31
	C3H/Sc Jax			13.2±0.3	43±1	30
	C57B1/6p Jax			13±0.3	43.3±0.8	30
	C57B1/6 Jax			13.3±0.2	44±0.4	30
	C57Br/cd Jax			14.6±0.2	47.4±0.9	29
	C57L/He Jax			14.9±0.2	50.6±0.4	29
	DBA/1Jax			13.2±0.2	43.8±0.6	30
	DBA/Wa Jax			12.5±0.2	43±0.6	29

Table 1.1 continued

Reference	Strain	Age	Sex	Hb (G/100 ml)	Haematocrit %	MCH %
Russell et al (1951) cont.	DBA/2 Jax			12.7±0.1	42.6±0.5	30
	I/Jax			13.5±0.1	46.8±0.7	29
	R111/Jax			13.7±0.2	44.5±0.6	31
	ST/Jax			12.1±0.2	44.1±1.1	31
Kienle and Strong (1959)	C57	6-16 weeks	M and F	14.2-15	---	---
	pBr			13.8-15.5	---	---
	F			13.1-14.5	---	---
	A			15.7-16.3	---	---
	C3H			14.7-15.1	---	---
Kuharcik and Forsthoefel (1963)	DP	Adult	M and F	15.4±0.3	53.5±0.4	---
	C57Bl/10			16±0.2	51.5±0.8	---

Table 1.1 continued

Reference	Strain	Age	Sex	Hb (G/100 ml)	Haematocrit %	MCH %
Ewing and Tauber (1964)	C57Bl	4-24 mo.	M	13.6±0.31- 16.2±0.22	42.2±0.68- 48.1±0.53	32.8±0.23- 33.7±0.22
Penny (1967)*	F_1 C57Bl/6 x A_2G		M	14.79±1.00	42.5±4.0	35.2±2.97

Values given as ranges or as means ± 1 standard error except references marked with an asterisk where means ± 1 standard deviation are given.

Table 1.2

RED CELL COUNT, MEAN CELL VOLUME AND MEAN CELL HAEMOGLOBIN IN ADULT MICE

Reference	Strain	Age	Sex	RBC x 10^6/cu mm.	MCV cu μ	MCH μμg.
Kamenoff (1937)	Bagg-Albino	6 weeks to 1 year	M	8.58-11.89	---	---
			F	7.46-10.66	---	---
	C58		M	9.01-10.11	---	---
			F	6.69-11.66	---	---
	Storrs-Little		M	8.63-11.61	---	---
			F	6.7-11.19	---	---
	d. br.		M	8.61-11.51	---	---
			F	7.6-10.45	---	---
Gowen and Calhoun (1943)	Bagg-Albino	10 to 100 days	M and F	6.95	---	---
	Silver			8.78	---	---
	Ervin			9.37	---	---
	Swiss			7.43	---	---
	Rockefeller Inst.			8.14	---	---
	selected			9.32	---	---

Table 1.2 continued

Reference	Strain	Age	Sex	RBC x 10^6/cu mm.	MCV cu μ	MCH μμg.
Endicott and Gump (1947)*	C57Br	2 mo.	F	11.09±1.02	---	---
	CFW		F	11.06±1.24	---	---
Russell et al (1951)	A/Jax	2-3 mo.	M and F	9.42±0.28	45.1±1.4	---
	A/He Jax			9.48±0.18	44.8±1	---
	AKR/Jax			9.38±0.24	48.5±1.6	---
	BALB/cAn Jax			10.14±0.15	45.9±1.1	---
	BALB/c Jax			10.51±0.16	45.7±1	---
	CBA/Jax			10.04±0.27	44.8±1.8	---
	C3H/Jax			8.79±0.24	44.9±1.4	---
	C3H/Sc Jax			9.63±0.26	44.7±1.6	---
	C57Bl/6p Jax			9.7±0.15	44.7±1	---
	C57Bl/6 Jax			9.66±0.09	45.5±0.6	---
	C57Br/cd Jax			10.54±0.17	47.4±0.9	---
	C57L/He Jax			9.82±0.20	51.5±1.1	---
	DBA/1 Jax			10.52±0.27	41.6±1.2	---

Table 1.2 continued

Reference	Strain	Age	Sex	RBC x 10^6/cu mm.	MCV cμ μ	MCH μμg.
Russell et al (1951) continued	DBA/Wa Jax			9.93±0.27	43.3±1.1	---
	DBA/2 Jax			10.3±0.25	41.4±1.1	---
	1/Jax			10.27±0.27	45.6±1.5	---
	RIII/Jax			9.63±0.25	46.2±1.3	---
	ST/Jax			9.88±0.19	44.6±1.4	---
Kienle and Strong (1959)	C57	6-16 weeks	M and F	9.2-10	---	---
	pBr			8.45-9.93	---	---
	F			8.95-9.35	---	---
	A			9.35-10.06	---	---
	C3H			8.77-9.45	---	---
Kuharcik and Forsthoefel (1963)	DP	"Adult"	M and F	10.9±0.22	50±1.1	---
	C57BI/10			10.48±0.19	49.2±1.2	---

Table 1.2 continued

Reference	Strain	Age	Sex	RBC x 10^6/cu mm.	MCV cu μ	MCH $\mu\mu$g.
Ewing and Tauber (1964)	C57B1	4-24 mo.	M	9.08±0.19- 9.97±0.14	45.6±0.59- 48.5±0.5	15±0.26- 16.3±0.18
Penny, R. H. C. (1967)*	F_1 C57B1/6 xA_2G		M	8.61±0.86	49.10±5.96	---

Values given as ranges or as means ± 1 standard error except references marked with an asterisk where means ±1 standard deviation are given.

Table 1.3

TOTAL[1] AND DIFFERENTIAL[2] WHITE CELL COUNTS IN ADULT MICE

Reference	Strain	Total White Cell Count	Lympho.	Neutro.	Mono.	Eos.
Fekete (1941)	C57Br	2.8-9.2	71%	14%	12%	3%
	C57Bl	4-5.6	66%	22%	11%	0.6%
	Bagg Albino	2.5-6.7	63%	23%	13%	0.3%
	dba	3.3-6.7	64%	25%	11%	0.4%
	C3H	2-6.3	63%	23%	14%	0.2%
	Leaden	1.7-10.5	80%	9%	8%	3%
Gowen and Calhoun (1943)	Bagg Albino	11.1	65.1%	18.2%	1.2%	2.1%
	Silver	11.6	74.1%	14.2%	1.3%	0.9%
	Ervin	14.6	69.4%	14%	2.6%	3.8%
	Swiss	16.1	75.3%	9.4%	1.2%	1.9%
	Rockefeller	22.5	67.3%	13.6%	0.7%	2.1%
	selected	19.6	72.8%	15%	0.7%	2%

Table 1.3 continued

Reference	Strain	Total White Cell Count	Lympho.	Neutro.		Mono.	Eos.
Endicott and Gump (1947)*	C57Br	19.8±5.6	17.3±5.3	2.1±1.6		---	0.33±0.26
	CFW	14.5±6.2	12.3±5.5	2.0±1.8		---	0.20±0.20
Russell et al (1951)†				Female	Male		
	A/Jax	8.7±1.4	---	10.7%	14.5%	---	---
	A/He Jax	6.1±0.7	---	19.8%	37.2%	---	---
	AKR/Jax	6.8±0.7	---	21.6%	23.8%	---	---
	BALB/cAn Jax	8.7±1.0	---	15.4%	29.6%	---	---
	BALB/c Jax	8.5±1.0	---	14.9%	15.3%	---	---
	CBA/Jax	6.4±0.3	---	26.8%	24.2%	---	---
	C3H/Jax	7.3±0.8	---	21.4%	27.5%	---	---
	C3H/Sc Jax	5.1±0.3	---	19%	22%	---	---
	C57Bl/6p Jax	10.6±0.6	---	12.1%	15.3%	---	---
	C57Bl/6 Jax	11.4±1.0	---	8.2%	10.4%	---	---
	C57Br/cd Jax	9.4±0.7	---	10.6%	11%	---	---
	C57L/He Jax	10.8±1.1	---	8.5%	6.7%	---	---
	DBA/1 Jax	8.6±1.6	---	16.5%	20.4%	---	---

Table 1.3 continued

Reference	Strain	Total White Cell Count	Lympho.	Neutro.		Mono.	Eos.
				Female	Male		
Russell et al (1951) cont.	DBA/Wa Jax	8.3±1.0	---	19.6%	17.2%	---	---
	DBA/2 Jax	9.3±0.3	---	16.7%	18.1%	---	---
	I/Jax	11.6±1.4	---	13.2%	18.1%	---	---
	R111/Jax	5.9±0.6	---	26.2%	18.6%	---	---
	ST/Jax	7.7±1.2	---	14.9%	19.4%	---	---
Penny (1967)*	F_1 $C57Bl/6$ x A_2G	4.51±2.09	77.6%	19.8%		1.7%	0.9%

[1]Total white cell count as thousands/cu mm; mean ± 1 standard deviation (indicated by asterisk), or ± 1 standard error (indicated thus †).

[2]Differential white cell count as percentage of the total or as mean absolute number in thousands/cu mm ± 1 standard deviation.

Table 1.4

PLATELET COUNTS IN NORMAL MICE

Reference	Strain	Age	Platelet Count per cu mm	Remarks
Petri (1933)	"White"	-	359,000-1,536,000	No age or sex differences found
Fekete (1941)	-	-	157,000-620,000	-
Copley and Robb (1942a)	-	-	248,120-338,800	-
Copley and Robb (1942b)	-	-	246,000-339,000	-
Jacobson (1944)	"White"	6-8 weeks	940,000-1,040,000	-
Platt and Zeller (1951)	A	-	389,000	Swiss and C57 stated to give similar values
Odell and McDonald (1960)	BDF_1	11-22 weeks	1,523,000±218,000	Mean ± 1 standard deviation
Andrew (1965)	-	-	157,000-412,620	-

Table 1.5

RED CELL SURVIVAL IN THE MOUSE

Reference	Method	Strain	Life Span in Days	Remarks
Burwell, Brickley and Finch (1953)	^{55}Fe	-	45-50 days	Random cell destruction
Von Ehrenstein (1958)	glycine-2- ^{14}C	TE C34	40-46 days (mean 42) mean 40 days	
Van Putten (1958)	DF ^{32}P	-	40.7±1.9 days	
Smith and Tohá	^{51}Cr	101 x C3H hydrids	$T_{\frac{1}{2}}$ ^{51}Cr 20 days	
Oliner, Schwartz and Dameshek (1961)	^{51}Cr	LAF	$T_{\frac{1}{2}}$ ^{51}Cr 14 days	
Goodman and Smith (1961)	^{51}Cr	Several strains	$T_{\frac{1}{2}}$ ^{51}Cr 15-20 days Disappearance time 50-55 days	Little different appreciable between strains
Möller (1965)	^{51}Cr	A strain	$T_{\frac{1}{2}}$ ^{51}Cr 8.6 days	
Ullman and Gordon (1965)	^{51}Cr	DBA/2	$T_{\frac{1}{2}}$ ^{51}Cr 20.2 days	
Lindsey, Donaldson and Woodruff (1966)	^{51}Cr	C57 Bl CBA	$T_{\frac{1}{2}}$ ^{51}Cr 10 days $T_{\frac{1}{2}}$ ^{51}Cr 14.1 days	
Brodsky et al (1966)	^{51}Cr	Swiss-Webster	$T_{\frac{1}{2}}$ ^{51}Cr 12-15 days	

Table 1.6

BLOOD FINDINGS IN EMBRYONIC MICE

Reference	Strain	Gestational Day	Hb G/100 ml	Haematocrit %	RBC x 10^6/cu mm	Mean cell volume cu μ
de Aberle (1927a)	---	16	---	---	2.6	---
Kamenoff (1935)	---	14	---	---	1.87	---
	---	15	---	---	2.20	---
	---	16	---	---	3.1	---
	---	17	---	---	3.3	---
	---	18	---	---	3.7	---
Russell and Fondal (1951)	C57 Bl/6	16	---	---	2.64 0.08	---
Kuharcik and Forsthoefel (1963)	DP	18--20	12.1±0.4	42.2±2.1	3.45±0.22	123±4.9
			10.6±0.5	43.7±1.8	3.87±0.13	115.4±6.6

Values given as means or means ± standard error.

Table 1.7

BLOOD FINDINGS IN YOUNG MICE

Reference	Strain	Age (days)	Hb (G/100 ml)	Haematocrit	RBC x 10^6/cu mm	MCHC %	MCV cu μ	MCH $\mu\mu$g	Reticulocytes %
Grüneberg (1939)	---	0-1	12.1	---	3.69	---	---	---	55.7
		3-10	9.6	---	4.39	---	---	---	20.8
		11-23	9.8	---	5.95	---	---	---	18.4
		33-88	13.7	---	9.03	---	---	---	5.5
	---	5	9.5	45	4.75	19.6	94.6	---	---
		10	10.4	42.5	5.70	22.7	74.6	---	---
		15	9.5	38.5	6.15	22.9	62.6	---	---
		31	12.7	53.5	9.38	22.2	57.0	---	---
Grüneberg (1942a)	CBA x Albino	0-1	10.26	38.5	3.7	26	103	27.6	97.7
		7	8.83	32.8	4.5	27	72	19.6	54.9
		13-14	9.98	37.4	6.2	27	61	16.2	23.8
		20-21	11.04	40.5	7.3	27	56	15.2	9.5
		21-28	13.36	49.4	8.5	27	59	15.8	6.8

Table 1.7 continued

Reference	Strain	Age (days)	Hb (G/100 ml)	Haematocrit	RBC x 10^6/cu mm	MCHC %	MCV cu μ	MCH μμg	Reticulocytes %
Kienle and Strong (1959)	C57	1	12.9	---	4.78	---	---	---	---
		10	12.2	---	5.13	---	---	---	---
		21	13.2	---	8.3	---	---	---	---
		35	14.6	---	9.03	---	---	---	---
	C3H	1	15.4	---	4.58	---	---	---	---
		10	12.7	---	5.15	---	---	---	---
		21	13.0	---	7.20	---	---	---	---
		35	13.9	---	8.39	---	---	---	---
Grewal (1962)	Mixed	0-1	13.7	44.1	4.00	31.2	109.7	34.1	83.0
		7	10.4	34.9	4.6	29.7	76.8	22.8	46.7
		14	10.0	34.2	5.6	29.1	62.0	18.1	31.4
		21	12.4	41.4	7.1	29.9	57.9	17.1	13.6
		28	12.7	41.0	6.5	31.0	62.8	19.5	11.2

Table 1.7 continued

Reference	Strain	Age (days)	Hb (G/100 ml)	Haematocrit	RBC x 10^6/cu mm	MCHC %	MCV cu μ	MCH $\mu\mu$g	Reticulocytes %
Kuharcik and Forsthoefel (1963)	DP	Newborn	12.7±0.4	40.1±0.7	3.96±0.09	---	97.4±3.6	---	---
	C57Bl 10	Newborn	13.1±1.1	42±1.5	4.4±0.11	---	87.6±2.7	---	---

Values given as means or mean ±1 standard error.

Table 1.8

THE MYELOGRAM OF THE NORMAL MOUSE

Cell Type	C57 Brown Strain	CFW Strain
Segmented neutrophils and metamyelocytes	20.7	24.4
Neutrophil myelocytes and premyelocytes	12.4	15.9
Eosinophils	10	7.5
Total granulocytes	42.9	47.8
Normoblasts	21.9	22.6
Pronormoblasts	8.6	4.6
Total erythropoietic cells	30.4	27.1
Blast cells	0.3	0.8
Lymphocytes	24.2	22.7
Miscellaneous cells	1.9	1.6

Mean percentages of each cell type in 20 C57 Br. and 40 CFW mice

After Endicott and Gump (1947)

Table 2.1

HEREDITARY ANAEMIAS OF MICE

Usual name	Gene symbol	Type of anaemia	Mechanism
Anaemias of dominant spotting	W	Macrocytic, hypoplastic	Stem cell defect
Steel	Sl	Macrocytic, hypoplastic	Defective erythropoietic stimulus and blood loss
Hertwig's anaemia	an	Macrocytic	? Stem cell defect
Flexed-tail	f	Hypochromic	ALA dehydratase deficiency
Sex-linked anaemia	sla	Hypochromic, microcytic	-
Microcytic	mk	Hypochromic, microcytic	Unknown
Spherocytosis	sp	Haemolytic	Intrinsic erythrocyte defect
Spherocytosis	sph	Haemolytic	Intrinsic erythrocyte defect
Jaundiced	ja	Haemolytic	Intrinsic erythrocyte defect
Haemolytic anaemia	ha	Haemolytic	Intrinsic erythrocyte defect
NZB/BL	-	Haemolytic	Autoimmunity
Luxoid	lst	Haemorrhagic	Blood loss
Hertwig's foetal anaemia	-	-	Unknown
Tail short	Ts	-	Unknown
Diminutive	dm	Macrocytic	Unknown

Table 2.2

PHENOTYPIC EFFECTS OF MUTANTS AT THE W-LOCUS

Reference Number	Genotype	Viability	Anaemia	Gonads	Pigment Intensity	Spotting
Russell and Fondal (1951)	$\underline{w/w}$	Normal	None	Normal	Full	None
	$\underline{W/w}$	Normal	None	Normal	Full	Belly spot
	$\underline{W/W}$	90% die in 7 days	Severe	Deficient	White	---
	$\underline{W^v/w}$	Normal	Slight reduction in red cell count and macrocytosis	Normal	Diluted	Belly spot
	$\underline{W^v/W^v}$	Normal	Less than $\underline{W/W}$ or $\underline{W^v/W}$	Deficient	White	---
	$\underline{W^v/W}$	50% die before 21 days age	Intermediate between $\underline{W^v/W^v}$ and $\underline{W/W}$	Deficient	White	---
Russell, Lawson and Schabtach (1957)	$\underline{W^j/w}$	Normal	Normal	Normal	Full	More extensive than $\underline{W/w}$ or $\underline{W^v/w}$ including dorsum
	$\underline{W^j/W^j}$	0-18 days	Similar to $\underline{W/W}$	Deficient	White	---

Table 2.3

FINDINGS IN W-ANAEMIC EMBRYONIC AND NEWBORN MICE

Reference Number	Genetic Background	Genotype	Mean red cell count in 16 day embryo ($\times 10^6$)	Mean red cell count in newborn ($\times 10^6$)
de Aberle (1927b)	Danforth stock	ww	2.6 (17)	4.7 (35)
		Ww	0.5 (19)	0.7 (28)
Grüneberg (1939)	Crosses with CBA	ww & W^vw	---	3.7 (8)
		W^vW^v	---	1.9 (8)
		WW^v	---	1.4 (4)
Russell and Fondal (1951)	C57 B1/6	ww	2.64 (10)	4.87 (10)
		W^vw	---	3.82 (16)
		W^vW^v	1.29 (7)	2.18 (10)
		W^vW	0.98 (7)	1.37 (10)
		WW	0.50 (8)	0.84 (10)

Figures in parentheses indicate numbers of mice in each group.

Table 2.4

FINDINGS IN 28 DAY OLD W-ANAEMIC MICE

(after Russell and Fondal, 1951)

Genotype	Red Cell Count x 10^6/cu mm.	Mean Cell Volume cu μ
$\underline{ww}$	8.44 ± 0.23 (13)	49.4 ± 1.3 (13)
$\underline{Ww}$	8.96 ± 0.28 (13)	47.4 ± 1.1 (13)
$\underline{W^vw}$	7.43 ± 0.25 (11)	53.5 ± 2.1 (11)
$\underline{W^vW^v}$	5.13 ± 0.13 (10)	61.7 ± 1.5 (10)
$\underline{W^vW}$	4.88 ± 0.25 (10)	65.6 ± 2.1 (10)

Figures in parentheses indicate the number of mice in each group.

Means ± 1 standard error of the mean.

Table 2.5

HAEMATOLOGICAL FINDINGS IN YOUNG AND ADULT

W^V/W^V MICE

(after Grüneberg, 1939)

Age (days)	Genotype	Red cell count x 10^6/cu mm.	Haemoglobin (per cent Haldane*)	Haematocrit %	C.I.	MCV cu μ	MCHC %
31	Normal	9.38	86	53.5	0.459	57	22.18
31	W^V/W^V	5.04	64	41.2	0.635	81.6	21.45
171	Normal	10.84	105	55.75	0.484	51.4	25.99
171	W^V/W^V	6.7	87	46	0.649	68.8	26.1

*100% Haldane = 13.8 G/100 ml.

Table 2.6

BLOOD FINDINGS IN DEER MOUSE SPHEROCYTOSIS

(after Anderson et al., 1960)

	Spherocytosis (sp/sp)	Normal (+/+)
Haemoglobin	13.1G/100 ml	13.6G/100 ml
Haematocrit	43.4%	48.3%
M.C.H.C.	30.6%	28.1%
Red cell count	11.1 m/cu mm	13.2 m/cu mm
M.C.V.	40.2 cu μ	36.8 cu μ
M.C.H.	12.1 μμg	10.6 μμg
Reticulocytes	11% 1.6 m/cu mm	1% 0.2 m/cu mm
Median corpuscular fragility	0.594% NaCl	0.427% NaCl
Red cell survival $T\frac{1}{2}$ ^{51}Cr	4.1 days	8.8 days

Table 2.7

A COMPARISON OF HUMAN AND DEER MOUSE SPHEROCYTOSIS

(after Anderson et al., (1960)

	Man	Deer Mouse
Neonatal Jaundice	Variable	Usually present
Adult Jaundice	Mild or absent	Absent
Anaemia	Mild or absent	Usually absent
Red cell appearance	Spherocytic	Spherocytic
Osmotic fragility	Increased	Increased
Red cell survival of sp/sp cells in normals and sp/sp animals	Decreased	Decreased
Red cell survival of normal cells in spherocytics	Normal	Normal
Bone marrow	Hyperplastic	Hyperplastic
Gallstones	High incidence	High incidence
Splenomegaly	Present	Present
Effect of splenectomy	Red cell survival normal	Red cell survival normal
Inheritance	Usually autosomal dominant; occasionally recessive?	Autosomal recessive

Table 2.8

A COMPARISON OF AUTO-IMMUNE HAEMOLYTIC ANAEMIA
OF NZB/BL MICE AND HUMAN SYSTEMIC LUPUS ERYTHEMATOSUS

Feature	NZB/Bl Mice		Human S.L.E. and haemolytic disease
Coombs test	Positive		Positive
Antibody	Incomplete 7S -globulin		Incomplete 7S -globulin
L.E. test	Positive in up to 50%		Positive
A.N.F.	Present in up to 50%		Present
Sex incidence	Equal: earlier onset in female		Female > male
Anaemia	Usually present		Usually present
Reticulocytosis	Present		Present
White cell and platelet count	Normal		Usually low
Splenomegaly	Usually present		Usually present
Renal lesions	Similar in NZB/Bl	and	human S.L.E.
Joint and skin lesions	Absent		Present
Response to splenectomy	Poor but consistent		Variable
Response to steroids	Remission		Frequent remission

Table 3.1

HAEMATOLOGICAL INVESTIGATIONS IN NORMAL MICE AND MICE WITH X-LINKED ANAEMIA

after Grewal, (1962)

a) Normal mice

Age (days)	Hb G/100 ml	P.C.V. %	Red cell count x 10^6/cu.mm.	M.C.H.C. %	M.C.V. cu μ	M.C.H. $\mu\mu$g
0-1	13.7	44.1	4.0	31.2	109.7	34.1
7	10.4	34.9	4.6	29.7	76.8	22.8
14	10.0	34.2	5.6	29.1	62.0	18.1
21	12.4	41.4	7.1	29.9	57.9	17.1
28	12.7	41.0	6.5	31.0	62.8	19.5
72	14.3	46.0	8.2	31.0	56.7	17.6
240	13.1	39.8	7.7	33.0	51.6	17.0

Table 3.1 continued

b) Anaemic mice

Age (days)	Hb G/100 ml	P.C.V. %	Red cell count x 10^6/cu.mm.	M.C.H.C. %	M.C.V. cu μ	M.C.H. μμg
0-1	9.4	33.9	3.1	27.8	109.8	30.6
7	7.0	23.8	3.2	29.4	68.5	20.1
14	7.2	26.9	4.4	27.0	61.8	16.6
21	9.5	32.9	5.4	28.9	60.0	17.3
28	8.1	26.8	5.0	30.1	53.0	16.1
72	8.2	30.4	6.2	27.1	49.7	13.4
240	10.6	32.4	6.6	32.8	49.2	16.1

Table 3.2

NORMAL MICE AND MICE WITH X-LINKED ANAEMIA COMPARED IN RESPECT OF THE HAEMOGLOBIN CONCENTRATION, PACKED CELL VOLUME, RED CELL COUNT AND RED CELL INDICES - ANAEMIC/NORMAL RATIOS

(after Grewal, 1962)

Age (days)	Hb	P.C.V.	Red cell count	M.C.H.C.	M.C.V.	M.C.H.
0-1	0.69	0.77	0.78	0.89	1.00	0.90
7	0.67	0.68	0.70	0.99	0.89	0.88
14	0.72	0.79	0.79	0.93	1.00	0.92
21	0.77	0.76	0.80	0.97	1.04	1.01
28	0.64	0.77	0.65	0.97	0.84	0.83
72	0.57	0.76	0.66	0.87	0.88	0.76
240	0.81	0.86	0.81	0.99	0.95	0.95

Table 4.1

THE CONSTITUTION OF ROCKLAND RAT CHOW

Ingredient	Stated content per cent
Protein	24.27
Fat	4.15
Fibre	4.86
Carbohydrate	56.23
Iron	0.019
Zinc	0.002
Copper	0.0012
Cobalt	0.000035
Manganese	0.0084
Other minerals	5.47
Pyridoxine	209-275 µg/100 G.
Vitamin C	3.6 mg/100 G.
Amino-acids	10.73
Calories	1193/lb.

Table 4.2

REAPPEARANCE OF RADIO-IRON IN THE PERIPHERAL BLOOD 5 DAYS AFTER ADMINISTRATION, CALCULATED FROM BLOOD VOLUME DETERMINATIONS WITH ^{51}Cr LABELLED RED CELLS AND RADIOIODINATED SERUM ALBUMIN (RISA)

Mouse number	Reappearance of ^{59}Fe in the peripheral blood (per cent) Blood volume determined by	
	^{51}Cr-labelled red cells	R I S A
1	74.5	122.4
2	66.2	108.6
3	64.4	105.8
4	66.0	108.4
5	63.3	104.2

Table 5.1

CHANGES IN M.C.H.C. IN RELATION TO AGE

Age (days)	sla/-	sla/sla	sla/	+/-
21-30	22.8 ± 0.46 (6)	23.3 ± 1.7 (4)	31 (1)	--
31-40	23.4 ± 1.0 (11)	24.4 ± 0.41 (14)	28.9 ± 0.63 (10)	29.7 ± 0.71 (3)
41-50	23 ± 0.91 (11)	24 ± 0.95 (5)	30.7 ± 1.9 (3)	29.8 ± 0.55 (4)
51-60	23 ± 0.83 (9)	25.8 ± 0.83 (6)	31.3 ± 0.85 (11)	31.8 ± 1 (5)
61-70	24.5 ± 1.3 (8)	26.1 ± 0.42 (11)	31.5 ± 0.85 (11)	29 ± 0.47 (3)
71-80	24.8 ± 0.97 (13)	25.6 ± 0.71 (18)	31.6 ± 0.83 (9)	30.9 ± 0.66 (12)
81-90	26 ± 1.4 (5)	25.6 ± 1.8 (5)	31.6 ± 0.81 (7)	29.7 ± 0.68 (6)
91-100	23.6 ± 1.1 (8)	26 ± 1.9 (4)	35 (1)	--
101-150	26.4 ± 0.66 (25)	28 ± 0.54 (29)	32.3 ± 0.43 (24)	30.5 ± 0.64 (11)
151-200	28.7 ± 0.58 (23)	27.6 ± 1.0 (12)	31.1 ± 0.48 (11)	30.9 ± 0.65 (7)
201-250	27.7 ± 0.71 (23)	31.8 ± 0.76 (11)	30.3 ± 0.52 (19)	30.6 ± 0.45 (18)
251-300	29.6 ± 0.78 (20)	29 ± 0.55 (24)	30.7 ± 0.58 (7)	30.2 ± 0.57 (9)
301-400	29.0 ± 0.69 (23)	28.6 ± 0.73 (17)	31.7 ± 0.44 (13)	31 ± 0.54 (11)

Means ± 1 standard error of the mean. Numbers of estimations are given in parenthesis.

Table 5.2

RED CELL COUNT IN MILLIONS/CU. MM.

Age (days)	Genotype			Ratio of means- anaemic/normal
	sla/sla & sla/-	sla/+	+/-	
0-100	8.11 ± 0.34 (11)	10.46 ± 0.81 (2)	9.84 ± 0.38 (11)	0.82
101-200	9.10 ± 0.81 (14)	11.16 ± 0.46 (4)	11.14 ± 0.17 (22)	0.82
201-300	10.36 ± 0.94 (9)	8.96 (1)	10.29 ± 0.17 (12)	1.01
301-400	10.00 ± 0.38 (8)	9.96 ± 0.7 (5)	9.38 ± 0.34 (8)	1.07

Means ± 1 standard error of the mean. Numbers in each group are given in parenthesis.

Table 5.3

RED CELL SIZE DISTRIBUTION

(red cell diameters in microns)

Mouse number	Genotype	Age (days)	Per cent of red cells in each size category							Film Score
			<3u	3-3.9u	4-4.9u	5-5.9u	6-6.9u	7-7.9u	>8u	
507	s1a/-	32	2	6	10	57	24	1	-	11
517	s1a/-	79	1	12	14	31	34	8	-	14
511	s1a/-	104	-	3	17	58	20	2	-	10
105	s1a/-	185	1	15	13	61	20	-	-	10
147	s1a/-	255	-	4	4	63	18	1	-	13
71	s1a/s1a	44	3	10	13	34	35	5	-	13
74	s1a/s1a	65	2	10	12	36	35	5	-	15
521	s1a/s1a	79	-	3	15	51	30	1	-	15
74	s1a/s1a	87	1	7	18	41	40	3	-	15
96	s1a/s1a	150	3	12	17	20	27	18	3	14

Table 5.3 continued

Mouse number	Genotype	Age (days)	Per cent of red cells in each size category							Film score
			<3u	3-3.9u	4-4.9u	5-5.9u	6-6.9u	7-7.9u	>8u	
522	sla/+	31	-	-	3	35	53	9	-	3
510	sla/+	56	-	-	2	42	55	1	-	5
38	sla/+	59	-	-	3	28	60	9	-	2
91	sla/+	66	-	-	2	37	54	7	-	2
32	sla/+	151	-	-	1	25	65	9	-	3
31	+/-	66	-	-	-	38	59	3	-	1
35	+/-	79	-	-	-	34	62	4	-	1
151	+/-	80	-	-	-	38	60	2	-	1
153	+/-	80	-	-	1	33	64	2	-	1
171	+/-	169	-	-	-	33	65	2	-	2

All blood films examined were from mice of the original mixed stock.

Table 5.4

RETICULOCYTE COUNTS

Genotype	Reticulocyte counts				Total number examined
	0-5%	6-10%	11-15%	over 15%	
sla/- and sla/sla	5	7	1	7	69
sla/+	10	2	1	0	13
+/-	12	1	0	0	13

Figures give the number of individual reticulocyte counts in each percentage range.

Table 5.5

TOTAL AND DIFFERENTIAL WHITE CELL COUNTS, AND PLATELET COUNTS IN ANAEMIC AND NORMAL MICE

	Normal (10)	Anaemic (8)
White cell count	5,900 ± 1062/cu.mm.	4,600 ± 656/cu.mm.
Neutrophil polymorphs	14-50% (900-3500/cu.mm.)	12-60% (500-3000/cu.mm.)
Lymphocytes	45-85% (1200-12,000/cu.mm.)	35-85% (2000-4000/cu.mm.)
Monocytes	1-5% (50-600/cu.mm.)	2-5% (50-250/cu.mm.)
Eosmophils	0-3% (0-200/cu.mm.)	0-4% (0-200/cu.mm.)
Platelet count	270,000 ± 25,300*/cu.mm.	290,000 ± 34,400/cu.mm.

For total white cell and platelet counts, means and standard errors are given, for the differential count ranges only are given. Nucleated red cells were not seen.

* Nine animals were examined.

Table 6.1

SPLEEN WEIGHTS

	Anaemic males and females (*sla*/- and *sla*/*sla*)	Carrier females (*sla*/+)	Normal males (+/-)
Number examined	26	7	33
Spleen weight (mg)	204.2 ± 22.8	83.4 ± 18.9	75.2 ± 5.7
Spleen weight as % of body weight	0.86 ± 0.10	0.32 ± 0.06	0.27 ± 0.02

Means ± 1 standard error of the mean for adult mice. When the spleen weights of anaemic and normal mice are compared, the differences are highly significant ($p < 0.001$). When the spleen weights of heterozygous carrier and normal mice are compared, there is no significant difference ($p > 0.1$). These comments apply equally whether the spleen weights are expressed as percentages of total body weight or in absolute values.

Table 6.2

SPLEEN WEIGHTS

Mouse number	Stock	Genotype	Body Weight G.	Spleen Weight mg.	Spleen Weight % of Body Weight
5'	O.M.S.	+/-	12.2	48.3	0.40
7	O.M.S.	sla/-	41.7	146	0.35
11	O.M.S.	sla/-	35.1	91.6	0.26
12	O.M.S.	sla/sla	29.1	208	0.71
14	O.M.S.	sla/sla	30.0	380	1.27
17	O.M.S.	sla/-	39.1	107.5	0.27
23	O.M.S.	sla/-	28.5	314	1.10
26	O.M.S.	sla/-	30.7	58.5	0.19
27	O.M.S.	+/-	14.2	42	0.30
27"	O.M.S.	sla/-	17.4	115	0.66
38	O.M.S.	sla/-	31.0	80	0.26
43	O.M.S.	sla/-	13.9	160	1.15
46"	O.M.S.	sla/-	35.5	183	0.51
64	O.M.S.	sla/	28.8	196	0.68
65	O.M.S.	sla/-	23.0	344	1.49
66	O.M.S.	sla/sla	36.0	257	0.71
68	O.M.S.	sla/sla	18.0	105	0.58
73	O.M.S.	sla/-	23.8	197	0.83
79	O.M.S.	+/-	15.1	32	0.21
125	O.M.S.	sla/-	21.1	283	1.34

Table 6.2 (continued)

SPLEEN WEIGHTS

Mouse number	Stock	Genotype	Body Weight G.	Spleen Weight mg.	Spleen Weight % of Body Weight
193	O.M.S.	sla/-	22.6	132	0.59
194	O.M.S.	sla/sla	20.4	151	0.74
196	O.M.S.	sla/sla	19.3	115	0.59
101	C57B1	+/+	19.2	66	0.35
102	C57B1	+/+	19.9	62	0.31
103	C57B1	+/+	18.3	55	0.30
104	C57B1	+/+	21.3	79	0.37
105	C57B1	+/+	18.5	34	0.18
106	C57B1	+/+	19.0	64	0.34
107	C57B1	+/+	19.7	61	0.31
108	C57B1	+/+	19.9	59	0.29
203	F_1	+/-	37.9	104	0.27
209	F_1	+/-	30.0	91	0.30
211	F_1	+/-	30.7	76	0.25
231	F_1	+/-	34.4	72	0.21
235	F_1	+/-	38.7	82	0.21
237	F_1	+/-	39.2	87	0.22
239	F_1	+/-	39.0	81	0.21
241	F_1	+/-	36.6	77	0.21
243	F_1	+/-	40.9	63	0.15

Table 6.2 (continued)

SPLEEN WEIGHTS

Mouse number	Stock	Genotype	Body Weight G.	Spleen Weight mg.	Spleen Weight % of Body Weight
245	F_1	+/-	34.7	90	0.25
250	F_1	sla/+	27.2	78	0.29
252'	F_1	sla/+	29.6	80	0.27
254'	F_1	sla/+	27.1	84	0.31
256	F_1	sla/+	26.2	54	0.21
258	F_1	sla/+	28.5	62	0.22
269	F_1	+/-	39.8	48	0.12
277	F_1	+/-	39.4	81	0.21
287	F_1	+/-	30.2	67	0.22
289	F_1	+/-	34.9	79	0.23
299	F_1	+/-	36.6	82	0.23
303	F_1	+/-	39.0	85	0.22
305	F_1	+/-	38.0	84	0.22
307	F_1	+/-	32.0	62	0.19
309	F_1	+/-	35.0	68	0.19
351	F_1	+/-	29.5	233	0.79
457	F_2	sla/-	15.6	154	0.99
491	F_2	sla/-	22.5	461	2.05
499	F_2	sla/-	16.5	147	0.82
522	O.M.S.	sla/+	10.5	30	0.29

Table 6.2 (continued)

SPLEEN WEIGHTS

Mouse number	Stock	Genotype	Body Weight G.	Spleen Weight mg.	Spleen Weight % of Body Weight
729	F_2	sla/-	24.5	59	0.24
735	F_1	+/-	35.5	84	0.24
737	F_1	+/-	32.3	83	0.26
805	F_2	sla/-	24.8	438	1.77
809	F_2	sla/-	21.2	388	1.83
811	F_2	sla/-	22.3	232	1.54

Abbreviations: O.M.S. - original mixed stock.

C57Bl - C57 Black/6 Jax.

F_1 - hybrid of original mixed stock crossed with C57Bl/6J strain.

F_2 - F_1 backcrossed with C57Bl/6J strain.

Table 6.3

PROPORTION OF SPLENIC TISSUE OCCUPIED BY RED PULP IN RELATION TO SPLEEN SIZE

Mouse number	Genotype	Spleen weight (% body weight)	Red pulp as % of splenic tissue
7	sla/-	0.35	25 - 50
11	sla/-	0.26	25 - 50
12	sla/sla	0.71	51 - 75
14	sla/sla	1.27	over 75
17	sla/-	0.27	less than 25
23	sla/-	1.10	over 75
26	sla/-	0.19	25 - 50
64	sla/+	0.68	25 - 50
73	sla/-	0.83	51 - 75
231	+/-	0.21	25 - 50
250	sla/+	0.29	less than 25
252'	sla/+	0.27	25 - 50
254'	sla/+	0.31	25 - 50
256	sla/+	0.21	25 - 50
258	sla/+	0.22	25 - 50
269	+/-	0.12	25 - 50
277	+/-	0.21	25 - 50

Table 6.3 (continued)

PROPORTION OF SPLENIC TISSUE OCCUPIED BY RED PULP IN RELATION TO SPLEEN SIZE

Mouse number	Genotype	Spleen weight (% body weight)	Red pulp as % of splenic tissue
287	+/-	0.22	25 - 50
289	+/-	0.23	25 - 50
457	sla/-	0.99	over 75
491	sla/-	2.05	over 75
499	sla/-	0.82	51 - 75
522	sla/+	0.29	25 - 50
729	sla/-	0.24	51 - 75
735	+/-	0.24	less than 25
737	+/-	0.26	25 - 50
805	sla/-	1.77	over 75
809	sla/-	1.83	over 75
811	sla/-	1.04	over 75

Table 6.4

PROPORTION OF SPLENIC TISSUE OCCUPIED BY RED PULP IN ANAEMIC, CARRIER AND NORMAL MICE

Proportion of spleen consisting of red pulp	Genotype		
	Anaemic sla/-, sla/sla	Carrier sla/+	Normal +/-
Less than 25%	3%	23%	20%
25 - 50%	29%	69%	68%
51 - 75%	34%	8%	12%
over 75%	34%	-	-
Number examined	35	13	25

Table 6.5

SPLEEN IRON STORES

Genotype	No. of animals examined	No. of animals whose spleen iron score was				
		0	1	2	3	4
Anaemic males and females sla/-, sla/sla	35	27	8	0	0	0
Carrier females sla/+	12	0	3	3	0	6
Normal males	25	0	0	0	0	25

Table 6.6

SPLEEN IRON STORES IN RELATION TO AGE AND HAEMATOCRIT IN ANAEMIC MICE

Mouse number	Haematocrit (per cent)	Age (days)	Spleen iron "score"
7	21	381	1
10'	17	41	0
11	31	439	0
12	20.5	159	0
14	30	518	0
15'	15	38	0
17	42	382	0
23	22	337	1
25	34	100	0
26	40	319	1
27'	28.5	147	0
32	35	27	0
46	44	319	0
49	15	280	0
53"	18.5	511	1
71	19.5	41	0
73	32	21	0
75	16.5	41	0
823	19	25	0

Table 6.6 (continued)

SPLEEN IRON STORES IN RELATION TO AGE AND HAEMATOCRIT IN ANAEMIC MICE

Mouse number	Haematocrit (per cent)	Age (days)	Spleen iron "score"
345	22	545	0
391	25.5	320	1
395	31.5	545	1
401	34	467	1
431	31.5	336	0
453	40.5	407	1
457	22	42	0
483	21.5	52	0
487	15.5	47	0
489	20	47	0
491	13.5	162	0
499	16.5	116	0
729	42	68	0
805	26	142	0
809	20	142	0
811	23	142	0

Table 6.7

IRON DEPOSITION IN THE DUODENAL MUCOSAL EPITHELIUM

Genotype	No. of animals examined	No. of animals whose grade of iron deposition was				
		0	1	2	3	4
sla/- and sla/sla (non-fasting)	17	1	2	1	3	10
sla/+ (non-fasting)	6	0	1	5	0	0
+/- (non-fasting)	22	15	6	1	0	0
sla/- (fasting)	7	0	1	0	2	4
sla/+ (fasting)	5	0	0	4	1	0
+/- (fasting)	6	6	0	0	0	0

Grading based on visual assessment of section stained with Prussian Blue; see Chapter 4.

