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NUTRITIONAL IRON-DEFICIENCY ANAEMIA OF PIGLETS.

SUMMARY.

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SUMMARY.

The literature relating to anaemia of piglets is reviewed. Many controversial findings have been noted. The haematological data recorded are not abundant.

Five experiments were conducted using artificial rearing methods and semi-synthetic diets involving a total of 62 piglets. The use of these methods allowed strict control of the iron intake and experimental conditions. The iron contents of the diets fed to the groups on low iron intake ranged from 2.42 - 4.48 mg. per 100 g. and were sufficiently low to cause profound haematological changes.

Studies were also made on a farm using piglets reared naturally, and a survey taken of the Hb. levels of unweaned piglets on 8 farms.

Some investigations were made also into the absorbtion of a radio-active iron dextran complex from the intestine of piglets. The haematology of normal and iron-deficient piglets was studied, and parameters established. Significant differences between groups of anaemic and non-anaemic pigs were noted.

The Hb. levels for piglets supplied with iron were usually between 9 and 12 g.%. In anaemic animals values as low as 5 g.% were readily produced but lower Hb. levels, of around 3 g.%, were less frequent.

The haematocrit values fell in anaemic piglets from the more normal level of around 40 ml.% to below even half this value.

The erythrocyte counts were markedly altered only in the more severe anaemias when they fell to about 3 million R.B.Cs. from around 5 million. The cell size decreased also, as shown by a fall in the M.C.V. and red cell diameter measurements.

In contrast little constant change was noted in the M.C.H.C. which remained about 30%.

The morphological changes were profound and R.B.Cs. exhibited mainly anisocytosis, with numerous microcytes and large numbers of red cells with mere rings of stained Hb. ("possary forms").

Except in the more severe cases poikilocytosis was not marked.

No regular increase of reticulocytes was noted in anaemic piglets.

A broad division in serum iron levels could be drawn about the 100 - 120 µg.% region below which many anaemic pigs fell though inconsistences were not unusual.

Parenchymal iron and copper values were recorded and in the femoral marrow hyperplasia with replacement of the fat cells was exhibited by the iron-deficient piglets.

Despite the profound clinical and pathological changes recorded in the literature diarrhoea, dullness, tachypnoea, dyspnoea or tachycardia and unthriftiness were not necessarily associated with the iron-deficiency anaemia and on autopsy no constant alterations, except in the bone-marrow, were noted. Pneumonia, hepatitis,

oedema and intestinal adhesions were not found in association with uncomplicated iron deficiency.

Though some anaemic piglets died, the deaths could be associated with concurrent infections, and no serious mortality was linked with Hb. levels as low as 4 g.%.

It is suggested that many of the changes recorded in the literature as typical of iron deficiency in piglets may have resulted from complicating factors such as cold or infections.

NUTRITIONAL IRON - DEFICIENCY ANAEMIA OF PIGLETS.

by

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INDEX.

59

NUTRITIONAL IRON - DEFICIENCY ANAEMIA OF PIGLETS.

INTRODUCTION.

Original ideas which are sufficiently revolutionary and which gain ready acceptance may remain unchallenged or their principles unaltered for many years after being propounded. It is only as knowledge is acquired on related problems that a more critical appraisal is made of these ideas and the initial concepts become untenable or are modified.

Thus it became apparent that with the advances in knowledge of the problems and diseases associated with the rearing of piglets reappraisal of the information on anaemia of suckling piglets, first comprehensively described over thirty years ago by Mc Gowan & Crichton(1923) was now required. Subsequent publications tended to lack haematological information and certain anomalies were obvious.

Keeping these points in mind this work was undertaken in order to assess the requirements of the young piglet for iron, to collect haematological data for the normal and the iron-deficient piglet and to study the clinical and pathological features of the deficient animal.

REVIEW OF LITERATURE.

Incidence and economic loss.

It would seem relevant to consider at the outset what has been written about the economic loss sustained through iron-deficiency anaemia.

Its ubiquity seems well established and reports of its occurrence in the United States, Canada, Britain and other European countries exist (Doyle et al 1927: Schofield 1929: Mc Gowan & Crichton 1924 $\dot{I}$ & $\dot{I}\dot{I}$: Adsersen 1932: Köhler 1956). No survey figures for the overall incidence of the disease in live pigs are available. Kernkamp (1935) stated that 91% of piglets in a series of experiments developed anaemia, the criterion of which was relative change in the haemoglobin (Hb), the red cells or their staining or morphology. This author also recorded that anaemia seldom occurred in the late spring, summer or autumn. Many authors considered that it seriously affected growth rate and weight gains (McGowan & Crichton 1924: Doyle et al 1927: Foot & Thompson 1947 - 48: Barber et al1955) though others took a less serious view (Moe et al1935: Howie et al 1949: Köhler 1956).

The mortality associated with the lack of iron, has also been in dispute. In groups of piglets under investigation from 9% (Kernkamp 1932) to 60% (Doyle et al 1927) of deaths have been recorded. According to McGowan & Crichton (1923) all piglets in affected litters may die. Though Adsersen (1932) considered the condition to be not necessarily fatal.

The post-natal mortality which occurs in pigs from all causes appears to

constitute a major problem. It has been estimated that about 18% of pigs die before weaning (Menzies - Kitchin 1938; Snedecor 1947; Gracie 1955); while of a 20.3% pre-weaning mortality noted by the National Pig Records survey 17.9% occurred in the first three weeks of life (National Pig Records Oct. 1954 - Sept.1955). The British Vet. Assoc. publication No.14 (1956) listed 3 main causes for an average pre-weaning mortality of 20%. These were lack of warmth, anaemia and crushing. On this estimate anaemia would constitute a major problem to the pig industry and since each piglet dying must represent a loss of about £3 - 5 the total sum involved may be calculated at over two million pounds annual loss as the result of anaemia.

Requirements of the Piglet for Iron.

Lintzel & Radeff (1931) found 8 mg. iron in the liver of a piglet at birth and in another 12 days old only 0.5 mg. From this evidence plus Hb estimations on three pigs they concluded that the pig is born with a low Hb content and a small iron reserve. Venn et al (1947) obtained figures for the total iron content of newborn pigs of from 1.92 to 3.48 mg. per 100 gm body weight. A figure which agreed closely with that of 36 mg. of iron in the body of a 1200 gm piglet obtained by Lintzel et al (1944) and quoted by Venn et al. These latter workers maintained that the liver contains insufficient iron to make any large contribution to the requirements of the piglet in the first three weeks of life. They found that only about 2.8mg was removed from the liver during this time; though for the young piglet to be four times its birth weight by the end of the third week they estimated it would require 7 mg of iron per day of which the

sow's milk can only supply a total of 23mg.

It is quite obvious that the very rapid weight increases made by the rapidly maturing piglet produced to-day makes great demands on the available sources of iron. A theoretical estimate of these requirements may be calculated. As the body weight increases the blood volume rises proportionately. For each kilo of body weight a piglet has about 74 ml. blood (Hansard et al 1959). Thus a piglet which at birth weighs one kilo and which at three weeks has attained a modest weight of four kilos will have increased its blood volume by 222 ml. As one gram of Hb. contains 0.34% iron (Fulton 1955), for the Hb. level to remain at 10 gm. per 100 ml., about 72 mg. of iron would be required during this period. Since most of the iron in the body is present in the Hb molecule (75% Fulton 1955; 69% Brown 1956) and only small additional amounts would be required to replenish that used by other tissues and replace that excreted little more would be required. Allowing about 100mg absorbed as the iron requirements of the piglet for the first three weeks of life it is obvious that iron must be obtained from sources other than milk. Venn et al (1947) estimated that a piglet needs to absorb and retain between 250 & 300 mg of iron duringthis period.

One large dose orally of 300mg. reduced iron(Schofield & Lloyd-Jones 1939) or two doses of 500 mg (Anon B.V.A. pub. No.14, 1956) have been considered adequate to meet the needs of the growing piglet. Hart et al (1929 - 30) found that 175 mg ferric oxide daily made little difference to anaemia but ferric chloride did.

Since the milk yield of the sow begins to decline after the third week

supplementary feeding from this time is required (Linton & Williamson 1943; Hammond 1944). It would seem reasonable that nutritional iron-deficiency is only likely to arise before the consumption ofsolid food becomes adequate.

Piglets should be encouraged to take solid food early and a "creep feed" mixture should be available from the second week of life. Sometimes from as early as 1 week piglets can be seen consuming food though it is generally considered that they start supplementing their diet by the third week so that 7 - 10 days later they will be consuming appreciable quantities (Linton & Williamson 1943) and when weaned at eight weeks should be eating between 1 - 2 lbs. each (Anon. B.V.A. Pub. 1947).

Clinical Signs Associated With Iron-Deficiency.

Certainly the first comprehensive and possibly the fullest description of the clinical and other features of iron-deficiency anaemia was given by M^{c}Gowan & Crichton (1923, 1924 $\dot{I}$ & $\ddot{II}$) and by M^{c} Gowan (1924). Earlier workers appear to have attributed this condition to cotton-seed meal poisoning (Wither & Carruth 1918, quoted by M^{c} Gowan & Crichton 1923), while in the publications by other authors which followed, much of the detail found in the original work is lacking. If authors have noted many of the features stressed by M^{c}Gowan & Crichton they have failed to record them.

The piglets affected with anaemia seen by M^{c} Gowan & Crichton (1923, 1924, $\dot{I}$ & $\ddot{II}$)were dull, listless, intensely white, hairy and of a stocky thick-set appearance, due they said to oedema of the skin (M^{c} Gowan & Crichton 1923). Diarrhoea was a prominent feature, as was respiratory distress with spasmodic

jerking of the diaphragm (the so-called "thumps"). Sudden death commonly resulted and survivors were frequently left stunted ("runts") with a chronic cough, and often became jaundiced. These authors also claimed to have seen the effects of iron-deficiency in sows which became very emaciated, though the usual age at which pigs became effected was 3 - 4 weeks.

A similar description, though less detailed was given by Doyle et al (1927) when in three experiments they compared outside and inside rearing. They noted that in the piglets reared inside the mortality rate was four times greater but in bad weather the death rate in those reared outside rose to 28% and even more significant these pigs were anaemic. Anaemia usually occurred in pigs over 3 weeks and sudden death was common. Another interesting feature recorded in this paper was that anaemic ones which survived recovered even without any change of experimental conditions or rations.

Clinical data were omitted from the paper by Hart et al (1929 - 30) though they state the changes were identical with those of M^{c} Gowan & Crichton (1924) & Doyle et al (1927). Hart et al (1929) also noted that anaemia could not be prevented by feeding iron to the sows, though later Doyle (1931) and Venn (1944) claimed that the feeding of certain green food to sows prevented anaemia in the piglets; Braude & Foot (1946) disputed this. Exposure to mercury - vapour - quartz lamps did not prevent anaemia (Mathews et al 1929).

Anaemia occurred in pigs between 1 and 3 weeks and spontaneous recovery took place at the 3rd and 4 th weeks according to Schofield (1929) which, he

maintained, was not due to the ingestion of solid food because of the marked lassitude of the anaemic piglets and their lack of desire to forage.

Skinner & Reid (1929 - 30) claimed that anaemic pigs were more prone to "bull-nose" infection.

About this time many workers concerned themselves about the best method of preventing anaemia, or administering iron with or without copper though no critical work had been done on the importance of the latter for pigs (Elvehjem & Steenbock 1930; Hamilton et al 1930; Willman and M^{c} Cay 1931; Anon Winsc. Ag. Exp. Bull.1931). Elvehjem & Hart (1932) claimed that iron alone was of little value and only after the administration of copper did improvement occur.

Adsersen (1932) thought nutritional anaemia was of great importance but not necessarily fatal and the circumstances which made the animals unable to survive he did not discover.

Milk anaemia according to Graham & Thorp (1932) was initiated by constipation and lethargy, but at three to five weeks the affected ones showed pale mucosae, were lethargic and had a "fatigued expression" though they were generally fat and had the appearance of fullness in the submaxillary region. Sudden death might occur or the symptoms recede gradually to be followed by retarded development for many weeks or months.

Nutritional anaemia was more prevalent in litters born in the winter and early spring, but was just as common in litters kept out of doors on concrete as in litters confined indoors according to the observations of Hamilton et al (1933). The onset was usually between the third and fifth weeks though it

could occur at the second week and persist as long as the sixth week. Pigs which lived beyond the fifth week usually recovered rapidly and the highest death rate occurred between the third and fifth weeks. The average live weight for all treated groups at week six was 19.5 lbs. which was exactly the figure recorded for those treated with iron.

Moe et al (1935) noted that the fattest pigs had the lowest Hb levels though after a time as growth slowed the Hb level appeared to rise.

Physical symptoms were less satisfactory than haematological examination according to Kernkamp (1935) and on many occasions a diagnosis of anaemia would not have been made without a blood examination. Recovery could occur in pigs with Hb as low as 2.7 gm per 100ml. Lemesh et al (1936) thought anaemia was particularly dangerous during the first fortnight of life and Baskett & Lamont (1936) recorded a high mortality in pigs given no iron supplement.

A check in the weight gains of anaemic pigs was noted by Fraser (1938) though he regarded the anaemia as transient and usually comparatively harmless. However Vestal and Doyle (1938) thought lasting damage was done to many. Lamont (1938) quoted Waldmann as expressing the opinion that anaemia is a symptom of piglet influenza, that the two are frequently confused and that a pathological anaemia does not occur. Lamont agreed that confusion has occurred but he believed anaemia could exist on its own though the symptoms of the two diseases were similar. Piglets affected with anaemia he noted as fat and stocky, with grey-white colour and prominent bristly hair; symptoms which show between three and six weeks of age. These piglets were not inclined to play and panted after

exertion. When the Hb fell below 5 gm % exaggerated respiratory movements ("thumps") and diarrhoea occurred and death took place within a few days. Pigs with a Hb as low as 4 gm % usually died.

Foot & Thompson (1947-48) noted that pigs reared on concrete became anaemic during their second week of life and developed pale extremities. By the third week they were flabby and lethargic though often sleek and fat, but by the fourth week they had become emasciated runts which usually died.

In a series of very interesting and valuable experiments performed in the same area as those conducted by M^{c}Gowan and Crichton (1923, 1924 $\dot{1}$ & $\ddot{11}$). Howie et al (1949) were able to show that iron failed to cure or prevent litters developing a condition with clinical or pathological features similar to those described by M^{c} Gowan & Crichton (1923, 1924 $\dot{1}$ & $\ddot{11}$). Iron deficiency anaemia they maintained did not of itself preclude good health and weight at weaning, and the syndrome observed was essentially one of cardiac failure. "Thumps" attributed by M^{c} Gowan & Crichton (1924) to spasmodic jerky breathing was regarded by Howie et al (1949) as being due to cardiac overaction.

In a later paper Naftalin & Howie (1949) described the clinical features of this disease which they associated with cold, wet housing conditions and which they claimed resembled oligaemic shock. Piglets were healthy till three weeks old but consistently they refused solid food; they then became listless and apathetic and did not lie contented but were continually struggling to lie on top of one another. Growth was variable; arching of

the back and abdominal distention due to ascites were usual. The hair was long and rough, the ear-tips blue, and intermittent diarrhoea occurred. Death took place either from cardiac overaction or in 12-36 hours in a coma. They finally concluded that this syndrome should be attributed no longer to iron deficiency.

An attempt to reproduce the condition by Lucas (1954) failed and he suggested that some unknown virus disease may have complicated the earlier work.

In 1951 Lemont expressed the view that anaemia could cause heavy losses in young pigs in cold damp houses. Some of these cases responded to iron but others did not, and since the condition was often associated with coughing a virus pneumonia may have some connection with the failure to respond to iron; and Santiago Lugue (1952) called for reappraisal of the position since a true lack of iron seemed unlikely to be the sole aetiological factor in anaemia.

Obel (1953) working on liver dystrophy in pigs, which occurred seldom before three weeks of age and usually about six weeks, noted clinical signs akin to those described by McGowan & Crichton (1924 $\dot{I}$ & $\dot{I}\dot{I}$). These were a short illness characterised by dyspnoea, diarrhoea or dysentery, vomiting, fever, icterus, loss of weight, and paralysis. Anaemia was frequently found.

Bringing its subject matter up to date the Brit. Vet. Assoc. Publication No.14 (Anon.1956) stated that anaemia was not easily diagnosed with confidence on clinical examination. Skin pallor may be masked by the use of heat lamps and "thumps" which is often said to be associated with anaemia is rare and

probably no part of the simple anaemia syndrome. Yellowish-white or grey diarrhoea accepted in Britain as a strong indication of anaemia has been little regarded in America and even in cases of severe anaemia may not be present. In the early stages anaemic pigs may be sleek and plump when the Hb level is about 5 gm% and they are about three to four weeks old. If, adequate iron is obtained growth continues unchecked, and even in severe anaemia slow weight increase is made, though eventually growth ceases and and piglets become pale and hairy. The syndrome it is claimed varies greatly according to whether the anaemia develops rapidly or slowly.

Piglets are at greatest risk from anaemia from birth to four or five weeks and the symptoms of classic anaemia are often followed by weakening of the constitution and susceptibility to infections though severe anaemia may of itself cause death, according to a report by the Organisation for European Economic Co-operation (Project No.345; 1947). A different view of anaemia was taken by Köhler (1956 $\frac{\cdot}{1}$ & $\frac{\cdot\cdot}{11}$), who regarded the most important factor in the aetiology of piglet anaemia as a deficiency of animal protein. He considered that deficiency of iron was at most transient and of subsidiary importance.

It proved so difficult for Widdowson & M^{c} Cance (1955) to obtain pigs rendered anaemic through deficiency of iron that they had to resort to venesection and eventually obtained pigs from a 'large white' colony "in which anaemia has become invariable." And Schultze et al (1936) in order to obtain anaemic pigs kept them for the first five weeks of life on wooden

floors with clean shevings and denied them access to the rations of the mother. M^{c} Nutt (1958) gave a description of anaemic piglets seen thirty years before to serve as an example of all such pigs. Affected animals were noticeable after two weeks from birth, and though still fat and plump they were pale or yellow particularly on the snout and above the hooves. When they played the heart and respiratory rates were tremendously increased, so much so that they ceased and stood semi-exhausted, weakened and dyspnoeic. When pulmonary oedema was severe the pigs were said to have "thumps". Pigs which survived became dehydrated, developed "all sorts of" dermatoses, lost weight, became stunted and were apt to develop diarrhoea. M^{c} Nutt however concluded that there are factors in piglet anaemia which are not understood such as those which allow piglets with Hb levels of 4 - 6 gm per 100 ml to appear fairly normal while others die.

Pathology.

As with the clinical features a full description of the pathology was given by M^{c}Gowan & Crichton particularly in their earliest paper (1923) and by M^{c}Gowen alone one yearlater (1924).

Those workers described a flagrant autopsical picture of a fat pig, with a greatly dilated heart, excess pericardial fluid, great peritoneal effusion and sometimes fluid in the chest and oedematous lungs. Haemopericardium and pericarditis were quite common and the lungs, crushed by the cardiac enlargement were often affected with a catarrhal pneumonia at the lower margins. The livers of affected piglets were pale and studded with irregular pale fool or irregular darker rings which they called the "fairy - ring" appearance. Mild

cases showed only slight and more advanced cases gross hepatomegaly.

The few piglets which survived were emasciated and hairy and on autopsy showed adhesions between the heart, pericardium, lungs and wall of the thorax. The lungs were collapsed, pneumonic and adherent to the ribs, diaphragm and vertebral bodies. Adhesions also existed between the liver lobes, the coils of the intestine and the enlarged spleen.

With such marked pathological changes occurring in the organs of affected piglets, one would expect that subsequent papers on anaemia would mention these features. Such however, is not the case and few authors have given other than a brief description of their findings.