Table 6.8

DUODENAL MUCOSAL IRON "SCORE" IN RELATION TO HAEMATOCRIT AND AGE IN ANAEMIC MICE

Mouse number	Haematocrit (per cent)	Age (days)	Duodenal iron "score"
14	30	518	4
53"	18.5	511	4
345	22	545	4
391	25.5	320	4
395	31.5	545	1
401	34	467	2
431	31.5	336	3
453	40.5	407	1
483	21.5	52	3
487	15.5	47	4
489	20	47	4
491	13.5	162	4
499	16.5	116	4
729	42	68	0
805	26	142	3
809	20	142	4
811	23	142	4
853*	31	163	4

Table 6.8 (continued)

DUODENAL MUCOSAL IRON "SCORE" IN RELATION TO HAEMATOCRIT AND AGE IN ANAEMIC MICE

Mouse number	Haematocrit (per cent)	Age (days)	Duodenal iron "score"
855*	29	163	3
833*	30	123	4
887*	28.5	127	1
911*	19.5	86	3
917*	34.5	84	4
919*	25.5	84	4

*Fasting for 6 hours prior to sacrifice.

Table 7.1

BLOOD VOLUME OF NORMAL AND ANAEMIC MICE

(ml/100G - means ± 1. S.E.)

Method	Normal (+/-)	Anaemic (sla/-)	p
^{51}Cr-labelled red cells	5.85 ± 0.07 (19)	8.93 ± 0.17 (9)	< 0.001
^{131}I-labelled albumin	9.61 ± 0.24 (7)	12.14 ± 0.52 (5)	< 0.001
p	< 0.001	< 0.001	---

Numbers of animals in each group are given in parenthesis.

Table 7.2

RED CELL MASS IN NORMAL AND ANAEMIC MICE

ml/100G - mean ± 1. S.E.

Method	Normal (+/-)	Anaemic (sla/-)	p
^{51}Cr-labelled red cells	2.69 ± 0.06 (19)	2.38 ± 0.13 (9)	< 0.02
^{131}I-labelled albumin	4.14 0.15 (7)	3.22 ± 0.22 (5)	< 0.005
p	< 0.001	< 0.005	---

Numbers of animals in each group are given in parenthesis.

Table 7.3

PLASMA VOLUME OF NORMAL AND ANAEMIC MICE

ml/100G - mean ± 1. S.E.

Method	Normal (+/-)	Anaemic (sla/-)	p
^{51}Cr-labelled red cells	3.15 ± 0.05 (19)	6.56 ± 0.45 (9)	< 0.001
^{131}I-labelled albumin	5.47 ± 0.17 (7)	8.92 ± 0.59 (5)	< 0.001
p	< 0.001	< 0.002	---

Numbers of animals in each group are given in parenthesis.

Table 7.4

REAPPEARANCE OF RADIO-IRON IN THE PERIPHERAL BLOOD 5 DAYS AFTER ADMINISTRATION, CALCULATED FROM BLOOD VOLUME DETERMINATIONS WITH LABELLED RED CELLS AND ALBUMIN

Mouse number	Reappearance of ^{59}iron in the peripheral blood (per cent) Blood volume determined by	
	^{51}Cr-labelled red cells	^{131}I-labelled albumin
1	74.5	122.4
2	66.2	108.6
3	64.4	105.8
4	66.0	108.4
5	63.3	104.2

Table 7.5

THE UTILIZATION OF TRACER DOSES OF ^{59}IRON FOR ERYTHROPOIESIS AFTER INTRAPERITONEAL INJECTION

Genotype	Utilization of iron (per cent) Means ± 1.S.E.				
	Day 1	Day 2	Day 3	Day 4	Day 5
Normal* (18)	30.4 ± 1.7	41.6 ± 3.0	43.3 ± 2.7	37.2 ± 2.8**	38.8 ± 2.7
Anaemic* (8)	44.3 ± 8.1	49 ± 3.9	49.8 ± 4.9	45.7 ± 4.3	47.6 ± 5.5
p	< 0.05	> 0.05	> 0.05	> 0.05	> 0.05

* Numbers of animals in each group are given in parenthesis.

** 14 animals in this group.

Table 7.6

THE UTILIZATION OF TRACER DOSES OF ^{59}IRON FOR ERYTHROPOIESIS 5 DAYS AFTER INTRAPERITONEAL INJECTION

Genotype	Utilization of iron (per cent) Means ± 1. S.E.	p
Normal* (9)	36.4 ± 2.5	
		< 0.02
Anaemic* (12)	53.9 ± 5.4	

* Numbers of animals in each group are given in parenthesis.

Table 7.7

THE UTILIZATION OF TRACER DOSES OF ^{59}IRON FOR ERYTHROPOIESIS

1 DAY AND 5 DAYS AFTER INTRAVENOUS INJECTION

Genotype	Utilization of iron at 1 day after injection (per cent) Means ± 1. S.E.	p
Normal* (11)	29.0 ± 1.43	
		< 0.001
Anaemic* (9)	72.2 ± 6.27	
---	Utilization of iron at 5 days after injection (per cent) Means ± 1. S.E.	---
Normal* (16)	58.6 ± 2.23	
		< 0.005
Anaemic* (14)	76.1 ± 4.70	

* Numbers of animals in each group are given in parenthesis.

Table 7.8

PLASMA IRON CLEARANCE HALF-TIME ($T\frac{1}{2}$ ^{59}Fe)

IN NORMAL AND ANAEMIC MICE

Means ± 1. S.E. and range

Genotype	$T\frac{1}{2}$ ^{59}Fe (min.)	p
Normal* (17)	46.1 ± 2.55 (31 - 65)	
		< 0.001
Anaemic* (8)	23.4 ± 4.66 (12 - 46)	

* Numbers of animals in each group are given in parenthesis.

Table 7.9

THE RETENTION OF FERROUS SULPHATE LABELLED WITH ^{59}IRON

Means ± 1. S.E.

Dose of ^{59}Fe (µg)	Genotype	Per cent retention on day					p**
		1	2	3	4	5	
0.1	normal*(10)	35.6 ± 6.78	30.5 ± 6.09	28.7 ± 5.65	24.3 ± 4.89	22.8 ± 4.63	< 0.02
	anaemic*(10)	29.2 ± 6.38	17.4 ± 4.32	12.9 ± 3.06	10.3 ± 2.39	9.6 ± 2.20	
1	normal*(26)	21.6 ± 2.59	17.6 ± 2.58	16.4 ± 2.31	15.5 ± 2.16	15.4 ± 2.36	< 0.005
	anaemic*(10)	17.7 ± 2.58	9.8 ± 1.29	7.0 ± 0.82	6.1 ± 0.66	5.7 ± 0.67	
10	normal*(11)	-- --	-- --	-- --	-- --	9.7 ± 2.35	< 0.025
	anaemic*(15)	-- --	-- --	-- --	-- --	5.0 ± 0.55	

* Numbers of animals in each group are given in parenthesis.

** Anaemic compared with normal on day 5.

Table 7.10

MEAN ABSOLUTE AMOUNTS OF IRON ABSORBED AT THE THREE ORAL DOSE LEVELS

Dose µg	Genotype	Mean absorption in µg.
0.1	Normal	0.023
	Anaemic	0.0096
1	Normal	0.167
	Anaemic	0.056
10	Normal	0.970
	Anaemic	0.500

Table 7.11

THE ABSORPTION OF ^{59}IRON-LABELLED HAEMOGLOBIN IRON

Means ± 1. S.E.

Genotype	Absorption of ^{59}Fe per cent	p
Normal* (7)	2.5 ± 0.67	
		> 0.1
Anaemic* (8)	2.3 ± 0.51	

Table 7.12

THE ABSORPTION OF RADIO-IODINATED TRIOLEIN, ^{65}ZINC CHLORIDE, ^{57}COBALTOUS CHLORIDE AND ^{64}CUPROUS NITRATE

Means ± 1. S.E.

*Genotype	Per cent retention of		p
+/-	^{131}I-	88.8 ± 2.69	> 0.1
sla/-	triolein	93.4 ± 1.46	
+/-	^{65}Zn	12.9 ± 1.98	> 0.05
sla/-		8.0 ± 1.30	
+/-	^{57}Co	1.6 ± 0.64	> 0.4
sla/-		1.8 ± 0.64	
+/-	^{64}Cu	15.1 ± 1.39	> 0.05
sla		11.2 ± 1.24	

* 5 male animals in each group.

Table 7.13

FAECAL ELIMINATION OF ORALLY ADMINISTERED CARMINE RED

Mouse number	Genotype	Age (days)	Haematocrit (per cent)	Carmine red in faeces on days after administration			
				1	2	3	4
1993	+/-	227	44.5	obvious	nil	nil	nil
1995	+/-	227	45.5	obvious	nil	nil	nil
1997	+/-	227	41.5	obvious	trace	nil	nil
2001	+/-	227	46.0	obvious	trace	nil	nil
1983	sla/-	253	30.5	obvious	nil	nil	nil
1987	sla/-	253	28.0	obvious	trace	nil	nil
1989	sla/-	227	22.5	obvious	nil	nil	nil
2051	sla/-	231	10.0	obvious	trace	nil	nil

Table 8.1

SERUM IRON CONCENTRATION AND TOTAL IRON BINDING CAPACITY (T.I.B.C.) IN NORMAL AND ANAEMIC MICE

Means ± 1. S.E.

Genotype	Serum iron concentration µg/100 ml	T.I.B.C. µg/100 ml
Normal	254 ± 8.3 (27)	420 ± 13.1 (8)
Anaemic	142 ± 26.2 (25)	612 ± 30.3 (9)
p	< 0.001	< 0.001

* Number of animals in each group are given in parenthesis.

Table 8.2

TOTAL BODY IRON CONTENT OF NORMAL AND ANAEMIC MICE AT DIFFERENT AGES

Means ± 1. S.E.

Age (days)	Genotype	Number of mice	Total body iron content (mg/100G)	p
less than 100	Normal	9	5.21 ± 0.43	< 0.01
	Anaemic	4	2.97 ± 0.36	
101-200	Normal	7	4.95 ± 0.12	< 0.025
	Anaemic	5	4.20 ± 0.64	
201-300	Normal	7	4.86 ± 0.26	< 0.001
	Anaemic	9	3.61 ± 0.22	
over 300	Normal	5	7.63 ± 0.76	< 0.001
	Anaemic	7	3.95 ± 0.55	

Table 8.3

ESTIMATIONS OF THE FREE ERYTHROCYTE PROTOPORPHYIN (F.E.P.) IN NORMAL AND ANAEMIC MICE IN µg/100 ml RED CELLS

Means ± 1. S.E.

Genotype	Number of Estimations	F.E.P.	p
Normal	11	49.7 ± 7.3	
			< 0.001
Anaemic	8	272.1 ± 57.2	

Table 8.4

HAEMATOLOGICAL FINDINGS IN DONOR MICE

Genotype	Hb. conc. G/100 ml	P.C.V. %	M.C.H.C. %	Red cell appearance
+/-	13.5	41.5	33.0	Normochromic and normocytic
sla/-	6.7	24.0	28.0	Hypochromic, microcytic, with target cells and poikilocytosis

Table 8.5

HAEMATOLOGICAL FINDINGS IN IRRADIATED RECIPIENT C57B1/10 JAX MICE 116 DAYS AFTER GRAFTING WITH NORMAL OR ANAEMIC (sla/-) HAEMOPOIETIC TISSUE

Genotype of Donor	Tissue grafted	Dose (cells x 10^6)	Recipient number	Hb G/100ml	PCV%	MCHC%
+/-	Bone marrow	7.2	1	17.2	51.5	33.4
"	"	"	2	16.0	51.0	31.4
"	"	"	3	13.5	44.5	30.3
"	"	"	4	14.7	46.0	32.0
"	"	"	5	14.7	45.0	32.7
sla/-	"	1.9	6	15.6	52.0	30.0
"	"	"	7	14.3	43.5	32.9
"	"	"	8	15.6	50.0	31.2
"	"	"	9	15.2	46.5	32.7
"	"	"	10	15.6	49.0	31.8
"	"	"	11	14.7	49.0	30.0
"	"	"	12	-	47.0	-
"	"	"	13	15.2	48.0	31.7
"	"	"	14	15.6	47.5	32.8
"	"	"	15	16.0	50.5	31.7
"	Spleen	8.96	16	-	52.0	-
"	"	"	17	-	45.5	-

The red cells in all recipients appeared morphologically normal. The white cells also appeared normal in number, form and proportion in all recipient animals.

Table 10.1

FACTORS STATED TO ENHANCE IRON ABSORPTION

Factor	Reference
Alcohol	Charlton et al, (1964); Sorensen, (1965).
α-keto-glutaric acid	Jacobs, Bothwell, and Charlton, (1966).
amino-acids	Kroe et al, (1966).
ascorbic acid	Moore and Dubach, (1951); Williams, (1959); Sorensen, (1965); Hallberg and Solvell, (1967).
blood loss	Hahn et al, (1943); Bothwell et al, (1958); Krantz et al, (1959); Pirzio-Biroli and Finch, (1960); Mendel et al, (1963); Weintraub, Conrad and Crosby, (1964); Jacobs, Bothwell and Charlton (1966).
carbohydrate	Sorensen, (1965).
cirrhosis of the liver	Callender and Malpas, (1963); Friedman et al, (1966); Williams et al, (1967).
citrate	Jacobs, Bothwell and Charlton, (1966).
cobalt	Krantz et al, (1959).
cysteine	Pollack, Kaufman and Crosby, (1964b).
erythropoiesis	Stewart et al, (1953); Bothwell et al, (1958); Krantz et al, (1959); Erlandson et al, (1962); Mendel et al, (1963); Chirasiri and Izak, (1966); Jacobs, Bothwell and Charlton, (1966).
erythropoietin	Krantz at al, (1959); Mendel, (1961).
fat	Sorensen, (1965).
ferrous iron	Tompsett, (1940); Moore et al, (1944); Hahn et al, (1945); Brise and Hallberg, (1962).
fructose	Pollack, Kaufman and Crosby, (1964b).
gastric juice (inorganic iron only)	Jacobs, Rhodes and Eakins, (1967).
Haemochromatosis	Bothwell et al, (1953); Pirzio-Biroli et al, (1958); Bannerman, Callender and Williams, (1962); Williams et al, (1966).

Table 10.1 continued

Factor	Reference
hog stomach	Turnberg, (1966).
hydroquinone	Pollack, Kaufman and Crosby, (1964b).
hypoxia	Krantz et al, (1959); Reynafarje et al, (1959); Greenberg et al, (1960); Mendel, (1961).
increased iron turnover	Weintraub, Conrad and Crosby, (1964); Wakisaka et al, (1965)
inosine, intravenously	Cheney and Finch, (1960).
intrinsic factor (?)	Waxman et al, (1966).
iron deficiency	Austoni and Greenberg, (1940); Hahn et al, (1943); Bothwell et al, (1958); Schulz and Smith (1958 a & b); Bonnet et al, (1960); Field et al, (1960); Bannerman, O'Brien and Witts, (1962); Conrad and Crosby, (1963; Bannerman, (1965b); Chirasiri and Izak, (1966); Jacobs, Rhodes et al, (1966); Hallberg and Solvell, (1967).
lactate	Pollack, Kaufman and Crosby, (1964b).
low iron intake	Wack and Wyatt, (1959); Bannerman, O'Brien and Witts, (1962); Pollack, Kaufman and Crosby, (1964a); Jacobs, Bothwell et al, (1966); Pearson et al, (1967).
low protein diet	Kinney, (1949); Kaufman et al, (1958).
low serum iron	Weintraub, Conrad and Crosby, (1964); Taylor and Gatenby, (1966).
Methylene blue	Jacobs, Bothwell and Charlton, (1966).
organic acids	Groen et al, (1947).
pancreatic insufficiency	Taylor et al, (1935); Kinney et al, (1955); Davis and Badenoch, (1962); Tonz et al, (1965); Brozovic et al, (196
pregnancy	Balfour et al, (1942); Hahn et al, (1951).
pyruvate	Pollack, Kaufman and Crosby, (1964b).
reducing agents	Groen et al, (1947).
unsaturated transferrin	Solvell, (1960); Taylor and Gatenby, (1966).

Table 10.2

FACTORS STATED TO SUPPRESS IRON ABSORPTION

Factor	Reference
Achlorhydria (inorganic iron)	Williams, (1959); Goldberg et al, (1963); Jacobs, Rhodes et al, (1966).
bacterial infection	Gubler et al, (1950).
bicarbonate	Benjamin et al, (1967).
bone marrow suppression	Saylor and Finch, (1953).
bromelain orally	Murray and Stein, (1967a).
copper deficiency	Chase et al, (1952); Gubler et al, (1952).
desferrioxamine (inorganic iron)	Hwang and Brown, (1963); Bannerman and Malpas, (1965); Hwang and Brown, (1965).
E.D.T.A.	Larsen et al, (1960).
gastrectomy	Stevens et al, (1959); Williams, (1959); Whitehead and Bannerman, (1964); Turnberg, (1966).
haemochromatosis	Chodos and Ross, (1953).
idiopathic steatorrhoea	Badenoch and Callender, (1954); Bonnet et al, (1960).
in vitro anoxia, cyanide, azide, iodacetamide	Jacobs, Bothwell and Charlton, (1966).
iron overload	Balfour et al, (1942); Saylor and Finch, (1953); Bothwell et al, (1953); Bothwell et al, (1958); Krantz et al, (1959); Field et al, (1960); Pirzio-Biroli and Finch, (1960).
iron supplemented diet	Bannerman, O'Brien and Witts, (1962); Pearson et al, (1967).
neomycin	Jacobson et al, (1960).
pancreatic extract or stimulation	Biggs and Davis, (1963); Callender and Malpas, (1963); Smith, (1964); Davis and Biggs, (1965); Brozovic it al, (1966).
phosphate	Hegsted et al, (1949).
phytate	McCance et al, (1943); Sharpe et al, (1950).

Table 10.2 continued

Factor	Reference
phytate (inorganic iron only)	Hallberg and Solvell, (1967).
polycythaemia	Krantz et at, (1959); Weintraub, Conrad and Crosby, (1965).
prior dose of oral iron	Hahn et al, (1943); Stewart et al, (1950); Hallberg and Solvell, (1967).
saturation of iron binding capacity	Solvell, (1960).
transfusion	Krantz et al, (1959).
turpentine abscess	Hahn et al, (1946); Saylor and Finch, (1953).

Table 10.3

FACTORS STATED NEITHER TO ENHANCE NOR SUPPRESS IRON ABSORPTION

Factor	Reference
Allopurinol	Davis and Deller, (1966); Kozma et al, (1967).
antacids	Moore and Dubach, (1951).
aplasia of bone marrow	Erlandson et al, (1962).
chronic pancreatitis	Balcerzak et al, (1967).
galactose	Pollack, Kaufman and Crosby, (1964b).
gastrectomy	Smith and Mallett, (1957).
gastric acidity (organic iron)	Callender et al, (1957).
gastric juice (organic iron)	Biggs et al, (1961).
glucose	Pollack, Kaufman and Crosby, (1964b).
haemolytic disease (mild)	Erlandson et al, (1962).
hydrochloric acid	Moore and Dubach, (1951).
low iron diet (in man)	Rush et al, (1966).
pancreas (powdered, by mouth)	Murray and Stein, (1967a).
pepsin	Schade and Schilling, (1967).
phytate	Cowan et al, (1966).
thalassaemia minor	(Erlandson et al, (1962).

APPENDICES

Abbreviations used:

+/- - normal genotype (male)

+/+ - normal genotype (female)

sla/+ - heterozygous female

sla/- - anaemic male

sla/sla - anaemic female

O.M.S. - original mixed stock

F_1 hybrid - cross of original mixed stock and C57Bl/6J strain

F_2 hybrid - backcross of F_1 and C57Bl/6J strain

F_3 hybrid - backcross of F_2 and C57Bl/6J strain

F_4 hybrid - backcross of F_3 and C57Bl/6J strain

F_5 hybrid - backcross of F_4 and C57Bl/6J strain

APPENDIX A

HAEMATOLOGICAL INVESTIGATIONS

1. Haemoglobin Estimations (Grams/100 ml)

All results were obtained from mice of the original mixed stock

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Normal male (+/-)	21-30	---	---	---	---
	31-40	15.2, 17.1, 15.0	15.8	0.93	0.55
	41-50	16.4, 16.6, 17, 16.2	16.5	0.12	0.06
	51-60	13.8, 15.1, 15.8, 16.4, 13.3	14.9	1.17	0.53
	61-70	13.6, 15.1, 15.2, 14.1, 14.2	14.4	0.62	0.28
	71-80	14, 17, 16, 15.7, 13.4, 14.8, 14.5	15.1	1.14	0.44
	81-90	15, 15.2, 15.2, 15.4, 13.8	14.9	0.57	0.26
	91-100	---	---	---	---
	101-150	13.8, 14.7, 15.6, 16.5, 13.8, 15.8, 13.0	14.7	1.17	0.45
	151-200	14.7, 15.5, 14.7, 15.2	15.0	0.34	0.17
	201-250	13.8, 14.3, 15.4, 13.0, 13.2, 13.8	13.9	0.79	0.33
	251-300	12.3, 13.8, 13.8	13.3	0.71	0.42
	301-400	12.5, 13.4, 13.4, 13.1, 13.8, 13.8	13.3	0.45	0.19

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic male (*sla*/-)	21-30	6.6, 7.8, 8.4, 7.6	7.6	0.59	0.30
	31-40	8.1, 8.5, 4.1, 7.2, 7.2, 6.5, 5.9, 4.3, 5.2, 4.8, 6.7	6.2	1.4	0.42
	41-50	5.2, 5.8, 5.9, 6.7, 6.7, 6.8, 7.2, 7.2, 7.7, 6.5, 8.1	6.7	1.0	0.30
	51-60	6.1, 6.4, 5.9, 9.1, 5.9, 7.2	6.8	1.1	0.46
	61-70	4.2, 4.8, 7.0, 7.2, 8.2, 8.2, 9.1, 10.3, 11	7.8	2.2	0.73
	71-80	4.8, 6.0, 7.2, 7.3, 8.6, 7.5, 10.8, 9.6 11, 8.5, 11.5	8.4	2.1	0.64
	81-90	9.6, 9.1, 6.5, 8.3, 10, 11.5, 10.9	9.4	1.55	0.62
	91-100	4.2, 7.4, 5.2, 10.5, 6.8	6.8	2.2	1.0
	101-150	4.3, 4.6, 5, 6.8, 7.2, 7.6, 7.6, 8.1, 8.2, 8.5, 9.1, 9.4, 9.6, 10, 10, 10, 5.7, 8.5, 8.1, 10, 10, 10.2, 10.5, 10.6, 11, 11.5, 11.7, 12.7, 13.8, 10.8, 5.3, 4.7, 4.8, 5.2	8.6	2.5	0.42

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic male (sla/-)	151-200	9.4, 9.6, 9.8, 10.0, 10.0, 10.4, 10.6, 11.0, 11.0, 11.5, 11.5, 12.0, 12.0, 12.3 12.9, 13.3, 13.5, 14.8, 15.2, 12.2	11.7	1.6	0.36
	201-250	4.7, 5.5, 9.2, 9.2, 9.4, 9.6, 10, 10, 10.6, 11.2, 11.9, 13.8, 14.2, 7.6, 11.2, 13.4, 13.0, 10.9, 13.3, 12.3, 12.5, 11.2, 5.0	10.4	2.6	0.54
	251-300	3.7, 9, 9.2, 10.5, 11, 11.2, 11.6, 11.9, 13.8, 14.1, 4.5, 14.9, 15.1, 9.0, 12.7, 13.6, 15.2	11.8	2.9	0.71
	301-400	6, 7.2, 8.4, 9.9, 10, 10.3, 10.5, 11, 14.4, 14.6, 15.2, 15.6, 9.6, 10.8, 15.2, 12.6, 15.2, 15.2, 16.5	12	3.1	0.70

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic female (sla/sla)	21-30	9.6, 7.6, 7.6, 9.1	8.5	0.89	0.45
	31-40	4.1, 4.4, 4.4, 4.5, 5.9, 6.5, 6.7, 7.2, 7.7, 8.1, 3.8, 7.2, 11.5, 10.7, 7.6, 7.2, 6.7, 6.5, 7.2	6.7	1.9	0.43
	41-50	4.2, 4.7, 7.4, 7.6, 12.8, 13.8, 7.2, 8.6 7.6, 7.2	8.2	3.0	0.94
	51-60	4.5, 8.2, 8.6, 7.6	7.2	1.6	0.80
	61-70	7.8, 11.0, 5.0, 3.5, 8.5, 9.6, 9.6, 9.8, 10, 10.4, 10.7, 11, 11, 9.1, 11, 9.6, 9.1	9.2	2.0	0.49
	71-80	3.0, 4.5, 6.5, 6.6, 6.7, 7.6, 8.2, 9.6, 9.6, 9.9, 10.5, 12.4, 14.3, 10.5, 5, 11.5, 9.1, 9.0, 8.5	8.6	2.7	0.61
	81-90	6.3, 11.5, 11.5, 11.8, 12.3, 13.3, 6.3, 11.0	10.5	2.5	0.89
	91-100	6.8, 12.3, 13.3, 11.0, 9.8	10.6	2.3	1.0
	101-150	5, 6.3, 8.5, 9.1, 9.5, 9.6, 9.6, 10.5, 10.5, 10.5, 11.4, 11.5, 11.6, 12, 12,	11.2	2.0	0.36

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic female (sla/sla) cont'd	101-150	12, 12, 11.7, 12.3, 12.3, 12.8, 13.8, 13.8, 9.9, 12.3, 13.8, 12.8, 13.3, 13.5, 11.2, 11.6, 11.7	11.2	2.0	0.36
	151-200	5.3, 5.4, 6.3, 7.2, 12.9, 15.1, 14.8, 14.2, 9.7, 11.9, 13.6, 12.2, 13.4	10.9	3.5	0.97
	201-250	4.9, 6.3, 10, 11.5, 11.9, 12.8, 13, 13.2, 13.6, 13.8, 14.2, 14.2, 14.4, 14.9, 15.2, 10, 13.3, 16.6, 15.2, 15.6, 16.0, 15.0, 14.3, 9.9	12.9	2.9	0.59
	251-300	4, 7.6, 9, 10, 11, 11, 11.5, 11.6, 11.9, 12.3, 12.3, 13.6, 13.6, 13.8, 13.8, 13.8, 13.9, 14.2, 14.6, 14.6, 15.2, 15.9, 13.9, 14.1, 14.2, 13.3, 14.8, 14.8, 14.3, 14.4	12.8	2.5	0.45
	301-400	4.8, 7.7, 7.7, 9.1, 9.3, 9.5, 11.0, 11.6, 11.9, 13, 13.6, 14.6, 14.8, 15.6, 17.2, 14.5, 14.1, 10.3, 11.2, 14.3, 11.2	11.8	3.04	0.65

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Carrier females (sla/+)	21-30	---	---	---	---
	31-40	13.8, 12.8, 12.6, 11.2, 12.9, 8.6 11.6, 11.8, 14.4, 14.7, 13.4	12.5	1.6	0.48
	41-50	16.1, 15.5, 15.3, 12.9, 16.2, 15.7 13, 14.9	14.9	1.22	0.44
	51-60	13.8, 15.2, 15.4, 15.2, 14.8, 14.8, 13.6, 12.8, 15.8, 15.6, 14.8, 13.8, 13.6, 16.1, 15.6	14.7	0.95	0.24
	61-70	17.8, 16.1, 16.5, 15.2, 14.8, 14.6, 14.6, 13.8, 13.8, 12.6, 16.6, 14.2, 15.7, 14.7, 12.2	14.9	1.46	0.37
	71-80	14.8, 15.6, 14.3, 14.8, 15.2, 13.3	14.7	0.73	0.30
	81-90	17.1, 16.2, 15.8, 15.7, 15.2, 14.2, 15.0, 15.2, 15.2, 14.4	15.4	0.81	0.25
	91-100	16.2, 15.7	16.0	0.25	0.18

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Carrier females (sla/+) cont'd	101-150	17.9, 17.6, 17.2, 16.9, 16.9, 16.2, 16, 16, 16.2, 15.7, 15.7, 15.7, 15.2, 15.2, 15.2, 15.2, 15.2, 15.8, 16, 14.7, 15.2, 13.8, 15.2, 16.2, 16.7, 13.4, 15.6, 16.3, 13.6, 15, 15.3	15.7	1.04	0.19
	151-200	17.6, 15.7, 13.2, 17.3, 15.6, 17.2, 16.6, 14.8, 16.2, 15.2, 15.6, 14.7, 15.2	15.8	1.07	0.30
	201-251	16.1, 15.2, 14.7, 18.1, 17.9, 17.2, 15.8, 15.6, 15.6, 14.9, 14.7, 13.7, 14.7, 12.3, 12.6, 15.2	15.3	1.53	0.37
	251-300	13.2, 15.2, 14.7, 15.2, 12.9, 13.0, 15.2	14.2	1.02	0.39
	301-400	13.8, 14.3, 14.3, 14.5, 14.7, 15.9, 15.6, 13.4, 14.8, 14.3, 15.1, 15.6, 15.8, 15.4	14.8	0.74	0.20

2. Packed Cell Volume Determinations (per cent)

All results were obtained from mice of the original mixed stock

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Normal males (+/-)	21-30	---	---	---	---
	31-40	44, 46.5, 47.5, 48,5, 49.5, 51, 55, 46, 53	49	3.3	1.1
	41-50	50.5, 52.5, 57.5, 57.5	54.5	3.1	1.6
	51-60	44, 53, 53.5, 47, 45	48.5	4.0	1.8
	61-70	51, 53.5, 49	51.2	1.5	0.88
	71-80	46.5, 50, 51, 46, 50, 46, 48	48.2	2.0	0.77
	81-90	51, 51.5, 54.5, 50, 49	51.2	1.9	0.86
	91-100	---	---	---	---
	101-150	41, 46, 46, 47.5, 48, 57.5, 47.5, 52, 48.5	48.2	4.2	1.4
	151-200	41, 53, 46, 45.5, 49	46.9	4.0	1.8
	201-250	43, 45.5, 53, 47.5, 46, 49.5	47.4	3.2	1.3
	251-300	43, 43, 44, 49, 51.5, 48.5	46.5	3.3	1.4
	301-400	41.5, 43.5, 43.5, 49, 43.5, 45, 44, 46	44.5	2.1	0.75

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-)	21-30	15.5, 25, 26.5, 30, 33, 35, 35, 38.5, 23.5	29.1	5.7	1.9
	31-40	26.5, 15.5, 19, 20.5, 25, 28, 28.5 32.5, 32.5, 37, 21.5, 30.5, 32, 25 21.5, 24, 22.5, 29.5	28.2	5.6	1.3
	41-50	21.5, 26, 26.5, 27, 27, 38, 38.5, 17, 20, 23.5, 24, 26, 27, 27, 29.5, 30, 30.5, 31, 31.5, 35, 36.5	28.3	5.7	1.2
	51-60	23, 27, 28.5, 30, 29.5, 21, 32, 27.5, 29.5, 29	27.8	3.2	1.0
	61-70	18.5, 21, 24, 24.5, 25.5, 27, 32, 31, 32.5, 34.5, 35, 35, 40, 30.5, 44	29.3	6.9	1.8
	71-80	42, 44, 39, 15.5, 17, 22.5, 29, 29.5, 30, 31, 32, 33, 34, 35, 36, 38, 40.5, 40	32.7	7.8	1.9
	81-90	12.5, 30, 31, 32.5, 33, 34, 35, 37, 41, 41	32.7	7.6	3.3
	91-100	18.5, 28, 24.5, 31, 43, 33	29.7	7.6	3.2

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-) cont'd	101-150	36.5, 35, 20.5, 23, 23.5, 24, 24.5, 25, 27, 27, 28.5, 30, 31.5, 33, 34, 34.5, 35, 33, 28, 33, 26.5, 30.5, 31, 39, 34, 36, 37, 37, 37, 37, 37, 38, 39, 39, 39.5, 40, 40, 40, 41, 42, 42, 44, 45, 45	34.2	6.0	0.91
	151-200	37, 38, 38, 38, 39, 39, 39, 40, 40, 40, 40.5, 40.5, 41.5, 41.5, 42, 42, 42, 42, 42.5, 43, 50, 39, 42.5, 39, 38	40.5	2.5	0.50
	201-250	24, 35.5, 36, 36.5, 38, 40, 40, 40.5, 41, 44, 45, 50, 29.5, 37, 42.5, 39, 37, 41, 38, 36.5, 23.5	38.2	6.0	1.3
	251-300	27.5, 31, 33.5, 35, 40, 42, 43, 43.5, 45, 47, 48, 48.5, 53, 33, 42, 47, 39, 30	40.1	6.8	2.0
	301-400	28, 30, 33, 35, 35.5, 37.5, 41, 42, 47, 48.5, 49,5, 55, 38, 37, 12, 40, 43.5, 51.5, 42.5, 47.5, 47, 45.5	40.4	9.2	2.0

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic females (sla/sla)	21-30	28, 34, 34, 37, 23, 32	31.3	4.6	1.9
	31-40	28, 30, 35, 16.5, 31, 30, 29, 39, 29.5, 26, 30.5, 18, 20, 21, 22, 24, 24, 24.5, 25.5, 26.5, 26.5, 26.5, 27.5, 27.5, 28, 24.5, 28.5	26.6	4.8	0.92
	41-50	13.5, 16, 16.5, 16.5, 18, 19.5, 21, 23, 26, 35, 34, 35, 30, 28, 29.5, 28, 26.5, 33	24.9	7.0	1.7
	51-60	13, 25, 37, 44, 23.5, 34, 33, 32	30.2	8.9	3.2
	61-70	31, 33.5, 34, 35, 38, 38, 39, 41, 43, 36, 40, 38, 34.5, 29.5	36.5	3.7	1.0
	71-80	37, 19, 28.5, 29, 30, 31, 32, 32, 32, 35.5, 41, 51, 25.5, 34, 46, 35, 39.5	34.0	7.4	1.8
	81-90	23.5, 30, 31, 39, 41, 41, 42, 47, 30, 41	36.8	7.0	2.2
	91-100	34, 40, 56, 57, 28, 37.5	42.1	10.8	4.5

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic females (sla/sla) cont'd	101-150	20, 30, 31, 34, 35, 35, 36, 36, 37, 37, 38, 39, 40, 40, 40, 40.5, 41, 41, 41, 43.5, 41.5, 42, 42, 42.5, 43, 43, 44, 46, 47, 29.5, 42, 44, 45, 43, 42, 51.5, 43, 40.5	39.7	5.5	0.89
	151-200	20, 24, 26, 29.5, 37, 37, 38, 42, 42, 49.5, 46, 46, 44, 33, 45, 44.5, 51.5, 43, 40.5	37.7	8.6	2.2
	201-250	21, 35, 38, 39.5, 43, 43, 44, 44.5, 45, 45, 46, 47, 47, 47, 48, 47, 48, 43, 44, 44, 47, 46.5, 47, 35, 45	43.8	5.2	0.91
	251-300	15, 35, 38, 39, 40, 41, 42, 42, 43 43, 43.5, 44, 44, 44, 45, 45, 55, 45.5, 46, 46, 46.5, 47.5, 49, 51, 45, 49, 45, 44, 44, 48, 51	43.7	5.3	0.98
	301-400	22.5, 29.5, 34, 34, 37.5, 38, 42, 42.5 42.5, 44, 44, 44.5, 46.5, 51.5, 46.5, 49, 53, 55, 46, 45, 48, 39, 49.5, 41.5	42.7	8.5	1.7

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Carrier females (sla/+)	21-30	35, 34, 35	34.7	0.46	0.27
	31-40	42.5, 36.5, 37.5, 39, 40.5, 43, 43.5, 44, 47, 48, 51, 34, 41, 35, 36, 43, 47, 49	42	4.9	1.2
	41-50	37, 40, 44, 44, 42.5, 51.5, 46.5,50, 45.5, 49, 49	45.3	4.2	1.3
	51-60	35.5, 49.5, 49, 49, 40.5, 44, 44.5, 44, 45.5, 48, 49.5, 47, 56, 46, 42, 48, 51, 58	47.1	4.9	1.2
	61-70	35.5, 45, 45, 49, 50, 50, 52.5, 51, 44, 48,42, 54, 42.5, 43.5	46.6	4.8	1.3
	71-80	50, 48, 47, 49, 46, 47.5	47.9	1.9	0.47
	81-90	45, 45, 48, 49, 50, 54, 50.5, 47, 48, 46	48.3	2.6	0.83

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Carrier females (sla/+) cont'd	91-100	43, 46, 44	44.3	1.2	0.71
	101-150	44, 45, 46, 46, 46, 50.5, 47, 48, 48, 48, 49, 49.5, 50, 50, 50.5, 57, 48.5, 51, 46, 50, 47, 48, 42, 44, 44.5, 47, 44, 46, 50.5, 46.5, 51, 48.5	47.5	2.9	0.53
	151-200	52, 52, 51, 42, 42, 48, 54, 55, 53, 42, 44, 50, 49, 47.5, 50.5, 51, 49.5,	49.0	4.1	1.0
	201-250	43.5, 46, 46, 48, 49, 49.5,50, 50, 53, 45, 49, 46.5, 51, 56.5, 48	48.7	2.8	0.72
	251-300	38, 45, 48, 46.5, 49, 42, 44, 41	44.2	3.5	1.3
	301-400	44.5, 45.5, 48, 42, 46, 46, 47.5, 47.5, 48, 52, 52, 44, 48, 41.5, 49.5, 40, 48, 48.5	46.6	3.2	0.76

3. Determinations of Mean Corpuscular Haemoglobin Concentration (per cent)

All results were obtained from mice of the original mixed stock

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Normal males (+/-)	21-30	---	---	---	---
	31-40	31, 30, 28	29.7	1.2	0.71
	41-50	30, 31, 30, 28	29.8	1.1	0.55
	51-60	31, 30, 36, 30, 32	31.8	2.2	1.0
	61-70	30, 28, 29	29.0	0.8	0.47
	71-80	30, 30, 28, 27, 31, 31, 32, 31, 31, 36, 34, 30	30.9	2.3	0.66
	81-90	29, 28, 31, 28, 32, 30	29.7	1.5	0.68
	91-100	---	---	---	---
	101-150	31, 29, 34, 33, 29, 32, 31, 27, 30, 31, 28	30.5	2.1	0.64
	151-200	32, 29, 28, 32, 31, 32, 32	30.9	1.7	0.65
	201-250	29, 30, 29, 32, 29, 32, 29, 31, 32, 30, 30, 35, 31, 33, 32, 32, 27, 28	30.6	1.9	0.45
	251-300	30, 30, 33, 29, 31, 31, 27, 32, 29	30.2	1.7	0.57
	301-400	29, 31, 31, 30, 34, 29, 35, 30, 31, 30	31.0	1.8	0.54

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-)	21-30	21, 24, 23, 24, 22, 23	22.8	1.1	0.46
	31-40	29, 23, 29, 24, 20, 27, 19	23.4	3.4	1.0
	41-50	20, 25, 25, 22, 23, 28, 27, 23, 22, 17, 21	23.0	3.0	0.91
	51-60	26, 23, 21, 23, 20, 22, 28, 24, 20	23.0	2.5	0.83
	61-70	23, 27, 27, 26, 30, 18, 25, 20	24.5	3.7	1.3
	71-80	27, 32, 27, 23, 28, 23, 29, 23, 23, 21, 24, 21, 22	24.8	3.3	0.92
	81-90	31, 22, 26, 27, 24	26.0	3.0	0.66
	91-100	24, 25, 30, 23, 22, 20, 21, 24	23.6	3.2	1.1
	101-150	33, 22, 24, 22, 28, 27, 28, 29, 31, 30, 26, 29, 29, 27, 28, 24, 23, 21, 19, 25, 24 27 28 26, 31	26.4	3.3	0.66

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-) cont'd	151-200	29, 30, 30, 27, 27, 30, 29, 26, 33, 33, 34, 31, 28, 27, 25, 29, 25, 26, 28, 35, 26, 27, 26	28.7	2.8	0.58
	201-250	33, 31, 21, 30, 30, 30, 27, 28, 28, 26, 28, 27, 23, 20, 24, 30, 32, 26, 27, 25, 33, 29, 30	27.7	3.4	0.71
	251-300	27, 35, 30, 28, 34, 31, 26, 32, 35, 30, 33, 22, 31, 25, 27, 24, 32, 32, 29, 28	29.6	3.5	0.78
	301-400	32, 28, 25, 29, 32, 32, 27, 22, 28, 30, 33, 29, 28, 30, 27, 29, 30, 25, 22, 30, 32, 31, 36	29.0	3.3	0.69

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic females (sla/sla)	21-30	24, 22, 19, 28	23.3	3.3	1.7
	31-40	24, 24, 23, 24, 21, 28, 24, 25, 23, 23, 24, 25, 26, 27	24.4	1.5	0.41
	41-50	25, 26, 22, 26, 21	24.0	2.1	0.95
	51-60	30, 26, 25, 24, 26, 24	25.8	2.0	0.83
	61-70	26, 27, 28, 26, 27, 25,26, 25, 28, 26, 23	26.1	1.4	0.42
	71-80	28, 27, 31, 23, 24, 21, 24, 23, 24, 26, 31, 28, 23, 31, 25, 26, 23, 23	25.6	3.0	0.71
	81-90	31, 28, 21, 21, 27	25.6	4.0	1.8
	91-100	20, 29, 26, 29	26.0	3.7	1.9
	101-150	32, 28, 27, 30, 30, 32, 29, 31, 30, 31, 32, 30, 29, 28, 27, 32, 25, 24, 24, 20, 28, 30, 27, 26, 25, 24, 27, 27, 28	28.0	2.9	0.54

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic females (sla/sla)	151-200	31, 21, 25, 21, 26, 28, 27, 29, 32, 31, 31, 29	27.6	3.6	1.0
cont'd	201-250	34, 31, 25, 30, 31, 33, 32, 34, 34, 32, 34	31.8	2.5	0.76
	251-300	28, 30, 31, 24, 23, 29, 27, 32, 27, 28, 32, 30, 28, 32, 34, 29, 28, 28, 32, 30, 31, 29, 31, 26	29.1	2.7	0.55
	301-400	26, 26, 21, 32, 26, 31, 31, 28, 33, 31, 27, 26, 32, 31, 29, 29, 27	28.6	3.0	0.73
Carrier females (sla/+)	21-30	31	(31)	---	---
	31-40	30, 30, 29, 31, 24, 27, 25, 28, 29	28.9	2.0	0.63
	41-50	35, 27, 30, 28, 27, 33	30.0	3.3	1.3
	51-60	34, 35, 34, 27, 34, 28, 32, 29, 27, 32, 32	31.3	2.8	0.85

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Carrier females (sla/+)	61-70	32, 36, 28, 28, 29, 33, 30, 29, 35, 31, 35	31.5	2.8	0.85
cont'd	71-80	35, 34, 28, 33, 30, 33, 31, 31, 29	31.6	2.5	0.83
	81-90	35, 30, 32, 32, 33, 31, 28	31.6	2.1	0.1
	91-100	35	(35)	---	---
	101-150	31, 33, 31, 33, 30, 33, 35, 35, 32, 31, 31, 35, 34, 31, 33, 28, 33, 34, 30, 28, 33, 35, 35, 31	32.3	2.1	0.43
	151-200	32, 30, 32, 28, 32, 34, 32, 31, 33, 29,	31.1	1.6	0.48
	201-250	36, 26, 34, 29, 30, 32, 28, 27, 30, 32, 30, 30, 30, 30, 29, 32, 29, 30, 31	30.3	2.3	0.52
	251-300	32, 32, 29, 31, 31, 28, 32	30.7	1.5	0.58
	301-400	31, 33, 32, 33, 30, 31, 35, 31, 32, 28, 33, 31, 32	31.7	1.6	0.44

4. Red Cell Count in Millions/cu. mm.

Results were obtained from mice of the original mixed stock and from hybrid animals (see below)

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Normal males (+/-)	0-100	9.9*, 7.52, 9.44, 7.92, 9.5*, 11.28, 11.48, 10.88, 11.16, 9.4, 9.76	9.84	1.25	0.38
	101-200	10.8, 10.44, 12.0, 12.88, 11.72, 11.76, 11.6, 11.76, 11.44, 12.92, 10.12, 10.6, 10.04, 11.36, 10.64, 10.24, 10.91, 10.88, 10,96, 11.4, 10.36, 10.32	11.14	0.79	0.17
	201-300	9.56*, 10.08, 10.44, 10.32, 10.32, 11.48, 9.64, 10.0*, 0.56, 10.44, 10.36	10.29	0.58	0.17
	301-400	8.2*, 8.24*, 10.16*, 8.68*, 9.2*, 10.64, 9.20, 10.72	9.38	0.95	0.34

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-) and females (sla/sla)	0-100	7.32*, 6.84*, 8.92*, 7.84*, 7.00*, 8.64, 9.32*, 6.56, 7.96, 8.48*, 10.28*	8.11	1.10	0.34
	101-200	10.0*, 11.76, 5.12, 5.08, 4.28, 6.32, 8.4, 8.0, 9.08, 8.95, 12.32*, 11.48*, 14.08*, 12.52*	9.1	2.99	0.81
	201-300	7.72, 7.16, 7.12, 7.08, 13.6, 12.44*, 12.04*, 13.92*, 12.16*	10.36	2.83	0.94
	301-400	9.08*, 10.36*, 9.96*, 8.92*, 8.96*, 12.36*, 10.48*, 9.84*	10.00	1.06	0.38
Carrier females (sla/+)	0-100	9.32*, 11.6*	10.46	---	---
	101-200	10.8*, 10.96*, 12.68*, 10.2	11.16	0.92	0.46
	201-300	8.96*	8.96		
	301-400	10.16, 9.32, 9.84, 10.46, 10.0	9.96	0.38	0.17

* Estimations from animals of the original mixed stock. Other values for the red cell count are taken from the second back cross generation hybrids of the original mixed stock and the C57Bl/6J strain.

5. Mean Cell Volume (cubic microns)

Results were obtained from mice of the original mixed stock and from hybrid animals (see below)

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Normal males (+/-)	0-100	49.1, 49.5, 50.5, 42.9, 43, 43.3, 46.9, 43.2, 42.1, 41.8, 43.9, 45, 45.6, 41.3, 41.4, 43.2, 45.2, 39.9, 42.5, 45.6, 45.2, 45.5, 44.5, 42.2	44.3	2.58	0.53
	101-200	44.4, 44.1, 41.7, 43.5, 39, 39.8, 38.1, 41.3, 41.4, 43.4, 48.9, 46.7, 44.3, 42.3, 44.6, 44.9, 46.3, 44.6, 48.3, 43, 48.3	43.8	2.96	0.64
	201-300	45*, 42.7*, 43.1, 45*, 44.6, 42.5, 44.6, 47.2, 45.1, 47, 49.5*, 43.9*	45.0	2.04	0.59
	301-400	50.6*, 48.5*, 48.4*, 48.0*, 46.3*, 48.9, 45.7, 44.8	47.7	1.91	0.67

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-) and females (sla/sla)	0-100	29.4*, 38*, 33.1*, 33.8*, 38.5*, 23.7, 38*, 33.2*, 30.9*, 35.5*, 33*, 29*, 43.4, 35.2, 31.3*, 35.2*, 32.1*	33.7	4.50	1.09
	101-200	30.5*, 32.3*, 36.5*, 34.4, 33.2, 42.3, 43.2, 41.9, 22.6*, 31.7*, 33.1*, 38.1, 39.4, 37.4	35.5	5.51	1.47
	201-300	39.5, 34.9, 38.6, 37*, 36, 29.8, 37.4 30.9*, 31.1*, 30.5*	38.4	6.19	2.17
	301-400	48*, 40.2*, 47.6*, 33.3*, 40.7*, 41.7*, 42.9*, 45.7*	42.5	4.78	1.68
Carrier females (sla/+)	0-100	52.2*, 50*, 40.3*, 45.9*	47.1	5.22	2.61
	101-200	46.8*, 47.4*, 41.0*, 49*	46.1	3.47	1.74
	201-300	48.5*	48.5	---	---
	301-400	42.8*, 47.7*, 46.2*, 45.9*, 47*	45.9	1.88	0.84

* Estimations from animals of the original mixed stock. Other values for the mean cell volume were obtained from the second back cross generation hybrids of the original mixed stock and the C57Bl/6J strain.

6. Assessment of Morphological Abnormality Seen in Blood Films

All results were obtained from mice of the original mixed stock

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
1'	sla/-	43	6.9	2	3	1	1	3	10
3'	sla/-	51	5.9	2	4	2	3	2	13
3'	sla/-	114	8.1	2	3	1	3	2	11
3'	sla/-	379	15.2	1	-	-	-	-	1
7	sla/-	238	10.0	1	2	2	2	2	9
12	sla/sla	79	5.0	3	3	2	3	2	13
12'	sla/+	186	17.3	1	-	-	-	-	1
15	sla/-	260	10.0	2	2	1	2	2	9
15'	sla/-	33	4.3	4	4	3	4	4	19
16'	sla/-	43	6.9	2	3	2	2	3	12
18	sla/+	337	14.8	3	1	-	-	1	5
20	sla/+	337	14.3	1	1	-	-	1	3
21	sla/-	80	2.8	2	4	3	4	4	17
22	sla/-	21	7.9	3	2	1	1	3	10

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
23	sla/-	59	7.3	2	3	2	3	2	12
23	sla/-	80	7.1	2	3	1	4	2	12
24	sla/+	270	13.8	1	-	-	-	-	1
25"	+/-	313	14.1	-	-	-	-	-	0
26'	sla/sla	342	14.5	1	1	-	-	1	3
31	+/-	44	16.4	1	1	1	1	1	5
31	+/-	66	15.1	1	-	-	-	-	1
32	sla/+	151	14.2	1	1	-	-	1	3
33	+/-	66	14.1	1	1	-	-	1	3
34	sla/sla	146	11.9	2	2	1	1	2	8
35	+/-	44	17	1	1	-	-	-	2
35	+/-	79	15.2	1	-	-	-	-	1
36	sla/+	183	14.8	1	1	-	-	1	3
37	sla/-	239	7.6	3	3	2	3	2	13
37	sla/-	311	9.6	3	3	2	2	3	13
38	sla/+	59	14.8	1	1	-	-	-	2
38	sla/+	78	14.8	1	-	-	-	-	1

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
38	sla/+	170	15.6	1	1	1	-	-	3
40'	sla/sla	284	13.9	1	1	-	-	-	2
41	sla/+	381	15.6	-	-	-	-	-	0
42	sla/sla	170	14.8	1	1	1	1	-	4
43'	sla/-	207	15.2	1	1	-	-	-	2
45	sla/+	27	14.0	1	2	1	1	1	6
45'	sla/-	244	10.6	2	1	1	1	2	7
45'	sla/-	278	14.5	3	1	2	1	3	10
46	sla/-	239	9.2	2	3	2	2	2	11
49	sla/sla	199	5.4	2	4	2	4	3	15
51'	sla/-	80	6.5	3	3	2	2	4	14
51'	sla/-	259	9.2	2	2	1	2	3	10
52	sla/+	247	14.2	1	-	-	-	2	3
53	sla/-	159	8.6	3	4	3	4	3	17
53'	sla/sla	75	6.7	2	2	2	1	3	10
53"	sla/-	292	8.0	2	2	2	2	4	12
54	sla/sla	146	4.9	1	4	2	4	3	14

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
55'	sla/-	43	8.4	3	3	2	3	3	14
56	sla/sla	222	14.3	2	1	-	-	-	3
58	sla/sla	59	8.2	3	3	2	3	3	14
58	sla/sla	156	9.7	4	3	2	3	2	14
58	sla/sla	520	14.9	-	1	-	-	1	2
61	sla/-	255	13.6	2	1	1	1	2	7
62	sla/+	246	18.6	1	1	-	-	1	3
63	sla/-	162	9.1	2	3	1	2	2	10
63	sla/-	74	7.7	1	3	2	2	1	9
63	sla/-	462	15.2	1	2	1	1	2	7
64	sla/+	162	16.2	2	1	-	-	1	4
65	sla/-	162	12.4	2	1	1	1	2	7
66	sla/sla	269	15.2	2	1	1	-	1	5
66'	sla/sla	279	12	1	-	-	-	1	2
71	sla/sla	41	4.7	3	3	2	3	2	13
71'	sla/-	308	12.6	-	1	-	-	1	2
72	+/+	84	14.3	1	2	-	-	1	4

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
73	sla/-	72	6.8	2	4	3	3	2	14
74	sla/sla	65	6.3	3	4	1	3	4	15
75'	sla/-	211	15.2	1	1	-	-	-	2
76	sla/sla	270	11	1	1	-	-	-	2
78	sla/+	39	12.7	3	2	1	1	2	9
78	sla/+	65	13.8	2	2	1	1	2	8
79	sla/-	292	13.4	1	1	1	-	1	4
80	sla/+	56	12.8	1	2	-	1	1	5
80	sla/+	128	16.2	1	1	-	-	-	2
82	sla/sla	66	9.7	2	2	1	3	4	12
82	sla/sla	160	11.9	2	2	1	3	3	11
82	sla/sla	284	16.0	-	-	-	-	1	1
83	+/-	162	15.5	1	-	-	-	-	1
84	sla/+	83	14.4	1	1	-	-	1	3
84	sla/+	146	16.3	1	1	-	-	-	2
85	+/+	272	13.9	1	-	-	-	1	2
90	sla/sla	222	14.3	1	1	-	-	2	4

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
91	sla/+	66	14.6	1	1	-	-	-	2
92	sla/+	38	8.6	3	2	1	2	2	10
92	sla/+	66	12.2	2	2	1	1	1	7
92	sla/+	207	15.2	1	-	-	-	-	1
94	sla/+	66	13.8	2	2	1	1	1	7
95	sla/sla	397	11.2	-	-	1	2	-	3
96	sla/sla	52	4.5	3	4	3	4	2	16
96	sla/sla	150	7.2	2	4	2	4	2	14
96	sla/sla	416	6.1	1	2	1	-	1	5
98	sla/sla	66	6.6	2	3	2	3	3	13
101	+/-	214	14.7	1	-	-	-	-	1
101	+/-	238	14.7	1	-	-	-	-	1
103	+/-	214	12.3	2	1	-	1	-	4
105	sla/-	185	10.6	2	2	1	3	2	10
107	sla/-	185	12.2	2	2	1	-	1	6
109	sla/-	266	14.4	1	-	-	-	-	1
123	+/-	130	13.8	-	1	-	-	1	2

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
123	+/-	156	14.7	-	1	-	-	-	1
132	sla/+	73	15.6	1	-	-	-	-	1
134	sla/+	214	15.2	1	1	-	-	1	3
136	sla/+	240	15.2	1	-	-	-	-	1
146	sla/+	196	14.7	1	1	-	-	1	3
147	sla/-	255	11.6	3	3	2	3	2	13
150	sla/sla	167	13.4	2	3	2	2	2	11
153	+/-	79	16	-	1	-	-	-	1
153	+/-	296	13.8	-	1	-	-	-	1
153	+/-	365	13.1	-	1	-	-	1	2
155	sla/-	267	14.1	-	1	-	-	-	1
159	+/-	296	13.3	-	-	-	-	-	0
165	sla/-	170	6.8	3	2	1	2	3	11
167	sla/-	95	7.4	3	4	3	4	3	17
171	+/-	75	14.4	1	-	-	-	1	2
171	+/-	169	14.5	-	1	-	1	-	2
178	sla/-	70	10.8	3	3	2	2	3	13

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
191	sla/-	172	9.9	1	3	2	4	3	13
193	sla/-	75	7.3	2	3	2	2	2	11
193	sla/-	109	9.6	2	3	1	2	3	11
194	sla/sla	75	6.6	2	4	3	3	4	16
195	+/-	216	13.8	1	1	-	-	1	3
196	sla/sla	75	8.6	2	3	2	2	2	8
196	sla/-	110	9.4	2	2	1	2	2	9
197	+/-	144	15.8	-	-	-	-	-	0
197	+/-	216	13.2	-	1	-	1	1	3
507	sla/-	32	7.2	2	3	1	2	3	11
507	sla/-	56	7.2	4	3	3	4	4	18
507	sla/-	104	8.5	4	3	1	3	3	14
508	sla/sla	32	7.2	2	3	1	1	3	10
510	sla/+	56	16.1	2	2	-	-	1	5
511	sla/-	32	6.5	2	3	2	2	2	11
511	sla/-	56	5.9	3	4	2	3	3	15
511	sla/-	104	8.1	2	2	1	2	3	10

Mouse Number	Genotype	Age (days)	Hb. (G/100 ml)	T*	A*	P*	F*	H*	"Film Score"
512	sla/+	32	11.8	1	2	-	1	1	5
512	sla/+	56	15.6	1	1	-	-	-	2
516	sla/+	50	13.8	1	1	-	-	-	2
517	sla/-	31	7.2	2	2	1	2	1	8
517	sla/-	79	8.5	3	4	2	3	2	14
521	sla/-	31	7.6	2	4	1	2	3	12
521	sla/-	79	8.5	3	3	2	3	4	15
525	sla/-	31	6.7	3	4	2	4	4	17
525	sla/-	79	9.0	2	3	2	3	3	13

* T - Target cells, A - Anisocytosis, P - Poikilocytosis, F - Fragmented cells, H - Hypochromia.

7. Reticulocyte Counts

All results obtained from the original mixed stock

a. Normal (+/-) mice:

Mouse Number	Age (days)	Hb (G/100 ml)	P.C.V.	Reticulocytes (%)
10"	305	16.5	50	1
33	39	15.0	53	5
35	31	--	47	9
101	142	15.6	46	1
101	295	14.7	45	1
109	206	14.8	49	2
141	228	14.7	46	4
143	218	13.6	48	1
157	235	14.3	46	2
159	236	13.0	48	1
161	219	13.4	51	2
195	80	14.5	48	4
197	80	14.8	46	5
b. Carrier (sla/+) female mice				
36	57	--	49	10
38	44	--	40	1
78	31	12.7	--	8
78	39	13.7	43	5
91	31	--	--	5
92	374	15.8	48	2
94	31	--	37	11

b. Carrier (sla/+) female mice (continued)

Mouse Number	Age (days)	Hb (G/100 ml)	P.C.V.	Reticulocytes (%)
94	38	--	48	5
112	142	16.3	50	1
146	139	15.0	46	2
146	150	15.3	51	2
164	235	14.7	50	1
166	235	16.5	54	1
c. Anaemic female (sla/sla) mice				
4'	284	16	46	2
4'	347	13.4	42	3
12	51	4.2	18	6
17'	265	13.8	44	1
26'	294	14.1	49	1
30	304	14.1	45	2
40'	284	13.9	45	2
40'	299	15.6	--	1
66'	236	15	46	1
66'	298	15.2	47	4
68	304	13.6	43	2
68'	352	13.8	45	3
69	305	13.6	44	1
69	352	14.4	44	2
74	52	--	25	4
82'	236	15.6	47	1
96	395	9.2	34	7

c. Anaemic female (sla/sla) mice (continued)

Mouse Number	Age (days)	Hb (G/100 ml)	P.C.V.	Reticulocytes (%)
150	139	11.7	42	2
194	75	6.6	29	6
194	122	--	--	1
194	127	--	31	3
196	75	8.6	36	3
196	117	--	32	3
196	122	--	--	4

d. Anaemic male (sla/-) mice

3'	349	14.5	50	1
5'	219	13.8	43	2
16	48	--	26	17
21'	372	15.2	55	1
33	215	4.7	24	1
37	275	9	33	8
37	347	10.8	37	3
43'	321	15.2	52	1
45'	244	10.6	38	3
46'	347	9.9	30	5
51'	295	9.0	34	3
53	57	--	25	6
53"	262	2.9	18	2
53"	328	8.1	38	5
63	59	--	--	23

d. Anaemic male (sla/-) mice

Mouse Number	Age (days)	Hb (G/100 ml)	P.C.V.	Reticulocytes (%)
67	140	5.7	31	1
71'	277	13.6	42	1
71'	343	15.2	48	1
73	57	--	22	12
75'	357	16.5	46	3
103	142	10.8	33	1
103	220	12.5	38	1
105	149	7.6	32	1
107	143	11.7	37	4
107	149	12.7	--	4
107	206	--	--	2
125	108	10.3	25	8
129	215	9.7	37	4
129	266	11.6	36	4
131	215	5.5	24	2
131	266	3.7	28	4
133	265	--	--	2
147	225	9.7	41	3
147	275	--	39	4
155	267	--	38	4
165	206	6.8	28	6
167	101	5.3	27	16
167	116	4.8	24	19
167	206	5.0	28	20
173	150	--	49	1

d. Anaemic Male (sla/-) mice (continued)

Mouse Number	Age (days)	Hb (G/100 ml)	P.C.V.	Reticulocytes (%)
191	186	--	37	5
193	75	7.3	--	21
193	89	9.6	40	17
193	117	--	35	5
193	122	--	--	2

The coefficient of correlation between the reticulocyte count and the packed cell volume in anaemic mice (r) = -0.48.

8. Counts of Polychromatophilic Cells and Reticulocytes (per cent)

All results obtained from mice of the original mixed stock

Mouse Number	Genotype	Polychromatophilic Cells	Reticulocytes
3'	sla/-	8	1
4'	sla/sla	1	2
21'	sla/-	1	1
26'	sla/sla	1	1
37	sla/-	3	8
37	sla/-	4	3
38	sla/+	1	1
40'	sla/sla	1	1
43'	sla/-	1	1
45'	sla/-	1	3
51'	sla/-	4	3
53"	sla/-	5	2
63	sla/-	20	23
66'	sla/sla	1	1
68'	sla/sla	3	3
69	sla/sla	1	1
71'	sla/-	2	1
73	sla/-	10	12
74	sla/sla	30	4
75'	sla/-	2	3
78	sla/+	2	5

Mouse Number	Genotype	Polychromatophilic Cells	Reticulocytes
94	sla/+	2	5
96	sla/sla	10	7
96	sla/sla	2	2
103	sla/-	1	1
105	sla/-	1	1
107	sla/-	1	2
109	+/-	1	2
112	sla/+	1	1
146	sla/+	1	2
147	sla/sla	1	3
150	sla/sla	1	2
155	sla/-	1	4
159	+/-	1	1
165	sla/-	11	6
167	sla/-	45	19
173	sla/-	1	1
191	sla/-	3	5
193	sla/-	5	21
193	sla/-	2	5
194	sla/sla	3	6
196	sla/sla	5	3

The correlation coefficient of the reticulocyte count and count of polychromatophilic cells (r) = +0.66.

9. Osmotic Fragility Determinations

Results were obtained from mice of the original mixed stock and from hybrid animals (see below)

Genotype	Haemolysis starts % NaCl	20% Haemolysis % NaCl	50% Haemolysis % NaCl	80% Haemolysis % NaCl	Haemolysis complete % NaCl
Normal	0.56	0.36	0.34	0.30	0.08
	0.82	0.47	0.43	0.39	0.14
	0.84	0.47	0.44	0.41	0.13
	0.60	0.47	0.43	0.39	0.13
	0.55	0.40	0.36	0.34	0.14
	0.54	0.38	0.36	0.31	0.18
	0.60	0.46	0.42	0.36	0.20
	0.59	0.49	0.46	0.43	0.15
	0.80	0.49	0.44	0.40	0.13
*	0.64	0.50	0.44	0.39	0.09

Genotype	Haemolysis starts % NaCl	20% Haemolysis % NaCl	50% Haemolysis % NaCl	80% Haemolysis % NaCl	Haemolysis complete % NaCl
Carrier*	0.70	0.50	0.45	0.38	0.16
*	0.82	0.50	0.46	0.42	0.12
Anaemic*	0.70	0.42	0.30	0.21	0.06
*	0.73	0.50	0.36	0.23	0.06
*	0.64	0.35	0.22	0.16	0.00
*	0.64	0.40	0.30	0.12	0.02
*	0.74	0.45	0.38	0.30	0.06
*	0.56	0.27	0.14	0.06	0.00
*	0.55	0.40	0.33	0.26	0.00

* Estimations from animals of the original mixed stock. Other values were obtained from first and second backcross generation hybrids of the original mixed stock and the C57Bl/6J strain.

10. Total and Differential White Cell and Platelet Counts (normal mice)

All results obtained from the original mixed stock

Genotype	Total white cell count /cu.mm.	Lymphocytes /cu.mm.(%)	Neutrophils /cu.mm.(%)	Monocytes /cu.mm.(%)	Eosinophils /cu.mm.(%)	Platelets /cu.mm.
+/-	3,200	1,664 (52)	1,400 (44)	128 (4)	---	230,000
+/-	4,500	3,465 (77)	945 (21)	45 (1)	45 (1)	240,000
+/-	4,100	2,747 (67)	1,066 (26)	205 (5)	82 (2)	260,000
+/-	4,600	3,220 (70)	1,196 (26)	92 (2)	138 (3)	300,000
+/-	7,400	3,552 (48)	3,404 (46)	370 (5)	74 (1)	340,000
+/-	15,300	12,852 (84)	2,142 (14)	612 (4)	---	210,000
+/-	4,200	2,856 (68)	1,218 (29)	42 (1)	84 (2)	170,000
+/-	7,000	4,060 (58)	2,730 (39)	210 (3)	140 (2)	380,000
+/-	5,500	3,355 (61)	1,815 (33)	165 (3)	165 (3)	420,000
+/-	3,300	1,245 (65)	1,023 (31)	66 (2)	66 (2)	220,000

Genotype	Total white cell count /cu.mm.	Lymphocytes /cu.mm.(%)	Neutrophils /cu.mm.(%)	Monocytes /cu.mm.(%)	Eosinophils /cu.mm.(%)	Platelets /cu.mm.
sla/-	4,900	3,185 (65)	1,470 (30)	245 (5)	---	120,000
sla/-	5,600	4,032 (72)	1,456 (26)	112 (2)	---	350,000
sla/-	4,900	3,381 (69)	1,421 (29)	98 (2)	---	210,000
sla/-	5,100	3,570 (70)	1,377 (27)	102 (2)	51 (1)	240,000
sla/-	3,500	3,010 (86)	420 (12)	70 (2)	---	340,000
sla/-	3,600	2,592 (72)	936 (26)	72 (2)	72 (2)	---
sla/-	4,700	2,124 (36)	2,773 (59)	94 (2)	188 (4)	480,000
sla/-	4,700	2,820 (60)	1,504 (32)	235 (5)	141 (3)	320,000

11. Determinations of Weight at Different Ages (in Gm.)

All results were obtained from the original mixed stock

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Normal males (+/-)	21-30	17	17	---	---
	31-40	22, 23	22.5	---	---
	41-50	23.2, 26.5, 25.8	25.2	1.4	0.84
	51-60	26, 27, 35.5, 26.7	28.8	3.9	1.9
	61-70	---	---	---	---
	71-80	30.5, 29.5, 29.5, 22.3, 34.5, 32.6	29.8	3.8	1.6
	81-90	---	---	---	---
	91-100	---	---	---	---
	101-150	30, 30.5, 29.7, 33.5, 33.9	31.5	1.8	0.82
	151-200	37.5, 34, 33.9, 30, 30.2	33.1	2.8	1.3
	201-250	29.7, 36.5, 40.5, 45, 35, 32.5, 36, 36.7	36.5	4.4	1.6
	251-300	42, 36, 36.3, 39.6, 42	39.2	3.1	1.4
	301-400	34, 36.9, 37.5, 40, 38	37.3	1.9	0.88

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-)	21-30	10, 10.5, 10.7, 12, 12, 12.2, 12.5 15.5, 16.5, 19.7	13.2	3.2	1.1
	31-40	10.9, 14.5, 16.7, 14.1, 15.2, 15.6, 13.5, 12.0, 9.5, 12, 12.7, 12, 13, 13.3, 12, 12.7, 14.5, 15, 16.5, 15.7, 15, 20, 20, 16.9	14.3	2.5	0.51
	41-50	10.5, 13.5, 16.2, 18, 18.5, 19.7, 16.1, 20.2, 21.8, 23, 22.6, 17.7, 21	18.4	4.2	1.2
	51-60	18.5, 9.8, 21.2, 24.4	18.5	5.4	2.9
	61-70	9, 9.5, 10, 11.5, 12.2, 10, 12.3, 13, 29.5, 24.5, 22.5	14.9	6.8	2.1
	71-80	19.5, 11, 26.3, 21.5, 13, 12.5, 26.5, 16, 20.2, 20.7	18.7	5.2	1.6
	81-90	19.8, 15.1	17.5	---	---
	91-100	14, 14.6, 15, 24, 29.8, 30.5, 32.5, 32.6, 34, 20.7, 21.7	23.5	7.4	2.3

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic males (sla/-)	101-150	25.2, 28.7, 17, 20.5, 22.5, 23.7, 26.5, 29.5, 30, 30, 30.5, 31, 31.5, 31.5, 29.9, 24.4, 24.7, 26.8, 25.3, 27, 32, 32.5, 33.4, 33.6, 33.5, 34.1, 34.5, 35.1, 35.4, 38, 40.1	29.6	5.0	0.89
	151-200	25.7, 27.2, 28, 29, 29.5, 30.1, 30.4, 31, 31.5, 32, 33.5, 34, 35.5, 33, 36.1, 31, 30	31.6	3.2	0.76
	201-250	26, 26.5, 27.2, 27.5, 28.5, 28.7, 29.6, 30, 30.7, 31, 31, 33, 35, 35, 35.5, 36, 37, 37.2, 39, 27.6, 35.2, 34, 36.5, 31.3, 28.5	31.9	3.8	0.76
	251-300	25, 28.5, 28.8, 29, 29.5, 30.1, 30, 31, 32.2, 32.6, 33, 33.2, 35.3, 35.7, 37.5, 38.5, 39, 28.5, 36, 36.5, 37.7, 31.4, 30, 31.4	32.5	3.7	0.76
	301-400	23, 22.5, 26, 26.5, 28.5, 31.2, 31.7, 33, 35, 35, 35.5, 36, 36, 38.3, 40.2, 27.3, 28.3, 30.3, 39.2, 38.2, 42.3	32.6	4.9	1.1

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic females (sla/sla) non-pregnant	21-30	8, 8.5, 10, 12, 13, 14.5, 18.2, 18.5, 11.5, 12.4	13.2	3.2	1.1
	31-40	6.5, 10.5, 11, 11.2, 11.2, 11.5, 12.5, 14, 14.5, 15, 15, 15.5, 15.5, 16.2, 19, 15.4, 14, 15.1, 16.3, 10.5, 16.5, 14.5, 12.3, 10.7, 8.6	13.9	3.1	0.67
	41-50	12.8, 16.1, 19.7, 18.5, 9.8, 7, 8.5, 9.5, 11.5, 12.4, 14.5, 15, 16.5, 17.5, 19.5, 20.5, 21, 19.3, 20.6, 18.8	15.2	4.7	1.0
	51-60	14, 15.8, 18.7, 20.2, 14.9, 19, 20, 21.7	18.0	2.6	0.91
	61-70	14.5, 19, 19.5, 20, 21, 19.5, 19.5, 22.6	19.5	2.0	0.71
	71-80	14.2, 15.6, 16.2, 20.2, 24.2, 25, 23.5, 23, 22, 26, 30	21.8	4.6	1.4
	81-90	18.6, 22.6, 24.5, 25.7, 26, 27.7, 27.8 23, 22.3, 21.7	23.7	3.4	1.1
	91-100	22, 26.8, 27.3, 28.5, 29.2, 24.8, 23.6	26.0	2.5	0.90

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Anaemic females (sla/sla) non-pregnant cont'd	101-150	12.3, 16, 16.5, 20.1, 22, 22.5, 24, 25.5, 26, 27.2, 27.5, 27.7, 28, 28, 24.4, 25.5, 28.7, 28.7, 29, 29.5, 29.5 32, 33, 24.3, 24.2, 22.5, 25, 26, 24.2	25.2	4.6	0.89
	151-200	23, 23, 23.5, 24, 26.5, 27.5, 28, 25.1, 31.5, 25.5, 31.2, 29.2, 34.6, 29.1	27.3	3.4	0.91
	201-250	23.7, 24.5, 25.2, 27.5, 28, 29, 30.5, 32, 20.1, 26.2, 30.7, 37, 24.7, 27.4	27.6	4.0	1.1
	251-300	23, 24, 24.5, 25, 25.6, 26.2, 26.9, 27, 27.5, 27.9, 28, 28.2, 33, 34, 38, 19.9, 25.8, 30, 38.4, 28.2, 27.3, 28.6, 27	28.1	4.3	0.90
	301-400	23.7, 23.8, 24, 24.5, 26.5, 30, 30.5, 31.2, 31.2, 32, 32.3, 35, 36, 36, 36.5, 38, 39.8, 40, 40.2, 45.8, 21, 24.6, 27.2, 31.4, 33.5, 29.1, 29.2	31.6	6.0	1.2

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Carrier females (sla/+) non-pregnant	21-30	9, 16, 10.5, 11.5	11.8	2.6	1.3
	31-40	19, 19.8, 13.4, 14.5, 19.9, 18.2, 14.4 14.7, 13.4	16.4	2.9	0.97
	41-50	17.2, 18.5, 18.8, 19.9, 17, 21.2, 13, 20, 18.7, 20.1, 22.2, 15.3	18.5	2.5	0.70
	51-60	20.3, 18, 20, 20, 20.5, 21, 21.2, 21.5, 24, 20.4, 18.5, 23, 22.8, 24.2, 20	21.0	1.8	0.45
	61-70	17, 20.2, 18	18.4	1.3	0.78
	71-80	22, 23, 26, 17.2, 23.1, 22.9, 20.1	22.0	2.5	0.98
	81-90	18.5, 23, 22.5, 24.5, 28.2, 24.5, 24.6	23.7	2.7	1.0
	91-100	19.5, 24, 25.7, 25.8, 22.7, 23.8	23.6	2.1	0.88

Genotype	Age (days)	Observations	Mean	Standard Deviation	Standard Error
Carrier females (sla/+) non-pregnant cont'd	101-150	18.5, 19, 21, 21.7, 22.5, 22.5, 23, 23, 23, 23.5, 24, 24.1, 24, 24.5, 24.5, 28.6, 23.7, 27.6, 31.7, 25, 25.5, 26, 29.5, 31.5, 33.5, 36, 27.1, 25.1, 25.7, 22, 26.5, 35, 27	25.4	4.1	0.72
	151-200	24, 33.5, 34, 34, 27.7, 23.4, 28.3, 28.6, 25.4	28.8	3.9	1.3
	201-250	37.6, 29.2, 23.5, 24, 24.5, 25, 25, 26, 29.7, 29.7, 36, 28, 28.1, 28.7, 26.6, 29.5	28.3	4.1	1.1
	251-300	24, 27.5, 33.6, 28, 38	30.2	4.8	2.2
	301-400	28, 30, 33.5, 34, 35, 38, 26.5, 34.9 33.3, 31.8, 34.5, 35.5, 28	32.5	3.3	0.92

12. Effect of Anaemia on Weight Gain During Period 30-85 Days of Age (hybrid animals)

Genotype and Number	Strain* and age in days at start		Mean Hb. conc. G/100 ml.**	Weight Gain (G.)	Period days	Weight gain/day (G.)
sla/- 1243	F_4	37	4.6	2.7	23	0.117
sla/- 1251	F_4	35	8.9	9.5	49	0.194
sla/- 1283	F_4	35	6.9	5.3	41	0.129
sla/- 1275	F_4	35	6.8	5.3	41	0.129
sla/- 1273	F_4	37	7.6	5.6	41	0.137
sla/- 1289	F_4	35	6.6	5.8	41	0.141
sla/- 1345	F_4	30	5.7	7.5	28	0.268
sla/- 1253	F_2	35	8.6	8.4	41	0.205
sla/- 1255	F_2	35	5.9	8.5	41	0.207
sla/- 1259	F_2	35	8.1	4.5	41	0.110
sla/- 1317	F_2	31	5.9	10.1	29	0.348
sla/- 1319	F_2	31	5.7	10	29	0.345

Genotype and Number	Strain* and age in days at start		Mean Hb. conc. G/100 ml.**	Weight Gain (G.)	Period days	Weight gain/day (G.)
sla/- 1329	F_2	31	6.3	8.7	29	0.300
sla/- 1335	F_2	32	6.9	9.4	29	0.324
sla/- 1339	F_2	31	7.7	10.7	29	0.367

*F_2 - F_1 hybrid of original mixed stock backcrossed to C57Bl/6J strain

F_4 - F_3 hybrid backcrossed to C57Bl/6J strain

**Mean of two readings at the beginning and end of the period of study

For all animals coefficient of correlation of weight gain and haemoglobin concentration (r) = -0.157