One of the most detailed was given by Lamont (1929) who indicated the percentage of cases showing pathological alteration in the various organs. Thus he stated that in 84 cases studied enlargement of the liver was present in 50%, 30% showed "typical 'nutmeg' appearance"; 44% had a pericardial effusion and 12% an adhesive pericarditis. The heart was enlarged in 70% of cases and in 88% of cases evidence of pneumonia was found.

The pneumonic lesions and the enlarged pale heart were mentioned again by Lamont (Baskett & Lamont 1936) though later he regarded the pneumonia as a complication factor (Lamont et al 1950).

A constant finding noticed by Doyle et al (1927) was liver damage. Hepatomegaly, liver haemorrhages and fatty infiltration werecommon and if the animal recovered fibrosis developed, even extensive enough to cause a "hobnail" appearance of the liver. Other features noted were, ascites and oedema of the folds of the mesentery, degeneration of the kidneys, increased thoracic

fluid, a dilated heart and frequently pneumonia. Fatty degeneration and later cirrhosis of the liver were common features noted in anaemic pigs by Craig (1930) together with cardiac dilatation, pneumonia and cachexia. Graham & Thorp (1932) also recorded degenerature changes of the liver though in the recovered case chronic myocarditis was the significant lesion. Sudden death of some anaemic piglets between the third and fourth week with Hb levels below 6 gm per 100 ml were attributed by Moe et al (1935) to unspecified pathological changes in the heart.

The pathological changes in the cases seen by Howie et al (1949) were essentially those of cardiac failure. The hearts were enlarged, dilated and flabby. Livers were large, congested and showed microscopic haemorrhages, and effusions were present in all the serous cavities. Similar autopsical features were again reported by Naftalin & Howie (1949) which they recorded as mainly ascites or adhesions and gross liver change. The lesions were essentially as described by M^{c} Gowan & Crichton (1923 & 1924 ††) but could not be prevented or cured by iron though they could be by improving the environment.

In a study of liver dystrophy in piglets, Obel (1953) noted that these piglets were often anaemic, occasionally icteric, had subcutaneous oedema, waxy degeneration of the muscles and serous effusions. The liver changes varied from hepatomegaly to, in chronic cases, fibrosis and patchy hyperplasia. These changes she attributed to toxic factors influenced by among other things diet and housing. Shanks (1953) too noticed the association between enlargement of the liver and anaemia, in a disease of unknown

aetiology affecting piglets, the pathology of which resembled many descriptions of iron-deficiency.

The details of the pathology of piglet anaemia given in the Brit. Vet. Assoc. Publication No.14 (1956) is roughly related to age. Thus it is stated that at about two weeks one of the most constant findings is anoxaemia of the liver. Irregular diffuse pale areas may be present and often miliary areas of necrosis. There is general palor of the carcase and sometimes slight cardiac enlargement. At about three to four weeks the carcase is fat and at this stage the heart is considerably enlarged and hypertrophied. Later the heart is thin-walled and dilated. Plasma exudates may be found in the serous cavities, with clots in the pericardial sac and strands of fibrin in the peritoneal cavity. By six to eight weeks organisation of the fluid in the pericardium causes a sand-like deposit on the surface of the heart. Oedema of the lungs may occur during the later phases, and death may result from the earlier damage.

Anthony (1955) gave a brief description of the pathology of nutritional anaemia and included oedema of the bowel, stomach wall, throat and eyelids, though he stated that these findings may be present also in other diseases.

The weights and cardiac outputs of the hearts of anaemic pigs were found by Widdowson & M^{c} Cance (1955) to be greater than those of normal piglets. The "hypertrophy" of the hearts was thought due to an increase in the numbers of normal muscle cells.

Haematology and Haemopoietic tissues.

Though the haematological data recorded by M^{c} Gowan & Crichton (1924 ••) 11)

and M^{c}Gowan (1923) were not extensive they deserve careful consideration since so many of our present concepts about piglet anaemia seem to stem from the work of these authors. They described a variable diminuation of Hb with, in advanced cases, a drop from a normal level of 80% to as low as 15%. Treatment with iron raised the Hb level from 20-30% to 70-80%. The red cells decreased less than the Hb but a reduction to about 3 million per c.mm was apparent from around the normal count of 4 - 5 million for pigs of this age. No change in the white cells was evident.

Blood film showed marked anisocytosis, with macrocytes, microcytes and sometimes severe poikilocytosis. Normoblasts were often numerous with a few megaloblasts in some cases. Polychromasia was marked, platelets abundant and "shadow red cells" found in large numbers.

The shafts of the femur and ribs contained cancellous tissue pale pink in colour but so dry and devoid of marrow that it was not found possible to prepare a film from them "by rubbing" the bone marrow "on a cover-glass while squeezing with forceps". Sections showed a more or less aplastic state, but the marrow elements were greatly reduced in numbers and showed no signs of activity.

The standards of anaemia, in piglets over two weeks old, laid down by Doyle et al (1927) here 3 million or fewer erythrocytes per c.mm. or 3.8 gm per 100 ml of Hb. They noted th t the red cells stained poorly with eosin and had a ragged outline. Normoblasts were not numerous. In contrast to M^{c} Gowan and Crichton (1924 ††) they noted active haemopoiesis of the bone

marrow. The same standards for the red cell count and the Hb were given by Craig (1930).

Schofield (1929) however was unable to discover a constant relationship between Hb and red cell numbers in anaemic piglets, and Kernkamp (1932) observed that the decrease in Hb was more marked than the erythrocyte count. He, as did Craft et al (1933) and Jespersen et al (1939) found marked variation in the Hb levels of piglets in the same litters, though no difference between the sexes was noticed by Draper & M^{c} Elroy (1949). Garry et al (1954) have pointed out that statements indicating that higher Hb values occur in male animals require further confirmation. Mays (1958) believed the sharp post-parturient decline in Hb noticed by himself and others (Schofield 1929; Hamilton et al 1930; Kernkamp 1932; Jespersen and Olsen 1939) was due to haemorrhage from the umbilicus and rapid hydration from being suckled. Craft & Moe (1933) noticed that the drop in Hb during the first week was not invariable though frequently present.

For ease of comparison of the various Hb levels quoted by authors a table of the values has been included. (Table 1). Other normal values for Hb which have been given for piglets include 10 gm % and over (Köhler 1956 †) and Tribe (1954) graphed the level of normal iron replete, pigs also as around 10 gm %. Braude (1954) gave the average Hb value for the pig as 12 mg/100 ml. of blood, with a range of from 8 - 16 mg/100 ml. though presumably the quantity has been erroneously quoted for gm. This author also gave the average normal haematocrit as "58 volumes % of R.B.Cs".

The other blood parameters have received less attention in the literature.

TABLE 1. Haemoglobin values of piglets quoted in the literature.

Author	Authors' comments and (age of piglet)	Haemoglobin Levels quoted	
		Anaemic	Normal
M^{c} Gowan (1924)	Advanced cases	15 %	80%
Hartet al (1929-30)	"Severe anaemia" (3 - 4 weeks).	3 - 4 gm%	8 - 10gm %
Graham & Thorp (1932)	-	30 - 40%	
Craig (1930)		≤ 3.8 gm %	
Hamilton et al (1930)	At birth	-	ave 10.75gm% (9 - 15 gm%)
Hamilton et al (1933)	Slightly anaemic Mod. " Severely " Extremely "	4.6 gm% 3.85gm% 1.7 " 1.0 "	
Kernkamp (1935)	Mild or slightly anaemic Definitely anaemic	6.0 gm% 4.0 "	
Lemont (1938)	Almost invariably fatal	4.0 "	
Klein and Kuhn (1940)	Lower level of Hb in anaemia	40 %	
Foot & Thompson (1947 - 48)	Level of anaemia (3 weeks)	4.15 gm %	
Howie et al (1949)	"Distinctly anaemic"(3 weeks)	39%	

Continuation of

TABLE 1 Haemoglobin values of piglets quoted in the literature.

Author	Authors' comments and (age of piglet)	Haemoglobin Levels quoted	
		Anaemic	Normal
Naftalin & Howie (1949)	Levels of anaemia (100% = 14.8 gm)	< 40%	80% (Adults)
Cartwright et al (1944)		mean 9 gm% range 7.4 - 9.7 gm %	14 ± 0.45 gm%
Anon (Brit.Vet. Assoc. Pub. 14. 1956)	"Borderline" anaemia	7.5 gm%	
	Definite anaemia	5.0 gm%	
Köhler (1956)			10 gm%
Keller (1958)	Subacute or marginal anaemia (24 - 36hrs)	6.5 - 9gm %	
M°Nutt (1958)	"Considered anaemic"	9 gm%	

The erythrocyte counts dropped to 3×10^6 per c.mm or less in the cases of anaemia seen by McGowan and Grichton (1923) Doyle et al (1927), Craig (1930) and Graham and Thorpe (1932). Kernkamp (1943) recorded levels of from 2.5 to 4.0 million per c.mm for the R.B.Cs of piglets between 3 and 6 weeks old reared on concrete, in contrast to counts of 5.5 to 7.0 millions per c.mm. for the same ages of pigs reared at grass. The mean count of three, apparently very anaemic, piglets when three weeks old was given by Fraser (1938) as 4,331,000 per c.mm., though this author gave the range for normal piglets as 4 - 8 millions, with an average of 4,881,000 R.B.Cs for 34 piglets aged 1 week to 1 month. Gardiner, Sippel and Mc Cormick (1953) noted a fall in erythrocyte counts and Hb levels of 26 piglets. A parallel decline took place during the first week but was corrected during the second week. The mean figures for 6 litters at 12 hours post-partum ranged from 8.57 - 10.35 gm% of Hb and 33.1 - 41% for the packed cells.

The erythrocyte count noted by Wintrobe (1943) was 7.93 millions per c.mm for normal pigs but no count for iron deficient pigs appeared to be given. Other parameters, however, were recorded for iron-deficient pigs by Wintrobe and co-authors in two main papers (Cartwright et al 1944; Wintrobe et al 1953). The prolific writings on anaemia in pigs of these authors contrast with the paucity of information imparted by others. One of the earliest descriptions was given by Hamilton et al (1933) who did record some data on the blood of anaemic piglets. He found the haematocrit values of slightly anaemic piglets were 23%, severely anaemic 16% and 10% in extreme cases. Erythrocyte counts dropped from about 3.5 millions in slight or moderate anaemia to 1.5 millions per c.mm.

in the extremely anaemic ones. With this range of values this author recorded small alterations in the erythrocyte volumes which remained around 65 c. microns.

The blood values obtained by Cartwright, Wintrobe and Humphries(1944) for five experimental iron-deficient piglets were :-

Volume of packed red cells (P.C.V)	Mean 29.8 ml per 100ml.
	range24 - 32.4 " "
Mean Corpuscular volume (M.C.V)	Mean 41.8 c μ
	range38 - 47"
Serum iron	Mean 48 * ± 2.31 μ g %.
	range 30 - 75 " "
Total White cells	Mean 16.6 x 10^3 per c.mm.

While Wintrobe, Cartwright and Gubler (1953) recorded the following values obtained from 10 iron-deficient piglets : -

P.C.V.	mean 21 ml per 100 ml.
M.C.V.	" 36 c. μ.
M.C.H.C.	" 28%

No ranges or standard deviations for these values were given

Köhler (1956 **11) after what he called very thorough investigations into anaemia caused by deficiency of iron recorded that the serum iron levels dropped below 110 - 130 μ g%. The fall was only transitory however, and rose again without any iron-supplements and the values reached were not only normal

* ± The standard error of the mean.

(approximately 200 μ g %) but could reach higher levels e.g. 400 μ g %. A reduction of the serum iron level Köhler maintained was the most characteristic indication of iron-deficiency anaemia, though Santiago Luque (1953) stated that although the total iron content of the blood is decreased there is an increase in the serum iron content in iron deficient piglets.

Some authors have recorded that no characteristic changes occurred in the white cells in anaemia (M^{c} Gowan 1924; Doyle et al 1927; Cartwright et al 1944) yet others noted, slight leucopoenia but no significant difference in the differential counts in anaemic pigs (Kernkamp 1932); a slight neutropoenia but no difference in the total counts (Lahey et al 1952); and a fall in the total white cells due to a decrease in the neutrophil leucocytes with an increase in the eosinophiles (Fraser 1938).

Variations in the white cells of piglets due to infection and to normal physiological changes (Venn 1944; Gardiner et al 1953) probably account for the differences in the interpretation of results.

An increase in the platelets to 1,311 x 10^{3} in iron deficiency from around the normal level of 615 x 10^{3} per c.mm has been given by Wintrobe et al (1953). M^{c} Gowan (1924) also noted abundant platelets in smears in some cases of anaemia.

The morphology of the red cells was recorded by some workers, of whom M^{c} Gowan (1924), M^{c}Gowan and Crichton (1924 $\dot{1}$ & $\ddot{11}$) and Doyle et al (1927) have been quoted. A hypochromasia of the cells in anaemia was noted by Kernkamp (1935), Santiago Luque (1952), Lahey et al (1952) and Cartwright et al (1944); though Fraser (1938) stated that in "the majority of cases the corpuscles are

not oligochromaemic." Fraser also noted marked anisocytosis with megalocytes predominating in the early cases. In more advanced cases microcytes became more frequent and in the final stages of fatal cases poikilocytosis and chlorosis occurred.

Santiago Luque (1952) described the anaemia of iron-deficient piglets as hypochromic microcytic with anisocytosis, a reduced reticulocyte countand polychromasis.

In experimentally produced iron deficiency in piglets Lahey et al (1952) recorded a severe microcytic (M.C.V. 36 ± 3.1 c μ) hypochromic (M.C.H.C. 28 ± 2.9%) anaemia, with hypoferraemia and an increased total iron-binding capacity of the plasma, Cartwright, Wintrobe and Humphries (1944) and Wintrobe, Cartwright and Cubler (1953) described the anaemia produced in piglets made deficient in iron as hypochromic and microcytic with hypoferraemia and hypercupraemia.

Crenation of R.B.Cs in piglets occurs readily due to alteration of temperature or mechanical damage (Köhler 1956 $\dot{1}$).

The average size of the erythron of piglets was given by Köhler (1956 $\dot{1}$) as 6.5 μ, who quotes the size recorded by other authors (6.2 μ Marek 1937; 6 - 6 . 2 μ, Wirth 1950; 6.2 μ Gutig 1907; 6.2 μ Lutje 1911; 6.2 - 10 μ, Senftlesen 1920). Wintrobe et al (1956) gave the red cell diameter of pigs as 5.5 μ and quoted a figure of 6.1 given by Scarborough (1931).

Examination of the bone marrow from piglets has received little attention and opinion is divided as to the type of change occurring in nutritional iron-deficiency of piglets. McGowan's (1924) remarks have been noted, and contrary

to the "more or less aplastic state" which he recorded, Doyle et al (1927) observed active haemopoietic centres in the bone-marrow. Fraser (1938) too classified the disease as an aplastic anaemia and Santiago Luque (1952) recorded a reduction in the erythro-blastic elements and immaturity of the cells of the bone marrow.

Hyperplasia of the marrow has been recorded however, by Graham and Thorp (1932); and explicitly stated as normoblastic by Lahey et al (1952) and Wintrobe et al (1953).

A normoblastic hyperplasia occurs in the bone marrow of humans deficient in iron (Whitby & Britton 1957; Wintrobe 1956).

Treatment of Nutritional Anaemia.

A useful review of the literature on piglet anaemia has been given by Seamer (1956) in which he included some of the abundant information on the treatment and prevention of this condition.

In order to complete the many facets of anaemia the subject of prophylaxis and treatment will be considered here, though briefly.

Though the administration of iron or greens to the sow has been suggested as prevention for anaemia in piglets (McGowan and Crichton 1923, Doyle 1931, Venn 1944). It is generally accepted that these have no direct influence on the post-parturient levels of Hb in piglets (Doyle et al 1927, Hart et al 1929, Foot and Thompson 1938, Braude and Foot 1946).

Various oral iron preparations have been suggested for administration to such as dialysed iron (Craig 1930), ferric citrate (Hamilton et al 1930), ferrous sulphate (Adsersen 1932), iron pyrophosphate (Foot and Thompson

1938) and reduced iron (Draper and McElroy 1949). Sometimes copper sulphate also has been recommended (Moe et al 1935; Harris 1938 - 39) and demonstrable improvement has been noticed when it was included with iron (Foot and Thompson 1938; Draper and McElroy 1949).

The amount of minerals required to prevent anaemia has been variously suggested as a weekly intake of about 175 mg of iron and 35 of copper (Hamilton et al 1930), 150 mg iron and 25 mg copper, (Anon 1931 Wiscon.Ag.Exp. Stat. Bull) and 210 mg of iron (Foot & Thompson 1938). Later Foot & Thompson (1947 - 48) recommended three doses of 30 mg of iron during the second week of life as being sufficient.

Tribe (1956) has stated that 10 - 15 mg iron daily is sufficient during the first six weeks and Venn et al (1947) said that 7 mg of iron must be absorbed and retained daily for the first 3 weeks for growth to proceed normally.

Soil has been suggested as a cheap and easy method of supplying the mineral requirements of piglets (Kernkamp 1935; Moe & Craft 1935) and recently iron administered parenterally has given favourable results (Brownlie 1955, Barber et al 1955, Kernkamp 1957).

That spontaneous recovery can occur without any obvious change in husbandry or management has been suggested (Schofield 1929; Tribe 1954) and some workers have indicated that the genetical pattern of the piglet has an influence on resistance or susceptibility to anaemia (Adsersen 1932; Craft & Moe 1933; Jespersen and Olsen 1939).

Most of the estimations of the iron requirements of the piglet have been

made on the empirical grounds of weight gains and Hb levels. Some determinations of the iron requirements have been made by carcase analysis however, (Venn et al 1947) and Brownlie (1955) and M^{c} Donald et al (1955) assessed iron retention from the increase in Hb and the weight of the piglets.

Anaemia in piglets due to causes other than deficiency of iron.

Within a few years of the publications of M^{c} Gowan & Crichton (1923, 1924 $\dot{1}$ & $\ddot{11}$) workers were considering the value of copper as a treatment or as a prevention for anaemia (Hart et al 1929-30; Moe et al 1935; Kernkamp 1935). The first real indication that copper was essential for proper erythropoiesis was given by the work of Schultze et al (1936 $\dot{1}$ & $\ddot{11}$) and this was later confirmed by Teague & Lawrence (1951), by Lahey et al (1952) and again by Gubler et al (1952) and Wintrobe et al (1953). These latter workers noted the interesting fact that diets deficient in copper which created anaemia in piglets did not do so when fed to infants. They also pointed out that the morphology of the anaemia in copper deficiency in no way differed from that of iron deficiency. The mean values for the blood parameters from about 70 experimental pigs deficient in copper were given as P.C.V. 22 ml per 100 ml; M.C.V 39 cμ ; M.C.H.C. 29 % ; and serum iron 30 μ g. It was thus a hypochromic microcytic anaemia with normoblastic hyperplasia of the marrow. Growth of the pigs was good and they were active until severe anaemia developed. The deficiency of copper acted by interfering with the absorption, transportation and mobilisation of iron.

Whether copper deficiency occurs naturally remains unanswered at the

moment though it was suggested by Brooksbank (1954) on rather tenuous grounds that it may.

Experimentally Birch, Chick & Martin (1937) produced an anaemia on a "pellagra - producing" diet. Later pyridoxine was shown to be an essential factor for erythropoiesis in pigs (Wintrobe et al 1943; Cartwright et al 1944), and deficiency caused anaemia which was also microcytic and hypochromic in type accompanied by bone-marrow hyperplasia. The following mean values were given for the blood attributes; Hb. 8.9 ± 0.89 gm%; P.C.V. 29.3 ± 2.51 ml per 100 ml ; M.C.V. 54.4 ± 1.84 c μ ; M.C.H.C. 28 - 33% ; serum iron 373.6 ± 14.31. There was impaired growth of the piglets, curled hair, diarrhoea, convulsions and ataxia.