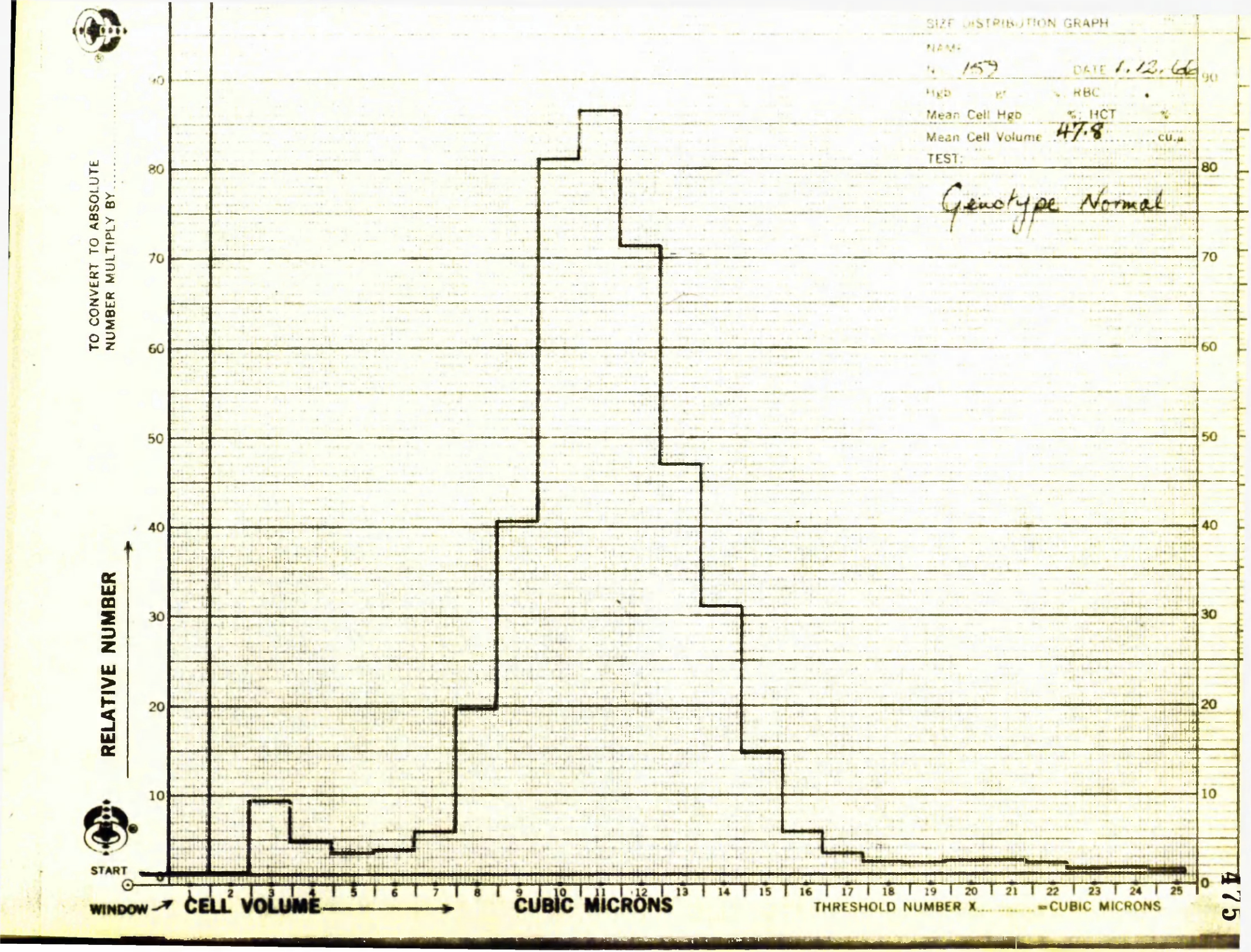
For F_2 animals r = -0.480

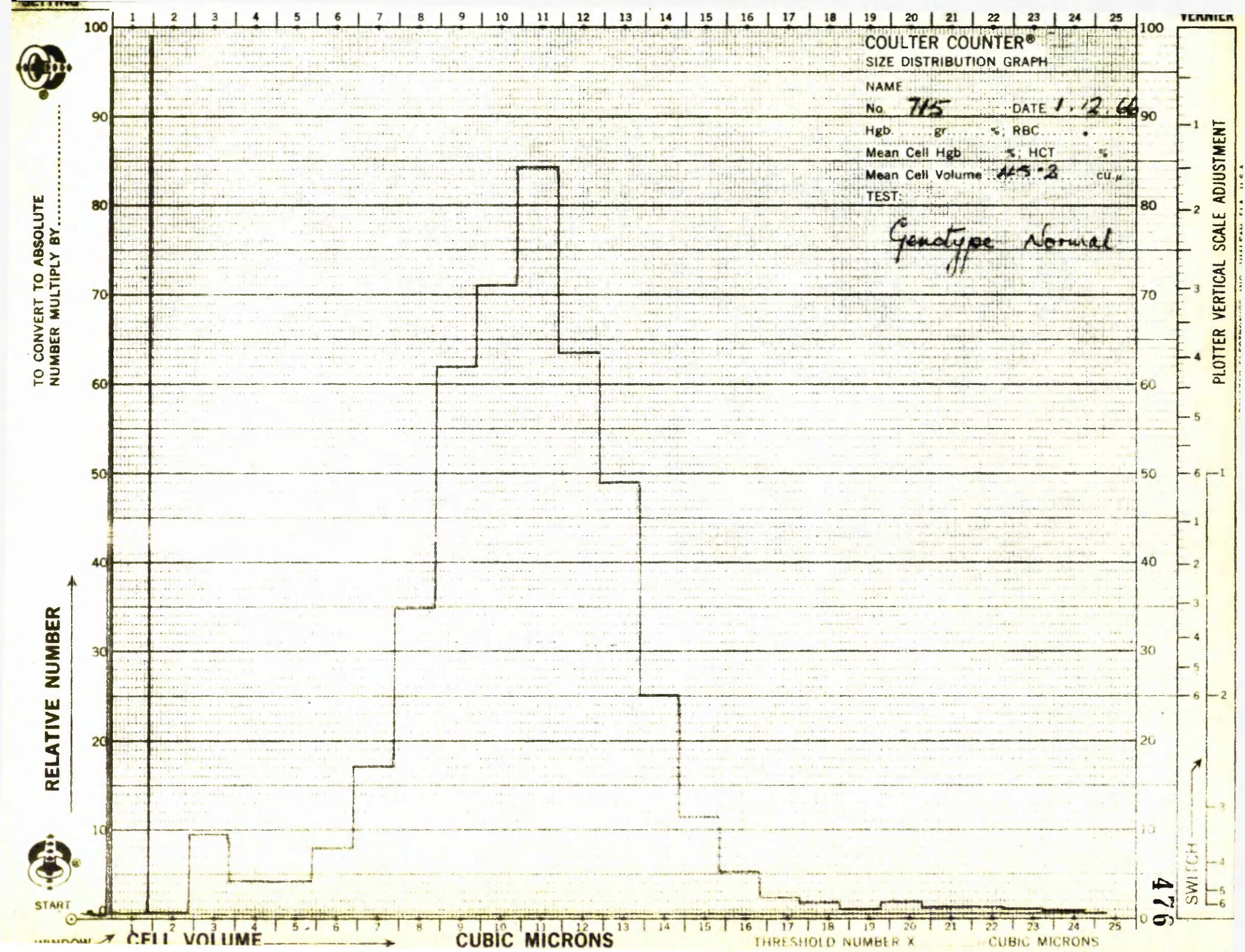
For F_4 animals r = +0.066

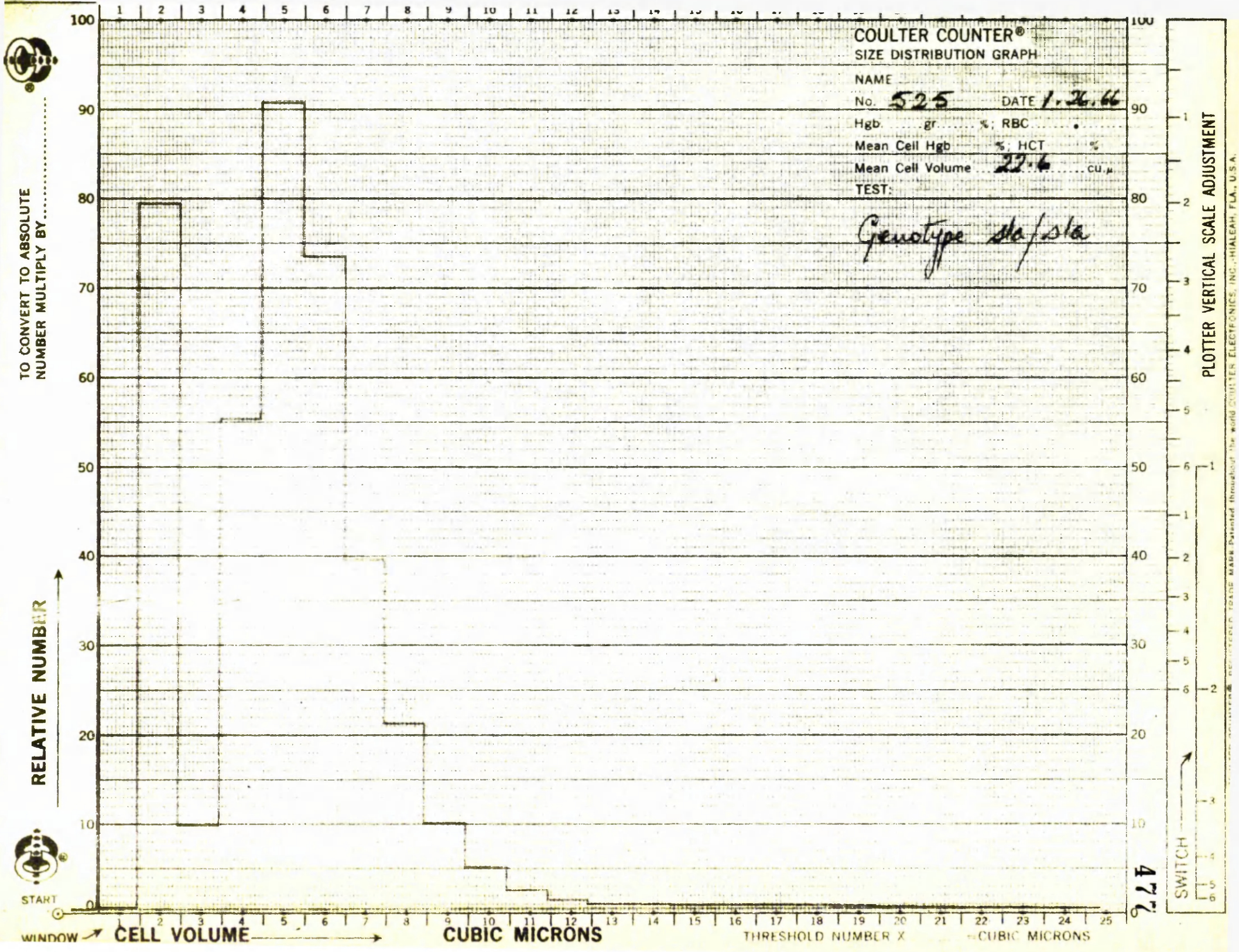
13. Representative examples of red cell size distribution curves obtained with the Coulter model J particle size analyser

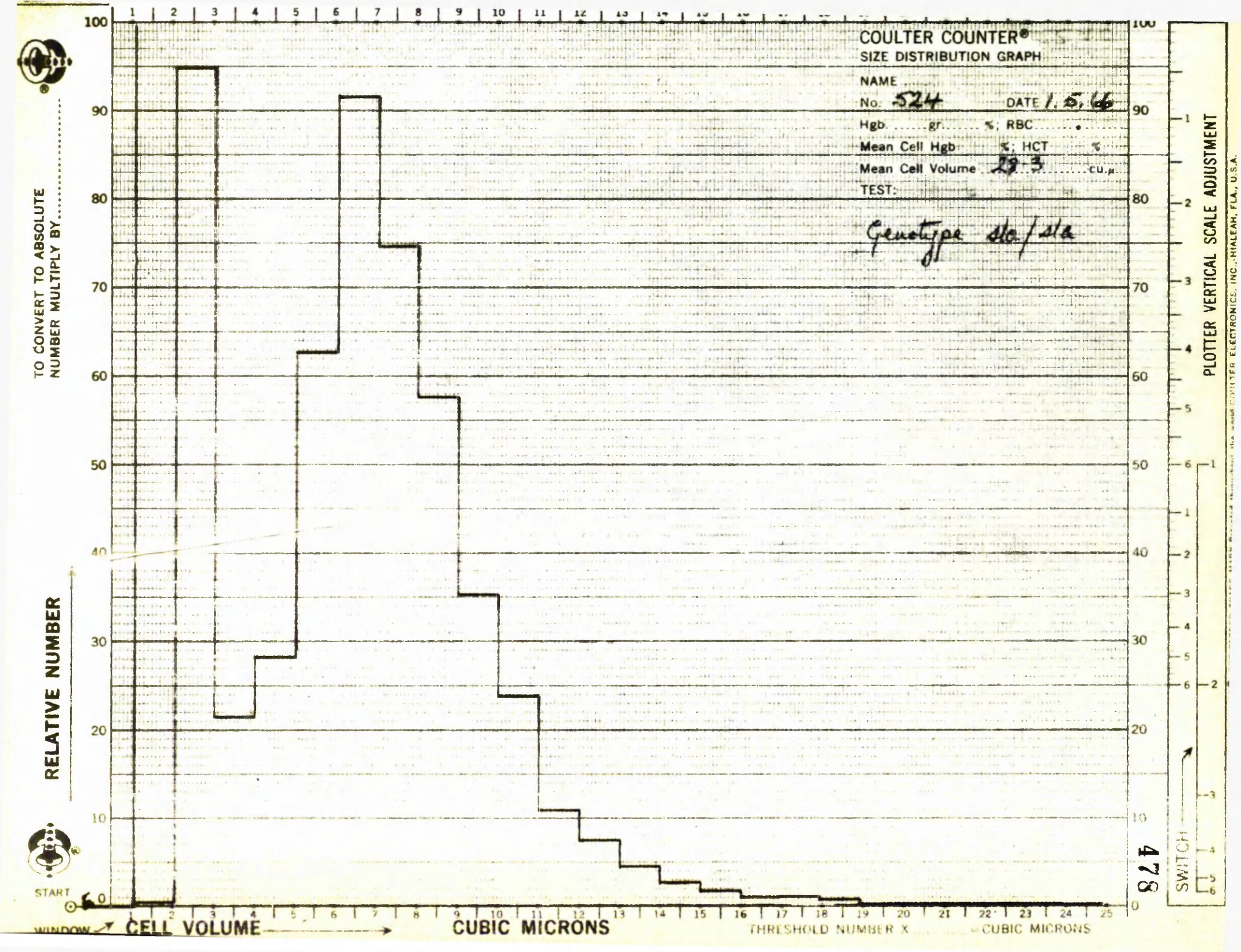
1. Normal male mouse, No. 159, +/-
   Mean cell volume, 47.8 cu. μ.
2. Normal male mouse, No. 715, +/-
   Mean cell volume, 45.2 cu. μ.
3.* Young anaemic female mouse, No. 525, sla/sla
   Mean cell volume, 28.3 cu. μ.
4.* Young anaemic female mouse, No. 524, sla/sla
   Mean cell volume, 28,3 cu. μ.
5.* Young heterozygous female mouse, No. 530, sla/+
   Mean cell volume, 45.9 cu. μ.
6.* Young heterozygous female mouse No. 528, sla/+
   Mean cell volume, 40.3 cu. μ.
7. Old "anaemic" male mouse after spontaneous recovery from anaemia (P.C.V., 44%), No. 173, genotype sla/-
   Mean cell volume, 47.3 cu. μ.

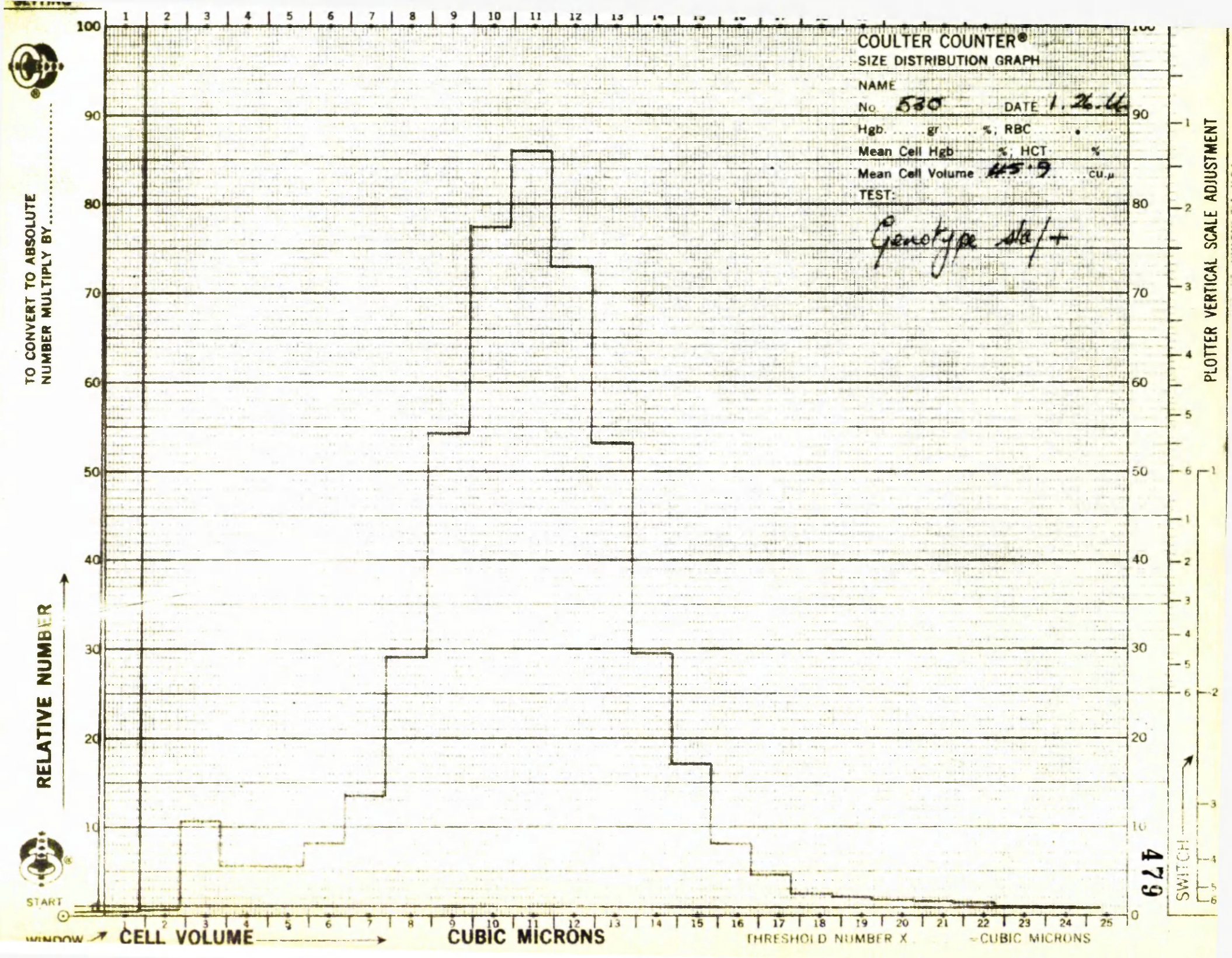
* Littermates

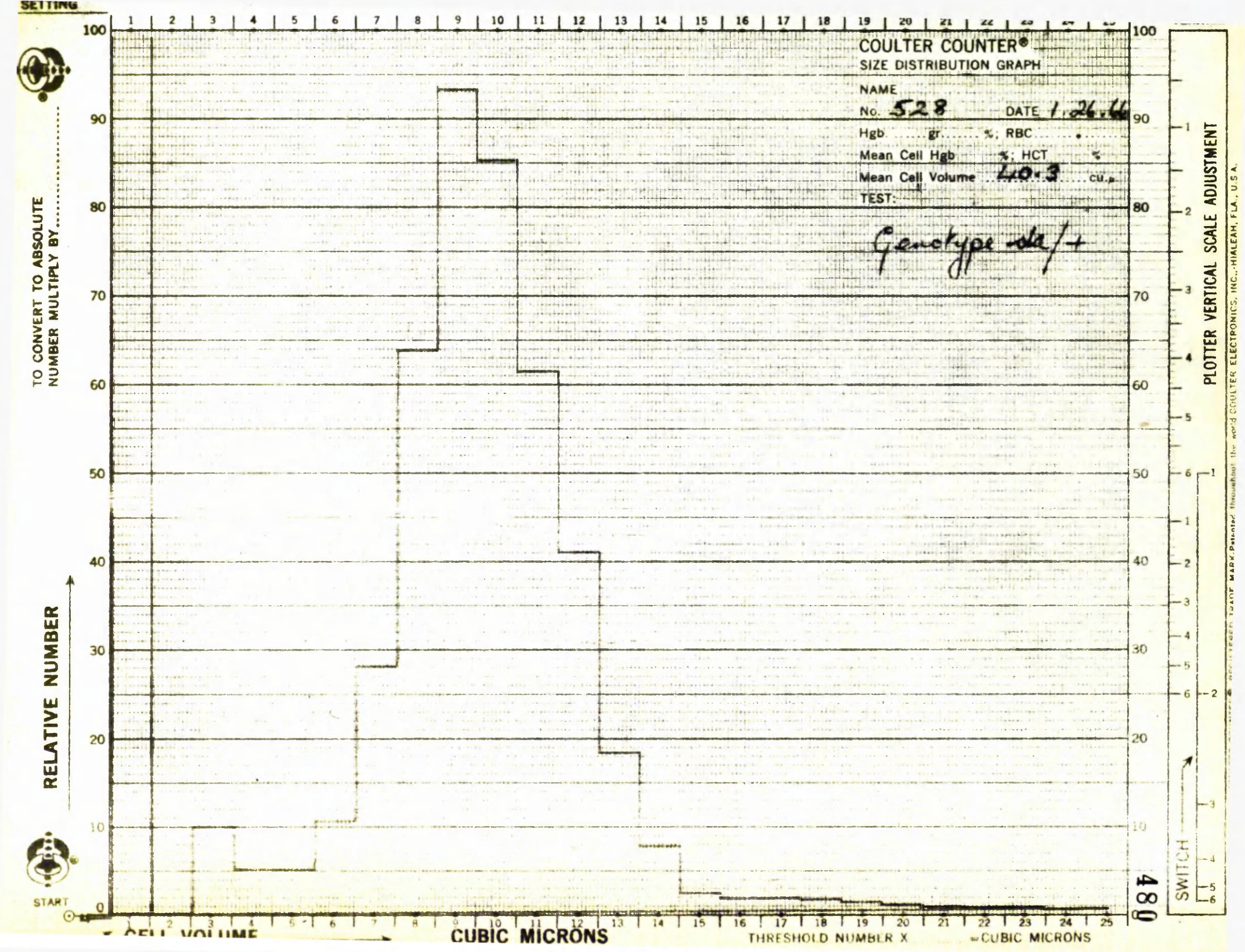

SIZE DISTRIBUTION GRAPH
NAME
No. 159 DATE 1.12.66
Hgb g% RBC
Mean Cell Hgb % HCT %
Mean Cell Volume 47.8 cu.μ
TEST:
Genotype Normal
TO CONVERT TO ABSOLUTE NUMBER MULTIPLY BY
RELATIVE NUMBER
0
10
20
30
40
50
60
70
80
90
START
WINDOW
CELL VOLUME
CUBIC MICRONS
1 2 3 4 5 6 7 8 9 10 11 12 13 14 15 16 17 18 19 20 21 22 23 24 25
THRESHOLD NUMBER X =CUBIC MICRONS

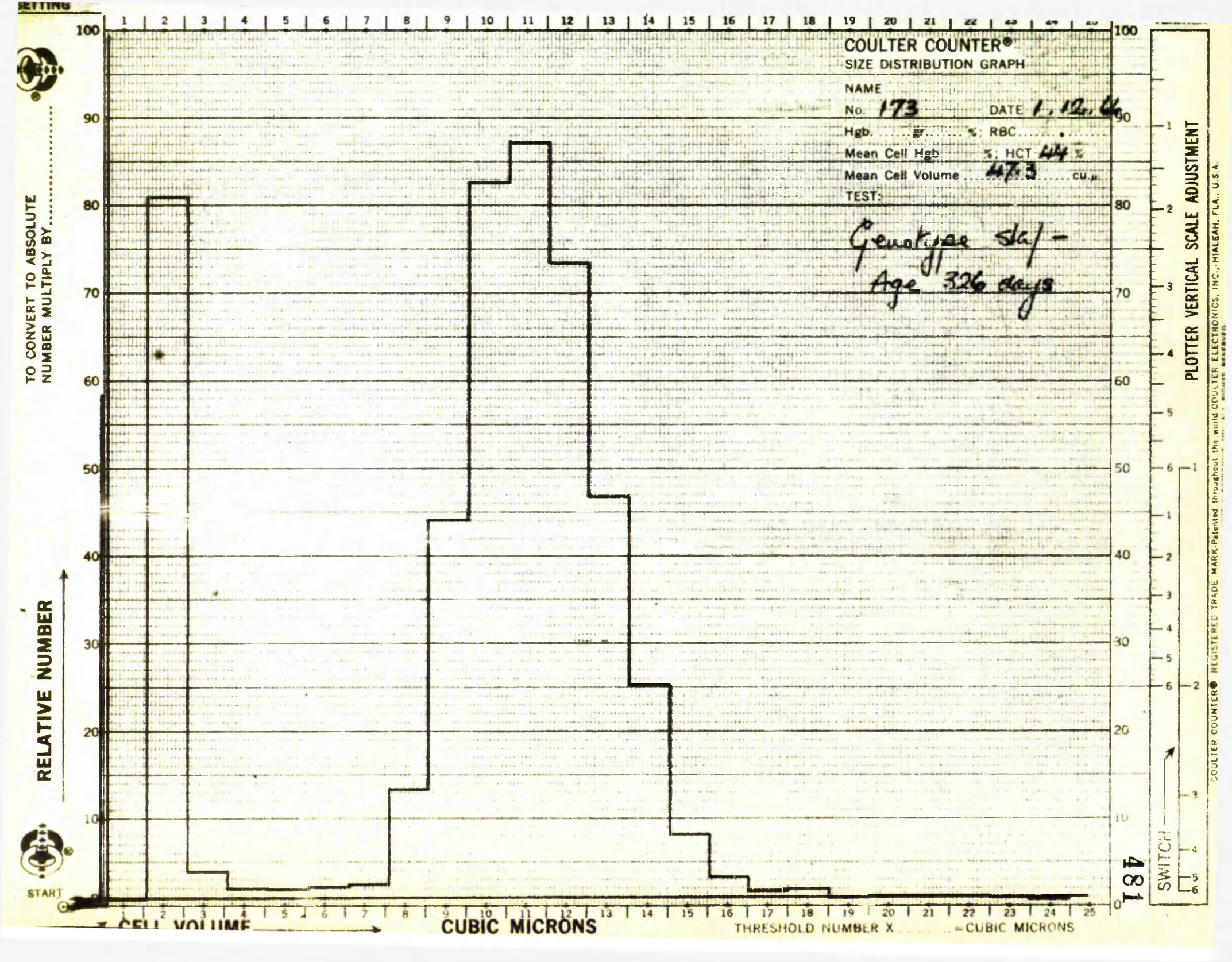

COULTER COUNTER®
SIZE DISTRIBUTION GRAPH
NAME
No. 715 DATE 1.12.66
Hgb. gr. %; RBC •
Mean Cell Hgb %; HCT %
Mean Cell Volume 115.2 cu.μ
TEST:
Genotype Normal
TO CONVERT TO ABSOLUTE NUMBER MULTIPLY BY.....
RELATIVE NUMBER
PLOTTER VERTICAL SCALE ADJUSTMENT
VERNIER
SWITCH
START
WINDOW
CELL VOLUME
CUBIC MICRONS
THRESHOLD NUMBER X CUBIC MICRONS
476


COULTER COUNTER®
SIZE DISTRIBUTION GRAPH
NAME
No. 525 DATE 1.26.66
Hgb. gr. %; RBC •
Mean Cell Hgb % HCT %
Mean Cell Volume 22.6 cu.μ
TEST:
Genotype sb/sb
TO CONVERT TO ABSOLUTE NUMBER MULTIPLY BY
RELATIVE NUMBER
PLOTTER VERTICAL SCALE ADJUSTMENT
SWITCH
START
WINDOW
CELL VOLUME
CUBIC MICRONS
THRESHOLD NUMBER X
CUBIC MICRONS
472


COULTER COUNTER®
SIZE DISTRIBUTION GRAPH
NAME
No. 524 DATE 1.5.66
Hgb. gr. %; RBC
Mean Cell Hgb %; HCT %
Mean Cell Volume 29.3 cu.μ
TEST:
Genotype sc/sc
TO CONVERT TO ABSOLUTE NUMBER MULTIPLY BY
RELATIVE NUMBER
PLOTTER VERTICAL SCALE ADJUSTMENT
SWITCH
START
WINDOW
CELL VOLUME
CUBIC MICRONS
THRESHOLD NUMBER X = CUBIC MICRONS
478


COULTER COUNTER®
SIZE DISTRIBUTION GRAPH
NAME
No. 530 DATE 1.26.66
Hgb. ..... gr ..... %; RBC .....
Mean Cell Hgb ..... %; HCT ..... %
Mean Cell Volume 45.9 ..... cu.μ
TEST:
Genotype dd/+
TO CONVERT TO ABSOLUTE NUMBER MULTIPLY BY.....
RELATIVE NUMBER
PLOTTER VERTICAL SCALE ADJUSTMENT
SWITCH
START
WINDOW
CELL VOLUME
CUBIC MICRONS
THRESHOLD NUMBER X ..... =CUBIC MICRONS
479


SETTING
COULTER COUNTER®
SIZE DISTRIBUTION GRAPH
NAME
No. 528 DATE 1.26.66
Hgb ..... gr..... %; RBC .....
Mean Cell Hgb ..... %; HCT ..... %
Mean Cell Volume 40.3 cu.μ
TEST:
Genotype da/+
TO CONVERT TO ABSOLUTE NUMBER MULTIPLY BY.....
RELATIVE NUMBER
START
CELL VOLUME
CUBIC MICRONS
THRESHOLD NUMBER X ..... = CUBIC MICRONS
PLOTTER VERTICAL SCALE ADJUSTMENT
SWITCH
480


COULTER COUNTER®
SIZE DISTRIBUTION GRAPH
NAME
No. 173 DATE 1, 12, 64
Hgb. ... %; RBC ...
Mean Cell Hgb ... %; HCT 44 %
Mean Cell Volume 47.3 cu.μ
TEST:
Genotype Std -
Age 326 days
TO CONVERT TO ABSOLUTE NUMBER MULTIPLY BY.....
RELATIVE NUMBER
START
CELL VOLUME
CUBIC MICRONS
THRESHOLD NUMBER X ... = CUBIC MICRONS
PLOTTER VERTICAL SCALE ADJUSTMENT
SWITCH
COULTER COUNTER® REGISTERED TRADE MARK-Patented throughout the world COULTER ELECTRONICS, INC., HIALEAH, FLA., U.S.A.
181

APPENDIX B

PATHOLOGICAL EXAMINATIONS

A. Normal mice

Mouse number 33; genotype +/-; age 546 days, Hb. concentration 13.4G/100 ml.; stock - O.M.S.

Spleen: The malpighian bodies appear normal and the red pulp occupies less than 25 per cent of the splenic tissue. Red cell precursors, granulocytes and megakaryocytes are present. Stainable iron is plentiful (grade 4), and is present in greater concentration in the malpighian bodies than in the red pulp.

Liver: The parenchyma is normal. One small haemopoietic focus is present. Stainable iron is not seen in Kupffer or parenchymal cells.

Mouse number 50; genotype +/-; age 502 days; haematocrit 31.5 per cent; stock - O.M.S.

Spleen: The malpighian bodies appear normal. The red pulp occupies 51-75 per cent of the splenic tissue. Red cell precursors, granulocytes and megakaryocytes are present. Stainable iron is plentiful (grade 4) and distributed predominantly in the malpighian bodies.

Liver: The parenchyma is normal. Haemopoietic foci are not seen and stainable iron is absent.

Mouse number 231; genotype +/-; age 593 days; Hb. concentration 15.1G/100 ml.; haematocrit - 52 per cent; stock - F_1 hybrid; body weight 34.4G.

Spleen: Weight 72 mg. or 0.21% of body weight. The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue; red cell precursors, granulocytes and megakaryocytes are present. Abundant stainable iron (Grade 4) is present, located mainly in the malpighian bodies.

Liver: The parenchymal cells are normal. Foci of haemopoiesis are not seen. Stainable iron is present in a few liver parenchymal cells, but not in Kupffer cells.

Bone marrow: The sternal marrow is actively haemopoietic and cells of the red cell, granulocytic and megakaryocytic series are easily identified. Vascular spaces are moderately prominent, but fat spaces are not seen. Traces of stainable iron are seen in reticuloendothelial cells. A normoblast/granulocyte ratio of 0.47:1 is found on examination of a smear of femoral marrow. Sideroblasts were not sought.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: This shows no abnormality on staining with haematoxylin and eosin (H & E). Occasional iron-containing cells are seen in the lamina propria, but stainable iron is not present in the epithelial cells.

Jejunum: No abnormality is seen. Stainable iron is not present.

Pancreas: The pancreas is nomal and stainable iron is not seen.

Heart: The myocardium is normal. Occasional iron-containing macrophages are present in the interstitital connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal. Scanty iron-containing macrophages are seen in the alveolar walls and in the peribronchial connective tissue.

Kidney: The glomeruli and tubules are normal. Traces of stainable iron are present in the perivascular connective tissue and in the capsule.

Testis: The interstitial cells are normal and active spermatogenesis is seen. Stainable iron is not present.

Mouse number 269; genotype +/-; age - 540 days; haematocrit 52%; stock - F_1 hybrid; body weight 39.8g.

Spleen: Weight - 48 mg. or 0.12% of body weight. The malpighian bodies are normal and the red pulp occupies between 25 and 50% of the splenic tissue. Red cell precursors, granulocytes and megakaryocytes are present. Stainable iron is abundant (Grade 4) and is present in slightly greater proportion in the malpighian bodies than in the red pulp.

Liver: The parenchymal cells are normal and haemopoietic foci are not seen. Traces of stainable iron are seen in parencymal cells, and moderate amounts are present in Kupffer cells.

Bone marrow: Sections of sternal marrow show active erythropoiesis, and granulopoiesis, and megakaryocytes are present. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is absent.

A normoblast/granulocyte ratio of 0.64:1 is found in femoral smears. Sideroblasts are not present.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: This shows no abnormality on staining with H. & E. Small amounts of stainable iron are present in the epithelial cells of the villous tips (Grade 2). Stainable iron is not seen elsewhere.

Jejunum: This shows no abnormality and stainable iron is not seen.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: The myocardium appears normal. Occasional iron containing macrophages are seen in the interstitial and perivascular connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal. Scanty iron containing cells are visible in the alveolar walls and peribronchial connective tissue.

Kidney: The glomeruli and tubules appear normal. Scanty stainable iron is seen in the perivascular and connective tissue.

Mouse number 277; genotype +/-; age - 540 days; Hb. concentration 13.4G/100 ml.; haematocrit, 46%; stock - F_1 hybrid; body weight - 39.4G.

Spleen: Weight 81 mg. or 0.21% of body weight. The malpighian bodies are normal and the red pulp occupies between 25 and 50% of the splenic tissue. Erythropoiesis and granulopoiesis are active and megakaryocytes are present. Plentiful stainable iron (Grade 4) is present and is located predominantly in the red pulp.

Liver: The parenchymal cells are normal and haemopoetic foci are not seen. Occasional parenchymal cells show a trace of stainable iron but none is seen in Kupffer cells.

Bone marrow: The sternal marrow is actively haemopoietic and cells of the erythroid, granulocytic and megakaryocytic series are readily identifiable. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is not present. In femoral marrow smears, the normoblast/granulocyte ratio is 0.54:1. Sideroblasts were not sought.

Stomach: The secretory gastric mucosa is normal and stainable iron is not present.

Duodenum: This shows no abnormality on staining with H. & E. A very faint haze of Prussian blue positive material is seen in a few of the mucosal cells of the tips of the villi (Grade 1). Scanty iron-containing macrophages are present in the lamina propria.

Jejunum: No abnormality is seen and stainable iron is absent.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: The myocardium is normal and iron deposition is not seen.

Lungs: The bronchi, alveoli and blood vessels are normal. Occasional iron-containing macrophages are present in the peribronchial connective tissue.

Kidney: The glomeruli and tubules are normal. Scanty iron-containing cells are seen in the perivascular and capsular connective tissue, and a few minute haemosiderin granules are present in the cells of the convoluted tubules.

Testis: Spermatogenesis is active and the interstitial cells appear normal. Stainable iron is absent.

Mouse number 287; genotype - +/-; age - 533 days; Hb. concentration 14.7G/100 ml.; haematocrit 50.5%; stock - F_1 hybrid; body weight - 30.2G.

Spleen: Weight 67 mg. or 0.22% of body weight. The malpighian bodies are normal and the red pulp occupies between 25 & 50% of the splenic tissue. Erythropoietic and granulocytic elements are easily identified, but megakaryocytes are rather scanty. Stainable iron is abundant (Grade 4), and is located predominantly in the white pulp.

Liver: The parenchymal cells are normal and foci of haemopoiesis are not seen. Small amounts of stainable iron are present in the parenchymal cells, but none is seen in Kupffer cells.

Bone marrow: The sternal marrow is actively haemopoietic. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is not present. In femoral marrow smears, the normoblast/granulocyte ratio is 0.42:1. Sideroblasts were not sought.

Stomach: The secretory gastric mucosa is normal. Scanty iron-containing macrophages are seen in the lamina propria.

Duodenum: This shows no abnormality on staining with H. & E. A very faint haze of Prussian blue positive material (Grade 1) is seen in the epithelial cells of the duodenal villi. Scanty iron-containing macrophages are present in the lamina propria.

Jejunum: No abnormality is seen and stainable iron is absent.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: The myocardium is normal. Occasional haemosiderin-containing phagocytes are present in the interstitial and perivascular connective tissue.

Lungs: The bronchi, alveoli, and blood vessels are normal. Macrophages containing stainable iron are fairly frequent in the alveoli.

Kidney: The glomeruli and tubules are normal. Scanty haemosiderin-containing phagocytes are present in the sub-capsular connective tissue, and occasional granules can be seen in the epithelium of the convoluted tubules.

Testis: Spermatogenesis is active and the interstitial cells appear normal. Stainable iron is absent.

Mouse number 289; genotype +/-; age - 533 days; Hb. concentration 14.3G/100 ml.; haematocrit 47.5%; stock - F_1 hybrid; body weight - 34.9G.

Spleen: Weight 79 mg. or 0.23% of body weight. The malpighian bodies appear normal and the red pulp occupies 25 - 50% of the splenic tissue. Erythropoiesis and granulopoiesis are active and occasional megakaryocytes are seen. Stainable iron is plentiful (Grade 4) and is located in the red and white pulp in more or less equal concentration.

Liver: The hepatic epithelium is normal and foci of haemopoiesis are not seen. Occasional parenchymal cells contain a trace of stainable iron, but none is seen in the Kupffer cells.

Bone Marrow: The sternal marrow is actively haemopoietic and cells of the erythroid, granulocyte and megakaryocyte series are readily identifiable. Vascular spaces are a prominent feature, but stainable iron is not present.

Stomach: The gastric secretory epithelium is normal and stainable iron is not seen.

Duodenum: This appears normal on staining with H. & E. Occasional fine granules of haemosiderin are seen in the epithelium of the duodenal villi (Grade 1) and macrophages containing iron are present in the lamina propria.

Jejunum: This shows no histological abnormality and stainable iron is not present.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: The myocardium shows no abnormality. Iron containing cells are present in the perivascular and interstitial connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal. Scanty iron-containing macrophages are seen in the alveoli and in the peribronchial connective tissue.

Kidney: The glomeruli and tubules are normal. Very scanty deposits of iron are present in the perivascular connective tissue.

Testis: Spermatogenesis and the interstitial cells are normal. Stainable iron is not seen.

Mouse number 347; genotype +/-; age 478 days; Hb. concentration 14.30/100 ml.; haematocrit 43.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp is small in amount (less than 25% of the splenic tissue) but shows active erythropoiesis and granulopoiesis, and megakaryocytes are present. Stainable iron is abundant (Grade 4) and is located mainly in the red pulp. The ratio of normoblasts to granulocytes in the splenic imprint is 3.5:1. Sideroblasts are not present.

Bone marrow: The sternal marrow is actively haemopoietic and cells of the erythroid, granulocytic and megakaryocytic series are present. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is absent.

In femoral marrow smears the ratio of normoblasts to granulocytes in 0.34:1. Scanty iron-containing reticulo-endothelial cells are present, and granules of haemosiderin are seen lying free. Sideroblasts are not identified.

Duodenum: This shows no histological abnormality. Stainable iron is not seen in the epithelium or in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Mouse number 359'; genotype +/-; age 503 days; haematocrit 46%; stock - F_2 hybrid.

Spleen: Imprints only were examined. The ratio of normoblasts to granulocytes is 3.3:1; sideroblasts are not present.

Bone marrow: Femoral marrow smears were examined. The ratio of normoblasts to granulocytes is 0.44:1. Occasional iron-containing macrophages are seen, together with iron granules lying free in the smear, but sideroblasts are not present.

Mouse number 367; genotype +/-; age 504 days, haematocrit 45.5%; stock - F_2 hybrid.

Spleen: Imprints only were examined and the ratio of normoblasts to granulocytes is 2.4:1. Sideroblasts are not present.

Bone marrow: Femoral marrow smears show a normoblast/granulocyte ratio of 0.17:1. Stainable iron is not seen and sideroblasts are not present.

Mouse number 373; genotype +/-; age 519 days; haematocrit 44%; stock - F_2 hybrid.

Spleen: Imprints only were examined. The ratio of normoblasts to granulocytes is 9.0:1. Sideroblasts are not seen.

Bone marrow: In femoral marrow smears, the ratio of normoblasts to granulocytes is 0.45:1. Stainable iron is not seen and sideroblasts are not identified.

Mouse number 377; genotype +/-; age 507 days; haematocrit 47%; stock - F_2 hybrid.

Spleen: Imprints only were examined. The ratio of normoblasts to granulocytes is 3.4:1; sideroblasts are not present.

Bone marrow: In femoral marrow smears, the normoblast/granulocyte ratio is 0.20:1. Traces of stainable iron are seen, but sideroblasts are absent.

Mouse number 397; genotype +/-; age 493 days; haematocrit 44%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and shows active erythropoiesis and frequent granulocytes; occasional megakaryocytes are seen. Stainable iron is plentiful (Grade 4) and is equally distributed in the red pulp and malpighian bodies. The ratio of normoblasts to granulocytes in the splenic imprints is 5.0:1. Sideroblasts are not present.

Bone marrow: Sections of sternal marrow show active red cell and granulocyte formation and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent. Femoral marrow smears have a normoblast/granulocyte ratio of 0.25:1; traces of stainable iron are seen but sideroblasts are not identified.

Pancreas: This organ appears normal and stainable iron is not seen.

Mouse number 479; genotype +/-; age 81 days, haematocrit 49%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal and the red pulp occupies 25 - 50% of the splenic tissue. Active erythropoiesis and granulopoiesis are present, but megakaryocytes are rather scanty. Stainable iron is plentiful (Grade 4) and is situated predominantly in the red pulp.

Liver: The parenchyma appears normal and haemopoietic foci are not seen. Scanty stainable iron is seen in the hepatic epithelial cells.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis, and megakaryocytes are present. The vascular spaces are not very prominent and no fat spaces are present. Traces of stainable iron are seen in reticulo-endothelial cells.

In smears of femoral marrow, the erythroid/granulocyte ratio is 1.04:1. Traces of stainable iron are also present, but sideroblasts are not identified.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: This shows no abnormality and stainable iron is not seen in the epithelium or elsewhere.

Jejunum: This is normal and stainable iron is not seen.

Pancreas: This organ appears normal and stainable iron is not seen.

Lungs: The bronchi, alveoli and blood vessels are normal and stainable iron is absent.

Kidney: The glomeruli and tubules appear normal. Stainable iron is not seen.

Mouse number 481; genotype +/-; age 81 days; haematocrit 49.5%; stock - F_2.

Spleen: The malpighian bodies are normal and the red pulp occupies less than 25% of the splenic tissue. Active erythropoiesis and granulopoiesis are seen and megakaryocytes are present in moderate numbers. Stainable iron is plentiful (Grade 4) and is situated predominantly in the red pulp. The normoblast/granulocyte ratio in splenic imprints is 4.1:1, and sideroblasts are not seen.

Liver: The hepatic epithelial cells are normal and haemopoietic foci are not seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and megakaryocytes are present. Vascular spaces are not a prominent feature and fat spaces are not seen. Stainable iron

is not present. In femoral marrow smears, the normoblast/granulocyte ratio is 0.46:1. Scanty stainable iron is seen, but sideroblasts are not present.

Stomach: The gastric secretory mucosa is normal and no stainable iron is present.

Duodenum: This appears normal and stainable iron is seen neither in the epithelium of the villi nor in the lamina propria.

Jejunum: This is normal and stainable iron is absent.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Kidney: The glomeruli and tubules are normal. Scanty stainable iron is present in the connective tissue of the capsule.

Mouse number 701; genotype +/-; age 110 days; haematocrit 33%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal and the red pulp occupies between 25 - 50% of the splenic tissue. Erythropoiesis and granulopoiesis are active and moderate number of megakaryocytes are present. Stainable iron is plentiful (Grade 4) and is located mainly in the red pulp.

Liver: Ths parenchyma is normal and no haemopoietic foci are seen. Stainable iron is absent.

Bone marrow: The sternal marrow shows active erythropoiesis and granulopoiesis and megakaryocytes are present. Vascular spaces are prominent but fat spaces are not seen. Stainable iron is absent.

Duodenum: This shows no abnormality and stainable iron is not seen in the epithelium or in the lamina propria.

Jejunum: The jejunum appears normal and stainable iron is absent.

Pancreas: The pancreas appears normal and stainalbe iron is not seen.

Lungs: The bronchi, alveoli and blood vessels are normal. Scanty iron-containing macrophages are present in the alveoli and in the peribronchial connective tissue.

Kidney: The glomeruli and tubules are normal. Stainable iron is not present.

Mouse number 703; genotype +/-; age 110 days; haematocrit 34%; stock - F_2 hybrid.

Spleen: The malpighian bodies appear normal and the red pulp occupies 25 - 50% of the splenic tissue. Erythropoiesis and granulopoiesis are active and a few megakaryocytes are seen. Stainable iron is plentiful (Grade 4) and is concentrated equally in the white and red pulp.

Liver: The parenchyma is normal and haemopoietic foci are not seen. Stainable iron is not present.

Stomach: The secretory gastric mucosa is normal and stainable iron is not present.

Duodenum: This shows no abnormality and stainable iron is not seen in the epithelium or in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not present.

Heart: The myocardium is normal and stainable iron is not present.

Lungs: The bronchi, alveoli and blood vessels are normal. Scanty iron containing cells are seen in the peribronchial connective tissue.

Kidney: The glomeruli and tubules are normal. Occasional haemosiderin deposits are present in the fibrous tissue of the capsule.

Testis: Spermatogenesis is active and the interstitial cells are normal. Stainable iron is not seen.

Mouse number 705; genotype +/-; age 110 days; haematocrit 38.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies 51 - 75% of the splenic tissue and shows active erythropoiesis and granulopoiesis. Megakarocytes are present but rather scanty. Stainable iron is abundant (Grade 4) and is equally distributed between the red and white pulp.

Liver: The parenchyma is normal and haemopoietic elements are not seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and megakaryocytes are present. Vascular spaces are not very prominent and fat spaces are absent. Stainable iron is not seen.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Jejunum: There is no abnormality of this tissue and stainable iron is not present in either the lamina propria or the mucosal epithelium.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: The myocardium is normal. Scanty iron-containing cells are seen in the interstitial connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal and stainable iron is not seen.

Kidney: The glomeruli and tubules are normal and scanty deposits of haemosiderin are seen in the capsular connective tissue.

Testis: Spermatogenesis is active and the interstitial cells appear normal. Stainable iron is not present.

Mouse number 707; genotype +/-; age 318 days; haematocrit 45.5%; stock - F_1 hybrid.

Spleen: Imprints only are available. The ratio of erythroblasts to granulocytes is 4.3:1, and sideroblasts are not present.

Duodenum: This organ shows no abnormality. Stainable iron is not present in the epithelium or in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not present.

Mouse number 709; genotype +/-; age 318; haematocrit 46.5%; stock - F_1 hybrid.

Spleen: Only spleen imprints are available. The erythroblast/granulocyte ratio is 2.2:1. Sideroblasts are not identified.

Bone marrow: Femoral marrow smears are available. The erythroblast/granulocyte ratio is 0.77:1. Stainable iron is present, as free granules and in reticulo-endothelial cells, but sideroblasts are not seen.

Mouse number 711; genotype +/-; age 318 days; haematocrit 39%; stock - F_1 hybrid.

Spleen: The malpighian bodies are normal. The red pulp is relatively scanty, forming less than 25% of the splenic tissue. Erythropoietic and granulopoietic activity is present, and scanty megakaryocytes are seen. Stainable iron is plentiful (Grade 4) and is more concentrated in the red, than in the white pulp. In splenic imprints, the ratio of erythroblasts to granulocytes is 2.3:1. Sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis, and megakaryocytes are present. Vascular spaces are not prominent and fat spaces are absent. Stainable iron is not seen.

Duodenum: This shows no histological abnormality. Stainable iron is not seen in the mucosal epithelium or in the lamina propria.

Pancreas: This shows no abnormality and stainable iron is not present.

Mouse number 713; genotype +/-; age 318 days; haematocrit 41%; stock - F_1 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue. Active erythropoiesis and granulopoiesis are present, together with moderate numbers of megakaryocytes. Stainable iron is plentiful (Grade 4) and is predominantly located in the red pulp. The erythroblast/granulocyte ratio is splenic imprints is 2.9:1. Sideroblasts are not present.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is absent.

Femoral marrow smears show an erythroblast/granulocyte ratio of 0.44.1. Traces of stainable iron are present but sideroblasts were not identified.

Duodenum: This presents no histological abnormality. Stainable iron is not seen in the mucosal epithelium or in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not present.

Mouse number 719; genotype +/-; age 318 days; haematocrit 42%; stock - F_1 hybrid.

Spleen: Only imprints have been examined. The ratio of erythroblasts/granulocytes is 2.5:1. Siveroblasts are not present.

Bone marrow: Femoral marrow smears have been examined. The erythroblast/granulocyte ratio is 0.58:1. Traces of stainable iron are present, but sideroblasts could not be identified.

Mouse number 721; genotype +/-; age 318 days; haematocrit 42%; stock - F_1 hybrid.

Spleen: The malpighian bodies appear normal. The red pulp occupies between 25 and 50% of the splenic tissue and shows active erythropoiesis and granulopoiesis; small numbers of megakaryocytes are present. Stainable iron is plentiful (Grade 4) and is a little more heavily deposited in the red pulp than in the white pulp.

Imprints show an erythroblast/granulocyte ratio of 3.1:1. Sideroblasts are not present.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent.

In femoral marrow smears the erythroblast/granulocyte ratio is 0.36:1. Traces of stainable iron are seen, but sideroblasts could not be identified.

Duodenum: This shows no histological abnormality. Stainable iron is not seen in the mucosal epithelium or in the lamina propria.

Pancreas: This organ shows no abnormality and stainable iron is not present.

Mouse number 723; genotype +/-; age 318 days; haematocrit 46%; stock - F_1 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and shows active erythropoiesis and granulopoiesis. Small numbers of megakaryocytes are present. Stainable iron is abundant (Grade 4), and is predominantly located in the red pulp.

Imprints show an erythroblast/ granulocyte ratio or 2.9:1. Sideroblasts are not present.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis, and megakaryocytes are present. Vascular spaces are not priminent and fat spaces are not seen. Stainable iron is absent.

Duodenum: No abnormality is seen on staining with H. & E. A very faint haze of Prussian Blue positive material is seen in the mucosal epithelium (Grade 1), and occasional iron containing cells are seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Mouse number 725; genotype +/-; age 330 days; haematocrit 45%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. There is active erythropoiesis and granulopoiesis and small number of megakaryocytes are present. The red pulp occupies 25 - 50% of the splenic structure. Stainable iron is plentiful (Grade 4), distributed mainly in the red pulp.

The ratio of erythroblasts/granulocytes in splenic imprints is 3.3:1. Sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active granulopoiesis and erythropoiesis and megakaryocytes are present. Vascular spaces are moderately prominent, but fat spaces are absent. Stainable iron is not seen.

In femoral marrow smears the ratio of erythroblasts/granulocytes is 0.44:1, and sideroblasts are not seen.

Duodenum: This shows no abnormality. Stainable iron is not seen either in the mucosal epithelium or in the lamina propria.

Pancreas; This organ appears normal and stainable iron is not present.

Mouse number 727; genotype+/-; age 298 days; haematocrit 46%; stock - F_2 hybrid.

Spleen: Only imprints were available. The erythroblast/granulocyte ratio is 0.7:1 and sideroblasts are not seen.

Mouse number 735; genotype +/-; age 220 days; haematocrit 46.5%; stock - F_1 hybrid. Body weight 35.5G.

Spleen: Weight 84 mg or 0.24% of body weight. The malpighian bodies are normal. The red pulp is rather inconspicuous, forming less than 25% of the splenic tissue. However, active erythropoiesis and granulopoiesis are seen and megakaryocytes are present. Stainable iron is plentiful (Grade 4), and is distributed in slightly greater concentration in the white pulp than in the red pulp.

Liver: The hepatic parenchyma is normal. Haemopoietic foci are not seen. Scanty stainable iron is seen in both parenchymal and Kupffer cells.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent.

Stomach: The secretory gastric mucosa is normal. Stainable iron is not seen.

Duodenum: The duodenal mucosa is normal in H. & E. stained sections. Scanty small haemosiderin granules are present in the mucosal epithelium (Grade 1), and occasional iron-containing macrophages are seen in the lamina propria.

Jejunum: This appears normal and stainable iron is not seen.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: The myocardium appears normal. Occasional haemosiderin-containing calls are seen in the interstitial connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal. Macrophages containing iron are present in moderate numbers in the alveoli and peribronchial connective tissue.

Kidney: The glomeruli and tubules are normal. Scanty haemosiderin granules are present in the epithelium of the convoluted tubules, and occasional iron-containing macrophages are seen in the capsule.

Testis: Spermatogenesis is active and the interstitial cells are not remarkable. Stainable iron is not seen.

Mouse number 737; genotype +/-; age 220 days; haematocrit 44%; stock - F_1 hybrid stock and body weight 32.2G.

Spleen: Weight 83 mg or 0.26% of body weight. The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and shows active haemopoiesis. Stainable iron is abundant (Grade 4), and is distributed mainly in the red pulp.

Liver: The hepatic parenchyma is normal and haemopoietic foci are not seen. Stainable iron is absent.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is absent.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: No abnormality is seen in sections stained with H. & E. A faint haze of Prussian blue positive material is seen in the mucosal epithelium (Grade 1). Scanty haemosiderin containing macrophages are seen in the lamina propria.

Jejunum: No abnormality is present and stainable iron is not seen.

Pancreas: The pancreas appears normal and stainable iron is not present.

Heart: The myocardium is normal. Scanty haemosiderin containing phagocytes are found in the interstitial connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal. Occasional iron-containing cells are seen in the peribronchial connective tissue.

Kidney: The glomeruli and tubules are normal and stainable iron is not seen.

Testis: Spermatogenesis is active and the interstitial cells appear normal. Stainable iron is not seen.

Mouse number 739; genotype +/-; age 292 days; haematocrit 40.5%; stock - F_1 hybrid.

Spleen: Only imprints are available. The erythroblast/granulocyte ratio is 2.4:1 and sideroblasts are not seen.

Bone marrow: Only femoral marrow smears are available. The erythroblast/granulocyte ratio is 0.51:1. Traces of stainable iron are seen lying free and in reticulo-endothelial cells, but sideroblasts are not present.

Mouse number 775; genotype +/-; age 214 days; haematocrit 48%; stock - F_3 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and shows active haemopoiesis. Stainable iron is plentiful (Grade 4), with a slightly greater concentration in the red than in the white pulp. In splenic imprints the erythroblast/granulocyte ratio is 3.3:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are not prominent and fat spaces are absent. Stainable iron is not present.

In femoral marrow smears, the erythroblast/granulocyte ratio is 0.22:1. Sideroblasts are not present but traces of stainable iron are seen lying free and in reticulo-endothelial cells.

Duodenum: This shows no histological abnormality and stainable iron is seen in neither the mucosal epithelium nor in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not present.

Mouse number 781; genotype +/-; age 193 days; haematocrit 41.5%; stock - F_3 hybrid.

Spleen: The malpighian bodies are normal. The red pulp is unusually prominent, occupying between 51 and 75% of the splenic tissue. Haemopoiesis is active and considerable amounts of stainable iron are present (Grade 4), mainly in the red pulp. In splenic imprints, the erythroblast: granulocyte ratio is 1.9:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis. Fat spaces are not seen and vascular spaces are not very prominent. Stainable iron is not present.

In femoral marrow smears the ratio of erythroblasts/granulocytes is 0.15:1. Stainable iron and sideroblasts are not seen.

Duodenum: This appears normal and stainable iron is not seen in the mucosal epithelium. Occasional iron containing macrophages are seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Mouse number 787; genotype +/-; age 193 days; haematocrit 52%; stock - F_3 hybrid.

Spleen: Imprints only are available. The erythroblast/granulocyte ratio is 1.8:1 and sideroblasts are not seen.

Bone marrow: Femoral marrow smears only were examined. The erythroblast/granulocyte ratio is 0.42:1. Stainable iron is present in traces in reticulo-endothelial cells and lying free, but sideroblasts are not present.

Mouse number 789; genotype +/-; age 180 days; haematocrit 46%; stock - F_3 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and shows active haemopoiesis. Stainable iron is plentiful (Grade 4) and is located principally in the red pulp.

The ratio of erythroblasts to granulocytes is 3.0:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Fat spaces are not seen and vascular channels are moderately prominent. Stainable iron is absent.

Smears of femoral marrow have an erythroblast/granulocyte ratio of 0.34:1. Traces of stainable iron are present but sideroblasts are not seen.

Duodenum: This shows no abnormality and stainable iron is not seen, either in the mucosal epithelium or in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 835; genotype +/-; age 111 days; haematocrit 49%; stock - F_3 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and active haemopoiesis is seen. Stainable iron is abundant (Grade 4) and is located mainly in the red pulp.

Splenic imprints show an erythroblast/granulocyte ratio of 4.9:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis. Fat spaces are not present and vascular spaces are not very prominent. Stainable iron is absent.

In femoral marrow smears the erythroblast/granulocyte ratio is 0.36:1. Traces of stainable iron are seen lying free and in reticulo-endothelial cells but sideroblasts are not seen.

Duodenum: The duodenum appears normal. Stainable iron is not present either in the mucosal epithelium or in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not present.

Mouse number 845; genotype +/-; age 173 days; haematocrit 48%; stock - F_4 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: No abnormality is seen. Stainable iron is not present, either in the mucosal epithelium or in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 863; genotype +/-; age 150 days; haematocrit 44.5%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: No abnormality is seen. Stainable iron is not present, either in the mucosal epithelium or in the lamina propria.

Mouse number 865; genotype +/-; age 150 days; haematocrit 45%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: The duodenum appears normal. Stainable iron is not seen, either in the mucosal epithelium or in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 877; genotype +/-; age 131 days; haematocrit 47%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: The duodenum appears normal. Stainable iron is not present, either in the mucosal epithelium or in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 879; genotype +/-; age 131 days; haematocrit 49.5%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: This appears normal. Stainable iron is not seen either in the mucosal epithelium or in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 881; genotype +/-; age 131 days; haematocrit 42%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: This appears normal. Stainable iron is not seen, either in the mucosal epithelium or in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not present.

B. ANAEMIC MICE

Mouse number 7; genotype sla/-; age 381 days; haematocrit 21%; stock - O.M.S.; body weight 41.7G.

Spleen: Weight 146 mg or 0.35% of body weight. The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue, and shows active erythropoiesis and granulopoiesis, with moderate numbers of megakaryocytes. Stainable iron is present in the red pulp, but is very scanty (Grade 1).

Liver: The parenchymal cells show no abnormality. Scanty small haemopoietic foci are present, but stainable iron is not seen either in the epithelial or in the Kupffer cells.

Mouse number 10'; genotype sla/sla; age 41 days, Hb. concentration 4.2G/100 ml; haematocrit 17%; stock - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp is prominent, occupying between 51 & 75% of the splenic tissue. Active erythropoiesis and granulopoiesis are evident and moderate numbers of megakaryocytes are seen. Stainable iron is absent.

Liver; The hepatic epithelium is normal and haemopoietic foci are not present. Stainable iron is not seen.

Mouse number 11; genotype sla/-; age 439 days; haematocrit 31%; stock - O.M.S.; body weight 35.1G.

Spleen: Weight 91.6 mg or 0.26% of body weight. The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue and shows active erythropoiesis and granulopoiesis with moderate numbers of megakaryocytes. Stainable iron is not present.

Liver: The parenchymal cells appear normal and haemopoietic foci are not seen. Stainable iron is not present.

Mouse number 12; genotype sla/sla; age 159 days; Hb. concentration 5.7G/100 ml; haematocrit 20.5%; stock - O.M.S.; body weight 29.1G.

Spleen: Weight 208 mg or 0.71% of body weight. The malpighian bodies are normal. The red pulp is prominent, occupying 51-75% of the splenic tissue, and shows active erythropoiesis and granulopoiesis;

megakaryocytes are very frequent. Stainable iron is absent.

Liver: The parenchymal cells are normal. Moderate numbers of haemopoietic foci are seen, but stainable iron is not present.

Heart: The myocardium is histologically normal, and stainable iron is not seen.

Lungs: The bronchi, alveoli and blood vessels are normal and stainable iron is not seen.

Kidney: The glomeruli and tubules are normal and stainable iron is not seen.

Mouse number 14; genotype sla/sla; age 518 days; Hb. concentration 6.7G/100 ml; haematocrit 30%; stock - O.M.S.; body weight 30.0G.

Spleen: Weight 380 mg or 1.27% of body weight. The malpighian bodies are normal, but the pulp is hyperplastic, forming more than 75% of the splenic tissue. Erythropoiesis is very active, and granulopoiesis and megakaryocytes are also very prominent. Stainable iron is absent.

Liver: The parenchyma is normal and haemopoietic foci are not seen. Stainable iron is absent.

Duodenum: This shows no abnormality on staining with H. & E. However, stainable iron is plentiful in the mucosal epithelial cells of the villi (Grade 4). None is seen in the lamina propria.

Jejunum: No abnormality is seen in sections stained with H. & E. Stainable iron is plentiful in the mucosal epithelial cells of the villi, but none is seen in the lamina propria.

Pancreas: This appears normal and stainable iron is not seen.

Mouse number 15'; genotype sla/-; age 38 days; Hb. concentration 4.30/100 ml; haematocrit 15%; stock - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and shows active erythropoiesis. Granulopoietic elements and occasional megakaryocytes are also seen. Stainable iron is absent.

Liver: The parenchymal cells are normal and haemopoietic foci are not seen. Stainable iron is absent.

Bone marrow: The sternal marrow is actively haemopoietic, and cells of the red cell, granulocyte and megakaryocyte series are readily identifiable. Vascular spaces are not prominent and fat spaces are not seen. Stainable iron is absent.

Mouse number 17; genotype sla/-; age 382 days; haematocrit 42%; stock - O.M.S.; body weight 39.1G.

Spleen: Weight 107.5 mg or 0.27% of body weight. The malpighian bodies appear normal and the red pulp

occupies less than 25% of the splenic tissue. Foci of erythropoiesis and granulopoiesis are present, together with occasional megakaryocytes. Stainable iron is absent.

Liver: The parenchyma shows no abnormality and haemopoietic foci are not seen. Stainable iron is absent.

Mouse number 23; genotype sla/-; age 337 days; Hb. concentration 3.9G/100 ml; haematocrit 22%; stock - O.M.S.; body weight 28.5G.

Spleen: Weight 314 mg or 1.10% of body weight. The malpighian bodies are normal but the red pulp is very prominent, occupying more than 75% of the splenic tissue. Haemopoiesis is very active and frequent megakaryocytes are seen. Traces of stainable iron (Grade 1) are seen in the malpighian bodies but none is seen in the red pulp.

Mouse number 25; genotype sla/-; age 100 days; Hb. concentration 10.3G/100 ml; haematocrit 34%; stock - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp occupies 51 - 75% of the splenic tissue and shows active erythropoiesis and granulopoiesis with frequent megakaryocytes. Stainable iron is absent.

Liver: The parenchymal cells are normal and scanty. Small haemopoietic foci can be found. Stainable iron is absent.

Mouse number 26; genotype sla/-; age 319 days; haematocrit 40%; stock - O.M.S.; body weight 30.7G.

Spleen: Weight 58.5 mg or 0.19% of body weight. The malpighian bodies are normal and the red pulp occupies between 25 & 50% of the splenic tissue. Active haemopoiesis and occasional megakaryocytes are seen. Scanty deposits of stainable iron (Grade 1) are seen in the malpighian bodies.

Liver: The parenchyma appears normal and haemopoietic foci are not present. Stainable iron is not seen in the epithelial or Kupffer cells.

Mouse number 27'; genotype sla/-; age 147 days; Hb. concentration 7.6G/100 ml; haematocrit 28.5%; stock - O.M.S.; body weight 17.4G.

Spleen: Weight 115 mg or 0.66% of body weight. The malpighian bodies appear normal. The red pulp is prominent, occupying between 51 & 75% of the splenic tissue, and shows very active erythropoiesis and granulopoiesis with frequent megakaryocytes. Stainable iron is absent.

Heart: The myocardium appears normal and stainable iron is not seen.

Lungs: The bronchi, alveoli and blood vessels appear normal. Fairly frequent iron-containing macrophages are present in the alveoli and bronchioles. None are seen in the peribronchial connective tissue.

Kidney: The glomeruli and tubules appear normal and stainable iron is not seen.

Mouse number 32; genotype sla/-; age 27 days; Hb. concentration 8.4G/100 ml; haematocrit 35%; strain - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp occupies between 51 and 75% of the splenic tissue, and shows active haemopoiesis and moderate numbers of megakaryocytes. Stainable iron is not present.

Liver: The parenchyma appears normal and haemopoietic foci are not seen. Stainable iron is absent.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis, and frequent megakaryocytes. Vascular spaces are not prominent and fat spaces are absent. Stainable iron is not seen.

Mouse number 43; genotype sla/-; age 109 days; haematocrit 22.5%; stock - O.M.S.; body weight 13.9G.

Spleen: Weight 160 mg or 1.15% of body weight.

Liver: The parenchyma is normal and haemopoietic foci are not seen. Stainable iron is absent.

Heart: The myocardium shows no histological abnormality and stainable iron is absent.

Lungs: The bronchi, alveoli and blood vessels appear normal. Scanty iron-containing macrophages are seen in the alveoli but none in the peribronchial connective tissue.

Kidney: The glomeruli and tubules show no abnormality. Stainable iron is not seen.

Mouse number 46; genotype sla/sla; age 319 days; haematocrit 44%; stock - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue. Foci of haemopoiesis are frequent are frequent and moderate numbers of megakaryocytes are also seen. Stainable iron is absent.

Liver: The parenchyma is normal and foci of haemopoiesis are not seen. Stainable iron is absent.

Kidney: The glomeruli and tubules are normal and stainable iron is not seen.

Mouse number 49; genotype sla/sla; age 280 days; Hb. concentration 4G/100 ml; haematocrit 15%; stock- O.M.S.

Spleen: The malpighian bodies are normal. The red pulp is very prominent and occupies over 75% of the splenic tidsue. Haemopoiesis is very active and megakaryocytes are frequent. Stainable iron is absent.

Liver: The hepatic parenchymal cells are normal. Frequent haemopoietic foci are seen, together with scattered haemopoietic elements. Stainable iron is absent.

Heart: The myocardium is normal. Stainable iron is not present.

Mouse number 53"; genotype sla/-; age 511 days; haematocrit 18.5%; stock - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp is prominent occupying more than 75% of the splenic tissue, and haemopoiesis is very active. Megakaryocytes are numerous. A very minute (Grade 1) amount of stainable iron is present in the red pulp.

Liver: The parenchymal cells show no abnormality. Scattered haemopoietic elements are seen but stainable iron is absent.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and megakaryocytes are plentiful. Vascular spaces are not prominent and fat spaces are absent. Stainable iron is not seen.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: No abnormality is seen on staining with H. & E. Stainable iron is plentiful (Grade 4) in the epithelium of the duodenal mucosa but none is seen in the lamina propria.

Jejunum: No histological abnormality is seen in sections stained with H. & E. A moderate amount of stainable iron is seen in the mucosal epithelial cells, but none is present in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not present.

Lungs: The bronchi, blood vessels and alveoli show no abnormality. Moderate numbers of iron-containing macrophages are present in the alveoli and bronchioles.

Kidney: A few glomeruli show early fibrosis and hyaline thickening of the capillary tufts but otherwise the glomeruli and tubules are not remarkable. Stainable iron is absent.

Mouse number 71; genotype sla/sla; age 41 days; Hb concentration 4.7G/100 ml; haematocrit 19.5%; stock - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp occupies between 51 and 75% of the splenic tissue and shows active haemopoiesis and moderate numbers of megakaryocytes. Stainable iron is absent.

Liver: The parenchyma appears normal and haemopoietic foce are not seen. Stainable iron is absent.

Mouse number 73; genotype sla/-; age 21 days; Hb. concentration 6.8G/100 ml; haematocrit 32%; stock - O.M.S.; body weight 23.8G.

Spleen: Weight 197 mg. or 0.83% body weight. The malpighian bodies are normal. The red pulp occupies between 51 and 75% of the splenic tissue, and shows active erythropoiesis and granulopoiesis. Megakaryocytes are numerous. Stainable iron is absent.

Liver: The parenchymal cells are not remarkable and very scanty haemopoietic foci are present. Stainable iron is not seen.

Jejunum: This shows no abnormality on staining with H. & E. Stainable iron is not seen in the mucosa or in the lamina propria. The level in the jejunum at which this section was taken is not known.

Pancreas: This organ appears normal and stainable iron is not seen.

Heart: The myocardium shows no histological abnormality. No stainable iron is seen.

Mouse number 75; genotype sla/sla; age 41 days; Hb. concentration 4.1G/100 ml; haematocrit 16.5%; stock - O.M.S.

Spleen: The malpighian bodies appear normal. The red pulp occupies between 25 and 50% of the splenic tissue and shows active haemopoiesis and moderate number of megakaryocytes. There is no stainable iron.

Liver: The parenchymal cells appear normal, and haemopoietic foci are not seen. Stainable iron is absent.

Mouse number 823; genotype sla/-; age 25 days; Hb. concentration 4.4G/100 ml; haematocrit 19%; stock - O.M.S.

Spleen: The malpighian bodies appear normal. The red pulp is very prominent and occupies more than 75% of the splenic tissue. Erythropoiesis is very active and granulopoiesis and moderate number of megakaryccytes are also seen. Stainable iron is absent.

Liver: The parenchymal cells appear normal and scanty small haemopoietic foci are present. Stainable iron is not seen.

Bone marrow: Sections of sternal marrow show active erythropoiesis, granulopoiesis and plentiful megakaryocytes. Vascular spaces are not a prominent feature and fat spaces are absent. Stainable iron is not seen.

Stomach: The secretory gastric mucosa appears normal. Stainable iron is not present.

Heart: The myocardium appears histologically normal. Stainable iron is not present.

Kidney: The glomeruli and tubules are normal, and stainable iron is not seen.

Mouse number 345; genotype sla/-; age 545 days; haematocrit 22%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue. Erythropoiesis is active and granulopoietic elements and occasional megakaryocytes are also seen. Stainable iron is absent. Examination of splenic imprints shows a normoblast/granulocyte ratio of 3.8:1. Sideroblasts are not present.

Bone marrow: Smears only were examined from the femoral marrow. The erythroid/mature granulocyte ratio is 0.45:1 and stainable iron and sideroblasts are not seen.

Duodenum: Sections stained with H. & E. show no abnormality. Stainable iron is present in the cytoplasm of the mucosal epithelial cells (Grade 4), but none is seen in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not present.

Mouse number 391; genotype sla/-; age 320 days; haematocrit 25.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp is prominent, occupying more than 75% of the splenic tissue. Erythropoiesis and granulopoiesis are active with moderate numbers of megakaryocytes present. Traces of stainable iron (Grade 1) are seen in both the white and red pulp. The ratio of normoblasts to granulocytes in splenic imprints is 1:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and frequent megakaryocytes. Vascular spaces are not prominent and fat spaces are not seen. Stainable iron is absent. Smears of femoral marrow have a normoblast/granulocyte ratio of 0.2:1. Sideroblasts are not seen and stainable iron is absent.

Duodenum: Sections stained with H. & E. show no abnormality. Stainable iron is abundant in the duodenal mucosal epithelium (Grade 4) but none is seen in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 395; genotype sla/-; age 545 days; haematocrit 31.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies between 51 and 75% of the splenic tissue and shows active haemopoiesis and moderate numbers of megakaryocytes. Minute amounts of stainable iron (Grade 1) are seen in the white pulp. The ratio of normoblasts/granulocytes in splenic imprints is 3.4:1. Sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent. Smears of femoral marrow show a ratio of normoblasts/granulocytes of 0.47:1. Sideroblasts are not seen and stainable iron is not present.

Duodenum: No abnormality is seen in sections stained by H. & E. stainable iron is present but is rather scanty (Grade 1) in the duodenal epithelial cells. None is seen in the lamina propria.

Mouse number 401; genotype sla/-; age 467 days; haematocrit 34%; stock - F_2 hybrid.

Spleen: The white pulp appears normal. The red pulp occupies 25 - 50% of the splenic tissue. Erythropoiesis and granulopoiesis are active and moderate numbers of megakaryocytes are present. Stainable iron is present in traces (Grade 1) in the white pulp. In splenic imprints the normoblast/

granulocyte ratio is 4:1. Sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present in normal numbers. Vascular spaces are not prominent and fat spaces are not seen. Stainable iron is absent. Smears of femoral marrow show a normoblast/granulocyte ratio of 0.39:1. Sideroblasts and stainable iron are absent.

Duodenum: Sections stained with H. & E. show no abnormality. Moderate amounts of stainable iron are seen in the duodenal epithelium (Grade 2) but none is seen in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 431; genotype sla/-; age 336 days; haematocrit 31.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp occupies between 51 and 75% of the splenic tissue and shows active haemopoiesis and frequent megakaryocytes. Stainable iron is not present.

Liver: The parenchyma is normal and haemopoietic foci are not seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent.

Stomach: The gastric secretory mucosa is normal and stainable iron is not seen.

Duodenum: Sections stained with H. & E. show no abnormality. Moderate quantities of stainable iron (Grade 3) are seen in the duodenal epithelium but none is present in the lamina propria.

Jejunum: No abnormality is apparent in sections stained with H. & E. Moderate amounts of stainable iron are seen in the mucosal epithelium, but none in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not present.

Heart: The myocardium shows no histological abnormality. Stainable iron is not seen.

Lungs: The bronchi, alveoli and blood vessels are normal. Scanty iron-containing macrophages are seen in the alveoli but none are present in the peribronchial tissues.

Kidney: The glomeruli and tubules appear normal and stainable iron is not present.

Testis: Spermatogenesis and the interstitial cells appear normal and stainable iron is absent.

Mouse number 453; genotype - sla/-; age 407 days; haematocrit 40.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies appear normal. The red pulp occupies between 51 and 75% of the splenic

tissue, and shows active erythropoiesis and granulopoiesis, with moderate numbers of megakaryocytes. A minute amount of stainable iron (Grade I) is seen in the white pulp. In spleen imprints the ratio of normoblasts to granulocytes is 3.9:1; sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis, and megakaryocytes are present. Vascular spaces are not very prominent and fat spaces are absent. Stainable iron is not seen. Smears of femoral marrow show a normoblast/granulocyte ratio of 0.68:1 and sideroblasts and stainable iron are not present.

Duodenum: There is no abnormality in sections stained with H. & E. Stainable iron is present, but scanty (Grade I) in the mucosal epithelium, but none is seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not present.

Mouse number 457; genotype sla/-; age 42 days; Hb. concentration 5.3G/100 ml; haematocrit 22%; stock - F_2 hybrid; body weight 15.6G.

Spleen: Weight 154 mg or 0.99% of body weight. The malpighian bodies appear normal. The red pulp is very prominent, occupying more than 75% of the splenic tissue. Erythropoiesis is highly active, and granulopoiesis and frequent megakaryocytes are also seen. Stainable iron is absent.

Liver: The parenchymal cells are normal and occasional haemopoietic foci are present. Stainable iron is not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular apaces are not very prominent fat spaces are absent. Stainable iron is not seen.

Mouse number 483; genotype sla/-; age 52 days; Hb. concentration 4.3G/100 ml; haematocrit 21.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp is very prominent and occupies more than 75% of the splenic tissue. Erythropoiesis is very active, and granulopoiesis and numerous megakaryocytes are also seen. Stainable iron is not present. The ratio of normoblasts to granulocytes is 5.9:1 in splenic imprints; sideroblasts are not present.

Liver: The parenchymal cells are normal and scanty haemopoietic foci are present. Stainable iron is not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent. Femoral marrow smears show a normoblast/granulocyte ratio of 0.65:1. Sideroblasts and stainable iron are not seen.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: Sections stained with H. & E. show a normal appearance. Stainable iron is moderately pleniful (Grade 3) in the mucosal epithelium, but none is seen in the lamina propria.

Jejunum: No abnormality is seen in sections stained with H. & E. Small amounts of stainable iron are seen in the mucosal epithelium.

Pancreas: This organ appears normal and no stainable iron is seen.

Heart: The myocardium shows no histological abnormality. Stainable iron is not seen.

Lung: This contains a single very small fibrous focus containing a few macrophages, some of which contain traces of stainable iron. The appearance is consistent with a healed granulomatous focus, but its causation is uncertain. Otherwise, stainable iron is absent, and the alveoli, bronchi and blood vessels are not remarkable.

Kidney: The glomeruli and tubules appear normal. Stainable iron is not present.

Mouse number 487; genotype sla/-; age 47 days; Hb. concentration 3.7G/100 ml; haematocrit 15.5%; stock - F_2 hybrid.

Spleen: The malpighian bodies appear normal. The red pulp occupies 25 - 50% of the splenic tissue, and shows active erythropoiesis and granulopoiesis and moderate numbers of megakaryocytes. Stainable iron is absent. In spleen imprints, the normoblast/granulocyte ratio is 5.7:1 and sideroblasts are not seen.

Liver: The parenchymal cells appear normal. Scanty small foci of haemopoiesis are present but stainable iron is not seen.

Bone marrow: Sections were not examined. Smears of femoral marrow show a normoblast/granulocyte ratio of 0.53:1. Sideroblasts are not seen and stainable iron is absent.

Stomach: The secretory gastric mucosa is normal. Stainable iron is not present.

Duodenum: The duodenum shows no abnormality in sections stained with H. & E. Stainable iron is abundant (Grade 4) in the mucosal epithelial cells but none is seen in the lamina propria.

Jejunum: The jejunum appears normal in sections stained with H. & E. Moderate amounts of stainable iron are seen in the mucosal epithelium, but none is present in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Heart: The myocardium shows no histological abnormality and stainable iron is not seen.

Lungs: The bronchi, alveoli and blood vessels are normal and stainable iron is not present.

Kidneys: The glomeruli and tubules appear normal and stainable iron is not seen.

Mouse number 489; genotype sla/-; age 47 days; haematocrit 20%; stock - F_2 hybrid.

Spleen: The malpighian bodies are normal. The red pulp is moderately prominent occupying between 51 and 75% of the splenic tissue. There is active erythropoiesis and leucopoiesis and megakaryocytes are frequent. Stainable iron is not present. In splenic imprints, the normoblast/granulocyte ratio is 3.3:1 and sideroblasts are not seen.

Liver: The parenchymal cells are normal. Scanty, small haemopoietic foci are present. Stainable iron is absent.

Sternal marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are prominent, but fat spaces are not seen. Stainable iron is not present. In smears of femoral marrow the normoblast/granulocyte ratio is 0.61:1. Sideroblasts and stainable iron are not seen.

Stomach: The secretory gastric mucosa appears normal. Stainable iron is not seen.

Duodenum: Sections stained with H. & E. show no abnormality. Stainable iron is plentiful (Grade 4) in the mucosal epithelium but none is present in the lamina propria.

Jejunum: There is no histological abnormality apparent and stainable iron is not seen.

Kidney: The glomeruli and tubules appear normal and stainable iron is not seen.

Mouse number 491; genotype sla/-; age 162 days; haematocrit 13.5%; stock - F_2 hybrid; body weight 22.5G.

Spleen: Weight 461 mg or 2.05% of body weight. The malpighian bodies are normal. The red pulp is very prominent and occupies more than 75% of the splenic tissue. Haemopoiesis is very active but megakaryocytes are rather infrequent. Stainable iron is absent.

Liver: The parenchymal cells are normal. Scanty haemopoietic foci and occasional megakaryocytes are present. Stainable iron is not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes. Vascular spaces are not prominent and fat spaces are absent. Stainable iron is not present.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: Sections stained with H. & E. reveal no abnormality. Stainable iron is plentiful (Grade 4) in the mucosal epithelium, but none is seen in the lamina propria.

Jejunum: Sections stained with H. & E. reveal no abnormality. Small amounts of stainable iron are present in the mucosal epithelium but none is seen in the lamina propria.

Heart: The myocardium shows no histological abnormality and stainable iron is not seen.

Lungs: The bronchi, alveoli and blood vessels show no abnormality. A few alveoli contain large iron-containing macrophages, but stainable iron is not seen in the mesenchymal tissue of the lung.

Kidney: The glomeruli and tubules are normal and stainable iron is not seen.

Testis: The interstitial cells appear normal and active spermatogenesis is seen. Stainable iron is not present.

Mouse number 499; genotype sla/-; age 116 days; haematocrit 16.5%; stock - F_2 hybrid; body weight 18.0G.

Spleen: Weight 147 mg or 0.82% of total body weight. The malpighian corpuscles appear normal; the red pulp is moderately prominent occupying between 51 and 75% of the splenic tissue. Erythropoiesis

and granulopoiesis are active and megakaryocytes are numerous. Stainable iron is not present.

Liver: The parenchymal cells appear normal and fairly frequent small haemopoietic foci are seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen and stainable iron is absent.

Stomach: The secretory gastric mucosa appears normal, and stainable iron is not present.

Duodenum: Sections stained with H. & E. show no abnormality. There is moderately heavy deposition of stainable iron (Grade 4) in the mucosal epithelium, but none is seen in the lamina propria.

Jejunum: Sections stained with H. & E. show no abnormality; moderate amounts of stainable iron are seen in the mucosal epithelium, but none is present in the lamina propria.

Heart: The myocardium shows no histological abnormality, and stainable iron is not present.

Lungs: The bronchi, alveoli and blood vessels are normal. Stainable iron is not seen in the mesenchymal tissues or in the alveoli or bronchi.

Kidney: The glomeruli and tubules are normal and stainable iron is not present.

Testis: The interstitial cells and spermatogenesis appear normal. Stainable iron is not seen.

Mouse number 729; Genotype sla/-; age 68 days; haematocrit 42%; stock - F_2 hybrid; body weight 24.5G.

Spleen: Weight 59.4 mg or 0.24% of body weight. The malpighian bodies are normal and the red pulp occupies between 25 and 50% of the splenic tissue. There is active haemopoiesis and moderately numerous megakaryocytes are seen. Stainable iron is not seen.

Liver: The hapatic parenchyma is notmal and haemopoietic foci are not seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are not prominent and fat spaces are not seen. Stainable iron is absent.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: Sections stained with H. & E. show a normal appearance, and stainable iron is not present either in the mucosal epithelium or in the lamina propria.

Jejunum: This shows no histological abnormality and stainable iron is not seen either in the mucosal epithelium or in the lamina propria.

Pancreas: This appears normal and stainable iron is not seen.

Heart: The myocardium is histologically normal and stainable iron is not seen.

Lungs: The bronchi, blood vessels and alveoli are normal and stainable iron is absent.

Kidney: The glomeruli and tubules are normal and stainable iron is not seen.

Testis: The interstitial cells are normal and spermatogenesis is active. Stainable iron is absent.

Mouse number 805; genotype sla/-; age 142 days; haematocrit 26%; stock - F_2 hybrid; body weight 24.8G.

Spleen: Weight 438 mg or 1.77% of body weight. The malpighian bodies are normal. The red pulp is very prominent, occupying more than 75% of the splenic tissue. Erythropoiesis is very active, and granulopoiesis and numerous megakaryocytes are also present. Stainable iron is not seen.

In the splenic imprints the normoblast/granulocyte ratio is 9.5:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are improminent and fat spaces are absent. Stainable iron is not seen. Smears of femoral marrow show a normoblast/granulocyte ratio of 1.63:1 and sideroblasts and stainable iron are not seen.

Duodenum: Sections stained with H. & E. appear normal. Moderate amounts of stainable iron (Grade 3)

are seen in the mucosal epithelium but none is present in the lamina propria.