Johnson & James (1948) incriminated choline as a factor essential for normal erythropoiesis and piglets deprived of it developed fatty infiltrations of the livers and Cartwright et al (1948 & 1952) created a macrocytic anaemia (hb. 6.5 gm%; M.C.V 69 c μ), a leucopoenia and a neutropoenia in swine made deficient in pteroylglutamic acid. The bone-marrow picture was macronormoblastic. A deficiency of vitamin B_{12} (cyanocobalamin) alone in pigs did not produce a macrocytic anaemia and irregularly created a normocytic anaemia (Cartwright et al 1951).

Shanks (1953) described a disease of piglets 3 - 8 weeks old usually, of unknown aetiology, in which the most consistent feature was hepatomegaly; which prompted him to call it "Big Liver Disease". On autopsy there were also effusions in the serous cavities, cardiac enlargement and splenomegaly.

Affected pigs showed anaemia, "thumping" and occasionally pneumonia. The morbidity and mortality rates were variable and if they survived the initial attack piglets were hairy, dirty, and thin and eventually succumbed. Iron neither prevented nor cured the disease.

Obel (1953) in her monograph on toxic liver dystrophies in swine recorded a variety of liver changes and stated that in hepatosis diaetetica anaemia was very frequent. No values were quoted however.

A protein deficiency was thought by Köhler (1956) to be the main cause of piglet anaemia. This he held responsible for a hypochromic type of anaemia accompanied by medullary fibrosis, and a disarrangement of blood proteins.

Various infections have also been incriminated as causes of anaemia. Splitter (1951) has described an infectious ictero-anaemia of pigs and in Britain, Jennings and Seamer (1956) have isolated a parasite which they called Eporythrozoon parvum, which caused anaemia in splenectomised but rarely in normal piglets.

What part infections such as polyserositis (Bohar et al1955), piglet influenza (Shope 1931) now apparently rare in Britain, so-called virus pneumonia (Betts 1952) and even piglet uraemia (Madsen et al 1944) play in influencing the course or onset of anaemia from iron-deficiency or otherwise, remains unknown. Neither is it known how much influence naturally occurring toxic factors or infections have on erythropoiesis though Wintrobe et al (1947) have shown that the uptake of radioactive iron is impaired by acute inflammation even in iron - deficient pigs.

Considerable information has been recorded on an acquired haemolytic disease of piglets, which has been produced experimentally (Bruner et al 1949; Saison et al ~~Goodwin~~ 1955; Goodwin and Saison 1955, 1956 & 1957) and has been seen in the field (Buxton & Brooksbank 1953; Doll and Brown 1954). The association with vaccination of the sow with swine fever crystal-violet vaccine has been established (Goodwin & Saison 1957). A breed difference in the reponse to the stimulus was noted; Essex/Wessex sows being more likely to develop iso-antibodies than those of the large white breed.

Goodwin et al (1955) have stressed the importance of the subclinical forms of the disease and the difficulty of detecting these forms clinically until the Hb has fallen below about 6 gm %. It would appear unlikely however, that acquired haemolytic anaemia is a frequent cause of anaemia in all breeds of pigs throughout this country.

METHODS AND TECHNIQUES.

Haematological Methods.

Samples of blood were withdrawn from piglets by two main routes. The ear vein was used when small quantities only were required, but larger samples were usually withdrawn from the anterior vena - cava (A.V.C.).

In withdrawing samples from the ear vessels an erythema was produced by gentle rubbing and swabbing with alcohol. In many pigs a vein follows the external edge of the pinna on its dorsal surface and by pricking with a sterile needle or scalpel blade one or two millimeters of blood, may be collected.

In adults or large pigs up to 20 ml have been withdrawn using a syringe, with eccentric nozzle and a needle about 16 - 17 gauge and 1 inch long. Hb. estimations, red cell and white cell counts, and smears may be made by taking blood directly from the vein. Provided the rate of flow from the haemorrhage was fast no difference was noted between estimations made thus compared with larger samples from the A.V.C.

The technique for bleeding pigs from the A.V.C has been described, (Sippel, 1949). The modifications made to this method were to use a 16 -17 gauge needle about two inches long and to place the pig on ~~a~~ dorsal recumbency when possible.

The anticoagulant usually used was the anticoagulant mixture, which has been described by Heller and Paul (1934).

A solution of 1.2 gm ammonium oxalate and 0.8 gm potassium oxalate was made in 100 ml of distilled water and 0.25 ml placed in bijoux bottles then

dried in an incubator oven at a temperature not exceeding 80° C. To each bottle 2.5 ml of blood was added.

Smears were made as rapidly as possible after mixing with blood and usually within an hour.

Occasionally heparin was used as the anticoagulant.

Estimations of Hb were determined as oxyhaemoglobin after the method described by Arnold (1949). The technique was to convert 0.02 ml of whole blood into oxy-Hb by diluting with 2 ml of 0.007 N. ammonia (0.04 ml ammonia solution, S.G. 0.88 to 100 ml with water; Szigeti 1940). The zero extinction value of an "E. E. L". colorimeter using an O G R 1 Filter (maximum transmission between 0.52 - 0.54 μ) and ammonia solution was determined. The galvinometric reading of the sample was then taken and from a previously prepared correlation chart, the corresponding Hb concentration was obtained.

Samples were estimated in duplicate whenever possible and occasional ones were estimated by the method of Bell et al (1945) using 0.2 ml of blood diluted to 25 ml with 0.007 N ammonia, and the readings converted on a suitable graph.

Blood samples of known Hb content were obtained regularly for comparison.*

The red cell counts were made by a standard technique (Whitby & Britton 1957) using a Thoma red cell pipette, Neubauer ruled counting chamber with 0.9% saline as diluting fluid. A dilution of 1 in 200 was made and the cells occupying five blocks of 16 x $^1/_{400}$ sq.mm.squares were counted and multiplied by 10,000. Repeat counts were made when possible.

* Obtained from C.Davis Keeler, Ltd.,
39, Wigmore Street,
LONDON. W.1.

The volume of packed red cells (P.C.V.) was estimated in a Wintrobe's haematocrit tube (Wintrobe 1956) spun at about 3,000 R.P.M.(approx 2,000xG). for 30 minutes.

Initial estimations of the P.C.Vs were made from blood samples withdrawn from ear veins and placed for centrifugation in microhaematocrit tubes, of the type open at both ends (Mc Inroy 1954) but because of leakage the results were unsatisfactory and this method was abandoned.

The total white cell counts were made in the manner described by Whitby & Britton (1957); counting the cells in a haemocytometer chamber with Neubauer ruling, after making a 1 in 10 dilution of the blood with diluting fluid containing glacial acetic acid and a 1% solution of gentian violet.

Differential white cell counts were made on the Leishman stained smears used for examining the morphology of the R.B.Cs.

The red-cell indices were calculated from the following formulae (Whitby & Britton 1957):-

Mean Corpuscular Haemoglobin:-

$$\text{M.C.H. (micromicrograms: } \gamma\gamma) = \frac{\text{Hb. in gm. per 1,000ml blood}}{\text{R.B.Cs in million per c.mm.}}$$

MeanMean Corpuscular Volume:

$$\text{M.C.V. (cubic microns : } c\mu) = \frac{\text{P.C.V. in ml. per 1,000 ml blood}}{\text{R.B.Cs in million per c.mm.}}$$

Mean Corpuscular Hb. concentration :-

$$\text{M.C.H.C. (gm \%) ..... } = \frac{\text{Hb. in gm per 100 ml. blood x 100}}{\text{P.C.V. in ml. per 100 ml.blood}}$$

Measurements of red cell diameters were made in all but the first experiment, by photographing the cells from smears together with a measuring scale. These were later projected on to graph paper from which the cell diameters could be read directly. The diameters were measured in two transverse directions and the means of 200 cells recorded on each occasion.

In the first experiment a modification of the above technique was used. The cells were photographed on to plates and measured by a suitably calibrated scale when trans-illuminated.

The osmotic or saline fragility of the R.B.Cs was estimated by using one of two techniques. Initially Creed's technique (1938) was used but this was replaced by the following method:-

Prepare a series of stock saline solutions (using 'Analar' reagents) ranging in concentration from 0.85% to 0.30% in intervals of 0.05%. Transfer 2.5 ml. of each of the solutions into corresponding tubes marked 1 - 12. Add 0.05 ml blood to each tube, mix and allow to stand for 10 minutes. Mix again and allow to stand for a further 10 minutes. Centrifuge lightly. Compare the haemolysis against standards prepared by adding 0.4 ml of blood to 10 ml. of distilled water. Dilute this standard in steps from 4/5 to 1/ 25 i.e. 80% to 4 % of the Hb content of the blood sample used. A normal control may also be set up.

Bone-marrow smears made ante-mortem were taken from the tibial marrow, which was found preferrable to the sternum or the femur. The site was chosen for withdrawing samples was just posterior to the ridge of the tibial tuberosity

on the medial aspect of the leg. It was found that an anaesthetic was not required. A needle of the Salah pattern was used, and though no difficulty was experienced in withdrawing marrow elements these were usually so diluted with blood as to make the interpretation difficult, despite withdrawing only small amounts (< 1 ml), ejecting into a watch glass and attempting to pick up the marrow granules. Since many pigs had to be sacrificed to allow of pathological examination, iron estimations and bone-marrow sections it was found easier to make smears at this time. All sections and smears were taken within 30 minutes of death.

Bone-marrow sections were fixed in Helly's fluid and stained with Leishmans (Whitby & Britton 1957), which was the stain generally used for blood and marrow smears and sections. Care was taken to check each new bottle of stain to ensure correct staining. Supravital staining for the reticulocyte counts were made by mixing one drop of blood with one drop of brilliant cresyl-blue stain on a slide. After five minutes in a moist atmosphere the mixture was smeared on a slide and when dry counterstained with Leishman, taking care not to allow longer then 30 seconds with the undiluted stain.

Bone-marrow and other tissues were stained by the following method for iron. Slides were fixed in methyl alcohol, washed and dried. Potassium ferrocyanide (10%) in distilled water, made up fresh using heat (< 56 °C) to dissolve the crystals, and 10% hydrochloric acid were mixed in equal amounts and poured on the slide. After twenty-five minutes the slide was washed in

running water for 30 - 60 minutes and then counterstained with dilute ($^1/10$) carbol fuschin for up to 15 seconds. The method was based on that described by Douglas and Dacie (1953).

Estimations of the iron contents of sera were based on the technique of Bothwell and Mallett (1955). This method was adapted for estimating the iron content of tissues. Approximately 2 gm of fresh material was digested with 1 ml sulphuric acid, 2 ml perchloric acid and 4 ml. nitric acid. When the digest was colourless it was diluted with water and transferred to a volumetric flask and further diluted to the 100 ml mark with water. Five millilitres of this was then taken and the technique for serum iron estimations followed, using thioglycolic acid and 4 % 2 : 2' dipyridyl.

Pig Husbandry Methods.

The young pig under suitable conditions of nutrition and environment, gains weight rapidly, and within three weeks of birth should have quadrupled its birth weight of between 2 and 3 lbs. As Bellis (1957) has pointed out such rapid growth is almost unique and makes exacting demands on nutrition and environment and inevitably presents many problems in artificial rearing. Yet artificial methods of rearing piglets have allowed investigation into problems in the laboratory which would have been impossible otherwise (M^c Crea and Tribe 1954). Early attempts to rear piglets artificially were unsuccessful since the pigs developed diarrhoea and died. (Bustad, Ham and Cunha 1948). In the last few years however the successful rearing of pigs removed from the sow shortly after birth has been reported (Becker et al 1954; M^c Crea and

Tribe 1954; Tribe 1954) and piglets have even been reared after hysterectomy

Tribe 1954; Tribe 1954) and piglets have even been reared after hysterectomy

(Young et al 1955) and after aseptic delivery (Done 1955).

It seemed that for the study of nutritional problems and in this instance iron-deficiency in piglets the use of artificial diets and semi-synthetic methods of rearing were useful experimental tools. The value of artificial over normal methods were thus:-

1. The husbandry was under a greater degree of control.
2. Blood sampling and other manipulations were executed more readily.
3. The amount of iron ingested could be estimated, and extraneous sources of iron eliminated.
4. The possibility of the piglets harbouring parasites or carrying infections was reduced or elimated.

The diet used in these experiments and husbandry methods adopted were based on those described by Tribe (1954) and Mc Crea and Tribe (1954: 1956).

The composition of the diet was :-

Lactose	20%	Sucrose	20%
Maize Starch	22%	Casein	20%
Dried Whole Milk	10%	Dried Yeast Powder	3%
Mineral Mixture	5%	* Aureomycin Feed Supplement	0.25%

ǂ Vitamin A .....200,000 I.U.per 100 lbs.

ǂ " D 3..... 50,000 " " " "

" E .....250 mgm " " "

* Aurofac 2 A (Lederle Labs.) containing 3.6 gm. aureomycin hydrochloride per lb.

ǂ A stable mixture of vitamins A & D_3 in powder form.

The casein used was unextracted lactic casein (Glaxo Labs. Ltd.,) with the approximate composition; moisture 10%; fat 2.1%; nitrogen 13.5% and calcium 0.04%.

Mc Collum's salt mixture number 185 was used as the mineral mixture. It was supplied with and without iron by The British Drug Houses Ltd., who gave the following composition:-

	With Iron.	Without Iron.
Ferric citrate	3.2%	-
Sod. chloride	4.5%	4.6%
Mag. sulphate exsicc	11.0%	11.4%
Sod.dihydrogen orthophosphate	9.0%	9.3%
di-Potassium hydrogen orthophosphate (K_2HPO_4).	24.7%	25.5%
Calcium hydrogen orthophosphate ($CaHPO_4$).....	14.0%	14.4%
Calcium lactate	33.6%	34.8%

The diet was made up in bulk mixed in quantities of about five cwt. for each formula. The respective iron contents on analysis of the diets for each experiment are given in Table 2. Since the initial contents of iron seemed rather high an analysis was made of the ingredients and the following amounts of iron noted :-

lactose 2.0	mg/100 gm.	yeast	8.1 mg/100 gm.
sucrose 1.25	" " "	aureomycin feed supp.	32.0 " " "
casein 4.0	" " "	vit. A & D_3 mixture	4.5 " " "

To reduce the iron content of the diet the aureomycin feed supplement was excluded from some experiments. ~~and while~~ This may have allowed a greater degree of iron-deficiency to arise, but probably influenced unfavourably the ability of the piglets to withstand enteric infections particularly since young piglets have difficulty in utilising sucrose. ~~and~~ The frequency with which they developed diarrhoea when fed sugar has been stressed (Johnson 1949; Becker et al 1954). It was found necessary also to obtain a supply of dried milk to which iron had not been added before drying.

The regimen adopted in rearing the piglets was to allow them to suck the sow for at least 24 hours. After removal from the sow the piglets were placed in the cages, in a warm environment and allowed no food or water for about six hours. In order to simplify the feeding it is important to encourage the piglets to eat solid food as soon as possible. Thus after the initial fast they were allowed in separate dishes, the dry diet, diet mixed to a thin paste with cold cows' milk, and water. After three to four days the paste was made with water and the mixture thickened. After about seven days the dry diet and water only were allowed. Both the diet and water were allowed ad libitum.

The water used was Glasgow tap-water and since there was a possibility of this containing an appreciable quantity of iron an enquiry was made at the Corporation of Glasgow, Chemist's and City Analyst's Department. The composition of samples drawn at the above department was as given as follows:-

Mineral Composition of Loch Katrine & Gorbals waters, Dec. 1954.

Results expressed in parts per million.

Hardness (E.D.T.A.Method).	Loch Katrine Water.	Gorbals Water.
Calcium Hardness ($CaCO_3$)	6.4	35.5
Magnesium " ($CaCO_3$)	2.5	9.5
Total " ($CaCO_3$)	8.9	45.0
Calcium (Ca)	2.40	14.90
Magnesium (Mg)	0.79	2.63
Iron (Fe)	0.03	0.08
Sodium (Na)	4.24	7.98
Manganese (Mn)	0.007	0.002
Aluminium (Al)	0.03	0.02
Silica (SiO_2)	0.30	3.20
Sulphate (SO_4)	4.70	14.87
Phosphate (PO_4)	0.007	0.06
Chloride (Cl)	7.00	11.50
Fluoride (F)	0.01	0.05
Total Alkalinity to Methyl Orange ($CaCO_3$)	5.5	32.5
Free Carbon Dioxide (CO_2)	2.7	3.0

Piglets were housed usually in groups of 2 - 4 in galvanised metal metabolic cages. The size of these cages was about 2' 8" x 2' 8" x 3' high. Three sides and the roof were of galvanised sheeting with a heavy mesh door occupying one side. The floor was also of heavy gauge wire mesh with a tray in the shape of an inverted cone beneath. The whole cage was mounted on legs. Feeding troughs were of earthenware. Initially both the control and experimental groups were allowed these dishes but because of the cost and the high rate of destruction metal dishes were used later for the piglets allowed iron.

The cages containing the pigs were housed in a 12' x 12' loose-box from

which all draughts were excluded. The environmental temperature was maintained around 60 - 70°F by a convector heater and in each cage an infra-red lamp. The pigs thus were kept warm and dry even though they upset their water. Thermographic records were taken regularly.

In the experiments on pigs reared naturally or included in the farm surveys "sow and weaner" meal was fed to the sows and sometimes to the piglets. Samples of one brand of this meal gave an iron content (expressed as Fe) for the Rearing Diet as 6.75 mg/100 gm. and for the Maintenance Diet as 4.35 mg/100 gm. In some experiments an early weaning diet * was supplied to the pigs. The stated total amount of active ferrous iron expressed as metallic iron was not less than 100 mg per lb.

A general analysis of four samples of the diet used in the first experiment gave the following composition; moisture 6%, ash 4.01%, crude protein 20.32%, ether extract 2.30%, n-free extractives 67.10%.

TABLE 2. Iron and Copper analyses of the Semi-Synthetic Diets.

Experiment.	Iron (Fe) & Copper (Cu) contents of Diets (mg. per 100 gm)			
	Control Groups		Experimental Groups	
	Fe	Cu	Fe	Cu
1	20.0	0.37	3.40	0.35
2	27.28	0.84	4.48 & 2.82	0.65
3	-	-	2.81	0.65
4	27.01	0.87	2.42	0.60
5	-	-	3.65	-

* Anvilac No.2. (Glaxo Lab. Ltd.,)

ARTIFICIAL REARING EXPERIMENT 1.

INTRODUCTION.

This was the first of a series of five experiments in which artificial methods of rearing piglets were used as a means of producing and studying the anaemia which resulted from deficiency of iron, and was started in January 1955.

The husbandry methods, diets and environment, have been described in the previous sections. Any details particularly relevant to this experiment or differing from those outlines, have been included at the appropriate point.

As this was the initial rearing experiment using semi-synthetic diets to rear pigs artificially, some difficulty with the method was expected. It was anticipated, however, from a perusal of the literature, that, under the conditions of the experiment, anaemia would be readily produced, with its attending group of clinical symptoms, resulting in death.

The piglets used in this experiment were farrowed by one sow (3587) and one first-litter gilt (G1).

The sow had been on the premises of the Veterinary Hospital for seven months prior to bearing this, her second, litter. She had been kept either indoors on concrete , in a pen with two brick and two galvanised tubular metal walls, or outside in a wooden pig ark fitted with a concrete run surrounded by painted metal railings, and fed on a commercial compound ration.

Of the thirteen piglets born only four lived longer than 12 hours post-partum. A catastrophy which could reasonably be attributed to chilling, to which recently born piglets are highly susceptible (Reid, 1954), since a sudden drop

in the ambient temperature accompanied by a snowfall coincided with farrowing.

The husbandry of the gilt prior to farrowing was unknown. She produced a small litter, seven days after purchase of which only three survived.