Mouse number 809; genotype sla/-; age 142 days; haematocrit 20%; stock - F_2 hybrid; body weight 21.2G.

Spleen: Weight 388 mg or 1.83% of body weight. The malpighian bodies appear normal; the red pulp is very prominent occupying more than 75% of the splenic tissue and shows extremely active erythropoiesis; granulopoiesis and fairly numerous megakaryocytes are also present. Stainable iron is absent. In splenic imprints, the normoblast/granulocyte ratio is 17.2:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active erythropoiesis and granulopoiesis, and megakaryocytes are present. Vascular spaces are moderately prominent, and fat spaces are not seen. Stainable iron is absent. Smears of femoral marrow show a normoblast/granulocyte ratio of 0.60:1, and sideroblasts and stainable iron are not present.

Duodenum: Sections stained with H. & E. show no abnormality. Prominent deposits of stainable iron (Grade 4) are seen in the mucosal epithelium, but none is seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not present.

Mouse number 811; genotype sla/-; age 142 days; haematocrit 23%; stock - F_2 hybrid; body weight 22.3G.

Spleen: Weight 232 mg or 1.04% of body weight. The malpighian bodies are normal; the red pulp is prominent, occupying more than 75% of the splenic tissue, and shows active haemopoiesis and very numerous megakaryocytes. Stainable iron is not seen. In splenic imprints the normoblast/granulocyte ratio is 3.2:1 and sideroblasts are not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are nor present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent. In femoral marrow smears the ratio of normoblasts to granulocytes is 0.44:1. Sideroblasts are not seen. Stainable iron is absent.

Duodenum: Sections stained with H. & E. show no abnormality. Plentiful stainable iron (Grade 4) is present in the mucosal epithelium but none is seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Mouse number 853: genotype sla/-; age 163 days; haematocrit 31%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: No abnormality is seen in sections stained with H. & E. Stainable iron is plentiful (Grade 4) in the mucosal epithelium but none is seen in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Mouse number 855; genotype sla/-; age 163 days; haematocrit 29%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: Sections stained with H. & E. show no abnormality. Moderate amounts of stainable iron (Grade 3) are seen in the mucosal epithelium but none is seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Mouse number 883; genotype sla/-; age 123 days; haematocrit 30%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: This shows no abnormality on staining with H. & E. The mucosal epithelium shows the presence of stainable iron in considerable quantities (Grade 4), but none is seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Mouse number 887; genotype sla/-; age 127 days; haematocrit 28.5%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: Sections stained with H. & E. show no abnormality. Small amounts of stainable iron (Grade 1) are seen in the mucosal epithelium but none is present in the lamina propria.

Pancreas: This organ appears normal stainable iron is not seen.

Mouse number 919; genotype sla/-; age 84 days; haematocrit 25.5%; stock - F_4 hybrid. Fasting for 6 hours prior to sacrifice.

Duodenum: Sections stained with H. & E. show no abnormality. Stainable iron is plentiful (Grade 4) in the mucosal epithelium but none is seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

C. Heterozygous carrier female mice:

Mouse number 20; genotype - sla/+; age 862 days; Hb. concentration 12.7G/100 ml; haematocrit 40.5%; stock - O.M.S.

Spleen: The malpighian bodies are normal. The red pulp occupies 25 - 50% of the splenic tissue and

shows foci of haemopoiesis and frequent megakaryocytes. Stainable iron is plentiful (Grade 4) and is concentrated equally in the malpighian bodies and in the red pulp.

Liver: The parenchyma is normal and foci of haemopoiesis are not seen. Stainable iron is present in small amounts in parenchymal and Kupffer cells.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are not prominent and fat spaces are absent. Stainable iron is not seen.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Jejunum: Sections stained with H. & E. show no abnormality. Stainable iron is not seen inthe mucosal epithelium, but occasional iron-containing macrophages are present in the lamina propria.

Heart: The myocardium shows no histological abnormality, and stainable iron is not seen.

Lungs: The alveoli, bronchi and blood vessels are normal. Occasional iron-containing macrophages are seen in the alveoli and in the peribronchial connective tissue.

Mouse number 48; genotype sla/+; age 37 days; haematocrit 51%; stock - O.M.S.

Liver: The parenchyma appears normal and there is no evidence of hepatic haemopoiesis. Stainable iron is not present.

Mouse number 64; genotype sla/+; age 710 days; Hb. concentration 14.4G/100 ml; haematocrit 48%; stock - O.M.S.; body weight 28.8G.

Spleen: Weight 196 mg or 0.68% of body weight. The malpighian bodies are normal. The red pulp occupies between 25 and 50% and shows foci of active haemopoiesis and occasional megakaryocytes. A moderate amount of stainable iron (Grade 2) is present, lying mainly in the malpighian bodies.

Liver: The parenchymal cells are normal and no foci of haemopoiesis are present. Scanty deposits of stainable iron are seen in occasional parenchymal and Kupffer cells.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are not prominent and fat spaces are not seen. Stainable iron is absent.

Heart: The myocardium shows no histological abnormality. Occasional cells in the interstitial connective tissue shows the presence of stainable iron.

Lungs: The bronchi, alveoli and blood vessels show no abnormality. Scanty iron-containing macrophages

are present in the alveoli and in the peribronchial connective tissue.

Kidney: The glomeruli and tubules show no abnormality. Stainable iron is not present.

Mouse number 250; genotype sla/4; age 555 days; haematocrit 56%; stock - F_1 hybrid; body weight 27.2G.

Spleen: Weight - 78 mg or 0.29% of total body weight. The malpighian bodies appear normal. The red pulp is not conspicuous oc cupying less than 25% of the splenic tissue, and shows foci of haemopoiesis and scanty megakaryocytes. Stainable iron is plentiful (Grade 4) and is equally concentrated in the red pulp and malpighian bodies.

Liver: The parenchyma is normal and haemopoietic foci are not seen. Faint tracts of stainable iron are seen in the parenchymal cells, but none is present in the Kupffer cells.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent and fat spaces are not seen. Stainable iron is absent.

Stomach: The secretory gastric mucosa is normal. Stainable iron is not seen.

Duodenum: Sections stained with H. & E. appear normal. Small amounts of stainable iron (Grade 2)

are seen in patches in the mucosal epithelium but none is seen in the lamina propria.

Jejunum: This appears normal and stainable iron is not seen.

Pancreas: The pancreas is normal and stainable iron is not seen.

Heart: The myocardium appears histologically normal. Occasional cells containing stainable iron are present in the perivascular and intersyncytial connective tissue of the heart.

Lungs: Thebronchi, alveoli and blood vessels are normal. Frequent iron-containing macrophages are seen in the peribronchial connective tissue and a few are also seen in the alveoli.

Kidney: The glomeruli and tubules are normal. Scanty deposits of stainable iron are seen in the capsular connective tissue.

Mouse number 252; genotype sla/+; age 572 days; haematocrit 52.5%; stock - F_1 hybrid; body weight 29.6G.

Spleen: Weight 80 mg or 0.27% of body weight. The malpighian bodies appear normal and the red pulp occupies between 25 and 50% of the splenic tissue. Foci of active haemopoiesis are seen and scanty megakaryocytes. Stainable iron is abundant (Grade 4) and is equally distributed in the malpighian bodies and red pulp.

Liver: The parenchyma appears normal and haemopoietic foci are not seen. Traces of stainable iron are seen in the parenchymal cells by none is present in the Kupffer cells.

Stomach: The secretory gastric mucosa shows no abnormality and stainable iron is not seen.

Duodenum: This shows no abnormality on staining with H. & E. Scanty granules of stainable iron (Grade 1) are present in the mucosal epithelium but one is seen in the lamina propria.

Jejunum: This appears normal and stainable iron is not seen.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: The myocardium is normal histologically. Occasional iron-containing macrophages are present in the perivascular and intersyncytial connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal. Moderate numbers of iron-containing macrophages are seen in the peribronchial connective tissue and in the alveoli.

Kidney: The glomeruli and tubules are normal. Traces of stainable iron are seen in the cells of the convoluted tubules and in the capsular connective tissue.

Mouse number 254'; genotype sla/+; age 572 days; haematocrit 52%; stock - F_1 hybrid; body weight 27.1G.

Spleen: Weight 84 mg, or 0.31% of body weight. The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue and shows foci of haemopoiesis and occasional megakaryocytes. Stainable iron is plentiful (Grade 4) and is equally concentrated in the red pulp and malpighian bodies.

Liver: The parenchyma appears normal and foci of haemopoiesis are not present. Stainable iron is absent.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent and occasional fat spaces are also seen. Stainable iron is not present.

Stomach: The secretory gastric mucosa is normal and stainable iron is not seen.

Duodenum: Sections stained with H. & E. appear normal. Patchy but distinct granules of stainable iron (Grade 2) are seen in the mucosal epithelial cells but none are seen in the lamina propria.

Jejunum: This shows no abnormality and stainable iron is not present.

Pancreas: The pancreas appears normal and stainable iron is not seen.

Heart: This appears normal histologically. Scanty iron-containing cells are seen in the perivascular connective tissue.

Lung: The bronchi, alveoli, and blood vessels are normal. Stainable iron is seen in small amounts in the peribronchial connective tissue but none is present in the alveoli.

Kidney: The glomeruli and tubules are normal. Traces of stainable iron are seen in the epithelial cells of the convoluted tubules and in the capsular connective tissue.

Mouse number 256; genotype sla/+; age 572 days; haematocrit 50.5%; stock - F_1 hybrid; body weight 26.2G.

Spleen: Weight 54 mg or 0.21% of body weight. The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue and shows foci of haemopoiesis and occasional megakaryocytes. Stainable iron is plentiful (Grade 4) and is evenly distributed between the malpighian bodies and the red pulp.

Liver: The parenchyma appears normal and haemopoietic foci are not seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are prominent and a few fat spaces are also seen. Stainable iron is not present.

Stomach: The secretory gastric mucosa appears normal and stainable iron is not seen.

Duodenum: There is no abnormality in sections stained with H. & E. Patchy, but distinct, granules of stainable iron are seen in the mucosal epithelium (Grade 2), and occasional iron containing macrophages are present in the lamina propria.

Jejunum: This shows no abnormality and stainable iron is not seen.

Pancreas: This appears normal and stainable iron is not seen.

Heart: The myocardium shows no histological abnormality. Occasional iron-containing cells are present in the interstitial and perivascular connective tissue.

Lung: The bronchi, alveoli and blood vessels appear normal. Frequent iron-containing macrophages are seen in the peribronchial connective tissue and a few are also seen in the alveoli and bronchioles.

Kidney: The glomeruli and tubules are normal. Stainable iron is clearly present in the epithelial cells of the convoluted tubules, and scanty iron containing macrophages are also seen in the capsular connective tissue.

Mouse number 258; genotype - sla/+; age 572 days; haematocrit 53%; stock - F_1 hybrid; body weight 28.5G.

Spleen: Weight 62 mg, or 0.22% of body weight. The malpighian bodies are normal. The red pulp occupies

between 25 and 50% of the splenic tissue. Foci of haemopoiesis and occasional megakaryocytes are seen. Stainable iron is plentiful (Grade 4) and is equally concentrated in the malpighian bodies and red pulp.

Liver: The parenchyma appears normal and foci of haemopoiesis are not seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active haemopoiesis and vascular spaces are moderately prominent. Fat spaces are not seen and stainable iron is not seen.

Stomach: The secretory gastric mucosa is normal and stainable iron is absent.

Duodenum: No abnormality is seen in sections stained with H. & E. Patches of distinct iron granulation (Grade 2) are seen in the epithelial cells of the mucosa and occasional iron containing macrophages are seen in the lamina propria.

Jejunum: This shows no abnormality. Stainable iron is not seen in the mucosal epithelium but scanty iron-containing macrophages are seen in the lamina propria.

Pancreas: This organ appears normal and stainable iron is not seen.

Heart: The myocardium shows no histological abnormality. Occasional macrophages containing stainable iron are present in the subendocardial, interstitial and perivascular connective tissue.

Lungs: The bronchi, alveoli and blood vessels are normal. A moderate number of iron-containing cells are present in the peribronchial connective tissue and a few are also seen in the alveoli.

Kidney: The glomeruli and tubules are normal. Traces of stainable iron are seen in the cells of the convoluted tubules, and in the capsular connective tissue.

Mouse number 292; genotype sla/+; age 471 days; haematocrit 37%; stock - F_1 hybrid.

Spleen: The malpighian bodies are normal; the red pulp is fairly prominent occupying between 51 and 75% of the splenic tissue. There is active erythropoiesis and granulopoiesis and megakaryocytes are frequent. Stainable iron is much reduced in quantity (Grade 1) and is situated mainly in the malpighian bodies.

Liver: The parenchyma appears normal and foci of haemopoiesis are not seen. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are not prominent, and fat spaces are not seen. Stainable iron is absent.

Stomach: The secretory gastric mucosa appears normal and stainable iron is not seen.

Duodenum: Sections stained with H. & E. appear normal. A small amount (Grade 2) of stainable iron is present in the mucosal epithelium and scanty iron-containing macrophages are seen in the lamina propria.

Jejunum: This shows no abnormality. Stainable iron is not seen in the mucosal epithelium but traces are present in macrophages in the lamina propria.

Pancreas: This organ shows no abnormality and stainable iron is not seen.

Heart: The myocardium appears histologically normal and stainable iron is not seen.

Lung: The bronchi, alveoli and blood vessels are not remarkable. Moderate numbers of iron-containing cells are seen in the peribronchial connective tissue and a few are also present in the alveoli.

Kidney: The glomeruli and tubules appear normal and stainable iron is not seen.

Mouse number 768; genotype sla/+; age 339 days; haematocrit 48%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Spleen: The malpighian bodies are normal; the red pulp is fairly prominent, occupying between 25 and 50% of the splenic tissue. There is active erythropoiesis and granulopoiesis, and megakaryocytes are frequent. Stainable iron is plentiful (Grade 4) and is situated mainly in the red pulp.

Liver: The parenchyma appears normal and haemopoietic foci are not seen. Stainable iron is not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are not prominent and fat spaces are not seen. Stainable iron is absent.

Duodenum: Sections stained with H. & E. appear normal. Stainable iron is widespread in the mucosal epithelial cells in moderate amounts (Grade 3), but none is seen in the lamina propria.

Pancreas: This shows no abnormality and stainable iron is not seen.

Mouse number 772; genotype sla/4; age 336 days; haematocrit 38%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Spleen: The malpighian bodies are normal; the red pulp occupies less than 25% of the splenic tissue. There is active erythropoiesis and granulopiesis and megakaryocytes are quite numerous. Stainable iron is plentiful (Grade 4) and is situated mainly in the red pulp.

Liver: The hepatic epithelium is not remarkable, but occasional very small foci of haemopoiesis are present. Stainable iron is not seen.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present.

Vascular spaces are not prominent and fat spaces are not seen. Stainable iron is absent.

Duodenum: Sections stained with H. & E. appear normal. Stainable iron is present in patches in the duodenal mucosal epithelium (Grade 2), but none is seen in the lamina propria.

Pancreas: This organ shows no abnormality and stainable iron is not seen.

Mouse number 774; genotype sla/+; age 336 days; haematocrit 36%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Spleen: The malpighian bodies are normal, the red pulp occupies between 25 and 50% of the splenic tissue, and shows active erythropoiesis and granulopoiesis, and megakaryocytes are fairly numerous. Stainable iron ir plentiful (Grade 4) and is situated mainly in the red pulp.

Liver: The liver parenchyma contains scanty small foci of haemopoiesis, but is otherwise unremarkable. Stainable iron is not present.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is absent.

Duodenum: Sections stained with H. & E. are normal. Stainable iron is present in patches in

the duodenal mucosal epithelium (Grade 2), but none is seen in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not present.

Mouse number 776; genotype sla/4; age 336 days; haematocrit 41%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Spleen: The malpighian bodies are normal. The red pulp occupies less than 25% of the splenic tissue and shows active haemopoiesis. Stainable iron is plentiful (Grade 4) and is located mainly in the red pulp.

Liver: The hepatic parenchymal cells are normal; occasional small foci of haemopoiesis are seen. Faint traces of stainable iron are present in the parenchymal cells, but none is seen in the Kupffer cells.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent but fat spaces are not seen. Stainable iron is absent.

Duodenum: Sections stained with H. & E. are normal. Stainable iron is present in patches in the duodenal mucosal epithelium (Grade 2) but none is seen in the lamina propria.

Pancreas: The pancreas appears normal and stainable iron is not present.

Mouse number 824; genotype sla/+; age 279 days; haematocrit 41%; stock - F_3 hybrid. Fasting for 6 hours prior to sacrifice.

Spleen: The malpighian bodies are normal. The red pulp occupies between 25 and 50% of the splenic tissue, and shows active haemopoiesis. Stainable iron is plentiful (Grade 4) and is situated mainly in the red pulp.

Liver: The parenchyma shows no abnormality and haemopoietic foci are not seen. Stainable iron is absent.

Bone marrow: Sections of sternal marrow show active haemopoiesis and megakaryocytes are present. Vascular spaces are moderately prominent, but fat spaces are not seen. Stainable iron is absent.

Duodenum: Sections stained with H. & E. are normal. Stainable iron is seen in the duodenal mucosal epithelium in patches (Grade 2), but none is seen in the lamina propria.

APPENDIX C

STUDIES WITH RADIO-ISOTOPES

A. Blood Volume Determinations

Experiment B.V.1

Isotope I 465643 CJS 1P. Sodium Chromate (^{51}Cr) solution, B.P. Radiochemical Centre, Amersham. Activity = 1 mc/ml. Chromium content 5.5 µg/ml.

Donor red cells from mice 1271 and 1285 (F_4, +/-); 0.1 ml of red cells was given intravenously.

Recipient mice — Standard = 66,594 c.p.m.

Number	Age (days)	Weight (G)	Genotype & strain	Haematocrit (per cent)	c.p.m. 20 µl of blood at 10 m. after injection
1239*	119	25.5	+/-,F_4	55	23,888
1277	95	21	+/-,F_4	53.5	26,429
1279	95	24.6	+/-,F_4	55	23,057
1281	95	22.3	+/-,F_4	51	23,461

* Received 0.11 ml red cells.

	Blood volume		Plasma volume		Red cell volume	
Number	ml	ml/100G	ml	ml/100G	ml	ml/100G
1239	1.5	6.0	0.8	3.1	0.7	2.9
1277	1.3	6.0	0.7	3.2	0.6	2.8
1279	1.4	5.9	0.7	3.1	0.7	2.8
1281	1.4	6.4	0.8	3.5	0.6	2.9

Experiment B.V.2

Isotope I 465643 CJS 1P. As for experiment B.V.1

Donor red cells from mice 1253 and 1317 (F_2, sla/-); 0.1 ml of red cells was given intravenously.

Recipient mice Standard = 55,716

Number	Age (days)	Weight (G)	Genotype & strain	Haematocrit (per cent)	c.p.m. 20 µl of blood at 10 m. after injection
1347	82	25.0	+/-, F_4	52	18,050
1353	80	24.6	+/-, F_4	49.5	17,822
1355	80	25.1	+/-, F_4	52	18,611
1357	80	24.4	+/-, F_4	53	19,372
1359	80	23.8	+/-, F_4	50	19,351

	Blood volume		Plasma volume		Red cell volume	
Number	ml	ml/100G	ml	ml/100G	ml	ml/100G
1347	1.5	6.2	0.8	3.0	0.7	3.2
1353	1.6	6.3	0.9	3.5	0.7	2.7
1355	1.5	6.0	0.8	3.3	0.7	2.7
1357	1.4	5.9	0.7	3.2	0.7	2.7
1359	1.4	6.1	0.8	3.4	0.6	2.7

Experiment B.V.3

Isotope I 468437 CJS 1P. Sodium Chromate (^{51}Cr) solution, B.P. Radiochemical Centre, Amersham. Activity = 1.03 mc/ml. Chromium content 9.3 µg/ml.

Donor red cells from mice 1237, 1375 (F_4, +/-); 0.1 ml of red cells was given intravenously.

Recipient mice Standard = 49,198 c.p.m.

Number	Age (days)	Weight (G)	Genotype & strain	Haematocrit (per cent)	c.p.m. 20 µl of blood at 10 m. after injection
1377	91	21.4	sla/-,F_4	28.5	14,274
1273	113	18.8	sla/-,F_4	28	13,026
1275	111	18.4	sla/-,F_4	27.5	14,852
1283	111	18.4	sla/-,F_4	29	14,274

	Blood volume		Plasma volume		Red cell volume	
Number	ml	ml/100G	ml	ml/100G	ml	ml/100G
1377	1.7	7.9	1.3	5.9	0.4	2.0
1273	1.9	10.0	1.4	7.5	0.5	2.5
1275	1.7	9.0	1.3	6.8	0.4	2.2
1283	1.6	8.9	1.2	6.6	0.4	2.3

Experiment B.V.4

Isotope I 472405 CJS 1P. Sodium chromate (^{51}Cr) solution, B.P. Radiochemical Centre, Amersham. Activity 1.07 mc/ml. Chromium content 4.3 µg/ml.

Donor red cells from mice 1349 and 1351 (F_4, sla/-); 0.1 ml of red cells was given intravenously.

Recipient mice — Standard = 70,296 c.p.m.

Number	Age (days)	Weight (G)	Genotype & strain	Haematocrit (per cent)	c.p.m. 20 µl of blood at 10 m. after injection
1101	197	21.8	sla/-,F_4	34	23,496
1083	202	29.3	sla/-,F_4	40.5	15,020
1497	97	14.5	sla/-,F_4	28	19,683
1647	76	15.2	sla/-,F_4	28	24,848
1649*	76	15.2	sla/-,F_4	29.5	16,050

* Received 0.06 ml red cells.

	Blood volume		Plasma volume		Red cell volume	
Number	ml	ml/100G	ml	ml/100G	ml	ml/100G
1101	1.5	6.9	1.0	4.8	0.5	2.1
1083	2.4	8.1	1.3	5.2	0.9	2.9
1497	1.8	12.4	1.4	9.3	0.4	3.1
1647	1.4	9.4	1.0	7.1	0.4	2.3
1649	1.2	7.8	0.9	5.8	0.3	2.0

Experiment B.V.5

Isotope I 472405 CJS 1P. As for experiment B.V.4.

Donor red cells from mice 1455 and 1469 (F_4, +/-).

Recipient mice Standard 45,466 c.p.m.

Number	Age (days)	Weight (G)	Genotype & strain	Haematocrit (per cent)	Vol. of donor cells injected (ml)
1451	93	24.0	+/-, F_3	53	0.09
1453	93	27.0	+/-, F_3	52	0.08
1457	96	28.0	+/-, F_3	52	0.09
1459	96	28.5	+/-, F_3	54.5	0.08
1461	95	28.2	+/-, F_3	51.5	0.08
1463	95	26.0	+/-, F_3	53	0.08
1465	95	26.5	+/-, F_3	53	0.05
1467	95	28.5	+/-, F_3	48.5	0.08
1571	75	22.5	+/-, F_3	53.5	0.07
1573	75	23.0	+/-, F_3	50	0.08

Number	c.p.m. 20 µl of blood at 10 m. after injection	Blood volume		Plasma Volume		Red cell volume	
		ml	ml/100G	ml	ml/100G	ml	ml/100G
1451	19,339	1.3	5.6	0.7	3.0	0.6	2.6
1453	13,290	1.6	5.9	0.9	3.2	0.7	2.7
1457	16,194	1.6	5.7	0.9	3.1	0.7	2.6
1459	12,453	1.7	5.9	0.9	3.1	0.8	2.8
1461	15,562	1.5	5.4	0.8	3.0	0.7	2.4
1463	18,135	1.3	5.0	0.7	2.7	0.6	2.3
1465	8,606	1.6	6.0	0.9	3.2	0.7	2.8
1467	14,177	1.6	5.6	0.9	3.2	0.7	2.4
1571	14,383	1.3	5.8	0.7	3.1	0.6	2.7
1573	16,169	1.3	5.7	0.7	3.2	0.6	2.5

Experiment B.V.6

Isotope Albumotope - I^{131} (Squibb); ^{131}I - labelled human serum albumin, 20 µc/ml.

0.1 ml of ^{131}I - labelled albumin solution was given intravenously.

Recipient mice — Standard = 55,930 c.p.m.

Number	Age (days)	Weight (G)	Genotype & strain	Haematocrit (per cent)	c.p.m. 20 µl of blood at 10 m. after injection
1473	102	26.0	+/-, F_3	54	10,866
1475	102	25.0	+/-, F_3	45	12,199
1515	92	25.5	+/-, F_3	51.5	13,001
1519	92	26.5	+/-, F_3	47.5	11,794
1521*	92	26.0	+/-, F_3	46	9,778
1567	73	24.5	+/-, F_3	48	10,210
1569*	73	21.5	+/-, F_3	50	11,581

*0.09 ml of ^{131}I-labelled albumin solution injected.

	Blood volume		Plasma volume		Red cell volume	
Number	ml	ml/100G	ml	ml/100G	ml	ml/100G
1473	2.6	10.0	1.4	5.3	1.2	4.7
1475	2.3	9.2	1.4	5.6	0.9	3.6
1515	2.2	8.6	1.2	4.6	1.0	4.0
1519	2.4	9.1	1.4	5.3	1.0	3.8
1521	2.6	10.0	1.5	5.9	1.1	4.1
1567	2.5	10.2	1.4	5.9	1.1	4.3
1569	2.2	10.2	1.2	5.7	1.0	4.5

Experiment B.V.7

Isotope Albumotope - I^{131} as for experiment B.V.6.

0.1 ml of ^{131}I-labelled albumin solution was given intravenously.

Recipient mice Standard = 74,473 c.p.m.

Number	Age (days)	Weight (G)	Genotype & strain	Haematocrit (per cent)	c.p.m. 20 µl of blood at 10 m. after injection
1329	130	23.0	sla/-,F_2	37.5	15,876
1339	130	23.0	sla/-,F_2	25.5	12,553
1625	60	17.5	sla/-,F_2	33.5	16,707
1629	63	21.5	sla/-,F_2	32.0	13,499
1637	63	17.5	sla/-,F_2	23.5	16,281

	Blood volume		Plasma volume		Red cell volume	
Number	ml	ml/100G	ml	ml/100G	ml	ml/100G
1329	2.4	10.2	1.5	6.8	0.9	3.4
1339	3.0	11.9	2.2	9.2	0.8	2.7
1625	2.2	12.7	1.5	9.0	0.7	3.7
1629	2.8	12.8	1.7	9.2	0.9	3.6
1637	2.3	13.1	1.8	10.4	0.5	2.7

B. Utilization of iron for haemopoiesis

1. Intraperitoneal administration of radioiron

Experiment MU7

Isotope FC - 424-1, Abbott Laboratories ^{59}Ferrous citrate, specific activity (S.A.) = 7.5 µc/µg. Elemental iron content = 4.3 µg/ml.

Each animal received intraperitoneally 0.025 ml of isotope solution, diluted to 0.25 ml with aline. Iron content 0.1 µg, radioactivity 0.75 µc.

Animals were sacrificed at day 5 and blood samples taken for counting.

Counts on day 5 were obtained on another Nuclear Chicago "TOBOR" by courtesy of Dr. R. S. Smith, Children's Hospital, Buffalo, since the instrument in my laboratory was temporarily out of order.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0	c.p.m. of blood day 5	Volume of blood counted (ml)	Total counts in peripheral blood (day 5)	Utilization of iron for haemopoiesis (per cent)
194 sla/sla	O.M.S.	127 F	20.4	1.82	34	24,571	4,965	0.60	15,069	65.4
196 sla/sla	O.M.S.	110 F	24.0	2.14	34	23,593	4,852	0.54	19,228	86.9
193 sla/-	O.M.S.	117 M	23.2	2.07	39	23,316	1,716	0.30	11,840	51.1

Standard day 0 = 24,272 c.p.m.

Standard day 5 = 22,767 c.p.m.

$$\frac{S_0}{S_5} = 1,066$$

Experiment MU8

Isotope Fe-424-1, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 7 µc/µg. Elemental iron content 4.3 µg/ml.

The dose was prepared and given as for MU7; each animal received 0.1 µg of iron, radioactivity, 0.7 µc.

Animals were sacrificed at day 5 and blood samples taken for counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0	c.p.m. of blood day 5	Volume of blood counted (ml)	Total counts in Peripheral blood day 5	Utilization of iron for haemopoiesis (per cent)
191 sla/-	O.M.S.	181 M	28.4	2.54	37	31,519	4,082	0.56	18,512	63.7
133 sla/-	O.M.S.	201 M	22.5	2.01	23.5	30,466	2,851	0.4	14,326	51.0
107 sla/-	O.M.S.	188 M	29.1	2.60	42	30,411	2,557	0.4	16,621	59.2
147 sla/-	O.M.S.	270 M	29.3	2.62	38	30,347	3,097	0.6	13,525	48.3
155 sla/-	O.M.S.	280 M	32.2	2.88	38	30,608	4,197	0.8	15,109	53.5
45' sla/-	O.M.S.	263 M	28.5	2.55	48	31,422	4,592	0.52	22,519	77.7
315 +/-	F_1	211 M	36.9	2.16	-	30,449	1,140	0.2	12,312	43.8
311 +/-	F_1	211 M	35.9	2.10	43	30,785	1,130	0.2	11,865	41.8
313 +/-	F_1	211 M	29.5	1.73	-	31,474	916	0.2	7,969	27.4
325 +/-	F_1	211 M	34.7	2.03	39.5	31,516	1,678	0.4	8,516	29.3
317 +/-	F_1	211 M	35.6	2.08	42	30,350	2,965	0.55	11,212	40.0
323 +/-	F_1	211 M	36.2	2.12	39.5	31,420	1,075	0.2	11,395	39.3

Standard day 0 = 30,910 c.p.m.

Standard day 5 = 28,504 c.p.m.

$$\frac{S_0}{S_5} = 1,084$$

Experiment MU9

Isotope Fe-463-17, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 10.3 μc/μg. Elemental iron content = 1.3 μg/ml.

Each animal received intraperitoneally 0.05 ml of isotope solution diluted to 0.2 ml with saline. Iron content = 0.065 μg, radioactivity 0.75 μc.

Animals were sacrificed at day 5 after injection and blood samples taken for radioactive counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0	c.p.m. of blood day 5	Volume of blood counted (ml)	Total counts in peripheral blood day 5	Utilization of iron for haemopoiesis (per cent)
701 +/-	F_3	105 M	28.9	1.69	45	30,560	3,586	0.77	7,871	27.6
703 +/-	F_3	105 M	25.5	1.49	42.5	30,911	5,197	0.85	9,110	31.5
705 +/-	F_3	105 M	30.0	1.76	49	25,525	5,739	0.90	11,223	47.0

Experiment MU10

Isotope FC-463-17, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 9.2 μc/μg. Elemental iron content = 1.3 μg/ml.

Each animal received intraperitoneally 0.05 ml isotope solution diluted to o.2 ml with saline. Iron content 0.065 μg radioactivity, 0.6 μc.

Animals were sacrificed at day 5 and blood samples taken for radioactive counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0	c.p.m. of blood day 5	Volume of blood counted (ml)	Total counts in peripheral blood day 5	Utilization of iron for haemopoiesis (per cent)
491 sla/-	F_2	162 M	22.5	2.01	13.5	30,513	4,742	1.1	8,664	30.9
499 sla/-	F_2	116 M	18.0	1.61	16.5	31,663	4,278	1.1	6,261	19.9
729 sla/-	F_2	68 M	24.5	2.19	42	26,171	4,780	1.1	9,517	39.6

Standard day 0 = 29,718 c.p.m.

Standard day 5 = 27,300 c.p.m.

$\frac{S_0}{S_5} = 1.089$

Experiment MU11

Isotope FC-463-17, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 8.7 μc/μg. Elemental iron content = 1.3 μg/ml.

Each animal received 0.1 ml of undiluted isotope solution intraperitoneally. Iron content 0.13 μg, radioactivity, 1.1 μc.

Serial blood samples of 20 ul were taken daily for 11 days (except for days 4 and 9) for counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood Volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0
257 +/-	F_1	291 M	39.3	2.30	43	46,516
259 +/-	F_1	291 M	37.0	2.16	47	45,294
349 +/-	F_1	263 M	30.9	1.81	45.5	44,038
357 +/-	F_1	263 M	31.3	1.83	47	58,837

	S_o/S_t
Standard - day 0 = 51,784 c.p.m.	
Standard - day 1 = 50,758 c.p.m.	1.020
Standard - day 2 = 50,502 c.p.m.	1.025
Standard - day 3 = 50,335 c.p.m.	1.029
Standard - day 4 - No results on day 4	-
Standard - day 5 = 50,330 c.p.m.	1.029
Standard - day 6 = 48,874 c.p.m.	1.060
Standard - day 7 = 48,986 c.p.m.	1.057
Standard - day 8 = 48,670 c.p.m.	1.064
Standard - day 9 - No results on day 9	-
Standard - day 10 = 48,572 c.p.m.	1.066
Standard - day 11 = 48,014 c.p.m.	1.079

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
257	1	139	15,985	35.1
259	1	137	14,796	33.3
349	1	234	21,177	49.0
357	1	240	21,960	38.1
257	2	217	24,955	54.9
259	2	198	21,384	48.4
349	2	290	26,245	61.1
357	2	293	26,886	46.8
257	3	170	19,550	43.2
259	3	163	17,507	39.8
349	3	315	28,507	66.6
357	3	238	21,777	38.1
257	5	139	15,985	35.4
259	5	143	16,445	37.4
349	5	263	23,801	55.6
357	5	273	24,980	43.7
257	6	134	15,410	35.1
259	6	146	15,768	36.9
349	6	185	16,743	40.3
357	6	226	20,679	37.3
257	7	132	15,180	34.5
259	7	138	14,904	34.8
349	7	230	20,815	50.0
357	7	215	19,673	35.3

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
257	8	156	17,940	41.0
259	8	146	15,768	37.0
349	8	239	21,629	52.2
357	8	272	24,888	45.0
257	10	137	15,755	36.1
259	10	127	13,716	32.3
349	10	214	19,367	46.9
357	10	220	20,130	36.5
257	11	145	16,675	38.7
259	11	121	13,068	31.1
349	11	158	14,299	35.0
357	11	199	18,208	33.4

* Based on 15 min. counts of 20 µl samples of blood.

Experiment MU12

Isotope FC-475-1, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 21.0 μc/μg. Elemental iron content 1.5 μg/ml.

Each animal received intraperitoneally 0.05 ml of isotope solution, diluted to 0.1 ml with saline. Iron content 0.075 μg, radioactivity 1.6 μc.

Serial blood samples of 20 μl were taken daily for 10 days for counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood Volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0
707 +/-	F_1	105 M	32.6	1.91	51	48,991
709 +/-	F_1	105 M	32.9	1.92	47	53,869
711 +/-	F_1	105 M	34.8	2.04	48	48,047
713 +/-	F_1	105 M	35.3	2.07	48.5	49,184

	S_o/S_t
Standard - day 0 = 56,503 c.p.m.	
Standard - day 1 = 56,947 c.p.m.	0.992
Standard - day 2 = 56,727 c.p.m.	0.996
Standard - day 3 = 55,426 c.p.m.	1.019
Standard - day 4 = 54,307 c.p.m.	1.040
Standard - day 5 = 53,647 c.p.m.	1.053
Standard - day 6 = 53,275 c.p.m.	1.061
Standard - day 7 = 53,268 c.p.m.	1.061
Standard - day 8 = 52,066 c.p.m.	1.085
Standard - day 9 = 51,882 c.p.m.	1.089
Standard - day 10 = 51,152 c.p.m.	1.105

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
707	1	114	10,887	22.0
709	1	121	11,616	21.4
711	1	158	16,116	33.3
713	1	108	11,178	22.5
707	2	130	12,415	25.2
709	2	217	20,832	38.5
711	2	162	16,524	34.3
713	2	196	20,286	41.0
707	3	160	15,280	31.8
709	3	250	24,000	45.3
711	3	194	19,788	42.0
713	3	220	22,770	47.2
707	4	157	14,993	31.8
709	4	250	24,000	46.3
711	4	199	20,298	43.6
713	4	220	22,770	48.1
707	5	152	14,516	31.2
709	5	234	22,464	43.9
711	5	183	18,666	41.8
713	5	213	22,045	47.2
707	6	143	13,656	29.6
709	6	211	20,256	39.9
711	6	174	17,748	40.0

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
713	6	197	20,389	44.0
707	7	142	13,561	29.4
709	7	219	21,024	41.4
711	7	165	16,830	37.9
713	7	180	18,630	40.2
707	8	127	12,129	26.9
709	8	195	18,720	37.7
711	8	162	16,524	38.1
713	8	175	18,113	40.0
707	9	122	11,651	25.9
709	9	194	18,624	37.6
711	9	160	16,320	37.0
713	9	159	16,457	36.4
707	10	109	10,410	23.5
709	10	175	16,800	34.5
711	10	117	11,934	27.5
713	10	128	13,248	29.8

* Based on 15 min. counts of 20 ul samples.

Experiment MU13

Isotope FC-475-1, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 17 μc/μg. Elemental iron content = 1.5 μg/ml.

Each animal received intraperitoneally 0.067 ml of isotope solution made up to 0.1 ml with saline. Iron content 0.1 μg, radioactivity, 1.7 μc.

Serial blood samples of 20 μl were taken daily for 10 days for counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0
527 sla/-	O.M.S.	103 M	24.3	2.17	36	74,531
529 sla/-	O.M.S.	103 M	25.2	2.25	34.5	61,645
533 sla/-	O.M.S.	103 M	24.6	2.20	24	53,760
- +/-	C57B1	200 M	25.0	1.46	-	64,755

	S_o/S_t
Standard - day 0 = 64,957 c.p.m.	
Standard - day 1 = 66,821 c.p.m.	0.972
Standard - day 2 = 64,918 c.p.m.	1.001
Standard - day 3 = 65,016 c.p.m.	0.999
Standard - day 4 = 66,714 c.p.m.	0.974
Standard - day 5 = 66,699 c.p.m.	0.973
Standard - day 6 = 67,836 c.p.m.	0.958
Standard - day 7 = 66,086 c.p.m.	0.983
Standard - day 8 = 66,989 c.p.m.	0.970
Standard - day 9 = 66,944 c.p.m.	0.970
Standard - day 10 = 67,744 c.p.m.	0.959

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
527	1	269	29,187	38.1
529	1	172	19,350	30.9
533	1	232	25,520	46.1
C57B1	1	198	14,454	21.7
527	2	325	35,263	47.4
529	2	207	23,288	37.8
533	2	201	22,110	41.2
C57B1	2	303	22,119	34.2
527	3	272	29,512	39.6
529	3	187	21,038	34.1
533	3	169	19,690	36.6
C57B1	3	288	21,024	32.4
527	4	276	29,946	39.1
529	4	188	21,150	33.4
533	4	181	19,910	36.1
C57B1	4	215	15,695	23.6
527	5	269	29,187	38.1
529	5	190	21,375	33.7
533	5	194	21,340	38.6
C57B1	5	169	12,337	18.5
527	6	219	23,762	30.5
529	6	178	20,025	31.1
533	6	172	18,920	33.7

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
C57B1	6	190	13,870	20.5
527	7	248	26,908	35.5
529	7	166	18,675	29.8
533	7	156	17,160	31.4
C57B1	7	162	11,826	18.0
527	8	231	25,064	32.6
529	8	172	19,350	30.4
533	8	152	16,720	30.2
C57B1	8	166	12,118	18.2
527	9	198	21,483	28.0
529	9	171	19,238	30.3
533	9	141	15,510	28.0
C57B1	9	166	12,118	18.2
527	10	216	23,436	30.2
529	10	173	19,462	30.3
533	10	138	15,180	27.1
C57B1	10	154	11,242	16.6

* Based on 15 min. counts of 20 µl of blood.

Experiment MU14

Isotope FC-489-11, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 21 µc/µg. Elemental iron content = 1.3 µg/ml.

Each animal received intraperitoneally 0.05 ml of isotope solution made up to 0.1 ml with saline. Iron content 0.065 µg, radioactivity 1.4 µc.

Serial blood samples of 20 µl were taken daily for 10 days for radioactive counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	^{59}Fe dose c.p.m. day 0
371 +/-	F_2	350 M	34.0	1.99	62,187
409 +/-	F_2	316 M	33.0	1.93	51,937
411 +/-	F_2	316 M	33.2	1.94	61,243
443 +/-	F_2	319 M	40.0	2.34	64,525
369 sla/-	F_2	350 M	33.5	2.99	64,090
445 sla/-	F_2	243 M	26.0	2.32	64,255

	S_o/S_t
Standard - day 0 = 58,799 c.p.m.	
Standard - day 1 = 57,844 c.p.m.	1.017
Standard - day 2 = 58,307 c.p.m.	1.008
Standard - day 3 = 57,764 c.p.m.	1.018
Standard - day 4 = 57,007 c.p.m.	1.031
Standard - day 5 = 55,917 c.p.m.	1.052
Standard - day 6 = 55,891 c.p.m.	1.052
Standard - day 7 = 54,806 c.p.m.	1.087
Standard - day 8 = 52,790 c.p.m.	1.114
Standard - day 9 = 55,235 c.p.m.	1.065
Standard - day 10 = 53,931 c.p.m.	1.090

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
371	1	79	7,861	12.9
409	1	102	9,843	19.3
411	1	119	11,543	19.2
443	1	112	13,104	20.7
369	1	109	16,296	25.9
445	1	146	16,936	26.8
371	2	122	12,139	19.7
409	2	146	14,089	27.3
411	2	168	16,296	26.8
433	2	156	18,252	28.5
369	2	218	32,591	51.3
445	2	326	37,816	59.3
371	3	132	13,134	21.5
405	3	165	15,923	31.2
411	3	204	19,788	32.9
433	3	210	24,570	40.8
369	3	238	35,581	56.5
445	3	340	39,440	62.5
371	4	125	12,438	20.6
409	4	153	14,765	29.3
411	4	175	16,975	28.1
433	4	159	18,603	29.7
369	4	164	24,518	39.4

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
445	4	238	27,608	44.3
371	5	125	12,438	21.0
409	5	146	14,089	28.5
411	5	149	15,423	26.5
443	5	155	18,135	29.6
369	5	139	20,781	34.1
445	5	216	25,056	41.0
371	6	128	12,736	21.5
409	6	161	15,537	31.5
441	6	176	17,072	29.3
443	6	168	19,656	32.0
369	6	161	24,070	39.5
445	6	217	25,172	41.2
371	7	102	10,149	17.7
409	7	113	10,905	22.8
411	7	158	15,326	27.2
443	7	177	20,709	34.9
369	7	159	23,771	40.3
445	7	191	22,156	37.5
371	8	109	10,846	19.4
409	8	113	10,906	23.4
411	8	140	13,580	24.7
443	8	120	14,040	24.2

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
369	8	137	20,482	35.6
445	8	177	20,532	35.6
371	9	134	13,333	22.8
409	9	116	11,194	22.9
411	9	147	14,259	24.8
443	9	141	16,497	27.2
369	9	102	15,249	25.3
445	9	178	20,648	34.2
371	10	144	14,328	25.1
409	10	156	15,054	31.5
411	10	127	12,319	21.9
443	10	138	16,008	27.0
369	10	126	18,837	32.0
445	10	175	20,300	34.4

* Based on 15 min. counts of 20 µl blood sample.

Experiment MU15

Isotope FC-520, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 21 μc/μg; Elemental iron content, 1.1 μg/ml.

Each animal received intraperitoneally 0.067 ml of isotope solution diluted to 0.1 ml with saline. Iron content 0.074 μg, radioactivity 1.6 μc.

Serial blood samples of 20 μl were taken daily for 6 days for counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	^{59}Fe dose c.p.m. day 0
837 +/-	F_3	152 M	35.2	2.06	31,921
807 +/-	F_1	166 M	36.0	2.10	27,687
839 +/-	F_3	132 M	28.8	1.68	32,344
843 +/-	F_3	148 M	28.5	1.67	37,363
813 +/-	F_1	166 M	27.2	1.59	31,529
815 sla/-	F_1	166 M	23.1	2.06	30,287
873 sla/-	F_3	114 M	24.5	2.19	29,460
875 sla/-	F_3	114 M	23.8	2.13	31,594

	S_o/S_t
Standard - day 0 = 33,361 c.p.m.	
Standard - day 1 = 34,546 c.p.m.	0.966
Standard - day 2 = 31,499 c.p.m.	1.059
Standard - day 3 = 29,834 c.p.m.	1.118
Standard - day 4 = 28,830 c.p.m.	1.157
Standard - day 5 = 28,272 c.p.m.	1.180
Standard - day 6 = 30,730 c.p.m.	1.086

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
837	1	122	12,566	38.0
807	1	113	11,865	41.4
839	1	154	12,936	38.6
843	1	187	15,614	40.4
813	1	166	13,197	40.4
815	1	87	8,961	28.6
873	1	244	26,718	87.6
875	1	217	23,111	70.7
837	2	135	13,905	46.1
807	2	137	14,385	55.0
839	2	171	14,193	46.5
843	2	229	19,038	54.0
813	2	225	17,808	59.8
815	2	108	11,124	39.0
873	2	122	13,359	48.0
875	2	190	20,235	67.8
837	3	149	15,244	53.3
807	3	140	14,700	59.3
839	3	170	14,280	49.3
843	3	188	14,863	44.5
813	3	214	17,013	60.3
815	3	139	14,317	52.8
873	3	102	11,169	42.4

Mouse number	Day	c.p.m. in blood	Total c.p.m. in blood	Utilization of iron for haemopoiesis (per cent)
875	3	196	20,874	73.9
837	4	92	9,476	34.3
807	4	93	9,765	40.8
839	4	132	11,088	39.7
843	4	177	15,576	48.2
813	4	164	13,038	47.8
815	4	115	11,845	45.2
873	4	137	15,070	59.2
875	4	176	18,744	68.8
837	5	111	11,433	42.2
807	5	119	12,495	53.2
839	5	172	14,448	52.7
843	5	203	17,560	55.5
813	5	194	15,423	57.7
815	5	133	13,699	53.4
873	5	156	17,082	68.4
875	5	185	19,703	73.6
837	6	110	11,330	38.5
807	6	113	11,865	46.5
839	6	155	13,020	43.7
843	6	218	18,203	57.4
813	6	185	14,707	50.6
815	6	98	10,094	36.2
873	6	114	12,483	46.0
875	6	180	19,170	65.9

2. Intravenous administration of radio-iron.

Experiment MU16

Isotope FC-550-16, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 15 μc/μg. Elemental iron content = 1.4 μg/ml.

Each animal received 0.05 ml of isotope solution, diluted to 0.1 ml with normal saline prepared with de-ionized water. Iron content 0.07 μg, radioactivity, 1.05 μc.

Whole body radioactivity on the day of administration was measured after the removal of samples for experiment P.1.C. 5, (Appendix C, Section C).

A blood sample of 20 μl was taken on the 5th day after administration, for counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0	c.p.m.* of blood day 5	Volume of blood counted (µl)	Total counts in peripheral blood day 5	Utilization of iron for haemopoiesis (per cent)
1129 +/-	F_4	107 M	24	1.40	46	29,643	270	20	18,900	66.0
1131 sla/-	F_4	107 M	20.1	1.79	15.5	30,130	177	20	15,842	54.4
1111 sla/-	F_2	100 M	25.8	2.30	28.5	30,278	222	20	25,530	87.3
1203 sla/-	F_2	88 M	27.4	2.45	22.5	27,900	134	20	16,415	60.9
1127 +/-	F_2	104 M	30.5	1.78	51.0	25,622	176	20	15,664	63.3

Standard day 0 = 31,126

Standard day 5 = 30,078

$\frac{S_0}{S_5} = 1.035$

* Based on 15 min. counts.

Experiment MU17

Isotope FC-550-16, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 11.7 μc/μg. Elemental iron content = 1.4 μg/ml.

Each animal received 0.1 ml of the undiluted isotope solution. Iron content 0.14 μg, radioactivity, 1.6 μc.

Whole body radioactivity on the day of administration was measured after the removal of samples for experiment P.1.C.6 (Appendix C, Section C).

A blood sample of 20 μl was taken on the 5th day after administration, for counting.

Mouse number and genotype	Stock	Age (days) sex	Weight (grams)	Blood volume (ml)	PCV %	^{59}Fe dose c.p.m. day 0	c.p.m.* of blood day 5	Volume of blood counted (µl)	Total counts in peripheral blood day 5	Utilization of iron for haemopoiesis (per cent)
1245 sla/-	F_5	114 M	19.3	1.72	29	54,615	417	20	35,862	70.6
1155 sla/-	F_3	116 M	21.2	1.89	28	42,514	243	20	22,964	58.1
1171 +/-	F_3	106 M	24.5	1.43	47	41,184	399	20	28,528	74.5
1153 +/-	F_3	114 M	29.1	1.70	48	46,384	336	20	28,560	66.2
1187 +/-	F_5	102 M	24.0	1.40	48	42,071	360	20	25,200	64.4

Standard day 0 = 43,319 c.p.m.

Standard day 5 = 40,286 c.p.m.

$$\frac{S_0}{S_5} = 1.075$$

* Based on 15 min. counts.

Experiment MU18

Isotope FC-575-4, Abbott Laboratories ^{59}Ferrous citrate, S. A.= 21.5 μc/μg. Elemental iron content, 1.3 μg/ml.

Each animal received intravenously 0.05 ml of isotope solution diluted to 0.1 ml with normal saline prepared from de-ionized water. Iron content 0.065 μg, radioactivity 1.4 μc.

Blood samples of 40 μl were taken on the 1st and 5th days after injection for counting.

Mouse number and genotype	Stock	Age (days)	Weight (G)	Blood volume (ml)	PCV (%)
1049 sla/-	F_2	243	28.7	2.56	31
1039 sla/-	F_2	243	26.2	2.34	25.5
995 +/-	F_2	250	34.6	2.02	49
991 +/-	F_2	250	35.3	2.07	51
989 +/-	F_2	255	42.7	2.50	47.5
1005 +/-	F_2	244	40.8	2.39	44.5
987 sla/-	F_2	255	27.4	2.45	30
997 sla/-	F_2	250	28.4	2.54	30
1003 sla/-	F_2	244	32.4	2.89	35.5
1035 sla/-	F_2	243	24.9	2.22	31
1563 +/-	F_4	118	27.3	1.60	42.5
1495 sla/-	F_4	132	18.5	1.65	20
1043 +/-	F_4	243	33.3	1.95	48.5
1053 +/-	F_2	237	26.5	1.55	44.5
1037 +/-	F_4	243	35.3	2.07	50
1557 sla/-	F_4	118	18.9	1.69	23.5
1041 +/-	F_4	243	34.6	2.02	46.5
1051 sla/-	F_2	237	23.2	2.07	22.5
1493 +/-	F_4	132	29.4	1.72	45
1559 +/-	F_4	118	26.5	1.55	47

Mouse number and genotype	^{59}Fe dose c.p.m. on day 0	c.p.m. in blood*		Total counts in blood		Utilization of iron (per cent)	
		day 1	day 5	day 1	day 5	day 1	day 5
1049 sla/-	43,253	451	688	28,864	44,032	68.1	85.5
1039 sla/-	45,167	368	526	21,528	30,771	48.7	57.2
995 +/-	40,491	243	602	12,272	30,401	28.0	57.1
991 +/-	45,256	262	496	13,559	25,668	30.6	47.6
989 +/-	43,917	203	529	12,688	33,063	29.5	63.2
1005 +/-	44,115	229	565	13,683	33,759	31.7	64.3
987 sla/-	45,967	507	703	31,050	43,059	69.0	78.7
997 sla/-	48,425	430	832	27,305	52,832	57.6	91.6
1003 sla/-	46,580	664	845	47,808	60,979	107.1	112.3
1035 sla/-	45,703	482	744	26,751	41,292	59.8	75.9
1563 +/-	42,976	353	899	14,120	35,960	32.1	67.2
1495 sla/-	45,867	866	1080	35,723	44,550	79.5	81.6
1043 +/-	40,805	183	464	8,921	22,620	22.3	46.6
1053 +/-	45,521	129	608	9,998	23,560	22.4	43.5
1037 +/-	42,575	281	544	14,542	28,152	34.9	55.5
1557 sla/-	44,087	658	693	27,801	29,279	64.4	55.8
1041 +/-	42,991	206	524	10,403	26,462	24.7	51.7
1051 sla/-	41,601	754	916	39,020	47,403	95.8	95.7
1493 +/-	46,165	280	623	12,040	26,789	26.6	48.7
1559 +/-	44,922	416	795	16,120	30,806	36.6	57.5

Standard day 0 = 46,158 c.p.m.

Standard day 1 = 45,213 c.p.m. $\frac{S_0}{S_1} = 1.021$

Standard day 5 = 54,957 c.p.m. $\frac{S_0}{S_5} = 0.840$

* Based on 15 min. counts (day 1) or 5 min. counts (day 5) on 40 µl blood samples.

† 20 µl sample only.

C. Plasma iron clearance determinations.

Experiment P.1.C.1

Isotope FC-536-10, Abbott Laboratories ^{59}Ferrous citrate - specific activity (S.A.) = 17.4 μc/μg. Elemental iron content = 1.8 μg/ml.

Each animal received 0.06 ml of isotope solution diluted to 0.1 ml with normal saline prepared with de-ionized water. Iron content 0.11 μg, radioactivity 1.9 μc.

10 μl samples of plasma were taken for counting at 10, 20 and 30 minutes after intravenous injection.

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent)	Counts in 5 min. 10 μl plasma at 10 m	20 m	30 m	P.1.C.* $T\frac{1}{2}$ mins.
895	156	+/-, F_3	51.5	2781	2471	-	46
897	156	+/-, F_3	50.5	2971	2711	2237	44
899	156	+/-, F_3	51	1731	1650	13.2	52
901	143	+/-, F_3	49	2324	2076	1672	40

* Plasma iron clearance, half time.

Experiment P.1.C.2

Isotope FC-536-10, Abbott Laboratories ^{59}Ferrous citrate. Prepared as for experiment P.1.C.1. Samples of plasma taken as for experiment P.1.C.1.

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent)	Counts in 5 min. 10 µl plasma at 10 m	20 m	30 m	P.1.C. $T_{\frac{1}{2}}$ mins.
907	111	+/-, F_3	49	2881	2379	2003	36
909	111	+/-, F_3	49	2652	2376	2040	60
913	116	+/-, F_3	48	2590	2043	1696	36
1007	96	+/-, F_3	51	2456	2136	2014	63

Experiment P.1.C.3

Isotope FC-545-2, Abbott Laboratories ^{59}Ferrous citrate - S.A. = 25 µc/µg. Elemental iron content = 1.2 µg/ml.

Each animal received 0.04 ml isotope solution diluted to 0.1 ml with normal saline prepared with de-ionized water. Iron content 0.05 µg, radioactivity 1.25 µc.

Samples of plasma taken as for experiment P.1.C.1.

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent)	Counts in 5 min. 10 µl plasma at 10 m	20 m	30 m	P.1.C. $T_{\frac{1}{2}}$ mins.
1011	92	+/-,F_4	50.5	2174	1758	1504	38
1013	92	+/-,F_4	54	1449	1307	1079	42
1115	61	+/-,F_4	46	1411	1233	1124	55
1201	62	sla/-,F_4	17.5	1077	658	426	14

Experiment P.1.C.4

Isotope FC-545-2, Abbott Laboratories ^{59}Ferrous citrate - S.A. = 22 μc/μg. Elemental iron content = 1.2 μg/ml.

Each animal received 0.05 ml of isotope solution, diluted to 0.1 ml with normal saline prepared with de-ionized water. Iron content 0.6 μg, radioactivity 1.3 μc.

10 μl samples of plasma taken as for P.1.C.1.

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent)	Counts in 5 min. 10 μl plasma at 10 m	20 m	30 m	P.1.C.* $T_{\frac{1}{2}}$ mins.
433	592	sla/-,F_2	25.5	1779	1515	1333	46
435	592	+/-, F_2	45	2807	2625	2340	65
1009	112	sla/-,F_3	26	1368	1163	934	35

Experiment P.1.C.5

Isotope FC-550-16, Abbott Laboratories ^{59}Ferrous citrate - S.A. = 15 μc/μg. Elemental iron content = 1.4 μg/ml.

Each animal received 0.05 ml of isotope solution, diluted to 0.1 ml with normal saline prepared with de-ionized water. Iron content 0.07 μg, radioactivity 1.05 μc.

10 μl samples of plasma taken as for P.1.C.1.

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent)	Counts in 5 mins. 10 μl plasma at 10 m	20 m	30 m	P.1.C. $T\frac{1}{2}$ mins.
1111	100	sla/-,F_2	28.5	1265	659	420	12
1127	104	+/-, F_2	51	1192	1108	946	55
1129	107	+/-, F_4	48	1655	1351	1067	31
1131	107	sla/-,F_4	15.5	1713	801	559	12
1203	88	sla/-,F_2	22.5	1640	1150	863	21

Experiment P.1.C.6

Isotope FC-550-16, Abbott Laboratories ^{59}Ferrous citrate - S.A. = 11.7 μc/μg. Elemental iron content = 1.4 μg/ml.

Each animal received 0.1 ml of the undiluted isotope solution. Iron content 0.14 μg, radioactivity, 1.6 μc.

10 μl samples of plasma taken as for P.1.C.1.

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent)	Counts in 10 min. 10 μl plasma at 10 m	20 m	30 m.	P.1.C. $T_{\frac{1}{2}}$ mins.
1153	114	+/-, F_3	48	3592	3005	-	40
1155	114	sla/-,F_3	28	5870	4680	3981	34
1171	106	+/-, F_4	47	3896	3202	2647	34
1187	102	+/-, F_5	48	4446	3888	3324	46
1245	116	sla/-,F_5	29	1720	910	619	13

D. Excretion or iron.

Experiment M.I.E.1.

Isotope FC-496-5, Abbott Laboratories ^{59}Ferrous citrate, specific activity, S.A. = 10 µc/ug. Elemental iron content = 25 µg/ml.

Each animal received intraperitoneally 0.1 ml of isotope solution containing 0.25 µg of elemental iron, radioactivity 2.5 µc.

Counts of the retained radioactivity were obtained on days 1, 3 and 6 after injection of ^{59}Fe.

Mouse number and genotype	Stock	Age (days)	P.C.V. (%)	^{59}Fe dose c.p.m. day 0
367 +/-	F_1	421	45.5	75,293
371 +/-	F_1	421	43.5	84,877
373 +/-	F_1	421	44	65,501
375 +/-	F_1	421	54	84,522
749 +/-	F_1	178	42.5	61,402
755 +/-	F_1	167	40.5	86,668
763 +/-	F_1	178	44.5	96,195
765 +/-	F_1	178	42.5	85,277

Mouse number	c.p.m. day 1	% retention of ^{59}Fe	c.p.m. day 3	% retention of ^{59}Fe	c.p.m. day 6	% retention of ^{59}Fe
367	73,143	100	67,736	98	60,398	93
371	80,040	97	76,612	98	71,432	97
373	61,779	97	54,958	91	49,725	88
375	84,173	102	74,934	97	68,712	94
749	65,314	109	57,206	101	56,001	105
755	86,540	102	78,966	99	71,251	95
763	95,469	102	87,921	99	75,020	90
765	82,846	100	75,919	97	69,027	94

Standard day 0 61,701 c.p.m.

Standard day 1 58,348 c.p.m. $S_o/S_1 = 1.057$

Standard day 3 56,515 c.p.m. $S_o/S_3 = 1.092$

Standard day 6 53,235 c.p.m. $S_o/S_6 = 1.159$

Experiment M.I.E.2

Isotope FC-520-4, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 21 μc/μg, elemental iron content = 1.1 μg/ml.

The isotope solution was diluted 2:1 with iron-free isotonic saline and 0.1 ml of diluted isotope solution given intraperitoneally, containing 0.074 μg of elemental iron, radioactivity 1.6 μc.

Counts of retained radioactivity were obtained on days 1, 3, 6, 11, 15, 23, 50, 147 and 178 after injection of ^{59}Fe.

Mouse number and genotype	Stock	Age (days)	P.C.V. (%)	^{59}Fe dose c.p.m. day 0
837 +/-	F_3	152	47	31,921
807 +/-	F_1	166	44.5	27,687
839 +/-	F_3	132	44.5	32,344
843 +/-	F_3	148	45.5	37,363
813 +/-	F_1	166	46.5	31,529
815 sla/-	F_1	166	17	30,287
873 sla/-	F_3	114	20	29,460
875 sla/-	F_3	114	33.5	31,594

Mouse number	c.p.m. day 1	% retention of ^{59}Fe	c.p.m. day 3	% retention of ^{59}Fe	c.p.m. day 6	% retention of ^{59}Fe
837	33,394	101	27,645	97	25,798	88
807	33,059	115	26,426	107	24,456	96
839	32,727	98	28,281	98	29,343	98
843	40,173	104	32,430	97	31,710	92
813	39,147	119	27,641	98	25,394	87
815	36,953	118	29,607	109	23,591	85
873	34,263	112	27,323	104	25,382	94
875	36,162	110	30,649	108	27,198	94

Mouse number	c.p.m. day 11	% retention of ^{59}Fe	c.p.m. day 15	% retention of ^{59}Fe	c.p.m. day 23	% retention of ^{59}Fe
837	18,828	80	18,290	85	14,623	78
807	18,003	89	16,472	88	12,967	80
839	20,286	85	19,229	88	15,273	81
843	24,270	88	22,668	89	17,778	81
813	19,006	82	17,708	83	14,234	77
815	14,734	66	12,909	63	8,642	49
873	18,958	87	16,908	85	11,874	69
875	22,342	96	21,846	102	16,234	88

Mouse number	c.p.m. day 50	% retention of ^{59}Fe	c.p.m. day 147	% retention of ^{59}Fe	c.p.m. day 178	% retention of ^{59}Fe
837	7,491	69	-	-	-	-
807	7,020	75	1,935	27	782	27
839	8,280	76	-	-	-	-
843	9,524	76	-	-	-	-
813	7,401	69	2,462	30	989	30
815	3,435	34	814	10	347	11
873	5,805	59	1,377	18	627	21
875	9,113	86	2,729	33	1,094	34

Standard day 0	33,361 c.p.m.	
Standard day 1	34,546 c.p.m.	$S_0/S_1 = 0.966$
Standard day 3	29,834 c.p.m.	$S_0/S_3 = 1.118$
Standard day 6	30,730 c.p.m.	$S_0/S_6 = 1.085$
Standard day 11	24,592 c.p.m.	$S_0/S_{11} = 1.356$
Standard day 15	22,599 c.p.m.	$S_0/S_{15} = 1.476$
Standard day 23	19,538 c.p.m.	$S_0/S_{23} = 1.707$
Standard day 50	11,222 c.p.m.	$S_0/S_{50} = 2.973$
Standard day 147	8,732 c.p.m.	$S_0/S_{147} = 3.821$
Standard day 178	3,384 c.p.m.	$S_0/S_{178} = 9.858$

Experiment M.I.E.3

Isotope FC-550-16, Abbott Laboratories ^{59}Ferrous citrate, S.A. = 11.7 μc/μg. Elemental iron content = 1.4 μg/ml.

Each animal received 0.1 ml of undiluted isotope solution intravenously, containing 0.14 μg of elemental iron, radioactivity, 1.6 μc.

Counts of retained radioactivity were obtained on days 50 and 69 after injection of ^{59}Fe.

Mouse number and genotype	Stock	Age (days)	P.C.V. (%)	^{59}Fe dose c.p.m. day 0
1129 +/-	F_4	107	46	29,643
1131 sla/-	F_4	107	15.5	30,130
1111 sla/-	F_4	100	28.5	30,278
1203 sla/-	F_4	88	22.5	27,900

Mouse number	c.p.m. day 50	% retention of ^{59}Fe	c.p.m. day 69	% retention of ^{59}Fe
1129	10,405	70	6,950	59
1131	7,330	49	4,542	38
1111	8,753	58	6,288	53
1203	6,325	46	4,089	37

Standard day 0 = 31,126 c.p.m.

Standard day 50 = 15,450 c.p.m. S_o/S_{50} = 2.015

Standard day 69 = 12,287 c.p.m. S_o/S_{69} = 2.533

E. Intestinal iron absorption determinations.

(1) Ferrous sulphate, 10 µg of elemental iron.

Experiment M.A.12

Isotope FS-266-5, Abbott Laboratories ^{59}Ferrous sulphate - specific activity (S.A.) = 14 µc/µg. Elemental iron content = 1.9 µg/ml.

Carrier - $FeSO_4, 7H_2O$, 248 mg in 500 ml, distilled water. (10 µg elemental iron in 0.1 ml).

Dose - 0.1 ml carrier 10 µg elemental iron.
0.04 ml isotope 0.08 µg elemental iron (1.1 µc).

Total dose per mouse = 10.08 µg iron in 0.14 ml dosing solution.

Mouse number	Genotype & strain	Age (days)	Haematocrit (per cent)	Counts (per mouse) on day 0	mins.	Counts per minute (c.p.m.) on day 0
Standard	-	-	-	47,785	2	23,893
805	F_1,*sla*/-	141	26	54,486	2	27,243
807	F_1, +/-	141	45	56,203	2	28,102
809	F1,*sla*/-	141	20	51,379	2	25,689
811	F_1,*sla*/-	141	23	51,218	2	25,609
813	F_1, +/-	141	46	53,022	2	26,511
815	F_1,*sla*/-	141	24.5	52,763	2	26,382
873	F_3,*sla*/-	50	25	41,462	2	20,731
875	F_3,*sla*/-	50	23.5	53,706	2	26,353

Mouse number	Counts (per mouse) on day 5	mins.	c.p.m. on day 5	% retention of ^{59}Fe on day 5
Standard	42,408	2	21,204	-
805	3,533	5	707	2.9
807	7,601	5	1,520	6.1
809	4,381	5	876	3.8
811	3,569	5	714	3.1
813	2,304	5	461	2.0
815	3,946	5	789	3.4
873	5,793	5	1,159	6.3
875	12,894	5	2,579	11.0

Experiment M.A. 16

Isotope FS-285-14, Abbott Laboratories ^{59}Ferrous sulphate - S.A. = 17.4 μc/μg. Elemental iron content = 1.5 μg/ml.

Carrier - $FeSO_4$, $7H_2O$, 620.5 mg in 1000 ml distilled water. (10 μg elemental iron in 0.08 ml).

Dose - 0.08 ml carrier 10 μg elemental iron.
0.02 ml isotope 0.03 ml isotope 0.03 μg elemental iron (0.5 μc).

Total dose per mouse - 10.03 μg elemental iron in 0.1 ml dosing solution.

Mouse number	Genotype & strain	Age (days)	P.C.V. %	Counts (per mouse) on day 0	Mins.	c.p.m. on day 0
Standard	-	-	-	31,479	2	15,740
1213	F_2, sla/-	73	23	24,400	2	12,200
1203	F_2, sla/-	81	26.5	27,076	2	13,538
1111	F_2, sla/-	93	32	28,177	2	14,084
1225	F_4, sla/-	78	20.5	28,284	2	14,142
1243	F_4, sla/-	73	17.5	26,643	2	13,322
1131	F_4, sla/-	100	22.5	25,768	2	12,884
1155	F_3, sla/-	91	20	29,919	2	14,960
1245	F_5, sla/-	93	20	30,099	2	15,050
1247	F_5, sla/-	93	18.5	29,431	2	14,716
1215	F_2, +/-	73	47.5	25,488	2	12,744
1207	F_2, +/-	73	45	23,782	2	11,891
1113	F_2, +/-	93	45.5	23,136	2	11,568
1299	F_2, +/-	44	42	26,984	2	13,492
1241	F_4, +/-	73	43	25,241	2	12,621
1129	F_4, +/-	100	46	28,213	2	14,107
1153	F_3, +/-	91	45.5	27,909	2	13,954
1185	F_5, +/-	80	33	24,366	2	12,183
1187	F_5, +/-	80	43	26,698	2	13,349

Mouse number	Counts (per mouse) on day 5	Mins.	c.p.m. on day 5	% retention of ^{59}Fe on day 5
Standard	29,928	2	14,964	-
1213	2,206	3	735	5.0
1203	2,653	3	884	5.8
1111	3,133	3	1,044	7.0
1225	2,292	3	764	4.6
1243	2,060	3	687	4.2
1131	1,767	3	589	3.6
1155	2,019	3	673	3.7
1245	3,410	3	1,137	6.9
1247	2,153	3	718	4.0
1215	2,314	3	771	5.1
1207	5,465	3	1,822	14.9
1113	2,367	3	789	5.8
1299	9,132	3	3,044	22.8
1241	1,541	3	514	3.0
1129	2,758	3	919	5.7
1153	3,870	3	1,290	8.6
1185	9,035	3	3,012	25.0
1187	3,226	3	1,075	7.3

(11) Ferrous sulphate, 1 µg of elemental iron

Experiment M.A.13

Isotope FS-266-5, Abbott Laboratories ^{59}Ferrous sulphate - S.A. = 11.4 µc/µg. Elemental iron content = 1.9 µg/ml.

Carrier - $FeSO_4 . 7H_2O$, 22.5 mg in 500 ml distilled water. (0.9 µg of elemental iron in 0.1 ml).

Dose - 0.1 ml carrier 0.9 µg elemental iron.
0.05 ml isotope 0.095 µg elemental iron (1.1 µc).

Total dose per mouse = 0.995 µg iron in 0.15 ml dosing solution.

Mouse number	Genotype & strain	Age (days)	P.C.V. %	Counts (per mouse) on day 0	Mins.	c.p.m. on day 0
Standard	-	-	-	74,167	3	24,722
851	F_3, sla/-	104	28	56,424	2	28,212
853	F_3, sla/-	104	31	55,383	2	27,692
855	F_3, sla/-	104	29	52,486	2	26,243
859	F_3, +/-	109	42	57,388	2	28,694
861	F_3, +/-	109	42.5	67,555	2	33,778
863	F_3, +/-	92	44.5	57,846	2	28,923
865	F_3, +/-	91	45	51,524	2	25,762
867	F_3, +/-	104	45.5	58,825	2	29,413
869	F_3, +/-	104	44.5	59,102	2	29,551
877	F_3, +/-	73	47	74,008	3	24,669
879	F_3, +/-	73	49.5	55,417	2	27,709
881	F_3, +/-	73	42	42,244	2	21,122
883	F_3, sla/-	66	30	53,937	2	26,969
885	F_3, +/-	69	48	55,165	2	27,583
887	F_3, sla/-	69	28.5	50,717	2	25,359

Mouse number	Counts (per mouse) on day 1	Mins.	c.p.m. on day 1	% retention of ^{59}Fe on day 1
Standard	68,031	3	22,677	-
851	6,839	2	3,420	13.2
853	8,688	2	4,344	17.1
855	9,814	2	4,907	20.4
855	9,814	2	4,907	20.4
859	9,777	2	4,889	18.6
861	14,863	3	4,954	16.0
863	4,015	2	2,008	7.6
865	6,831	2	3,416	14.5
867	22,413	3	7,471	27.7
869	7,980	2	3,990	14.7
877	6,014	2	3,007	13.3
879	14,217	2	7,109	27.6
881	12,725	2	6,363	32.8
883	9,676	2	4,838	19.6
885	30,233	2	15,117	59.7
887	15,720	2	7,860	33.8

Mouse number	Counts (per mouse) on day 2	Mins.	c.p.m. on day 2	% retention of ^{59}Fe on day 2	Counts (per mouse) on day 3	Mins.	c.p.m. on day 3	% retention of ^{59}Fe on day 1
Standard	48,466	2	24,233	-	44,503	2	22,252	-
851	3,343	2	1,672	6.7	3,732	3	1,244	4.9
853	5,108	2	2,554	10.5	5,191	3	1,730	6.9
855	5,096	2	2,548	11.0	5,593	3	1,864	7.9
859	12,716	3	4,239	16.8	11,613	3	3,871	15.0
861	8,445	2	4,208	14.1	11,607	3	3,869	12.7
863	3,240	2	1,620	6.4	4,625	3	1,542	5.9
865	5,736	2	2,868	12.7	7,903	3	2,634	11.4
867	19,408	3	6,469	25.0	17,473	3	5,824	22.0
869	6,942	2	3,471	13.4	9,800	3	3,267	12.3
877	3,738	2	1,869	8.6	5,103	3	1,701	7.7
879	13,214	2	6,607	27.1	17,697	3	5,899	23.6
881	10,610	2	5,305	28.6	14,951	3	4,984	26.2
883	4,729	2	2,365	10.0	5,507	3	1,836	7.6
885	27,788	2	13,894	57.3	27,299	2	13,650	55.0
887	6,733	2	3,367	15.1	4,735	3	1,645	7.2

Mouse number	Counts (per mouse) on day 4	Mins.	c.p.m. on day 4	% retention of ^{59}Fe on day 4	Counts (per mouse) on day 5	Mins.	c.p.m. on day 5	% retention of ^{59}Fe on day 5
Standard	44,916	2	22,458	-	42,195	2	21,098	-
851	3,411	3	1,137	4.4	3,001	3	1,000	4.2
853	4,375	3	1,458	5.8	4,002	3	1,334	5.6
855	5,173	3	1,724	7.2	4,325	3	1,442	6.4
859	11,015	3	3,672	14.1	10,203	3	3,401	13.9
861	11,413	3	3,804	12.4	10,616	3	3,539	12.3
863	4,808	3	1,603	6.1	3,781	3	1,260	5.1
865	7,286	3	2,429	10.4	7,149	3	2,383	10.8
867	16,889	3	5,630	21.1	15,697	3	5,232	20.8
869	8,929	3	2,976	11.1	8,904	3	2,968	11.8
877	5,077	3	1,692	7.6	4,339	3	1,446	6.9
879	16,314	3	5,438	21.8	15,531	3	5,177	21.9
881	15,177	3	5,059	26.3	14,624	3	4,875	27.0
883	5,033	3	1,678	6.9	4,364	3	1,455	6.3
885	37,988	3	12,663	50.5	37,619	3	2,539	58.3
887	3,626	3	1,209	5.3	3,093	3	1,031	4.8

Mouse number	Counts (per mouse) on day 6	Mins.	c.p.m. on day 6	% retention of ^{59}Fe on day 6	Counts (per mouse) on day 7	Mins.	c.p.m. on day 7	% retention of ^{59}Fe on day 7
Standard	41,176	2	20,588	-	40,481	2	20,241	-
851	2,878	3	966	4.1	2,648	3	895	3.9
853	3,681	3	1,227	5.3	4,075	3	1,358	6.0
855	4,173	3	1,391	6.4	4,270	3	1,423	6.6
859	9,630	3	3,210	13.4	9,487	3	3,162	13.5
861	10,387	3	3,462	12.3	9,844	3	3,281	11.9
863	3,565	3	1,188	4.9	3,413	3	1,138	4.8
865	6,697	3	2,232	10.4	6,589	3	2,196	10.4
867	14,879	3	4,960	20.3	14,049	3	4,683	19.4
869	8,758	3	2,919	11.7	9,844	3	2,663	11.0
877	4,222	3	1,407	6.9	4,040	3	1,347	6.7
879	14,754	3	4,918	21.3	15,007	3	5,002	22.0
881	14,264	3	4,755	27.0	13,732	3	4,577	26.5
883	4,124	3	1,375	6.1	4,113	3	1,371	6.2
885	36,304	3	12,101	52.7	35,348	3	11,782	52.2
877	2,768	3	923	4.4	2,584	3	861	4.1

Experiment M.A.14

Isotope FS-268-9, Abbott Laboratories ^{59}Ferrous sulphate - S.A.= 22 µc/µg. Elemental iron content = 1 µg/ml.

Carrier - $FeSO_4 . 7H_2O$, 24 mg in 500 ml distilled water (0.96 µg of elemental iron 0.1 ml).

Dose -	0.1 ml carrier	0.96 µg elemental iron.
	0.06 ml isotope	0.06 µg elemental iron (1.3 µc).

Total dose per mouse = 1.02 µg iron in 0.16 ml dosing solution.

Mouse number	Strain & genotype	Age (days)	P.C.V. %	Counts (per mouse) on day 0	Mins.	c.p.m. on day 0
Standard	-	-	-	51,693	2	25,847
775	F_3, +/-	202	48	59,588	2	29,794
781	F_3, +/-	180	41.5	51,965	2	25,983
787	F_3, +/-	180	52	67,912	2	33,956
789	F_3, +/-	180	46	63,785	2	31,893
835	F_3, +/-	98	49	56,563	2	28,282
345	F_2, sla/-	497	22	65,665	2	32,833
347	F_2, +/-	497	43.5	57,348	2	28,674
359	F_2, +/-	492	46	58,475	2	29,238
367	F_2, +/-	493	45.5	66,396	2	33,198
453	F_2, sla/-	424	40.5	69,276	2	34,638
373	F_2, +/-	507	44	82,600	2	41,300
377	F_2, +/-	482	47	66,546	2	33,273
391	F_2, sla/-	514	25.5	62,765	2	31,383
395	F_2, sla/-	485	31.5	59,913	2	29,957
397	F_2, +/-	482	44	70,603	2	35,302
401	F_2, sla/-	455	34	56,749	2	28,375
405	F_2, +/-	452	43	65,772	2	32,886
497	F_2, +/-	350	49	60,876	2	30,438
725	F_2, +/-	319	45	66,457	2	33,229
727	F_2, +/-	287	46	64,731	2	32,366
731	F_2, +/-	302	40.5	55,219	2	27,610

Mouse number	Counts (per mouse) on day 1	Mins.	c.p.m. on day 1	% retention of ^{59}Fe on day 1
Standard	50,242	2	25,121	-
775	21,207	2	10,604	36.6
781	13,510	2	6,755	26.8
787	16,768	2	8,384	25.4
789	15,100	2	7,550	24.4
835	3,827	2	1,914	7.0
345	15,193	2	7,596	23.8
347	20,934	2	10,467	37.6
359	3,561	2	1,781	6.3
367	22,363	2	11,182	33.7
453	4,563	2	2,282	6.8
373	35,259	2	17,630	43.9
377	8,516	2	4,258	13.2
391	12,206	2	6,103	20.0
395	7,609	2	3,804	13.1
397	11,463	2	5,732	16.7
401	4,955	2	2,478	9.0
405	3,363	2	1,682	5.3
497	11,576	2	5,788	19.6
725	7,795	2	3,898	12.1
729	5,044	2	2,522	8.0
731	7,329	2	3,665	13.7

Mouse number	Counts (per mouse) on day 2	Mins.	c.p.m. on day 2	% retention of ^{59}Fe on day 2
Standard	42,006	2	21,003	-
775	15,173	2	7,586	30.5
781	7,565	2	3,783	17.4
787	11,904	2	5,952	20.9
789	10,956	2	5,478	20.5
835	3,015	2	1,508	6.4
345	8,700	2	4,350	15.8
347	2,776	2	1,388	5.9
359	2,081	2	1,041	4.4
367	15,508	2	7,754	25.9
453	2,185	2	1,092	3.7
373	27,901	2	13,950	40.4
377	6,188	2	3,094	11.1
391	6,551	2	3,275	12.4
395	4,228	2	2,114	8.4
397	9,514	2	4,757	16.1
401	2,110	2	1,055	4.4
405	1,590	2	795	2.9
497	8,364	2	4,182	16.4
725	6,379	2	3,189	11.5
729	2,993	2	1,497	5.2
731	5,874	2	2,937	12.7

Mouse number	Counts (per mouse) on day 3	Mins.	c.p.m. on day 3	% retention of ^{59}Fe on day 3
Standard	47,968	2	23,984	-
775	15,452	2	7,726	27.9
781	5,688	2	2,844	11.8
787	12,427	2	6,214	19.7
789	11,355	2	5,678	19.2
835	3,140	2	1,570	6.0
345	7,365	2	3,682	12.1
347	4,081	3	1,360	5.1
359	2,083	2	1,042	3.9
367	17,565	2	8,783	28.5
453	2,215	2	1,107	3.4
373	43,690	3	14,563	38.0
377	6,087	2	3,044	9.9
391	5,494	2	2,747	9.4
395	3,550	2	1,775	6.4
397	9,975	2	4,988	15.2
401	2,035	2	1,018	3.9
405	2,057	3	686	2.3
497	9,666	2	4,833	17.1
725	7,278	2	3,639	11.8
727	3,290	2	1,645	5.5
731	6,158	2	3,079	12.0

Mouse number	Counts (per mouse) on day 4	Mins.	c.p.m. on day 4	% retention of ^{59}Fe on day 4
Standard	47,912	2	23,956	-
775	21,634	3	7,211	26.1
781	7,937	3	2,646	11.0
787	17,754	3	5,918	18.8
789	16,778	3	5,593	18.9
835	4,760	3	1,587	6.1
345	9,141	3	3,047	10.0
347	3,490	3	1,163	4.5
359	3,011	3	1,004	3.7
367	26,703	3	8,901	28.9
453	2,958	3	986	3.1
373	39,556	3	13,185	34.4
377	8,602	3	2,867	9.3
391	7,054	3	2,351	8.1
395	5,142	3	1,714	6.1
397	13,889	3	4,630	14.2
401	2,814	3	938	3.6
405	2,008	3	669	2.2
497	13,960	3	4,653	16.5
725	10,126	3	3,375	11.0
727	4,221	3	1,407	4.7
731	9,315	3	3,105	12.1

Mouse number	Counts (per mouse) on day 5	Mins.	c.p.m. on day 5	% retention of ^{59}Fe on day 5
Standard	48,511	2	24,256	-
775	17,972	3	5,991	21.4
781	7,276	3	2,425	10.0
787	22,200	3	7,400	23.2
789	16,439	3	5,480	18.3
835	4,705	3	1,568	5.9
345	9,337	3	3,112	10.0
347	3,336	3	1,112	4.1
359	2,897	3	996	3.5
367	25,681	3	8,560	27.5
453	2,893	3	964	2.9
373	38,132	3	12,711	32.8
377	7,685	3	2,561	8.2
391	6,807	3	2,269	7.7
395	5,149	3	1,716	6.1
397	13,085	3	4,361	13.2
401	2,668	3	889	3.3
405	1,891	3	630	2.1
497	12,839	3	4,280	15.0
725	9,178	3	3,059	9.8
727	4,477	3	1,492	4.9
731	8,109	3	2,703	10.4

Mouse number	Counts (per mouse) on day 6	Mins.	c.p.m. on day 6	% retention of 59Fe on day 6
Standard	45,091	2	22,546	-
775	Animal Missing			
781	5,891	3	1,964	8.7
787	18,772	3	6,257	21.1
789	13,809	3	4,603	16.5
835	4,050	3	1,350	5.5
345	8,022	3	2,674	9.3
347	2,989	3	996	4.0
359	2,736	3	912	3.6
367	23,821	3	7,940	27.4
453	2,610	3	870	2.9
373	30,822	3	10,274	28.5
377	6,950	3	2,317	8.0
391	6,373	3	2,124	7.8
395	4,596	3	1,532	5.9
397	11,981	3	3,994	13.0
401	2,499	3	833	3.4
405	1,765	3	588	2.1
497	9,770	3	3,257	12.3
725	7,979	3	2,650	9.2
727	3,940	3	1,313	4.6
731	7,241	3	2,414	10.0

(iii) Ferrous sulphate, 0.1 µg of elemental iron.