Both litters were allowed to suckle for 72 hours before being placed on experiment. The relevent data pertaining to each pig are given in the following table.

TABLE 1 A. Data Concerning Allocations of Piglets In Experiment 1.

Piglet No.	Weight (lbs)	Sex	Dam	Group Allocated	Iron Content of Diet mg/100 gm.
1	2⅜	M	3587		
2	2⅛	F	"	IRON	20.2
5	2⅜	M	01	CONTROL	
6	3	F	01		
3	2⅜	M	3587		
4	3	F	"	NON - IRON	3.4
7	2⅛	F	01	EXPERIMENTAL	

Piglet 2 died two days after weaning, and has been deleted from the experiment, resulting in the number being reduced to 3 per group.

Each piglet was bled twice a week usually. Difficulty was experienced initially in evolving a satisfactory technique for obtaining the quantity of blood necessary for a full haematological examination. Some of the earlier

results were probably affected by this, though the adoption of the technique of bleeding from the anterior vena-cava almost eliminated the problem. As a routine about 2.5 ml. of blood was withdrawn, though when serum-iron estimations were required about 10 ml. was necessary. These quantities would appear to be so small as not to interfere with normal erythropoiesis.

In this and subsequent experiments using artificial methods of rearing all data are presented according to the age of the piglet, which corresponds to the period of time on the experimental diet, plus the initial period suckled. The duration of the experiment was 10 weeks.

HAEMATOLOGICAL RESULTS.

The data obtained on the various blood parameters are presented separately.

Haemoglobin.

The Hb values which were obtained for each of the piglets during the ten weeks of the experiment are given in the accompanying table (1B).

The mean values and standard deviations (S.D.) for each group on each week of the experiment as calculated from the individual readings are given, together with the standard error(S.E.) of the means in Table 1C. It can be seen that the means for the two groups approximated closely during the first three weeks of the experiment. Provided bias was not exercised in the selection of the piglets and until the effect of the difference in the content of iron in the two diets became apparent, these results would be expected. By the fourth week, however, the experimental group showed a marked fall in the Hb level, amounting to about 3 gm. per 100 ml. and the reduction continued until it reached the lowest point at the sixth week. Thereafter a slight rise became evident.

TABLE 1 B. Hb. attributes of pigs in gm. per 100 ml.

Week	CONTROL GROUP			EXPERIMENTAL GROUP		
	PIGLET NUMBER					
	1	5	6	3	4	7
1	9.0 -	12.6 -	13.6 -	9.2 -	11.0 -	13.6 -
2	13.9 12.6	13.9 13.6	13.6 14.2	12.2 10.5	14.4 13.6	13.6 13.5
3	12.4 12.6	12.7 13.5	11.2 13.1	12.4 10.5	14.6 13.8	12.4 10.8
4	12.4 13.4	12.7 12.0	13.9 11.6	10.9 7.9	10.2 9.2	9.4 10.8
5	11.6 -	13.5 11.6	11.6 12.4	7.9 7.4	9.0 8.5	8.2 -
6	11.6 12.4	11.2 14.4	10.5 11.0	6.4 -	7.1 -	9.4 8.8
7	12.0 11.3	11.6 11.3	14.4 13.0	7.2 8.6	8.0 8.6	9.5 9.5
8	11.5 12.0	13.6 11.8	14.1 15.1	7.7 8.0	8.8 8.8	9.0 7.4
9	13.6 12.8	13.1 -	13.0 -	8.2 7.8	9.2 9.0	9.5 -
10	12.6 -	13.4 -	13.0 -	8.8 -	9.3 -	- -

TABLE 1 C. Group Mean Hb values for each week.

Week	Control Group		Experimental Group	
	Mean	S.E.	Mean	S.E.
1	11.73 ± 2.42	± 1.30	11.27 ± 2.21	± 1.27
2	13.60 ± 0.55	± 0.22	12.95 ± 1.40	± 0.57
3	12.56 ± 0.78	± 0.31	12.41 ± 1.60	± 0.65
4	12.63 ± 0.86	± 0.35	9.73 ± 1.14	± 0.46
5	12.12 ± 0.84	± 0.37	8.18 ± 0.60	± 0.27
6	11.83 ± 1.40	± 0.57	7.92 ± 1.41	± 0.70
7	12.23 ± 1.22	± 0.50	8.56 ± 0.90	± 0.37
8	13.0 ± 1.46	± 0.60	8.26 ± 0.67	± 0.27
9	13.12 ± 0.34	± 0.17	8.74 ± 0.71	± 0.32
10	13.0 ± 0.40	± 0.23	9.05 ± 0.36	± 0.25

These variations can be appreciated readily by referring to Fig.1A. It will be observed that only relatively minor fluctuations in the Hb. level of the control group occurred during the ten weeks of the experiment, and the calculated mean for all the readings obtained for the pigs comprising this group, over the whole experimental period was:

$$12.61 \pm 1.17 \text{ gm. per 100 ml. blood.}$$

The lowest point reached by the mean Hb. level for the three piglets on the iron - poor diet occurred at the 6th week, when the mean value was :

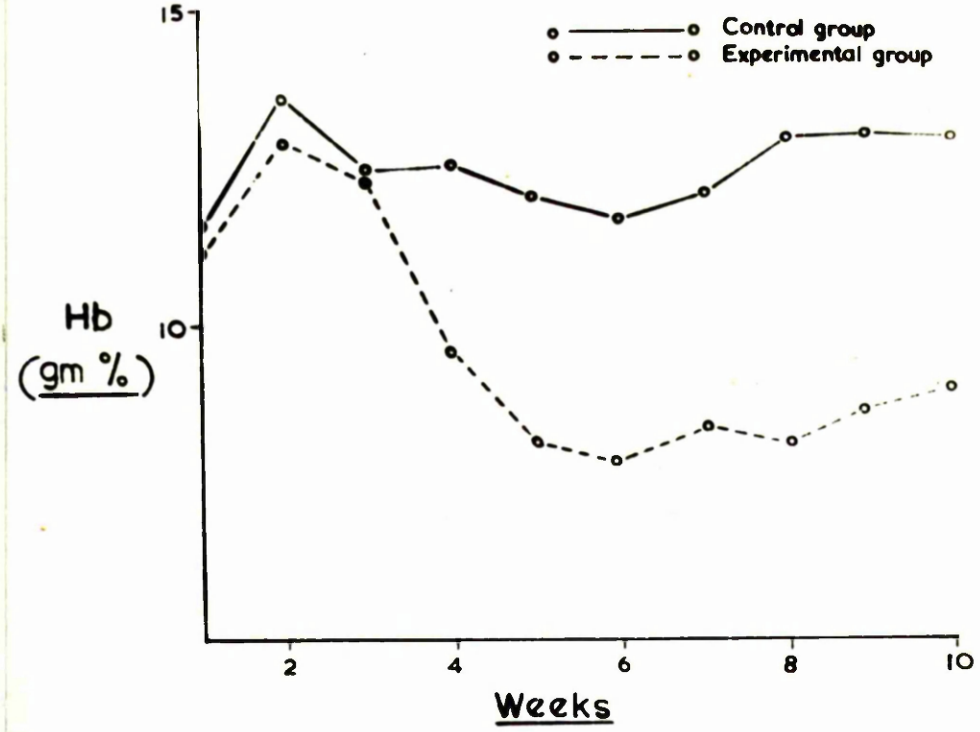
$$7.92 \pm 1.41 \text{ gm. per 100 ml. blood}$$

In order to compare these figures it is useful first to test the variances obtained for these two means to find if they can be pooled. Comparing the variances the greater being the numerator the value 1.44 was obtained which for the appropriate degrees of freedom 34 & 50 at the 5% level shows no significance (Fisher & Yates 1948). Further analysis of the data can now be made as indicated.

Applying the ratio 't' in comparing the samples the resultant is clearly outside the 0.01 level of probability and thus the difference between the level of Hb. for the control group over the duration of the experiment and that for the experimental group at the fifth week was highly significant. For the interpretation of significance the terminology suggested by Finney (1953) has been adopted.

It is informative to analyse the figures for the experimental group at other weeks comparing them with that for the control group as before.

FIG 1A



Week 1 $P > 0.1$ Week 4 $P < 0.01$ Week 6 $P < 0.01$

" 3 $P > 0.1$ " 5 "

Only after the third week did the two sample means differ significantly.

When the mean value for the control group at the fifth week only, was tested against that for the experimental group, the result showed that the probability of such a result occurring by chance was less than 1%.

The method used to obtain the Hb values recorded can be tested by calculating the variance (F) ratio from, A the variance between the sample means and, B the variance within the samples.

The sum of the squares of deviation (S_2) for A, from which the variance (σ_2^2) may be obtained can be calculated from the formula:-

$$S_2 = \frac{t_1^2}{n_1} + \frac{t_2^2}{n_2} + \frac{t_3^2}{n_3} - \frac{T^2}{N}$$

where t = the sum of the Hb values for the individual pigs.

T = " " " " " " " all the pigs of the group.

N & n = appropriate degrees of freedom

B canbe obtained by subtracting S_2 from the total sum of squares (S_1)

i.e. $S_1 - S_2 = S_3$

& the variance $\sigma_3^2 = \dfrac{S_3}{n - 1 - (\text{no. of pigs} - 1)}$

S_1 canbe calculated thus -

$$S_1 = \sum_1 x^2 - \frac{T}{N}$$

where $\sum_1 x^2$ = the sum of squares of all the values.

Dividing the variances obtained for A & B, the greater being the numerator,

gives the F ratio. Calculation of the F ratio for the Hb values for the control group at the fifth week gives a product which is smaller than the value given at the 5% level of significance.

While it can be concluded from this analysis of the method that it appeared to be completely acceptable further weight may be added to this reasoning by similar analysis of the results obtained for the experimental group at the same week. This too gives a variance ratio which is within the 0.05 significance limit, for the appropriate degrees of freedom.

It is now clearly evident that the methods employed have been justified and that the differences in the Hb. values while without significance for the first 3 weeks of the experiment, were highly significant thereafter. A result which can be reasonably explained on the basis of depletion of the reserves of iron in the group which received no additional iron.

Haematocrit values (P.C.V).

The following tables (Tables 1D & 1E) summarise the results of the haematocrit readings.

TABLE 1D. The mean Haematocrit values (ml.per 100 ml. blood) for each pig on each week.

Week	CONTROL GROUP			EXPERIMENTAL GROUP		
	PIGLET NUMBER					
	1	5	6	3	4	7
1	30	40	39	26	-	30
2	-	37	39.2	29.5	41	-
3	29.5	36.7	35.2	39	-	29.5
4	36.5	33.5	35.5	26.5	29.2	27.5
5	36	37.5	39	22.7	23.2	30.5
6	40	35	38.2	19	28.5	31
7	33	36.5	43.5	31	32	28.5
8	38	38	44.5	27.5	31.	27
9	40	40.5	41	29.2	32.2	34
10	40	41	41	35	36	-
11	40	-	-	-	-	-

From these values the group mean figures and the standard deviations have been obtained (Table 1 E).

TABLE 1 E. Group Mean Haematocrit values (ml. per 100 ml.) for each week.

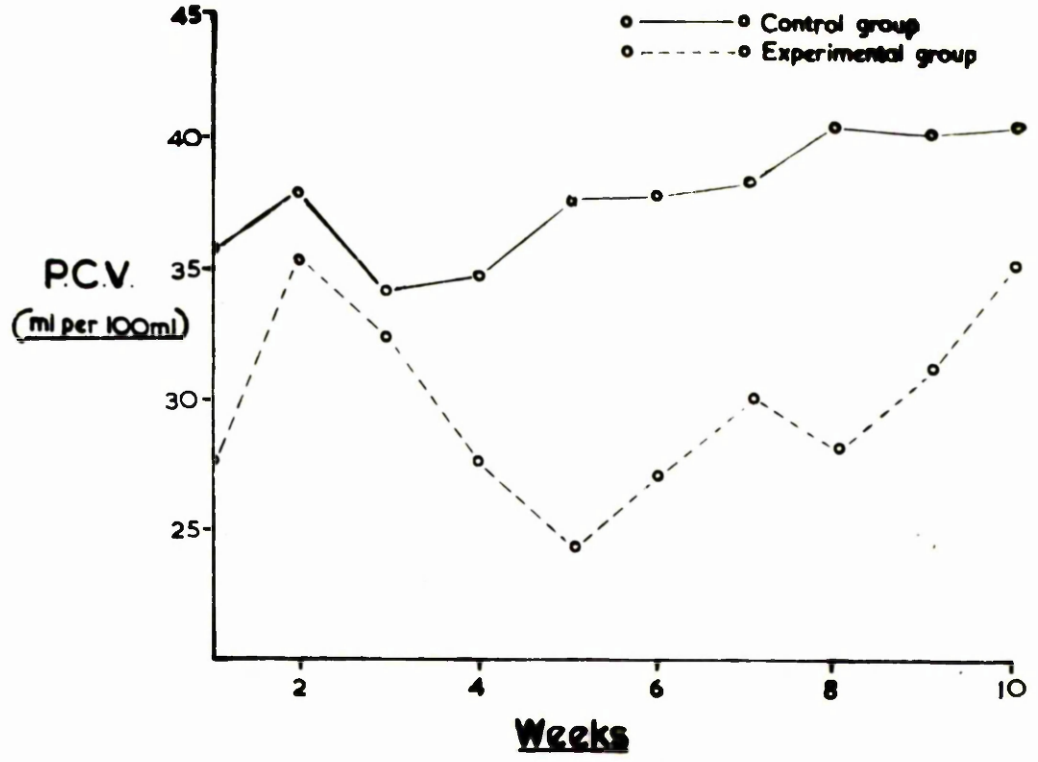
WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
1	36.33 ± 5.5	28.0 ± 2.82
2	38.10 ± 1.27	35.25 ± 8.15
3	34.70 ± 2.95	32.66 ± 5.48
4	34.90 ± 1.35	27.98 ± 1.19
5	37.80 ± 1.40	24.46 ± 3.38
6	38.28 ± 2.04	27.37 ± 5.70
7	38.60 ± 4.70	30.50 ± 1.61
8	40.60 ± 3.60	28.50 ± 1.95
9	40.37 ± 0.94	31.36 ± 4.40
10	40.66 ± 0.58	35.50 ± 0.708

From Table 1E it can be seen that the volumes of packed red cells for the pigs in the experimental and control groups did not differ markedly until the fourth week. An analysis calculated for this week gave a value for 't' which showed that the difference between the two samples was highly significant ($P < 0.01$).

Graphing the results gives a ready method of appreciating the divergence of the two sample means. (Fig. 1 B).

Three points relating to the haematocrit readings must be the subject of comment. First it should be pointed out that as this was the first experiment, certain techniques which at the outset seemed most useful later had to be

FIG Ib


Control group
Experimental group
45
40
35
30
25
P.C.V.
(ml per 100ml)
2
4
6
8
10
Weeks

abandoned or altered. Thus in this experiment haematocrit readings were made initially with capillary haematocrit tubes. These, however, proved awkward mainly because some blood not infrequently escaped despite all precautions. This technique was therefore replaced by one using Wintrobe's haematocrit tubes as has been described (vide - Haematological Methods). The lower average readings for both groups at the start of the experiment may have resulted in part from the use of the capillary method.

Second it is noteworthy that the drop which started in the haematocrit values of the experimental group by the third week continued till the fifth week and resembled that noted for the Hb levels but not that for the R.B.C's as will be seen.

Finally the rise in the haematocrit indices from the lowest point at week five also seems to be linked more closely with the Hb levels than with the R.B.C's. Further discussion will be given to these points.

Erythrocyte counts.

The first table (Table 1F) presents the erythrocyte counts for each pig on each week of the experiment, as a single value or as the mean of two to three counts during each week from which the group mean values have been calculated. It was usual, as has been indicated earlier, to bleed the pigs at the start and towards the end of each week. The mean group counts are given in Table 1 G.

TABLE 1 F Erythrocyte counts for each pig (R.B.C's x 10^6 per c.mm.)

Week	CONTROL GROUP			EXPERIMENTAL GROUP		
	PIGLET NUMBER.					
	1	5	6	3	4	7
1	3.8	6.45	6.1	2.7	4.5	4.6
2	5.4	6.85	6.1	4.7	5.46	6.4
3	5.4	5.5	5.15	5.8	7.0	6.15
4	7.0	6.0	5.6	5.65	6.5	5.9
5	6.2	5.55	5.65	5.45	5.75	5.6
6	6.35	5.8	5.4	4.8	8.2	5.35
7	6.15	5.45	6.45	6.15	7.35	5.5
8	6.4	5.82	7.25	6.2	6.4	5.45
9	7.85	5.9	6.7	6.7	7.0	6.6
10	6.8	6.4	5.9	6.8	7.2	-

TABLE 1 G. Mean Erythrocyte Counts for each group on each week.

(R.B.C's x 10^6 per c.mm).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
1	5.75 ± 1.11	4.1 ± 0.93
2	6.01 ± 0.65	5.41 ± 0.70
3	5.35 ± 0.16	6.45 ± 0.55
4	6.20 ± 0.64	6.01 ± 0.39
5	5.72 ± 0.27	5.6 ± 0.15
6	5.85 ± 0.42	5.92 ± 1.54
7	6.01 ± 0.46	6.33 ± 0.84
8	6.49 ± 0.64	6.01 ± 0.45
9	7.07 ± 0.95	6.80 ± 0.18
10	6.36 ± 0.45	7.00 ± 0.28

By inspection it is obvious that a significant difference in R.B.C's at any one week did not exist either among the pigs composing either group or between the two groups. This observation while perhaps surprising was of interest since it has been shown that the difference between the Hb levels of the two groups was highly significant, and will be commented on in the discussion.

Red Cell Indices.

The Mean Corpuscular Volume (M.C.V).

TABLE 1 H. Mean values for the M.C.V of control and experimental groups on each week (μ^3).

Week	Control Group	Experimental Group
1	69.25 ± 13.5	86.60 ± 13.7
2	65.56 ± 4.61	58.30 ± 6.86
3	64.72 ± 10.67	48.15 ± 2.61
4	60.16 ± 6.62	45.52 ± 2.41
5	66.32 ± 5.83	44.10 ± 7.96
6	65.68 ± 6.00	47.55 ± 12.15
7	64.94 ± 5.28	47.73 ± 5.10
8	62.42 ± 3.92	47.76 ± 4.86
9	58.10 ± 8.82	46.52 ± 6.15
10	64.86 ± 6.54	50.75 ± 1.06

Though it is acknowledged that this calculation is inherently inaccurate due to the variation which can occur in the red cell count, (Wintrobe 1956; Briggs and Macmillan 1948), the additional difficulty experienced with capillary haematocrit tubes may have been responsible in part for the wide variation in the first indices. Despite this the difference between the means of the two groups tested at the fifth week was significant, ($P<0.05>0.01$).

Mean Corpuscular Haemoglobin Concentration. (M.C.H.C.).

The M.C.H.C. mean indices for the two groups are given in table 1 I. These figures were obtained by taking the mean of the individual results.

TABLE 1 I M.C.H.C. (%) : Mean values for each group on each week.

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
2	35.5 ± 0.60	34.35 ± 1.76
3	35.64 ± 2.91	36.73 ± 5.96
4	36.22 ± 4.09	36.12 ± 5.37
5	32.12 ± 2.34	33.94 ± 5.11
6	31.52 ± 5.63	29.32 ± 3.67
7	32.32 ± 3.01	28.25 ± 3.96
8	32.48 ± 2.12	29.11 ± 1.37
9	32.42 ± 1.11	27.80 ± 1.86
10	33.30 ± 2.98	25.45 ± 0.35

The similarity between the group means which is obvious from the figures quoted above gave a t value which was without significance at the 5 th week ($P > 0,05$) and will be commented on in the discussion.

The Mean Corpuscular Haemoglobin (M.C.H.).

Table 1 J gives the group mean M.C.H. indices as obtained from the individual results for Hb and R.B.C's, expressed as micromicrograms ($\gamma\gamma$).

TABLE 1 J. Group Mean M.C.H. Indices ($\gamma\gamma$).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
1	21.93 ± 1.64	28.0 ± 5.22
2	22.83 ± 3.44	25.98 ± 5.85
3	23.53 ± 1.56	19.34 ± 1.55
4	20.56 ± 2.72	16.20 ± 2.76
5	21.22 ± 1.58	14.74 ± 2.04
6	20.0 ± 2.50	13.68 ± 3.66
7	20.43 ± 2.17	14.16 ± 2.91
8	20.03 ± 1.23	13.81 ± 3.68
9	18.85 ± 2.74	12.86 ± 1.41
10	20.46 ± 1.78	12.90 ± 0.0

The probability of the two sample means, tested at the fifth week only, arising from the same population sample was less than 1%. The M.C.H's for the controls appeared to remain around 20 $\gamma\gamma$, and index lower than the normal value for men (Wintrobe 1956) - whereas the experimental group indices dropped steadily till they reached a figure which would be consistent with severe hypochromic anaemia in men. (Wintrobe 1956).

Reticulocyte Counts.

TABLE 1 K. Group Mean Reticulocyte counts (% of erythrocytes).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
1	1.20 ± 0.67	6
2	2.70 ± 1.81	1.65 ± 1.48
3	4.70 ± 2.94	6.85 ± 2.75
4	6.50 ± 1.37	4.83 ± 0.38
5	4.70 ± 1.27	7.40 ± 2.96
6	2.35 ± 0.87	7.43 ± 2.74
7	3.76 ± 1.54	3.80 ± 0.60
8	4.50 ± 0.79	3.46 ± 0.24
9	3.56 ± 0.57	3.20 ± 0.91
10	3.8 *	2.85 ± 0.36

* One count only made.

By inspection there would appear to be no significant difference between the mean counts of the two groups at any week of the experiment. The mean count over the period was 3.7% for the controls and 4.8% for the experimental group. The difference between the group means at week 6 was not significant, ($P > 0.05$). Reticulocytes are present in normal numbers in iron deficiency in man (Whitby & Britton 1957).

Cell Morphology.

In the first two weeks of the experiment, smears of the blood from all pigs showed the red cells to be of even size and staining with a variable number of larger basophilic cells present and an occasional normoblast. No significant alteration in the cell morphology was noted in the control group at later weeks. In contrast those of experimental pigs 3 and 4 showed noticeable anisocytosis with numerous microcytes, almost no poikilocytosis but marked ring staining ("pessary forms") with only some of the cells fully haemoglobinised. The cells of piglet 7 showed almost no alteration until about the eighth week when pessary forms became more frequent.

Normoblasts and Howell - Jolly bodies were observed in many smears though no special frequency for these nuclear remnants or early forms was noticed.

Serum - Iron Estimations.

Few serum - iron estimations were attempted in this experiment. It was considered at this stage that to remove 20 ml. of blood at intervals might exacerbate or alter the characteristics of the anaemia. A view which would appear to be incorrect considering the blood volume of piglets. Later it was found that as little as 8 - 10 ml. of whole blood usually allowed two separate estimates of the serum - iron level.

The results are given in the accompanying table (1 L).

Though the results would appear to be too few to allow significant conclusions to be drawn it is of interest to note that the lowest figure recorded by an individual in the control group was 95 μ g per 100 ml. blood while the highest figure for one of the other group was 82.5 μ.g. and this

before the influence of the diet could have lowered the serum iron. It might seem that a rough line could be drawn about the level of 90 - 100 μ g. below which iron deficiency might be indicated. The mean S.D. and S.E. of all the serum-iron values for each group, after the first week were; -

Control Group 265.0 ± 133.00 (S.E. ± 77.34)

Experimental Group 65.41 ± 13.35 (S.E. ± 5.47)

The high serum-iron levels recorded by piglets 1 at week 7, and 6 at week 6, were thought to have been due possibly to contamination by the glass syringes boiled in tap water, or dirt on the skin of the animals, and stricter measures to avoid this were taken in future experiments.

TABLE 1 L. Serum - Iron Estimations (μ g per 100 ml. blood).

Week	Piglet Number.					
	1	5	6	3	4	7
1	95	-	-	82.5	-	-
3	162.5	-	-	77.5	-	-
6	-	150	317.5	45.0	-	72.5
7	430	-	-	75.0	-	-
10	-	-	-	52.5	70	-

Measurement of Red Cell Diameters.

The techniques used to obtain the measurement of red cell diameters have been described in the chapter on methods, and were based on that described by Price - Jones (1933). In this experiment measurement of the cells from a negative photographic plate was used to obtain the values used in drawing the accompanying graphs, and in calculation the percentage of microcytosis. Measurements were obtained for pigs 1 (Control) and 3 (Experimental) at the 2nd, 5th, 6th and 8th weeks.

Figure 1 C gives the curves of red-cell distribution for control piglet 1, and Figure 1 D gives similar curves for experimental piglet 3 at the same weeks as those given for pig 1.

Superimposing the important area of Figure 1 D on 1 E illustrates the extent of the microcytosis which occurred in piglet 3 at the sixth week of the experiment. It can be seen from the graph Figure 1 E that from about the class - interval 6 μ the curve for the experiment piglet differs from that of control and the difference between the left side of the curves delineates the extent of microcytosis enhibited by the experimental piglet.

The extent of the microcytosis was calculated, and is given for weeks 6 & 8, in Table 1 M.

FIG. Ic

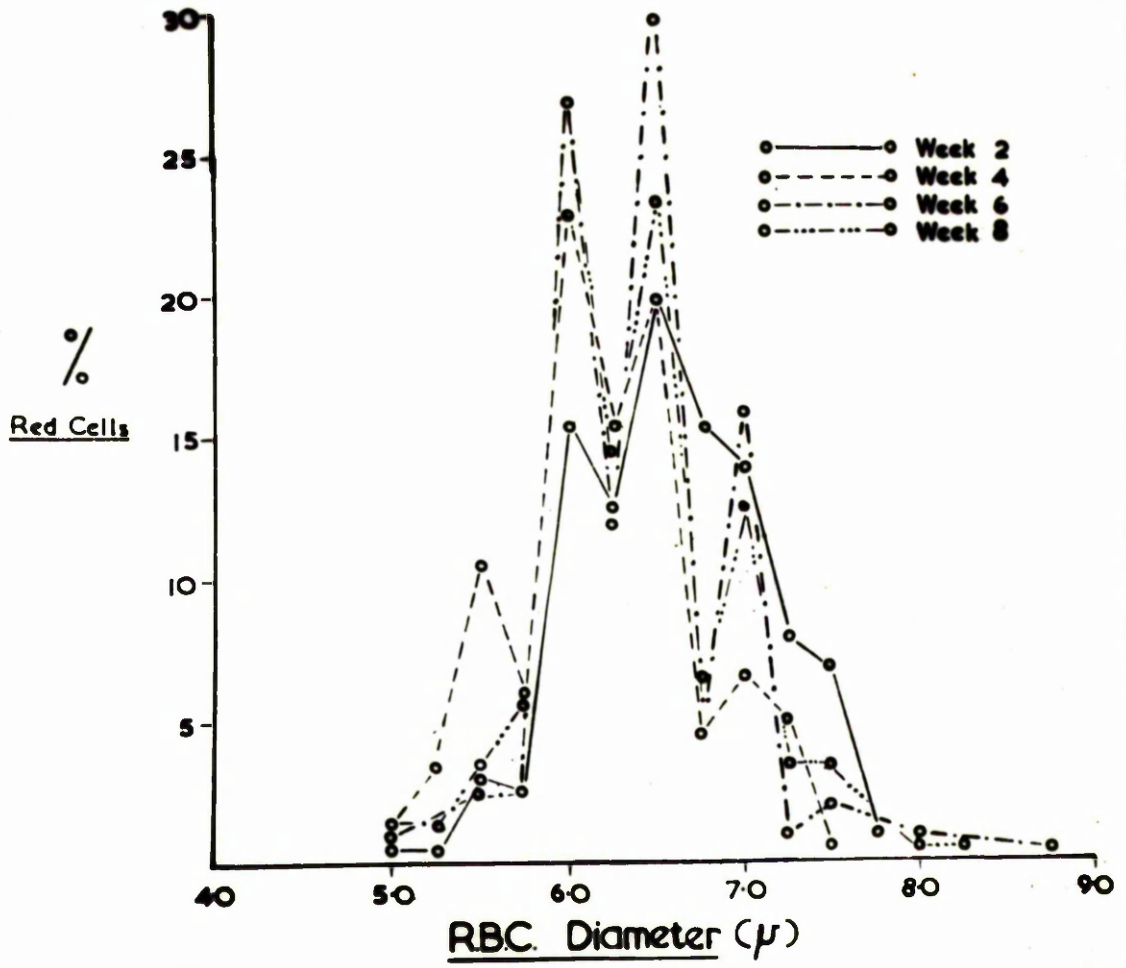

30
25
20
15
10
5
%
Red Cells
Week 2
Week 4
Week 6
Week 8
4·0
5·0
6·0
7·0
8·0
9·0
RBC. Diameter (μ)

FIG. 1D

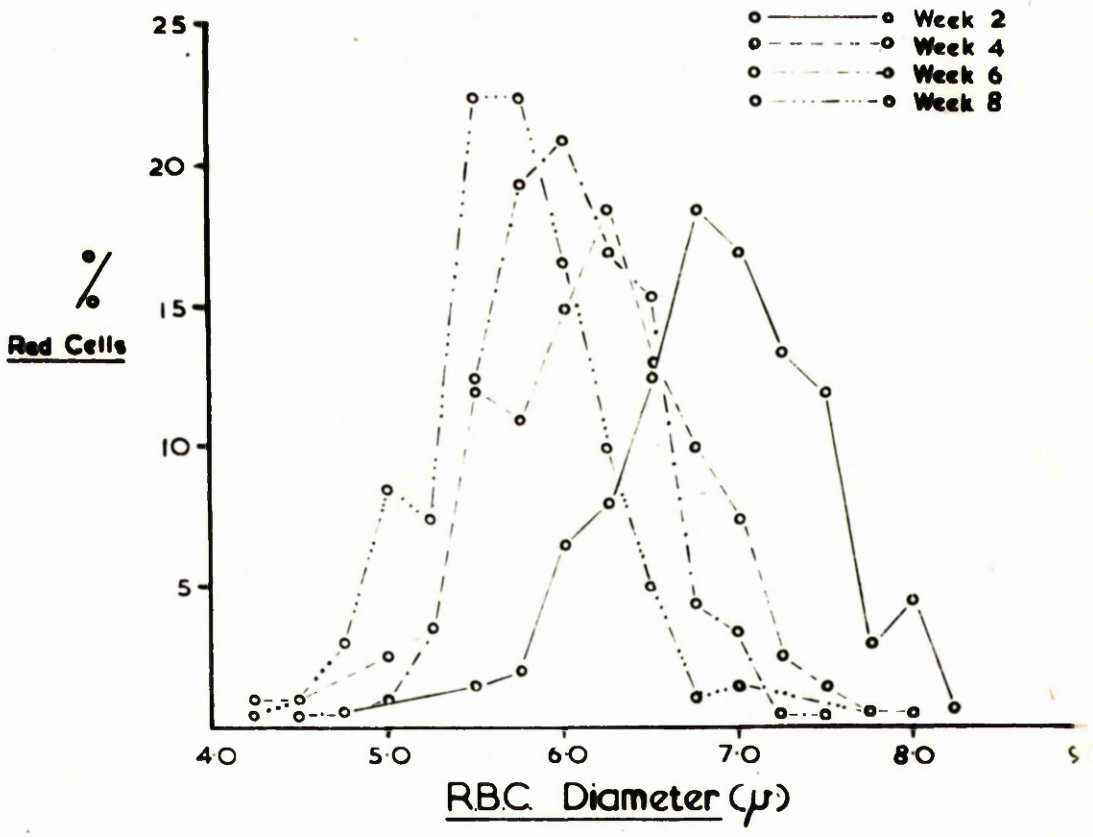

Week 2
Week 4
Week 6
Week 8
25
20
15
10
5
%
Red Cells
4·0
5·0
6·0
7·0
8·0
RBC Diameter (μ)

FIG. 1E

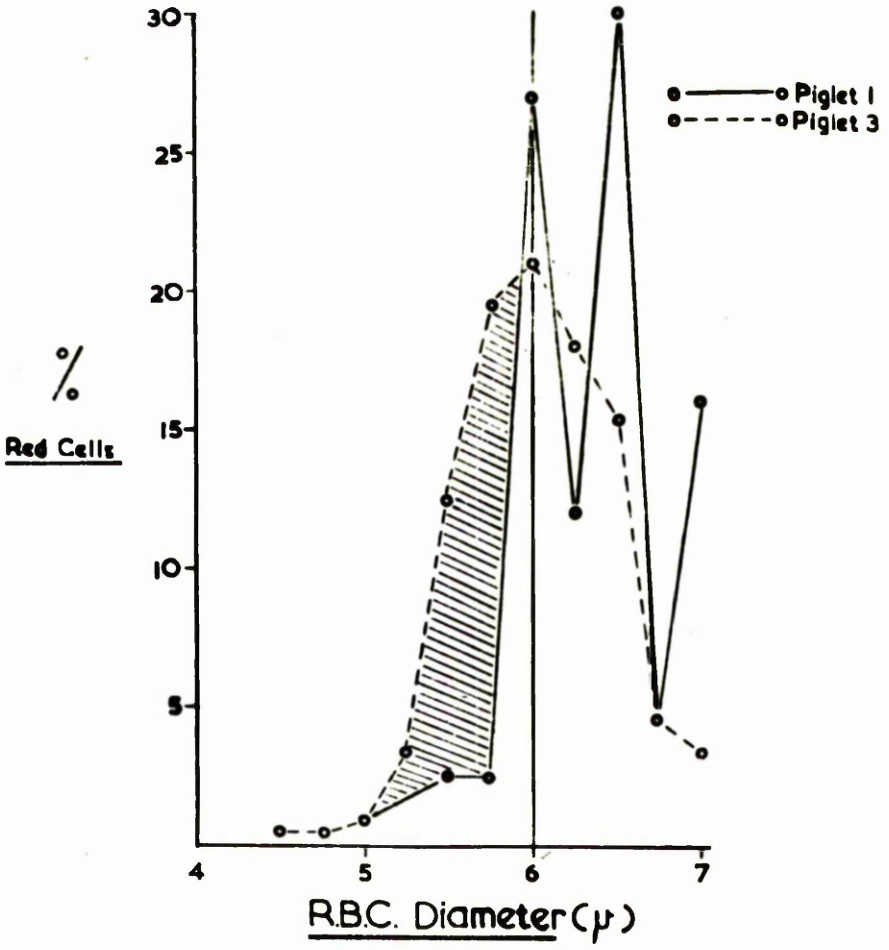


TABLE 1 M. Percentage of Microcytosis.

MID-POINT OF CLASS INTERVALS.	CONTROL PIGLET NO.1		EXPERIMENTAL PIGLET NO.3	
(0.25 μ)	% cells. Week 6	Week 8	% cells Week 6	Week 8
6.0	27.0	23.0	21.0	16.5
5.75	2.5	5.5	19.5	22.5
5.5	2.5	3.5	12.5	22.5
5.25	0	1.5	3.5	7.5
5.0	1.0	1.5	1.0	8.5
4.75	0	0	0.5	3.0
4.5	0	0	0.5	1.0
4.25	0	0	0	0.5
4.0	0	0	0	0
TOTAL	33.0	35.0	58.5	82.0

Percentage of microcytosis :-

Week 6 $\quad$ 58.5 - 33.0 = 25.5%

" 8 $\quad$ 82.0 - 35.0 = 47%

The amount of microcytosis exhibited by the cells from the experimental piglet was considerable and the distribution curves illustrate this alteration.

White Cell Count.

Total and differential white cell counts were made on several occasions. The accompanying tables (1H and 1 O) record the group mean and S.D. figures for these parameters.

Inspection of the figures in these tables did not reveal any significant features.

TABLE 1 H. Group Mean Total White Cell Counts.

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
1	7.40 ± 1.90	7.50 ± 2.26
2	10.16 ± 1.89	10.73 ± 2.04
3	12.40 ± 1.53	10.10 ± 1.70
4	10.63 ± 1.96	13.26 ± 2.60
5	14.90 ± 2.06	13.50 ± 3.02
6	13.56 ± 2.34	13.0 *
7	12.36 ± 1.40	13.66 ± 1.64
8	13.4 ± 1.64	13.86 ± 0.83
9	12.23 ± 1.70	14.50 ± 3.16
10	11.66 ± 1.02	11.65 ± 1.20

* One count only made .

TABLE 1. 0. Individual or Group mean differential white cell counts.(%). *

	Cell Type	Week. 1	2	3	4	5	6	7	8	9	10
Control Group	Band	-	2.5	4	2	2	3	1.5	1.13 ±1.37	-	-
	Poly	56	39	40	41	30	30	47.5 ±24.7	43.8 ±9.9	35.6 ±9.9	38
	Lymph	40	56.5	55	54	68	63	50 ±25.4	53.1 ±9.4	61.3 ±7.5	59
	Eosin	-	1	-	1	-	1	0.5	0.6	1.6 ±1.5	-
	Baso	2	-	-	-	-	-	-	-	-	-
	Mono	2	2	1	2	0	3	0.5	1.33 ±1.5	2.0 ±1.7	3
Experimental Group	Band	-	1.5 ±0.7	-	-	-	-	-	1.0 ±1.0	-	2
	Poly	42.0 ±4.6	38.0 ± 6.4	-	-	-	-	-	30.0 ±1.0	33.0	32
	Lymph	50.5 ±5.0	58.0 ±8.4	-	-	-	-	-	67.3 ±1.2	77	60
	Eosin	0.5	-	-	-	-	-	-	0.6	-	4
	Baso	1.5	-	-	-	-	-	-	-	-	-
	Mono	5.5 ±1.5	2.0 ±0.0	-	-	-	-	-	1.0 ±1.0	-	2

x Where no S.D. is quoted only one value was available.

PATHOLOGICAL DETAILS.

No piglet died during the period under experiment. One piglet (number 1) was destroyed at the termination of the experiment; no gross pathological change existed. Evidence of pneumonia was not present.

Liver biopsies were taken from piglets 1, 3 and 4 at the termination of the experiment.

Marrow biopsy specimens were removed several times, but no very satisfactory smears were obtained, though several sternal and tibial punctures were made. Marked dilution of the marrow elements with blood seemed to occur.

The liver samples, were analysed for their iron content and the following figures obtained.

TABLE 1 P. Iron & Copper Estimations of the Livers.

PIGLET NUMBER	SPECIMEN	IRON (mgm. per 100 gm.)		COPPER (parts per million).
		Dry Tissue	Wet Tissue	Dry Tissue
1 Control	Biopsy	19.7	5.7	-
"	Autopsy	20.3	5.8	17.6
3 Exp.	Biopsy	10.48	2.9	22.4
4 "	"	19.7	5.3	-

These results are discussed along with the others obtained in the final section on these experiments.

CLINICAL SIGNS & MORTALITY.

As stated earlier one piglet (No.2) died within 48 hours of receiving the artificial diet. The remaining six piglets survived the duration of the 10 week experiment, and following liver biopsy of numbers 1, 3, & 4 were fattened and sold some weeks later.

Few signs of illness were observed in either group. Considering the reduction in the Hb levels of the experimental group more flagrant evidence of disease was expected. Since the lowest individual reading obtained for Hb was 6.4 gm it is possible that the lower values would have to be achieved before clinical signs became evident. In the review of the literature however it was noted that difference of opinion existed as to the levels which constituted anaemia in piglets. Levels of 6.8 gm Hb are a feature of hypochromic anaemias of man. (Stitt et al 1948).