Experiment M.A.15

Isotope FS-279-1, Abbott Laboratories ^{59}Ferrous sulphate - S.A. = 16 µc/µg. Elemental iron content = 1.8 µg/ml.

Carrier - none.

Dose - 0.055 ml isotope 0.1 µg. Elemental iron (1.6 µc).
0.045 ml deionized water.

Total dose per mouse = 0.1 µg iron in 0.10 ml dosing solution.

Mouse number	Strain & genotype	Age (days)	P.C.V. %	Counts (per mouse) on day 0	Mins.	c.p.m. on day 0
Standard	-	-	-	267,144	2	133,572
891	F_4, +/-	149	44.5	245,985	2	122,992
893	F_4, +/-	149	42.5	227,240	2	113,620
921	F_4, +/-	119	49.5	251,711	2	125,855
923	F_4, sla/-	113	33	236,262	2	118,131
925	F_4, +/-	119	47.5	235,451	2	117,725
927	F_4, sla/-	113	28.5	275,008	2	137,504
929	F_4, sla/-	83	20	272,629	2	136,314
931	F_4, +/-	83	44	231,069	2	115,534
935	F_4, sla/-	83	24.5	234,485	2	117,242
941	F_4, sla/-	83	20	279,674	2	139,837
943	F_4, sla/-	83	13	302,770	2	151,385
945	F_4, sla/-	83	18	320,683	2	160,341
947	F_4, sla/-	83	14.5	194,870	2	97,243
955	F_4, +/-	88	46.5	283,272	2	141,636
957	F_4, +/-	88	49.5	247,271	2	123,635
959	F_4, +/-	88	48	282,741	2	141,370
961	F_4, +/-	88	48	220,546	2	110,273
963	F_4, +/-	88	46	254,907	2	127,453
969	F_4, sla/-	84	22.5	244,595	2	122,297
975	F_4, sla/-	84	25	274,275	2	137,137

Mouse number	Counts (per mouse) on day 1	Mins.	c.p.m. on day 1	% retention of ^{59}Fe on day 1
Standard	276,605	2	138,302	-
891	84,072	2	42,036	33.0
893	22,048	2	11,024	9.4
921	16,999	2	8,499	6.5
923	89,382	2	44,691	36.5
925	125,718	2	62,859	51.6
927	18,232	2	9,116	6.4
929	42,203	2	21,101	15.0
931	63,416	2	31,708	26.5
935	26,687	2	13,343	11.0
941	188,796	2	94,398	65.2
943	85,872	2	42,913	27.4
945	88,218	2	44,109	26.6
947	47,902	2	23,951	23.8
955	102,825	2	51,412	35.1
957	164,031	2	82,015	64.1
959	132,127	2	66,063	45.1
961	154,110	2	77,055	67.5
963	46,002	2	23,001	17.4
969	157,286	2	78,643	62.1
975	49,717	2	24,858	17.5

Mouse number	Counts (per mouse) on day 2	Mins.	c.p.m. on day 1	% retention of ^{59}Fe on day 2
Standard	279,915	2	138,857	-
891	67,627	2	33,813	26.4
893	19,439	2	9,719	8.2
921	12,582	2	6,291	4.8
923	54,624	2	27,312	22.2
925	102,828	2	51,414	42.0
927	9,442	2	421	3.3
929	19,120	2	9,560	6.7
931	49,567	2	24,788	20.6
935	14,940	2	7,470	6.1
941	112,984	2	56,492	38.9
943	45,040	2	22,520	14.3
945	41,911	2	20,958	12.6
947	29,380	2	14,690	14.5
955	83,060	2	41,530	28.2
957	147,482	2	73,741	57.4
959	107,999	2	53,999	36.7
961	139,964	2	69,972	61.0
963	40,387	2	20,193	15.2
969	110,416	2	55,208	43.4
975	33,291	2	16,645	11.7

Mouse number	Counts (per mouse) on day 3	Mins.	c.p.m. on day 3	% retention of ^{59}Fe on day 3
Standard	273,934	2	136,967	-
891	63,603	2	31,802	25.2
893	17,005	2	8,502	7.3
921	11,216	2	5,608	4.4
923	41,243	2	20,621	17.0
925	100,254	2	50,127	41.5
927	6,919	2	3,459	2.5
929	14,664	2	7,332	5.2
931	46,765	2	23,382	19.7
935	12,404	2	6,202	5.1
941	82,508	2	41,254	28.7
943	38,878	2	19,439	12.5
945	35,086	2	17,543	10.7
947	21,203	2	10,601	10.6
955	86,829	2	43,414	29.9
957	141,420	2	70,710	55.8
959	100,353	2	50,176	34.6
961	120,522	2	60,261	53.3
963	39,283	2	19,641	15.0
969	75,308	2	37,654	30.0
975	17,717	2	8,858	6.3

Mouse number	Counts (per mouse) on day 4	Mins.	c.p.m. on day 4	% retention of ^{59}Fe on day 4
Standard	236,705	2	118,353	-
891	47,460	2	23,730	21.8
893	13,709	2	6,855	6.8
921	8,771	2	4,386	3.9
923	28,590	2	14,295	13.6
925	74,000	2	37,000	35.5
927	5,346	2	2,673	2.2
929	10,195	2	5,098	4.2
931	32,597	2	16,299	15.9
935	8,232	2	4,116	4.0
941	57,219	2	28,610	23.1
943	26,670	2	13,335	9.9
945	24,906	2	12,453	8.8
947	15,983	2	7,992	9.3
955	64,571	2	32,286	25.7
957	94,698	2	47,349	43.2
959	69,752	2	34,876	27.9
961	98,718	2	49,359	50.5
963	26,138	2	13,069	11.6
969	50,031	2	25,016	23.1
975	17,716	2	5,858	4.8

Mouse number	Counts (per mouse) on day 5	Mins.	c.p.m. on day 5	% retention of ^{59}Fe on day 5
Standard	240,610	2	120,305	-
891	44,483	2	22,242	20.1
893	16,132	2	5,377	5.3
921	8,403	2	4,202	3.7
923	28,564	2	14,282	13.4
925	72,131	2	36,066	34.0
927	4,429	2	2,215	1.8
929	11,549	2	5,775	4.7
931	30,421	2	15,211	14.6
935	7,783	2	3,792	3.6
941	52,054	2	26,027	20.6
943	23,388	2	11,694	8.6
945	23,015	2	11,508	8.0
947	14,508	2	7,254	8.3
955	59,748	2	29,874	23.4
957	93,631	2	46,816	42.0
959	69,970	2	34,985	27.5
961	91,543	2	45,774	46.1
963	26,973	2	13,487	11.7
969	48,280	2	24,140	21.8
975	11,788	2	5,894	4.8

(iv) Haemoglobin, 10 µg of elemental iron.

Experiment M.A.10

C57Bl/6J mouse given 0.9 ml of ^{59}Ferrous citrate FC-489-11 (Abbott Laboratories), containing 1.17 µg of iron and 17.6 µc of radioactivity.

Haemolysate of strength 5GHb/100 ml, was prepared 5 days later. Iron content 170 µg Fe/ml. 10 µg of elemental iron is thus contained in 0.06 ml of haemolysate.

Dose given per mouse = 0.06 ml haemolysate diluted with an equal quantity of saline, i.e. 0.12 ml.

Mouse number	Strain & genotype	Age (days)	P.C.V. %	Counts (per mouse) on day 0	Mins.	c.p.m. on day 0
Standard	-	-	-	4,659	5	932
397	F_2, +/-	363	44	5,512	5	1,102
405	F_2, +/-	333	43	4,722	5	944
423	F_2, +/-	320	45	5,318	5	1,064
393	F_2, sla/-	366	25.5	4,997	5	999
395	F_2, sla/-	366	32	5,278	5	1,056
401	F_2, sla/-	336	34	5,931	5	1,186
345	F_2, sla/-	378	22	5,712	5	1,142

Mouse number	Counts (per mouse) on day 5	Mins.	c.p.m. on day 5	% retention of ^{59}Fe on day 5
Standard	8,531	10	853	-
397	261	20	13	1.3
405	298	20	15	1.7
423	297	20	15	1.5
393	317	20	16	1.7
395	221	20	11	1.1
401	263	20	13	1.2
345	275	20	14	1.3

Experiment M.A.11

C57Bl/6J mouse given ^{59}Ferrous citrate, FC-496-5, (Abbott Laboratories). No further details available.

Haemolysate prepared on the 5th day after injection, haemoglobin content 15.6G/100 ml. Thus, 10 µg elemental iron is contained 0.02 ml of haemolysate.

The haemolysate was then diluted with 4 parts of de-ionized water and a total dose of 0.1 ml given to each mouse, containing 10 µg elemental iron as haemoglobin.

Mouse number	Strain & genotype	Age (days)	P.C.V. %	Counts (per mouse) on day 0	Mins.	c.p.m. on day 0
Standard	-	-	-	9,615	2	4,808
415	F_2, +/-	380	47.5	23,048	4	5,762
417	F_2, +/-	380	46	12,103	2	6,052
435	F_2, +/-	381	50	13,558	2	6,779
451	F_2, +/-	317	48.5	10,486	2	5,243
391	F_2, sla/-	407	25.5	10,948	2	5,474
413	F_2, sla/-	380	31.5	18,978	4	4,745
433	F_2, sla/-	380	33	21,279	5	4,256
453	F_2, sla/-	317	40.5	31,456	5	6,291

Mouse number	Counts (per mouse) on day 5	Mins.	c.p.m. on day 5	% retention of ^{59}Fe on day 5
Standard	9,184	2	4,592	-
415	3,129	10	313	5.7
417	1,455	10	146	2.5
435	1,594	10	159	2.5
451	1,099	10	110	2.2
391	1,565	10	157	3.0
413	1,023	10	102	2.3
433	2,219	10	222	5.5
453	1,349	10	135	2.2

F. Determinations of the absorption of radioiodinated triolein.

Experiment M.A.17

Isotope K89R96 "Trioleotope", Squibb.

0.5G radioiodinated triolein, activity 80 μc, was suspended in 8 ml commercial margarine.

Dose - 0.1 ml margarine containing 1 μc of radioactivity and 6.25 mg triolein.

Mouse number	Age (days)	Genotype & strain	Haematocrit (per cent)	c.p.m. day 0 whole mouse
Standard	-	-	-	34,559
1281	197	+/-, F_4	51	46,714
1283	197	sla/-, F_4	29	33,547
1289	197	sla/-, F_4	27	53,897
1305	192	sla/-, F_2	20	45,379
1321	182	+/-, F_2	41	29,362
1323	182	+/-, F_2	41	44,955
1357	179	+/-, F_4	53	51,459
1359	179	+/-, F_4	50	43,310
1377	177	sla/-, F_4	28.5	46,357
1379	177	sla/-, F_4	17	46,401

Mouse number	c.p.m. faeces* (5 day collection)	% c.p.m. in faeces	% radio-iodinated triolein retained on day 5
Standard	12,744	-	-
1281	1,527	8.7	91.3
1283	635	5.1	94.9
1289	1,115	5.6	94.4
1305	1,293	7.7	92.3
1321	447	4.1	95.9
1323	2,636	15.9	84.1
1351	3,567	18.8	81.2
1359	1,343	8.4	91.6
1377	1,985	11.6	88.4
1379	512	3.0	97.0

*Based on 5 min. counts.

$$\frac{\text{Standard day 0}}{\text{Standard day 5}} = 2.712$$

G. Determinations of the absorption of zinc, cobalt and copper.

Experiment M.A.18a Zinc

Isotope 481470, Z.A.S.1, Zinc Chloride (^{65}Zn) Radiochemical Centre, Amersham, England. Specific activity, 0.56 µc/ug. Elemental zinc content 1.5 mg/ml.

0.1 ml of isotope solution was diluted to 4 ml with distilled water.

Dose - 0.1 ml of diluted isotope containing 3.5 µg of elemental zinc and 2 µc of radioactivity.

Mouse number	Age (days)	Genotype & strain	Haematocrit (per cent)	c.p.m. day 0 whole mouse	c.p.m. day 5 whole mouse	% retention of ^{65}Zn on day 5
Standard	-	-	-	80,332	78,087	-
1425	192	+/-, F_4	41	73,697	6,787	9.5
1427	192	+/-, F_4	44	91,613	8,421	9.6
1489	192	+/-, F_4	41.5	63,903	11,502	18.5
1491	192	+/-, F_4	44	83,621	8,033	9.9
1553	178	+/-, F_4	43	86,000	14,196	16.9
1565	203	sla/-,F_4	18.5	83,183	6,544	8.1
1649	171	sla/-,F_4	29.5	93,774	10,738	11.8
1683	156	sla/-,F_4	20	85,850	4,882	5.9
1497	192	sla/-,F_4	28	90,534	8,404	9.6
1555	178	sla/-,F_4	23	70,790	3,094	4.5

Experiment M.A.18b, Cobalt

Isotope I 481468, C.T.S.1. Cobaltous chloride (^{57}Co). The Radiochemical Centre, Amersham, England. Specific activity 3.3 mc/µg. Elemental cobalt content 0.28 µg/ml.

0.1 ml of isotope solution was diluted to 5 ml with distilled water.

Dose - 0.1 ml of diluted isotope solution containing 0.0006 µg of elemental cobalt and 2 µc of radioactivity.

Mouse number	Age (days)	Genotype & strain	Haematocrit (per cent)	c.p.m. day 0 whole mouse	c.p.m. day 5 whole mouse	% retention of ^{57}Co on day 5
Standard	-	-	-	92,354	89,777	-
1729	143	+/-, F_4	43.5	83,301	1,506	1.9
1745	157	+/-, F_4	44.5	90,183	1,247	1.4
1749	157	+/-, F_4	44.5	81,455	282	0.4
1747	157	+/-, F_4	45.5	77,563	2,911	3.9
1731	143	+/-, F_4	45	86,330	451	0.5
1753	136	sla/-,F_4	23	105,052	906	0.9
1755	136	sla/-,F_4	27.5	74,176	3,069	4.2
1775	127	sla/-,F_4	25.5	105,647	571	0.6
1685	156	sla/-,F_4	26	79,120	1,390	1.8
1777	127	sla/-,F_4	26	90,910	1,126	1.3

Experiment M.A.18c, Copper

Isotope 435, 1967 Cuprous nitrate (^{64}Cu), Nuclear Science and Engineering Company. Specific activity, 9.85 μc/μg. Elemental copper content - 1.53 mg/ml.

0.1 ml Isotope solution was diluted to 15.5 ml with distilled water.

Dose - 0.1 ml of diluted isotope solution containing 10 μg of elemental copper and 100 μc of radioactivity.

Mouse number	Age (days)	Genotype & strain	Haematocrit (per cent)	c.p.m. day 0 whole mouse	c.p.m. day 4* whole mouse	% retention of ^{64}Cu on day 4
Standard	-	-	-	224,939	1,495	-
1955	49	+/-, F_4	39.5	210,941	151	10.7
1957	49	+/-, F_4	46	155,932	173	16.7
1959	86	+/-, F_4	47.5	157,316	194	18.5
1961	86	+/-, F_4	49	171,723	155	13.6
1963	86	+/-, F_4	48	150,522	160	16.0
Standard	-	-	-	229,531	1,344	-
1965	63	sla/-, F_4	28	223,455	150	11.5
1967	63	sla/-, F_4	27.5	177,670	115	11.1
1969	63	sla/-, F_4	16.5	234,762	125	9.1
1971	63	sla/-, F_4	19.0	238,860	120	8.6
1899	105	sla/-, F_4	28.5	201,059	184	15.6

* Based on 5 min. counts.

APPENDIX D

RESULTS OF MISCELLANEOUS INVESTIGATIONS

1. Determinations of serum iron concentration

a) Normal mice (genotype, +/-)

Mouse number	Age (days)	Stock	Haematocrit per cent	Volume of serum used (ml)	Serum iron (µg/100 ml)
347	478	F_2	43.5	0.41	284
359	503	F_2	46	0.52	217
367	504	F_2	45.5	0.50	154
373	518	F_2	44	0.62	217
377	507	F_2	47	0.37	271
397	493	F_2	44	0.37	305
711/719	318	F_1	39/42	0.60	246
713/723	318	F_1	41/46	0.90	223
721/739	318/292	F_1	42/40.5	0.70	247
725	330	F_1	45	0.41	278
775	214	F_3	48	0.40	231
781	193	F_3	41.5	0.34	251
787	192	F_3	52	0.36	257
789/835	180/111	F_3	46/49	0.47	260
841	173	F_2	46	0.19	272
845	173	F_3	48	0.27	254
847	172	F_3	40.5	0.34	280
859	109	F_3	42	0.40	210
861	109	F_3	42.5	0.30	190
863	92	F_3	44.5	0.32	249
865	91	F_3	45	0.15	298

Mouse number	Age (days)	Stock	Haematocrit per cent	Volume of serum used (ml)	Serum iron (µg/100 ml)
867	150	F_3	45.5	0.20	345
869	104	F_3	44.5	0.21	272
877	131	F_3	47	0.38	269
879	131	F_3	49.5	0.21	322
881	131	F_3	42	0.50	170
885	123	F_3	48	0.21	281

b) Anaemic mice (genotype, sla/-)

Mouse number	Age (days)	Stock	Haematocrit per cent	Volume of serum used (ml)	Serum iron (µg/100 ml)
53'	511	O.M.S.	18.5	0.80	223
345	545	F_2	22	0.42	73
391	320	F_2	25.5	0.79	491
395	545	F_2	31.5	0.52	73
401	467	F_2	34	0.40	80
453	407	F_2	40.5	0.48	111
491	162	F_2	13.5	0.60	215
805	142	F_2	26	0.37	80
811	142	F_2	23	0.50	564
855	104	F_3	29	0.56	220
883	123	F_3	30	0.49	97
887	69	F_3	28.5	0.52	69
911	86	F_3	19.5	0.46	43
917	84	F_3	34.5	0.60	132
919	84	F_3	25.5	0.48	76
987	269	F_2	30	0.44	72
997	264	F_2	30	0.42	171
1003	258	F_2	35.5	0.34	240
1035	257	F_2	31	0.27	101
1039	257	F_2	25.5	0.48	48
1049	257	F_2	31	0.44	62
1051	251	F_4	22.5	0.38	64
1329	161	F_2	37.5	0.29	64
1495	146	F_4	20	0.32	31
1557	132	F_4	23.5	0.60	143

2. Determinations of total serum iron binding capacity

a) Normal mice (genotype, +/-)

Mouse number	Age (days)	Stock	Haematocrit (%)	Volume of serum used (ml)	T.I.B.C. µg/100 ml serum
1007	197	F_3	48.0	0.45	432
1015	173	F_3	50.0	0.34	426
1087	108	F_3	45.5	0.25	480
1115	140	F_3	44.0	0.40	385
1241	149	F_4	43.0	0.30	432
1279	134	F_4	55.0	0.28	360
1307	115	F_3	42.0	0.29	399
1347	109	F_4	52.0	0.30	442

b) Anaemic mice (genotype, sla/-)

Mouse number	Age (days)	Stock	Haematocrit (%)	Volume of serum used (ml)	T.I.B.C. µg/100 ml serum
1009	197	F_3	24.5	0.52	566
1017	173	F_3	19.0	0.40	549
1085	108	F_3	22.5	0.32	603
1117	140	F_3	29.0	0.44	541
1201	146	F_3	29.0	0.38	606
1243	134	F_2	17.5	0.58	488
1259	133	F_2	32.0	0.25	691
1275	134	F_4	27.5	0.46	678
1345	109	F_4	23.5	0.48	786

3. Determinations of total body iron content

a) Normal mice (genotype +/-)

Mouse number	Age (days)	Weight (G)	Stock	G/100 ml	Haematocrit (%)	Total body iron content	
						mg	mg/100G
79	338	42.2	O.M.S.	15.6	-	3.17	7.51
109	634	20.5	O.M.S.	14.4	-	2.25	10.90
195	330	30.1	O.M.S.	13.8	-	2.13	7.06
301	222	30.4	F_1	15.4	-	1.73	5.69
311	216	32.7	F_1	13.3	43	1.35	4.13
313	216	29.5	F_1	13.3	-	1.20	4.07
315	216	36.9	F_1	14.0	-	2.26	6.12
317	216	35.6	F_1	12.5	42	1.57	4.41
323	216	35.0	F_1	12.5	39.5	1.51	4.31
325	216	35.0	F_1	12.5	39.5	1.83	5.23
353	338	28.2	F_1	15.4	-	1.73	6.13
363	599	29.8	F_1	14.7	-	1.95	6.54
447	55	7.4	F_2	-	43.5	0.46	6.22
757	73	25.0	F_1	-	38	1.35	5.40

3. a) continued

Mouse number	Age (days)	Weight (G)	Stock	G/100 ml	Haematocrit (%)	Total body iron content	
						mg	mg/100G
759	73	24.4	F_1	-	40	1.35	5.53
761	39	7.1	F_2	-	43	0.25	3.52
769	73	28.2	F_1	-	40	1.25	4.43
791	124	27.1	F_3	13.9	-	1.33	4.91
793	123	22.0	F_3	13.7	-	1.13	5.14
797	133	25.9	F_3	13.9	-	1.30	5.02
799	127	27.1	F_3	12.9	-	1.18	4.35
801	139	31.5	F_3	13.5	-	1.60	5.08
803	139	30.0	F_3	13.1	-	1.60	5.33
817	94	29.1	F_3	11.8	-	1.60	5.50
819	94	28.2	F_3	13.3	-	2.00	7.09
821	104	30.0	F_3	13.9	-	1.44	4.80
857	54	12.8	F_3	11.4	-	0.78	6.09
1301	44	10.0	F_2	12.4	-	0.313	3.13

b) Anaemic mice (genotype sla/-, and *sla/sla)

Mouse number	Age (days)	Weight (G)	Stock	G/100 ml	Haematocrit (%)	Total body iron content	
						mg	mg/100G
*0'	60	10.0	O.M.S.	-	15	0.40	4.00
*4'	347	24.7	O.M.S.	13.4	-	1.60	6.48
33	546	38.1	O.M.S.	13.4	-	1.14	2.95
*40'	299	29.5	O.M.S.	15.6	-	1.44	4.88
45'	293	28.5	O.M.S.	14.5	-	0.99	3.47
*68'	352	29.9	O.M.S.	13.8	-	1.38	4.62
*69	352	29.9	O.M.S.	14.4	-	1.48	4.95
81	739	27.9	O.M.S.	9.2	-	1.35	4.83
103	368	22.5	O.M.S.	11.2	-	0.50	2.22
105	206	25.5	O.M.S.	11.6	-	0.85	3.33
107	205	29.6	O.M.S.	12.2	-	1.07	3.61
125	108	20.4	O.M.S.	10.3	-	1.14	5.59
129	266	32.4	O.M.S.	11.6	-	1.20	3.70
131	266	28.7	O.M.S.	3.7	-	1.15	4.01

b) continued

Mouse number	Age (days)	Weight (G)	Stock	G/100 ml	Haematocrit (%)	Total body iron content	
						mg	mg/100G
133	265	26.4	O.M.S.	4.7	-	0.68	2.58
147	275	28.7	O.M.S.	11.6	-	1.03	3.59
155	286	38.5	O.M.S.	14.1	-	1.11	2.88
167	315	23.0	O.M.S.	5.0	-	0.45	1.96
178	90	23.6	O.M.S.	11.2	-	0.69	2.92
191	186	27.9	O.M.S.	9.9	-	1.39	4.98
193	123	22.6	O.M.S.	9.6	-	0.78	3.45
*194	132	20.4	O.M.S.	9.2	-	0.39	1.91
*196	123	19.3	O.M.S.	9.4	-	0.98	5.08
1217	98	13.0	F_4	5.5	-	0.33	2.50
1303	44	8.7	F_2	5.7	-	0.21	2.45

4. Free erythrocyte protoporphyrin concentration

Mouse number	Age (days)	Genotype and stock	Haematocrit (%)	Free erythrocyte protoporphyrin µg/100 ml red cells
-	300	+/-, C57B1/6J	39.5	63.7
-	300	+/-, C57B1/6J	40.5	70.4
-	300	+/-, C57B1/6J	46	54.9
701	110	+/-, F_1	45	102.7
703	110	+/-, F_1	42.5	42.4
705	110	+/-, F_1	49	49.5
292	471	+/-, F_1	37	37.5
53"	511	sla/-, O.M.S.	18.5	200.0
729	68	sla/-, F_2	42	112.7
491	162	sla/-, F_2	13.5	211.7
499	116	sla/-, F_2	16.5	421.5
335	434	+/-, F_1	40	14.0
337	431	+/-, F_1	37.5	35.3
339	431	+/-, F_1	38	21.9
805	141	sla/-, F_2	26	182.4
809	141	sla/-, F_2	20	336.6
811	141	sla/-, F_2	23	128.6
479/481*	81	+/-, F_2	45	54.4
483/487/489*	81	sla/-, F_2	19	583.5

* Samples pooled.

5. Haemoglobin and transferrin electrophoresis

Mouse number	Age (days)	Genotype and stock	Haemoglobin pattern	Transferrin type
20	800	sla/+, O.M.S.	single band	-
32'	375	sla/+, O.M.S.	single band	b
33	476	+/-, O.M.S.	single band	-
46'	387	sla/-, O.M.S.	single band	a/b
82'	531	sla/sla, O.M.S.	-	b
90'	490	sla/sla, O.M.S.	-	b
193	123	sla/-, O.M.S.	-	b
237	294	+/-, F_1	-	b
245	287	+/-, F_1	-	b
405	246	+/-, F_2	-	b
446	253	+/-, F_2	-	b
510	176	sla/+, O.M.S.	single band	b
527	131	sla/-, O.M.S.	-	b
528	89	sla/+, O.M.S.	single band	a/b
529	103	sla/-, O.M.S.	-	b
530	89	sla/+, O.M.S.	single band	b
533	89	sla/-, O.M.S.	single band	b
537	80	sla/-, O.M.S.	-	b
823	196	+/-, F_3	-	b
825	196	+/-, F_3	-	b
827	196	+/-, F_3	-	b
829	197	+/-, F_3	-	b

5. continued

Mouse number	Age (days)	Genotype and stock	Haemoglobin pattern	Transferrin type
831	195	+/-, F_3	-	b
833	195	+/-, F_3	-	b
1101	247	sla/-, F_4	single band	b
1103	247	+/-, F_4	single band	b
1685	111	sla/-, F_4	single band	b
1729	98	+/-, F_4	single band	b
1731	98	+/-, F_4	single band	-
1745	112	+/-, F_4	single band	b
1747	112	+/-, F_4	single band	-
1749	112	+/-, F_4	single band	-
1753	91	sla/- F_4	single band	b
1755	91	sla/-, F_4	single band	b
1775	82	sla/-, F_4	single band	b
1777	82	sla/-, F_4	single band	b

6. Electrophoresis of ^{59}Fe - labelled transferrin in starch gel. The section of gel containing the transferrin band is marked with an asterisk.

Mouse number, 32'; age, 375 days; genotype, sla/+; stock, O.M.S.; haematocrit, 49.4%; transferrin type b.

Section number	c.p.m.	Section number	c.p.m.
1	49	5	81
2	191	6	54
3*	641	7	61
4	317	Total	1394

Mouse number, 46'; age, 387 days; genotype, sla/-; stock, O.M.S.; haematocrit, 22%; transferrin type, ab.

Section number	c.p.m.	Section number	c.p.m.
1	9	6	53
2	11	7	8
3*	138	8	13
4*	187	9	4
5	102	Total	516

Mouse number, 82'; age, 536 days; genotype, sla/sla; stock, O.M.S.; haematocrit, 45%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	337	7	53
2	867	8	47
3	766	9	42
4*	3948	10	30
5	828	11	23
6	115	Total	7056

Mouse number 90'; age, 494 days; genotype, sla/sla; stock, O.M.S.; haematocrit, 47%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	45	7	35
2	80	8	43
3	110	9	23
4*	1268	10	33
5	972	11	32
6	68	Total	2699

Mouse number, 193; age, 123 days; genotype, sla/-; stock, O.M.S.; haematocrit, 39%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	27	6	9
2	53	7	12
3*	580	8	17
4	175	9	4
5	26	Total	860

Mouse number, 237; age, 294 days; genotype, +/-; stock, F_1 hybrid of O.M.S. and C57Bl/6J strain; haematocrit, 50.5%, transferrin type b.

Section number	c.p.m.	Section number	c.p.m.
1	8	6	4
2	9	7	13
3*	349	8	4
4	92	9	3
5	25	Total	446

Mouse number, 245; age, 287 days; genotype, +/-; stock, F_1 hybrid of O.M.S. and C57Bl/6J strain; haematocrit, 42%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	70	6	13
2	78	7	12
3*	636	8	13
4	159	9	14
5	45	Total	1007

Mouse number, 405; age, 254 days; genotype, +/-; stock, F_1 hybrid of O.M.S. and C57Bl/6J strain; haematocrit 44%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	23	7	33
2	40	8	22
3	180	9	33
4	1967	10	27
5	620	11	23
6	52	Total	3020

Mouse number, 446; age, 261 days; genotype, +/-; stock, F_1 hybrid of O.M.S. and C57Bl/6J strain; haematocrit, 45.5%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	142	7	33
2	547	8	33
3	1896	9	27
4*	5612	10	28
5	1858	11	20
6	125	Total	10,321

Mouse number, 510; age, 176 days; genotype, sla/+ ; stock, O.M.S.; haematocrit, 52%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	66	5	96
2	196	6	48
3*	625	7	60
4	354	Total	1445

Mouse number, 530; age, 70 days; genotype, sla/+ ; stock, O.M.S.; haematocrit, 49%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	37	5	49
2	158	6	33
3*	291	7	54
4	123	Total	745

Mouse number 533; age, 70 days; genotype, sla/-; stock, O.M.S.; haematocrit, 27%; transferrin type, b.

Section number	c.p.m.	Section number	c.p.m.
1	131	5	52
2	272	6	20
3*	548	7	37
4	121	Total	1161

APPENDIX E

EFFECT OF THERAPY

A. Parenteral iron dextran ("Imferon") in a single dose

(1) Dose of elemental iron, 0.5 mg

Mouse number, 1'; genotype, sla/-; stock, O.M.S.; age, 41 days.

Haematocrit before treatment:	30%
Haematocrit 1 week after treatment:	51%
Haematocrit 2 weeks after treatment:	46%
Haematocrit 3 weeks after treatment:	49%
Haematocrit 7 weeks after treatment:	37%
Haematocrit 15 weeks after treatment:	44%

Mouse number, 16'; genotype, sla/-; stock, O.M.S.; age 41 days.

Haematocrit before treatment:	29.5%
Haematocrit 1 week after treatment:	50.5%
Haematocrit 2 weeks after treatment:	45%
Haematocrit 3 weeks after treatment:	44%
Haematocrit 13 weeks after treatment:	43%
Haematocrit 15 weeks after treatment:	44%

Mouse number, 25; genotype, sla/-; stock, O.M.S.; age 40 days.

Haematocrit before treatment:	23%
Haematocrit 1 week after treatment:	47%
Haematocrit 2 weeks after treatment:	44.5%
Haematocrit 3 weeks after treatment:	43%
Haematocrit 7 weeks after treatment:	34%

Mouse number, 28; genotype, sla/sla; stock, O.M.S. age 39 days.

Haematocrit before treatment:	29%
Haematocrit 1 week after treatment:	47%
Haematocrit 3 weeks after treatment:	50%
Haematocrit 6 weeks after treatment:	47%
Haematocrit 19 weeks after treatment:	57%

Mouse number 32'; genotype, sla/sla; stock, O.M.S.; age 103 days.

Haematocrit before treatment:	27%
Haematocrit 1 week after treatment:	50%
Haematocrit 3 weeks after treatment:	42%
Haematocrit 20 weeks after treatment:	48%

Mouse number, 65; genotype, sla/-; stock, O.M.S.; age 41 days.

Haematocrit before treatment:	27%
Haematocrit 1 week after treatment:	47%
Haematocrit 3 weeks after treatment:	49%
Haematocrit 6 weeks after treatment:	42%
Haematocrit 16 weeks after treatment:	44.5%
Haematocrit 32 weeks after treatment:	36.5%
Haematocrit 36 weeks after treatment:	26.5%
Haematocrit 53 weeks after treatment:	29%

Mouse number, 68; genotype, sla/sla; stock, O.M.S.; age 41 days.

Haematocrit before treatment:	28%
Haematocrit 1 week after treatment:	29%
Haematocrit 3 weeks after treatment:	41%

Mouse number, 88; genotype, sla/sla; stock, O.M.S.; age 39 days.

Haematocrit before treatment:	29%
Haematocrit 1 week after treatment:	42%
Haematocrit 3 weeks after treatment:	46%
Haematocrit 6 weeks after treatment:	41%
Haematocrit 21 weeks after treatment:	49.5%
Haematocrit 32 weeks after treatment:	43%
Haematocrit 36 weeks after treatment:	45%
Haematocrit 43 weeks after treatment:	45.5%

Mouse number, 98; genotype, sla/sla; stock, O.M.S.; age 38 days.

Haematocrit before treatment:	24.5%
Haematocrit 1 week after treatment:	43%
Haematocrit 2 weeks after treatment:	41%
Haematocrit 8 weeks after treatment:	28%
Haematocrit 15 weeks after treatment:	27.5%
Haematocrit 19 weeks after treatment:	15%

Mouse number, 56; genotype, sla/sla; stock, O.M.S.; age 112 days.

Haematocrit before treatment:	28%
Haematocrit 3 weeks after treatment:	48%
Haematocrit 5 weeks after treatment:	43%
Haematocrit 6 weeks after treatment:	37%
Haematocrit 16 weeks after treatment;	47%
Haematocrit 32 weeks after treatment:	50%
Haematocrit 57 weeks after treatment:	42.5%

Mouse number	Genotype & strain	Age (days)	Per cent haematocrit at Before treatment	1 week	2 week	3 week	4 week	12 week
493	sla/-, F_2	143	26.5	29	37	37.5	-	-
495	sla/-, F_2	143	18.5	28	35.5	40	-	-
531	sla/-, OMS	180	22	35	39	39	30	-
537	sla/-, OMS	73	27	35	-	-	-	42.5
539	sla/-, OMS	120	29	37	38	38.5	42.5	-
543	sla/-, OMS	115	22	26.5	28.5	27.5	29	-
549	sla/-, OMS	115	22	19	22	25	26	-

(ii) Dose of elemental iron, 5 mg.

Mouse number*	Age (days)	Per cent haematocrit at Before treatment	1 week	2 week	3 week	4 week	8 week	12 week
923	167	34	43.5	45	43	42	40	43
927	167	31	45	47	43	44	40	42
929	131	30	43.5	45.5	41.5	42	40.5	40.5
935	132	23.5	46	49	40.5	42.5	36	43
941	132	30.5	45.5	42	42.5	39	39	41.5
943	132	34	47	43	41	42.5	40.5	45
945	132	27	46.5	47	46	47	42.5	45
947	132	25	44	43	43	42.5	40.5	44

*All animals of genotype sla/- and F_4 hybrids.

B. Parenteral pyridoxine hydrochloride in a single dose.

(i) 0.1 mg

Mouse number*	Per cent haematocrit at Before treatment	1 week	2 week	3 week	4 week
543	28	29.5	25	27	22
545	26.5	30	28.5	26.5	25.5
547	29	29	29	22	24.5
549	23	23.5	21.5	22	14

*All animals of genotype sla/-, of the original mixed stock aged 75 days.

(ii) 5 mg

Mouse number*	Genotype	Per cent haematocrit at Before treatment	1 week	2 week	3 week	4 week
23	sla/-	27	33.5	-	28	33
50'	sla/sla	30	-	29	30.5	-
74	sla/sla	25	25	-	29	31
3'	sla/-	30	31	-	25	-

*All animals of original mixed stock, aged between 43 and 55 days.

C. Parenteral vitamin B_{12} in a single dose of 100 µg

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent) Before treatment	Haematocrit (per cent) 1 week after treatment
393	356	sla/-, F_2	16	25
401	326	sla/-, F_2	39	34
431	329	sla/-, F_2	31	31.5
527	117	sla/-, O.M.S.	32	26.5
529	117	sla/-, O.M.S.	32.5	27
533	117	sla/-, O.M.S.	24	23

D. Parenteral folic acid in a single dose of 0.5 mg

Mouse number	Age (days)	Genotype and strain	Haematocrit (per cent) Before treatment	Haematocrit (per cent) 1 week after treatment
345	368	sla/-, F_3	28	22
395	356	sla/-, F_3	32	31.5
527	131	sla/-, O.M.S.	26.5	35
529	131	sla/-, O.M.S.	27	32
531	131	sla/-, O.M.S.	20.5	18.5
533	131	sla/-, O.M.S.	23	23

E. Parenteral "delatestryl" (Squibb) - Testosterone enanthate in a single dose of 20 mg.

Mouse number*	Age (days)	Haematocrit (per cent) Before treatment	Haematocrit (per cent) 1 week after treatment
55'	47	31.5	35
165	497	32.5	29.5
527	180	22	29.5
529	180	31.5	32.5
531	180	24	18
533	180	26.5	24

*All animals of genotype sla/-, of the original mixed stock.

F. Histochemical demonstration of iron stores following treatment with iron dextran.

(i) 0.5 mg dose of elemental iron.

Mouse number, 65; genotype, sla/-; original mixed stock; time lapse between treatment and sacrifice, 450 days; age at death, 491 days, haematocrit, 29%.

Spleen: Stainable iron is not present. The red pulp is hyperplastic (occupying 50-75% of the splenic tissue) and shows very active haemopoiesis.

Heart: Stainable iron is not present.

Lung: Stainable iron is not present.

Mouse number, 68; genotype, sla/sla; original mixed stock; time lapse between treatment and death, 23 days; age at death, 64 days; haematocrit, 41%.

Spleen: Stainable iron is not present. The red pulp shows moderate hyperplasia occupying 25-50% of the splenic tissue. Active haemopoiesis is seen.

Liver: Stainable iron is not present. Haemopoietic elements are not seen.

Heart: Stainable iron is not present.

Kidney: Stainable iron is not seen.

Mouse number, 163; genotype, sla/-; original mixed stock; time lapse between treatment and death, 3 days; age at death, 118 days; haematocrit, 18.5%.

Spleen: Stainable iron is not present; there is hyperplasia of the red pulp which occupies 50-75% of the spleen. Very active haemopoiesis is seen.

Liver: Stainable iron is not present. Haemopoietic foci are not seen.

(ii) 5 mg dose of elemental iron.

All mice studied were F_4 hybrids of genotype sla/-, sacrificed 84 days after treatment. Age at time of death is given below.

Mouse number	Age (days)	Haematocrit %	Spleen iron stores	Red pulp (% of spleen)
923	251	43	Grade 4	25
927	251	42	Grade 4	25
929	215	40.5	Grade 4	25-50
935	216	43	Grade 4	25
941	216	41.5	Grade 4	25
943	216	45	Grade 4	25
945	216	45	Grade 4	25-50
947	216	44	Grade 4	25

	Liver iron stores		Bone marrow (sternum)		
Mouse number	Parenchyma	Kupffer cells	Appearance	Iron	Duodenal mucosa iron
923	***	**	Normal	0	3
927	***	**	no tissue taken		3
929	***	**	Normal	0	3
935	***	**	Normal	0	3
941	***	**	Normal	0	2
943	***	**	Normal	0	2
945	***	**	Normal	0	2
947	***	**	no tissue taken		4

*** = Stainable iron present in all or nearly all parenchymal cells.

** = Kupffer cells contain abundant stainable iron.