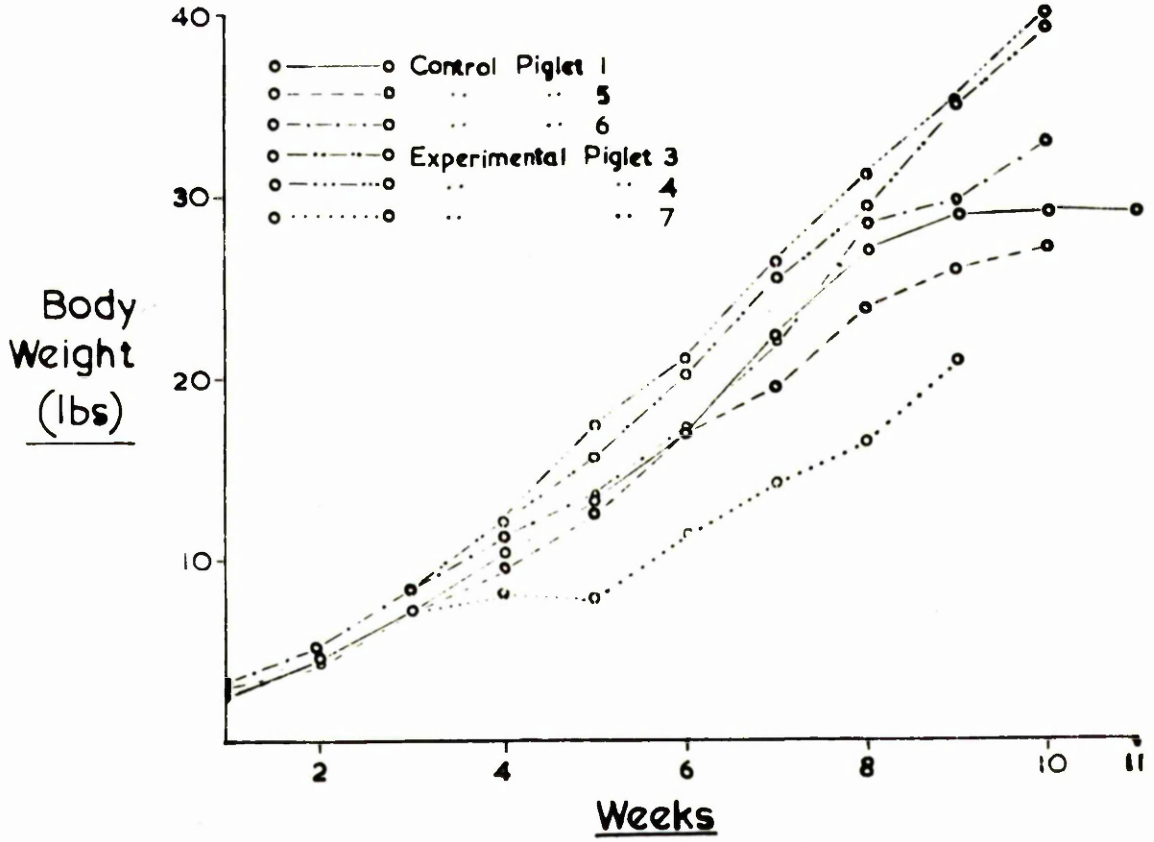
Of the classical signs of anaemia reported in the literature namely palor, unthriftiness, loss of weight, hairiness, and diarrhoea, only the latter was evident and this no more frequent or severe in the experimental than in the control group. Initially on weaning to the artificial diet a bout of diarrhoea, characterised by pale thin stools developed which lasted about 10 days, and was evident in all piglets. After that the control pigs suffered more frequent attacks of diarrhoea than the experimental ones in the latter the stools were of a black colour, attributed to the ingestion of the ferric citrate.

Evidence of respiratory disease was never noticed, despite frequent and careful examinations of the piglets. Occasionally sneezing was heard which seemed due to inhalation of the dry powder during feeding.

A slight lameness, accompanied by some curvature of the long bones of the limbs developed in one or two of the piglets. The lesion appeared to be unassociated with the lack of iron (Teague & Lawrence, 1951) and occurred in individuals of both groups.

Both groups gained weight as can be seen from the graph (Fig. 1 F). It will be noted that reasonable body weights were reached at 8 weeks when pigs should average about 30 - 40 lbs: (Linton & Robertson 1943: B.V.A. Pub. No.14 1956) and that some of the experimental pigs gained weight more readily than the control ones, although at week 8 the mean body weights were 27.0 lbs. for the controls and 25.6 lbs. for the experimental group. It would appear, however, that the most satisfactory gains were made by the piglets from the dam C.1. so that no conclusions can be made beyond stating that unthriftiness did not result when the iron intake was reduced.

FIG IF


Control Piglet 1
" " 5
" " 6
Experimental Piglet 3
" " 4
" " 7
Body Weight (lbs)
40
30
20
10
2
4
6
8
10
11
Weeks

DISCUSSION.

As this was the first experiment using methods which were subsequently adopted for other four artificial rearing experiments difficulties were expected and experienced. One of the most notable was in obtaining satisfactory samples of blood both for quantity and freedom from clots and extraneous sources of iron which could have interfered with the estimations of iron in the sera. This difficulty was largely overcome as has been referred to earlier.

Despite the small numbers of the population samples the marked differences in the levels of Hb and P.C.V. between the two groups were actually highly significant. ($P < 0.01$). This was a very satisfactory result which increased the importance of the close approximation of the erythrocyte counts of the pigs in both groups. It would seem that while the type of anaemia produced was microcytic and hypochromic (as noted from the Hb., P.C.V. and red cell indices) the number of red cells was not reduced to any measurable extent. Even though the red cell life in normal pigs is approximately 63 ± 16 days (Jensen et al 1956) it was not possible for the count to remain up in the anaemic ones despite even cessation of production since the pigs gained weight, and thus increased their blood volume. Alternatively the numbers produced were only sufficient to keep pace with the destruction. This would have necessitated sufficient iron being utilised from the food to meet the needs of the body aided by attempts to make best use of the small quantity available for example by a reduction in the size of the red cells. Since the "classical" clinical signs of anaemia were not produced and as the lowest Hb reading was 6.4 gm it would appear that sufficient iron was obtained from the food to prevent a catastrophic anaemia developing.

One other unusual feature was the close parallel of the M.C.H.C in both groups. Whitby & Britton (1957) stated that in hypochromic anaemia in man the reduction in the M.C.H.C. is the true indication of anaemia due to iron deficiency. It is obvious from the method used to obtain this value that should the Hb and P.C.V. be reduced proportionately (an occurrence which would appear to be infrequent in humans) the resultant will be the same as for the normal subject. In this respect the pig suffering from iron deficiency may differ from man.

The colour - index (colour - count ratio) as obtained from the group means was 0.81 for the control group and 0.51 for the experimental lot for week five. This too indicates a lower relative amount of Hb in each cell. A conclusion which is justified also from the M.C.H. indices.

Parsons (1938) stated that an "obvious microcytosis and a low colour-index mean essentially a deficiency of iron in the body". This author also noted that iron-deficiency proceeds in three stages. First there results a microcytosis and a compensatory polycythaemia. Then with greater deficiency a hypochromic microcytic anaemia develops and it is only in the late stages that a reductionin the numbers of erythrocytes occurs and to a lesser extent than with the Hb. Perhaps the same stages may be noted in pigs; in this experiment the second stage only would have been produced.

ARTIFICIAL REARING EXPERIMENT 2.

INTRODUCTION.

The initial experiment had justified the methods adopted and had enabled techniques to be modified where necessary.

It was decided that a larger number of pigs should be used so that the additional numbers would be helpful when analysing the results, though it meant that fewer readings were obtained. It was hoped by placing more pigs in the experimental than in the control group to find some pigs which would develop the signs so frequently recorded in the literature. Housing, management and methods were as have been described. As only 5 pigs bred on the premises were available piglets from two different sources were obtained within the first 72 hours after birth. On breed conformation these piglets could be classified into three lots. Four piglets died within the first week of the experiment, and these have been deleted.

Haematological data were obtained for 17 piglets which were distributed as indicated in Table 2 A.

TABLE 2 A.

	Control Group			Experimental Group			
No.	Source	Sex	Ave.Wt(lbs)	No.	Source	Sex	Ave.Wt.(lbs).
2	'P'	2M	3.0	5	'P'	4M 1F	3.1
2	'WC'	2F	3.5	4	'WC'	2M 2F	2.9
1	"	F	3.0	3	'J'	2M 1F	3.0

From experience it is now appreciated that more equal distribution might have been attempted or alternatively random distribution would have placed no selection bias on either group.

In the light of the knowledge gained in the first experiment it appeared that the initial six weeks were the important period for the production of anaemia and results were recorded after the seventh week in only a few pigs, to indicate that further important changes did not occur.

The iron contentsof the diets fed have been described in the appropriate section. The diet in this experiment was altered in an endeavour to reduce the iron-content of the iron- poor diet; 2 piglets received this diet containing 4.48 mg. per 100 gm diet and compose the first experimental group. The others, making up the second group got only 2.82 mg. of iron per 100 gm of food. This decrease in the dietary iron was effected by changing the make of dried milk and excluding antibiotics.

HAEMATOLOGICAL RESULTS.

Unless where stated otherwise the standard deviation is quoted with each group mean.

Haemoglobin.

The tables following give each Hb reading for the control and the experimental piglets. (Tables 2B, C, D).

TABLE 2 B. Individual Hb. value for control piglets.

Week	Piglets.									
	1		2		8		10		11	
1	12.4	9.1	12.0	8.9	10.9	10.1	12.7	11.2	12.0	12.0
2	10.8	10.1	7.8	7.8			8.9		9.1	
3	10.1		7.9		9.5		9.5		10.5	
4	11.4		9.5		10.1		10.0		10.5	
5	12.0	12.6	9.9	11.0	10.5		10.0		11.4	
6	12.0	12.2	9.9	9.5	10.1		11.4		10.5	
7	12.0		8.2		-		-		11.0	
8	12.0		8.9		-		-		11.2	
9	12.2		8.7		-		-		-	
10	12.6		10.3		-		-		-	

TABLE 20 Individual Hb. values for the experimental groups.

Group 1 Piglets.			Group 2. Piglets.									
Week	3	4	5	6	7	12	14	15	16	32	33	35
1	13.5 9.7	13.5 10.5	10.4	11.7	10.1	12.7 12.1	-	8.2	7.6	9.7	-	-
2	8.0 8.4	9.5 8.1	10.1	9.7	10.9	7.4		7.0	7.8	8.2	6.0	6.1
3	6.6	7.6	13.0 8.7	9.0 6.6	8.2 5.9	7.0	7.6	6.1	7.6	-	6.3	6.3
4	7.8	7.4	8.0	6.8	8.0	5.7	6.3	4.5	6.5	7.4	5.0	7.0
5	6.0	8.2 5.5	7.6	6.3	7.0	6.3	5.7	4.7	6.5	5.5 7.8	7.6 7.0	6.3
6	4.5 3.7	8.0	6.3	6.1	8.2	7.2 7.2	6.3	4.7 5.0	6.5 7.0	5.5	7.0	6.1
7	6.1 7.6	9.5	8.2	8.2	8.9	6.8	-	5.5	6.1 5.7	6.1	6.3	6.3
8	8.4	-	-	-	-	-	-	-	-	5.5	-	-
9	-	-	-	-	-	6.8	-	5.1	6.1	5.9	-	-
10	9.8	-	-	-	-	-	-	5.1 5.7	5.7	4.6	-	-
11	-	-	-	-	-	-	-	4.6	-	3.8	-	-
12	-	-	-	-	-	-	-	4.4	-	-	-	-
13	-	-	-	-	-	-	-	3.8 5.9	-	4.0	-	-
14	-	-	-	-	-	-	-	-	-	4.4 4.2	-	-
15	-	-	-	-	-	-	-	-	-	4.4 5.5	-	-

TABLE 2 D. Group Mean Hb. values for first 7 weeks of Experiment 2.

Week	Control Group		Experimental Group 1		Experimental Group 2	
	Mean	SE	Mean	SE	Mean	SE
1	11.15 ± 1.35	±0.42	11.80±1.99	±0.99	10.31 ± 1.81	±0.64
2	8.92 ± 1.17	±0.44	8.50±0.69	±0.34	8.24 ± 2.23	±0.74
3	9.50 ± 0.99	±0.44	7.10±0.70	±0.49	7.60 ± 2.01	±0.58
4	10.30 ± 0.71	±0.31	7.60±0.28	±0.20	6.52 ± 1.17	±0.36
5	11.05 ± 1.01	±0.38	6.56±1.43	±0.82	6.52 ± 0.93	±0.26
6	10.80 ± 1.06	±0.40	5.40±2.28	±1.31	6.39 ± 0.95	±0.26
7	10.40 ± 1.96	±0.88	7.73±1.70	±0.98	6.81 ± 1.03	±0.32

A clostridial infection of both piglets in the first experimental group was evident at the fifth and sixth week and this coincided with a noticeable reduction in the Hb level.

Several of the pigs showed high Hb values in the first few days oflife; a feature which has been noticed in pigs (Craft & Moe, 1932: Doyle, 1932: Gardiner et al 1953) and in human infants. Some of the neo-natal piglets seemed to have higher Hb values than others, but only in piglet number 16 and perhaps in number 15 were the Hb values low. The group means, however, during the first week were not appreciably different, and only at the third week did the difference become noticeable. A drop amounting to about 2 gm occurred after the first week in the mean for the control group. This may

have been due in part to the pigs failing to ingest sufficient food.

The relative differences, and fluctuations in the group mean values can be viewed readily by studying the accompanying graph, (Fig. 2 A.).

It is obvious that no significant difference existed between the Hb levels of the control group and either experimental group at week 1 or 2, as would be expected. Finding the ratio t in testing the means of the groups at week 3 gave a value which was significant ($n = 5 : P < 0.05$) for the difference between the means for the control and experimental Group 1. The mean for Group 2, however, was not significant at the 5% but was at the 10% level of probability.

At weeks 4 and 5 the difference between the mean for the control and each experimental group was greater and the t test of the values showed a highly significant difference ($P < 0.01$) in each case, which by inspection would appear to have existed at least till week seven.

It is thus of considerable interest to note the close similarity between the groups at the start of the experiment and the widening gap in the levels of Hb of the controls and the other groups as the influence of the shortage of dietary iron became evident, until after the third week the difference became highly significant.

Haematocrit values.

The tables which follow give the P.C.Vs for each pig comprising the control and experimental groups (Table 2 E and F), and in Table 2 G the group mean values are recorded.

FIG 2A

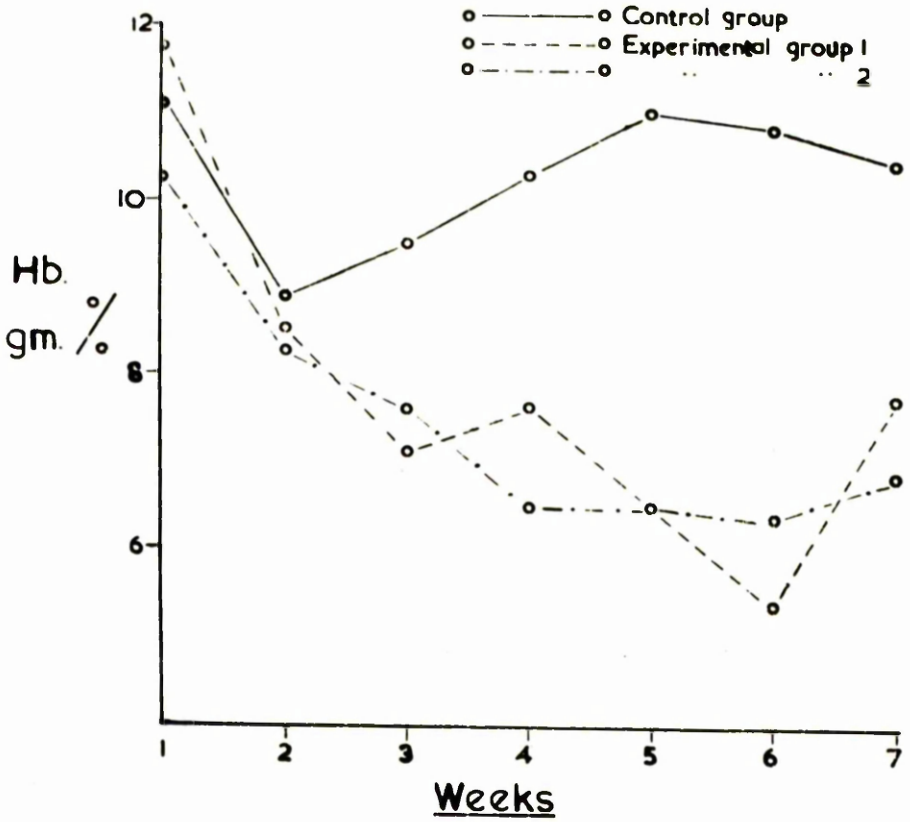

Control group
Experimental group 1
.. 2
12
10
8
6
Hb. gm. %
1
2
3
4
5
6
7
Weeks

TABLE 2 E. Mean weekly P.C.Vs (ml per 100 ml) for the control piglets.

Week	PIGLET NUMBER				
	1	2	8	10	11
1	26	31	33	34.2	24
2	30	25	26	30	30
3	-	-	31	34	37
4	40	36	37	37	38
5	40.5	37	33	34	42
6	42.5	34.5	36	37	29
7	41	27	-	-	40
8	41	28	-	-	36
9	41.5	30	-	-	-
10	47	38	-	-	-

TABLE 2 F. Mean weekly P.C.V for the experimental piglets (ml per 100 ml).

Week	Group 1		Group 2									
	Piglet No.		Piglet Number									
	3	4	5	6	7	12	14	15	16	J2	J3	J5
1	32	35	-	-	-	37	31	24	-	33	-	-
2	34	31	-	-	-	24	36	23	24	33	17	22
3	24	-	31	24.5	25	25	24	20	23	-	23	23
4	23	27	26.5	22.5	26.5	21	19	15	20	24	-	24
5	19	21	24	20	27	21	19	16	20	21.5	26.5	23.5
6	12.25	29	23	20	23	24.5	24	20.75	25.7	22	24	23
7	15	33	26	26	31	25	-	25	23.2	22	24	24
8	28	-	-	-	-	-	-	-	-	19	-	-
9	-	-	-	-	-	23	-	20	22	20	-	-
10	34	-	-	-	-	-	-	21.5	24	15	-	-
11	-	-	-	-	-	-	-	19	-	12	-	-
12	-	-	-	-	-	-	-	18	-	-	-	-
13	-	-	-	-	-	-	-	18	-	17	-	-

TABLE 2 G. Group Mean P.C.Vs (ml per 100ml).

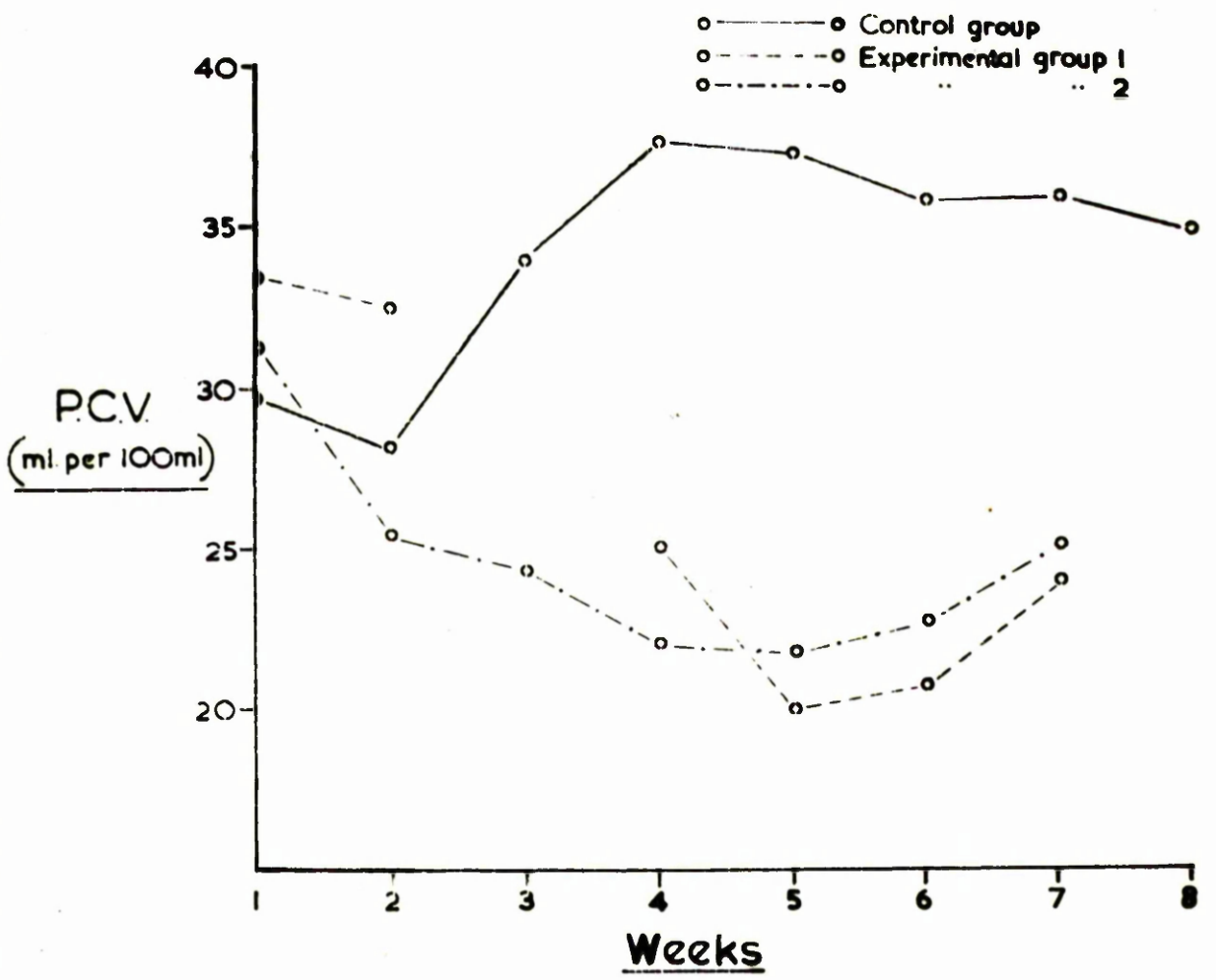
Week	Control Group	Exper. Group 1	Exper. Group 2
1	29.64 ± 4.43	33.50 ± 2.12	31.25 ± 6.65
2	28.20 ± 2.49	32.50 ± 2.12	25.50 ± 6.60
3	34.00 ± 3.0	-	24.27 ± 2.94
4	37.60 ± 1.51	25.00 ± 2.82	22.05 ± 3.92
5	37.30 ± 3.92	20.00 ± 1.41	21.85 ± 3.42
6	35.80 ± 4.84	20.65 ± 11.7	22.79 ± 1.46
7	36.00 ± 7.81	24.00 ± 12.7	25.13 ± 2.55
8	35.00 ± 6.55	-	-

On a few occasions P.C.Vs have not been recorded often due to the impossibility of repeating the samples in which clots appeared because of other commitments.

No difference appeared to exist between the groups for the first two weeks. At week 3 only one P.C.V was made for the first experimental group so that no mean can be obtained. A t test for significance of the means for the controls and group 2 showed the difference between these to be highly significant ($P < 0.01$). At week 4 the difference between the mean P.C.Vs of each experimental group and the control was also highly significant.

Fig. 2 B. illustrates the fluctuations and differences in the group mean P.C.Vs.

FIG 2B


Control group
Experimental group 1
" " 2
40
35
30
25
20
P.C.V.
(ml. per 100ml)
1
2
3
4
5
6
7
8
Weeks

Erythrocyte Counts.

The tables of the erythrocyte counts for the individual pigs record the count, where only one, or the mean where two or more counts were made during any week, (Tables 2 H & 2 I).

TABLE 2 H. R.B.Cs ($\times 10^6$ per c mm.) for the control pigs.

Week	Piglet Number				
	1	2	8	10	11
1	5.3	4.5	4.9	5.6	5.9
2	4.5	3.7	4.9	5.1	4.9
3	5.0	4.0	5.2	5.6	5.9
4	5.3	5.0	6.6	5.3	5.8
5	5.5	5.0	5.1	5.0	6.6
6	6.1	5.0	5.1	5.7	5.5
7	6.2	4.4	-	-	5.9
8	6.7	5.2	-	-	-
9	6.3	5.0	-	-	-
10	7.7	5.2	-	-	-

TABLE 2 I. R.B.Cs ($\times 10^6$ per c.m.m.) for the piglets comprising the experimental group.

Week	Group 1 Piglet No.		Group 2 Piglet Number									
	3	4	5	6	7	12	14	15	16	J2	J3	J5
1	5.9	5.6	5.1	6.5	5.0	5.8	5.8	4.3	3.7	4.9	-	-
2	4.7	4.6	5.1	5.1	6.2	4.1	5.3	4.5	3.8	5.7	3.6	3.2
3	3.7	3.6	5.4	3.4	3.9	3.6	3.9	4.2	4.9	-	3.8	4.3
4	3.6	4.2	4.4	3.7	4.4	4.2	3.4	3.5	3.0	5.2	4.5	5.1
5	3.1	3.4	4.8	4.3	5.0	4.2	3.8	2.8	4.0	4.5	5.3	5.4
6	4.1	3.6	4.5	3.8	6.4	4.6	5.0	3.9	4.4	3.9	4.7	4.9
7	6.8	4.1	5.3	5.3	5.8	4.5	-	4.1	4.0	4.6	4.3	3.0
8	8.4	-	-	-	-	-	-	-	-	3.9	-	-
9	-	-	-	-	-	4.2	-	3.5	4.9	3.8	-	-
10	9.8	-	-	-	-	-	-	4.5	4.3	2.3	-	-
11	-	-	-	-	-	-	-	3.0	-	2.2	-	-
12	-	-	-	-	-	-	-	3.9	-	-	-	-
13	-	-	-	-	-	-	-	3.7	-	3.2	-	-
14	-	-	-	-	-	-	-	-	-	3.8	-	-
15	-	-	-	-	-	-	-	-	-	3.1	-	-

From the tables it is evident that many individuals in the experimental group showed a reduction in the R.B.Cs when compared with the controls, particularly after the third week. The over-all differences can be appreciated most readily by studying the table giving the group means (Table 2 J).

While only slight differences existed between the means of the group samples in the first two weeks, from the third to the sixth week a much wider gap existed. At the fourth week, a significant difference existed at the 5% level between the control and each experimental lot. In this respect these group samples differed from the erythrocyte count results obtained for the samples in experiment 1.

TABLE 2 J. Group Mean Erythrocyte Counts ($x10^6$ per c.m.m).

Week	Control Group	Exper. Group 1	Exper. Group 2.
1	5.22 ± 0.56	5.75 ± 0.22	5.13 ± 0.88
2	4.62 ± 0.57	4.65 ± 0.10	4.66 ± 0.97
3	5.14 ± 0.72	3.65 ± 0.10	4.15 ± 0.64
4	5.60 ± 0.62	3.90 ± 0.42	4.14 ± 0.72
5	5.44 ± 0.68	3.25 ± 0.36	4.61 ± 0.78
6	5.48 ± 0.44	3.85 ± 0.36	4.61 ± 0.75
7	5.50 ± 0.96	5.45 ± 1.91	4.54 ± 0.84

Red - Cell Indices.

The Mean Corpuscular Volume.

TABLE 2 K. Group Mean M.C.Vs (μ^3).

Week	Control Group.	Exp. Group 1 .	Exp. Group 2.
1	60.95 ± 10.48	68.45 ± 1.62	62.34 ± 4.44
2	62.48 ± 6.20	69.60 ± 3.61	57.41 ± 6.84
3	61.10 ± 1.55	-	61.85 ± 10.40
4	67.66 ± 10.22	64.05 ± 0.22	54.38 ± 8.31
5	69.68 ± 6.70	61.30 ± 2.75	49.65 ± 3.83
6	68.90 ± 2.02	65.73 ± 12.90	51.56 ± 4.51
7	65.03 ± 3.33	63.50 ± 16.20	56.97 ± 10.25

At week 4 which was the earliest that any marked difference appeared to be present, only the mean of the second experimental lot showed a significant difference ($P < 0.05$) from the mean of the control group. Statistically significant differences can be shown, however, between the mean of each experimental group and that of the controls at the fifth week.

Except for those of the second experimental group the reduction in the M.C.V did not seem as great as experienced in experiment 4.

The Mean Corpuscular Haemoglobin Concentrations.

The accompanying tables give the group mean figures for the M.C.H.C.

TABLE 2 L. M.C.H.C. Group means (%).

Week	Control Group	Exp. Group 1	Exp. Group 2
1	36.11 ± 6.18	30.15 ± 0.22	32.60 ± 2.21
2	31.42 ± 4.50	26.42 ± 3.24	28.96 ± 3.32
3	28.53 ± 1.95	27.5	29.25 ± 3.27
4	27.94 ± 1.35	30.65 ± 4.59	30.35 ± 1.77
5	29.83 ± 2.23	32.20 ± 0.65	29.52 ± 2.91
6	27.87 ± 1.46	31.46 ± 4.24	27.66 ± 3.37
7	29.03 ± 1.45	40.60 ± 11.08	27.18 ± 2.99

By inspection of the mean figures it can be seen that any difference between the control and either experimental group was without significance. At weeks 5 & 7 the t values were well within the limits of significance even at the 10% level of probability.

The Mean Corpuscular Haemoglobin.

Table 2 M gives the M.C.H as group mean values.

TABLE 2 M. M.C.H. indices group means. (yy).

Week	Control Group	Exp. Group 1.	Exp. Group 2.
1	21.38 ± 2.97	20.25 ± 1.57	20.01 ± 1.11
2	20.01 ± 3.11	18.15 ± 1.40	17.48 ± 2.42
3	18.54 ± 1.37	19.45 ± 2.33	17.45 ± 2.86
4	18.54 ± 2.28	19.60 ± 2.82	15.99 ± 3.34
5	20.60 ± 1.94	19.73 ± 1.11	14.63 ± 1.44
6	19.61 ± 0.56	20.76 ± 2.94	14.48 ± 2.34
7	18.83 ± 0.40	25.30 ± 1.27	15.35 ± 2.64
8	17.59 ± 0.54	-	-
9	18.35 ± 1.34	-	14.62 ± 1.62
10	18.15 ± 2.61	-	14.27 ± 3.86

Except for the first experimental group the M.C.H values agree with those obtained in the first experiment.

Reticulocyte Counts.

The mean group reticulocyte counts are recorded in Table 2 N.

TABLE 2 N. Group Mean Reticulocyte Counts * (% of erythrocytes).

WEEK	CONTROL GROUP	EXP. GROUP 1.	EXP. GROUP 2
1	3.80 ± 1.32	4.45 ± 0.10	3.20 * - 0.28
2	3.58 ± 0.76	3.75 ± 0.10	3.68 ± 0.75
3	3.98 ± 1.08	5.05 -	4.11 * - 0.93
4	4.02 ± 1.01	7.40 ± 2.25	4.48 ± 1.29
5	5.20 ± 0.42	10.15 ± 5.72	4.90 ± 1.17
6	3.90 ± 1.58	6.50 -	4.30 ± 0.91
7	5.00 -	6.90 -	4.76 ± 0.85
8	6.10 ± 1.55	6.20 -	-
9	-	-	-
10	-	6.40	6.00 ± 0.28

* Where only one count was made, this is recorded without any standard deviation.

Somewhat high reticulocyte counts were obtained for the first experimental group at the fourth and fifth week of the experiment. These were due to increased counts for piglet 4 which had a super-imposed clostridial infection evident at the end of the fifth week, and which necessitated its destruction at the end of week 6. The reticulocytes of piglet 4 rose from 4.3% of the red cell count at

the start of the fifth week to 24.1% five days later. Counts for the remaining pig are recorded out of interest. Apart from the weeks mentioned, at no other week did the group mean reticulocyte counts appear, by inspection to differ significantly.

Cell Morphology.

Throughout the experiment the red cells of the control piglets maintained a uniform size and even staining. Those of the experimental piglets varied in the severity of the changes they exhibited depending on the individual pig. The cells of some pigs showed mainly anisocytosis with many microcytes (e.g. number 7) whereas incompletely haemoglobinised cells were numerous as well in the blood smears from other pigs (e.g. number 15).

Many platelets were observed in some smears from pigs of the experimental group.

Osmotic Fragility of Erythrocytes.

In this experiment the ability of the red cells to withstand varying concentrations of Na Cl was studied since no information on this point had been noted in the literature on nutritional iron-deficiency, anaemia in piglets. The methods used in arriving at the figures quoted for the saline (osmotic) fragility of the R.B.Cs have been described.

The fragility of the cells was not estimated for every pig on each week, but estimates were made on the samples from all the pigs except number 14. Some of these values have been quoted in Table 2 0. The first figure in each case being the concentration of saline necessary to initiate haemolysis follow-

ed by that required for complete haemolysis.

More frequent readings of the fragility of R.B.Cs were made from the second experimental group of pigs because any alteration in the cell resistance resulting from the anaemia might have been evident. The fragilities obtained for the first experimental group were infrequent and since these pigs suffered from a complicating infection have not been recorded. The number of readings for the control group pigs were few but, no marked differences were noted, between these and those of the experimental groups.

TABLE 20. Osmotic Fragility of the R.B.Cs (% salt concentration initial/complete haemolysis.)

Piglet Number	Weeks. 1	2	3	4	5	6	7	8	9	10	11	13
1	-	-	-	-	-	-	-	-	-	0.56/ 0.40	-	-
2	-	-	-	-	/0.45	-	-	-	-	-	-	-
8	-	-	-	-	-	0.55/ 0.40	-	-	-	-	-	-
10	-	-	-	0.60/ 0.45	-	-	-	-	-	-	-	-
11	-	-	-	0.50/ 0.35	-	0.60/ 0.45	0.70/ 0.45	-	-	-	-	-
5	-	-	-	-	-	-	0.65/ 0.35	-	-	-	-	-
6	-	-	-	-	-	-	0.66/ 0.35	-	-	-	-	-
7	-	-	-	-	-	-	0.70/ 0.40	-	-	-	-	-
12	-	-	-	-	-	-	-	-	0.65/ 0.35	-	-	-
15	-	-	-	-	-	-	0.55/ 0.35	-	0.45/ 0.35	0.50/ 0.35	0.60/ 0.30	0.50/ 0.30
16	-	-	-	-	-	-	0.70/ 0.35	-	0.50 0.35	-	-	-
J2	0.75/ 0.40	0.75/ 0.40	-	0.55/ 0.35	0.60/ 0.35	0.65/ 0.35	-	0.55/ 0.30	-	-	-	-
J3	-	0.65/ 0.40	-	-	0.50/ 0.35	0.45/ 0.35	0.55/ 0.30	-	-	-	-	-
J5	-	0.65/ 0.35	0.65/ 0.35	0.50/ 0.35	-	0.45/ 0.35	0.55/ 0.30	-	-	-	-	-

Serum - Iron Estimations.

Estimates of the iron content of serum samples, were made on several occasions, from most of the pigs under experiment and these are recorded in table 2 P.

TABLE 2 P. Serum - Iron Levels (µg per 100 ml).

Groups	Control 1		Exp.1	Experimental 2.							
Piglet No.	1	2	3	5	6	7	12	14	15	16	J2
Weeks 2	-	-	-	-	-	-	85.6	79.2	57.6	109.4	74.8
3	-	-	-	49.2	72.8	70.4	-	-	-	72.8	-
7	182.0	363.6	231.2	-	-	-	74.8	-	77.2	-	-
8	100.1	62.0	209.0	-	-	-	-	-	-	-	-
11	-	-	-	-	-	-	-	-	-	-	160.4
14	-	-	-	-	-	-	-	-	-	-	74.8

Three serum-iron values, seemed to be unusual, namely both values for piglet 3 and that for J2 at the eleventh week. Contamination by iron-containing materials, was unlikely since every precaution was taken. It is worth noting however, that piglet 3 had recently suffered a clostridial infection from which its partner died, and that the treatment with sodium and procaine penicillin had been administered.

Measurement of Red Cell Diameters.

Direct measurements of the diameters of the red cells of piglets 2 and 10 (control group) and 12 and 15 (experimental group 2) were made, using the strip film technique projected on graph paper.

The graphs (Fig. 2 C and 2 D) illustrate the interesting alterations which occurred in the red-cell diameters of these piglets during the period under experiment.

In contrast to the noticeable swing towards a microcytosis noted in piglet 12 particularly at week 4 and piglet 15 at week 3 , the cells of the control piglets examined showed no such alteration. The wide spread of the curves for the cell diameters of the experimental pigs over the period of the experiment contrasts with the relatively close grouping of the diameters of the R.B.Cs for the controls around the 6 μ region.

For table 2 Q the "normal" distribution has been taken as the curve shown by piglet 2 at the second week, which is approximately centre for the curves for the control pigs.

FIG 2C

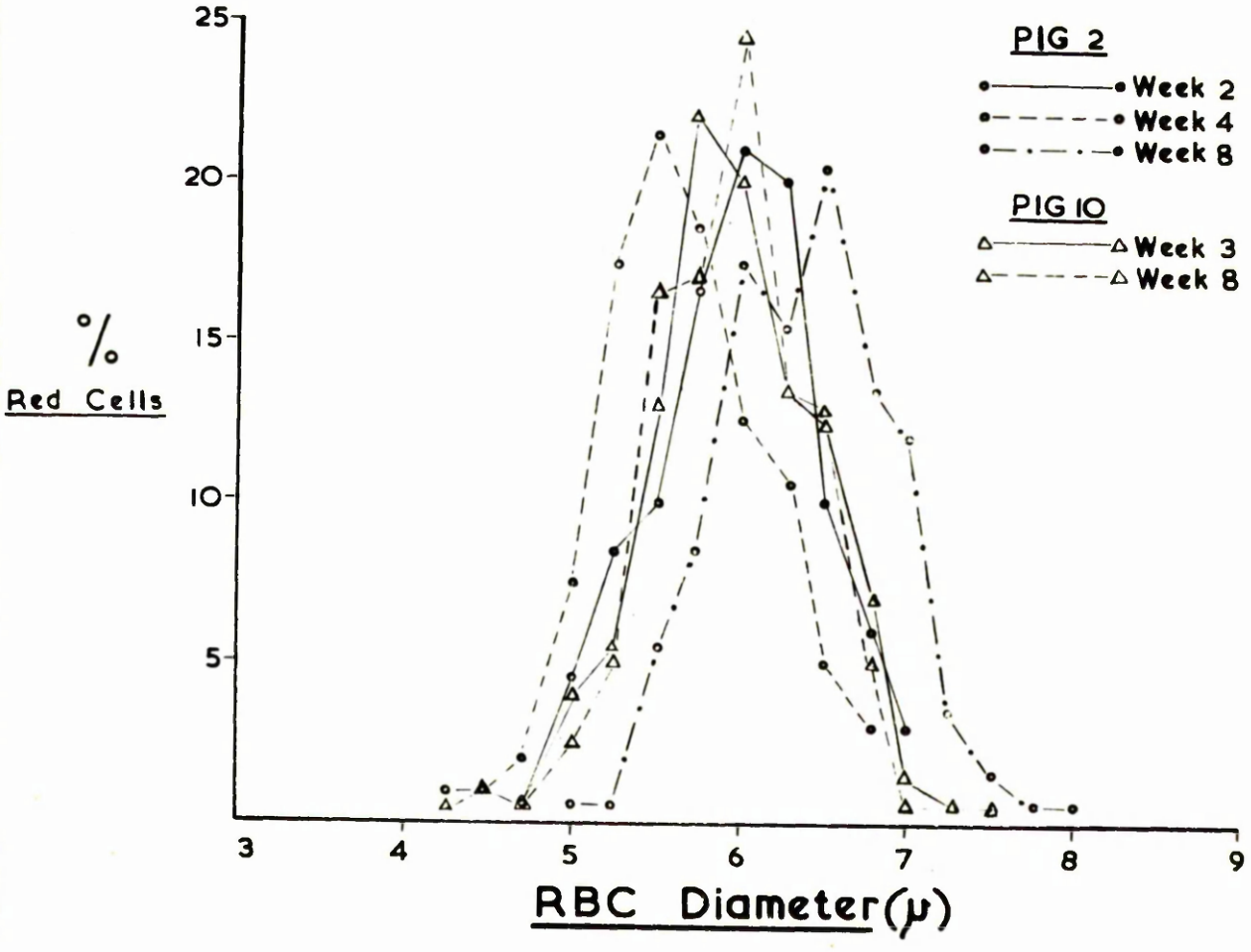

25
20
15
10
5
%
Red Cells
3
4
5
6
7
8
9
RBC Diameter(μ)
PIG 2
Week 2
Week 4
Week 8
PIG 10
Week 3
Week 8

FIG 2D

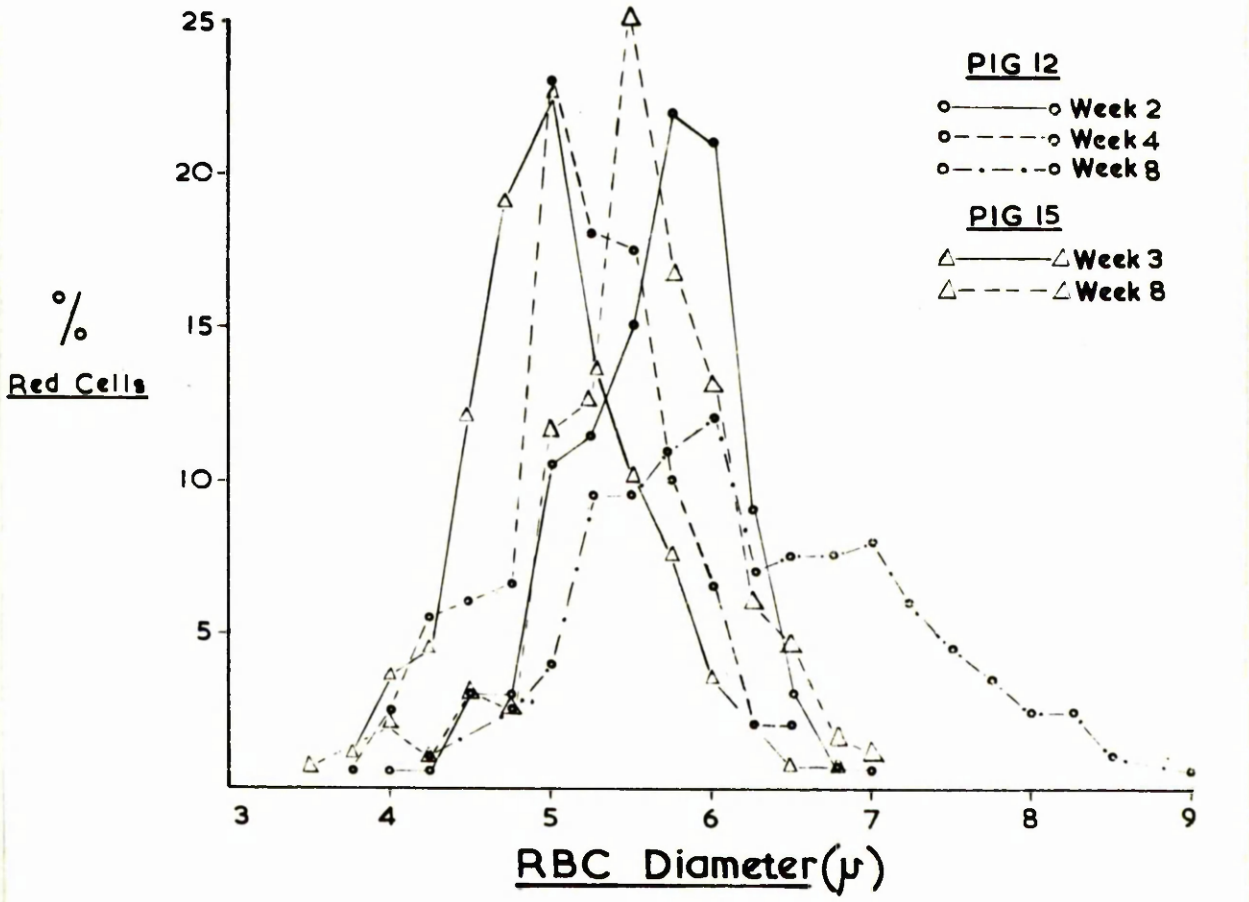

25
20
15
10
5
% Red Cells
3
4
5
6
7
8
9
RBC Diameter(μ)
PIG 12
Week 2
Week 4
Week 8
PIG 15
Week 3
Week 8

TABLE 2 Q. Percentage of Microcytosis.

MID-POINT OF CLASS INTERVALS (0.25 μ).	CONTROL PIGLET 2 (%)	EXP. PIGLET 12 (%)
5.5	10.0	17.5
5.25	8.5	18.0
5.0	4.5	23.0
4.75	0.5	6.5
4.5	0	6.0
4.25	0	5.5
4.0	0	2.5
3.75	0	0.5
TOTAL:	23.5	79.5

Percentage microcytosis : - 79.5 - 23.5 = 56%

WHITE CELLS.

The group mean counts for the total and different W.B.Cs are given in tables 2 R and 2 S.

TABLE 2 R. Group Mean * White Cell Counts (x 10^3 per c.mm).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP 1	EXPERIMENTAL GROUP 2
1	11.14 ± 1.64	10.35 ± 1.48	14.55 ± 3.42
2	10.75 ± 1.49	12.10 ± 0.14	10.71 ± 2.23
3	11.68 ± 0.65	12.45 ± 2.90	12.34 ± 1.96
4	11.74 ± 1.62	9.35 ± 0.64	13.62 ± 3.36
5	12.96 ± 0.89	14.75 ± 0.50	12.76 ± 3.33
6	16.44 ± 3.34	18.10 * 0.98	14.84 ± 5.33
7	15.93 ± 0.46	16.0	15.84 ± 6.51
8	22.65 ± 2.33	12.8	22.40
9	19.50 ± 2.40	-	22.75 ± 9.49
10	15.60 ± 0.70	11.1	28.43 ± 9.33

* Where no S.D. is quoted the count for one pig only is given.

Towards the end of the experiment some pigs showed increased white cell counts, which may have indicated that some infection was present since about this time several of the pigs suffered from diarrhoea.

One piglet (J 2) showed a large number of young polymorphs at week 10.

TABLE 2 S. Group Mean & S.D. for the differential White Cell Counts (%).*

	Cell	Weeks. 1	2	3	4	5	6	7	8
Control Group.	Band	1.2 ± 2.0	4.0 ± 2.8	- -	- -	4.0 ± 2.8	2.0 ± -	- -	5.5 ± 0.7
	Poly	54.6 ±18.0	58.7 ± 8.1	49.0	50.0	53.5 ± 4.9	54.0	-	78.5 ± 2.1
	Lymph	38.4 ±19.0	35.5 ±10.6	49.0	48.0	40.0 ± 5.6	43.0		14.0 ± 1.4
	Eosin	0.2	0.75	-	1.0	-	1.0	-	-
	Baso	-	-	-	-	-	-	-	1.0
	Mono	1.0 ± 1.0	3.0 ± 0.0	2.0	1.0	2.0	-	-	1.0
Experimental Group 1.	Band	3.5	1.0	-	4.0	3.0	3.5 ± 2.1	3.0	-
	Poly	55.5 ± 16.2	58.0 ± 2.8	-	60.0	62.0	51.5 ±19.1	63.0	-
	Lymph	39.0 ± 21.1	38.2 ± 2.4		30.0	35.0	43.5 ±20.5	29.0	-
	Eosin	-	1.0 ± 0.0	-	2.0	-	1.0 ± 0.0	1.0	-
	Baso	-	-	-	-	-	-	-	-
	Mono	2.0 ± 0.0	1.7 ± 0.3	-	4.0	-	0.5	4.0	-
Experimental Group 2.	Band	0.75	2.0 ± 0.0	3.0 ± 2.8	6.0	-	8.3 ±9.6	15.5 ± 20.5	9.0
	Poly	33.0 ± 2.8	59.5 ± 2.1	65.0 ±11.3	65.0	68.0	50.0 ±5.0	49.0 ± 1.4	52.0
	Lymph	62.5 ± 4.9	37.5 ± 3.5	30.0 ± 5.6	27.0 -	31.0 -	38.3 ±13.3	34.0 ±19.8	31.0 -
	Eosin	1.0 ± 1.4		1.0		1.0 0.0	0.0	0.5	
	Baso	-	-	-	-	-	-	-	-
	Mono	0.5	1.0	1.0	2.0	-	3.5 ± 1.2	1.0 ± 0.0	8.0

* Where no S.D. is given the values quoted are for one individual.

PARENCHYMAL IRON & COPPER.

The copper and iron contents of the livers and some of the spleens and kidneys from the pigs which died were estimated and are recorded in Table 2 T.

TABLE 2 T. Parenchymal Iron and Copper Content.

GROUP	PIGLET NUMBER	ORGAN	IRON mgm.per 100 gm. WET TISSUE	COPPER p.p.m DRY TISSUE.
Control	8	Liver	23.5	-
		Spleen	21.4	-
		Kidney	6.3	-
Experiment 1.	4	Liver	15.2	21.2
	14	Liver	6.37	56.0
Experiment 2	16	Liver	6.0	28.0
		Spleen	18.3	-
	15	Liver	4.1	18.9
		Spleen	12.0	-
		Kidney	4.6	-

Except for piglet number 4 which died from a clostridial infection, all the experimental piglets showed liver iron contents well below that for the control piglet, even number 15 which had been allowed sow and weaner meal. The iron content of the livers of the control piglet and number 4 seemed high.

BODY WEIGHTS AND CLINICAL SIGNS.

The mean body weights for the groups at weeks 3 & 5 were :-

	WEEK 3	WEEK 5
Control Group	6.9 lbs.	10.9 lbs.
Experimental Group 1.	8.0 lbs.	16.0 lbs.
" " 2.	6.8 lbs.	9.6 lbs.

Some of the pigs failed to thrive well and a few pigs particularly in the control and the second experimental group showed diarrhoea, which was not apparently related to the haemoglobin level of the pig.

At the fifth week the heaviest pig in the control group (no.24) weighed 20.25 lbs. and lightest 9 lbs. Whereas J 3 which was the best pig in the second experimental group weighed 16 lbs. Piglets 14, 15, & 16, all of which eventually died or were destroyed, made little or no increase in weight during the course of the experiment.

It is likely that the exclusion of antibiotics from the diet influenced the ability of these pigs to thrive.

DISCUSSION.

In assessing the results obtained in this experiment, it is considered that little emphasis should be placed on the results for the various blood parameters for the first experimental group not only because it comprised of two pigs only but also as a result of the clostridial infection which was accidentally introduced. Both pigs were noticed to be dull during the sixth week and have numerous large dark blue haematoma - like swellings involving the cutis and sub-cutis. One pig, No.4 was killed in extremis at the sixth week and the other was treated with pencillin and recovered. Inflammation and infection of the body wall was apparent at the autopsy of No.4 from which Cl. welchi was isolated.

Two other piglets died during the experiment. One (No.14) died, when six weeks old and the other (no.16) when 10 weeks old. From Table 2 C it can be seen that neither had particularly low Hb values prior to death. Autopsy of the former showed the cause of death to be peritonitis arising from a necrotic enteritis, while the latter had an acute pleurisy involving only the right side of the thorax. This lesion may have arisen from penetration of the thorax in withdrawing blood from the anterior vena cava. No lung damage attributable to so- called virus pneumonia or liver lesions suggestive of the changes noted by Mc.Gowan and Crichton (1924) were present in either pig. The femoral marrow of no.14 looked pale pink in colour, but because death had occurred the previous day no sections were taken, even though the body had been stored at $^{+}4$ C.

One pig (no.15), 2 weeks after the termination of the experiment was destroyed when moribund. The main features on autopsy were those of necrotic enteritis.

The Hb values of some pigs in the experimental group were lower than those achieved in Experiment 1 ; values about 4 gm % were recorded in some pigs. Many of the lower values were noted in the pigs which had been kept on the diets for long periods. The pigs from both control and experimental groups showed a noticeable failure to continue to gain in weight, and even some loss of general condition. These pigs tended to be the ones which suffered from necrotic enteritis and showed a great desire for more fibrous diet. It was not always possible to completely satisfy their appetites with the pure diet as they became larger.

The probability of the difference between the mean erythrocyte counts for the groups occurring by chance was greater than with those for Hb or the haematocrit values. This was taken to indicate that the reduction in the red cell population proceeded at a slower rate than the hypochromia and microcytosis. Again the M.C.H.C showed no significant difference between the sample means.

The osmotic fragilities recorded were too few to allow definite interpretation but some variation appears to have occurred even in the same pigs at different weeks. These values too seem lower than those noted by Hudson (1955). Wintrobe (1956) has emphasised that temperature, p.H. and the anticoagulant used are among the factors which influence the result.

ARTIFICIAL REARING EXPERIMENT 3.

INTRODUCTION.

In this experiment the format used in the two antecedent experiments was altered. An inspection of the literature on piglet anaemia had made it abundantly clear that iron-deficiency anaemia was usual in piglets reared naturally, unless some suitable source of iron was allowed. This condition of anaemia was indicated by a group of clinical signs eventually leading to death. Since the first two experiments had failed to reproduce this 'classical' syndrome though anaemia had undoubtedly been produced it was considered important to compare the haematological changes noted in the piglets reared on the semi-synthetic diet, with a similar group reared naturally, but without access to soil and as free as possible from other sources of iron.

No change from the housing and management used in the previous experiment was made in rearing the group maintained on the iron-poor semi-synthetic diet.

The piglets allowed to suckle the sow were housed in a wooden ark, with run attached formed of concrete paving-slabs, surrounded by painted metal rails and galvanised wire. Each day the exercise area was cleaned of superfluous excreta and food. The sow was fed wet mash twice daily from a heavy earthenware trough, and bedding consisted of oat straw. The piglets were given no solid food till they were five weeks old, but for at least seven days before had been observed nibbling at any food left by the sow. These piglets thus were maintained under conditions which from a perusal of the literature were considered adequate to create an iron-deficient state.

The offspring of one large white sow which had been kept in a concrete

pen for several months, were used in this experiment. This sow (K3587) farrowed eleven piglets, of which one was dead. The remaining 10 were divided into 3 groups. No selection bias was used in allocating the pigs to any group, either with regard to sex, which from previous experience was not considered to be important, or body weight.

Four pigs were placed, on the third post-natal day, on the iron-poor semi-synthetic diet. These were numbered K1 to K4. Four others (K7 - 10) were allowed to suckle as described and the remaining two (K5 & 6) were placed on commercial food used for rearing pigs artificially, with an adequate iron-content, of about 20 mg per 100 g. Unfortunately, one of the latter two died shortly after being placed on the diet, and while the results for the remaining pig are recorded, no cognisance was taken of them in recording means or significances. The object of the experiment was thus two-fold. First to compare the haematology and clinical features of naturally reared piglets with those artificially reared under conditions favourable to the production of nutritional iron-deficiency anaemia and less important, to check how pigs reared on a diet containing adequate iron compared with those in either of the first two groups.

It will be noted that for the first two weeks of the experiment few data were recorded for the piglets reared by the sow ("naturally-reared group"). This omission arose from the difficulty in collecting the piglets from the ark during the first period, an attempt to reduce the volume of work and mainly a belief that, while it would be of interest, no vital changes were likely to occur during the initial two post-natal weeks.

Haematological examinations were made on piglets K1 and K3 over thirteen weeks to follow the changes which occurred during a prolonged period.

HAEMATOLOGICAL RESULTS.

Except where otherwise stated group mean values for the blood parameters are given with their appropriate standard deviations.

Haemoglobin.

The Hb values recorded for the pigs in the experiment are given in the following tables, divided, as explained, into the three groups, viz. the group reared artificially on an iron-poor diet; those which were suckled by the sow and the single pig reared using artificial methods on a diet containing sufficient iron.

TABLE 3 A. Hb values for the piglets reared on a diet with a low content of iron (gm%).

Weeks.	Piglet.			
	K1	K2	K3	K4
1	8.2	8.2	8.7	7.2
2	8.0	7.6	8.9	7.2
3	7.0	7.2	8.2	6.3
4	7.0	5.1 4.7	8.2	4.9 4.4
5	6.3	4.2 3.8	6.3 7.2	4.7 4.2
6	6.3	3.2	7.4	4.6
7	4.9	3.2	5.9	-
8	-	-	6.8	-
9	5.1	-	6.5	-
10	4.9	-	5.9	-
11	4.8 5.7	-	6.3 7.2	-
12	4.2	-	6.8	-
13	5.0	-	7.8	-

TABLE 3 B. Hb.values for the piglets reared by the sow and that reared by the artificial methods on a diet containing adequate iron (K6) (gm%).

Week	Piglet				Piglet
	K7	K8	K9	K10	K6
1	-	-	-	-	8.4
3	9.1	6.1	7.8	8.0	11.2
4	11.2	9.8	11.0	11.2	-
5	11.2	10.1	10.3	-	10.8
6	-	11.0	10.7	10.7	11.0
7	-	-	-	-	11.2

The means for the two main groups viz. artificially reared (iron-poor diet) and naturally reared are given in Table 3 C.

TABLE 3 C. Group mean Hb values (gm %).

Week	Artificially Reared Group		Naturally Reared Group (IronPoor)	
	Mean	S.E.	Mean	S.E.
1	8.07 ± 0.62	± 0.31	-	-
2	7.92 ± 0.76	± 0.38	-	-
3	7.17 ± 0.67	± 0.33	7.75 ± 1.23	± 0.61
4	5.71 ± 1.52	± 0.62	10.80 ± 0.67	± 0.33
5	5.24 ± 1.33	± 0.50	10.53 ± 0.58	± 0.33
6	5.37 ± 1.85	± 0.92	10.80 ± 0.17	± 0.10
7	4.66 ± 1.37	± 0.79	---	-

A graph of the Hb values is given in Fig. 3 A.

It is of great interest to see the marked divergence of the levels beyond the third week, after the close similarity at week three. Regrettably the values for the group maintained on the sow have not been recorded during week one and two but it would appear that they were as deficient in Hb as the group on the iron-poor diet. After the third week the rise in Hb of the piglets with the sow could be explained by the ability of the now active piglets to supplement their diet of milk with any extraneous material and thus presumably ingest a sufficient quantity of iron to allow normal erythropoiesis. In contrast the Hb values of the group held on the iron-poor diet showed a continued decline.

No significant difference existed between the group means at the third week but by the fourth week, and subsequent weeks recorded, the difference became highly significant, ($P \leqslant 0.01$).

Haematocrit Values.

Table 3 D. gives the mean haematocrit value for the individuals in experiment 3 and Table 3 E the group means.

FIG 3A

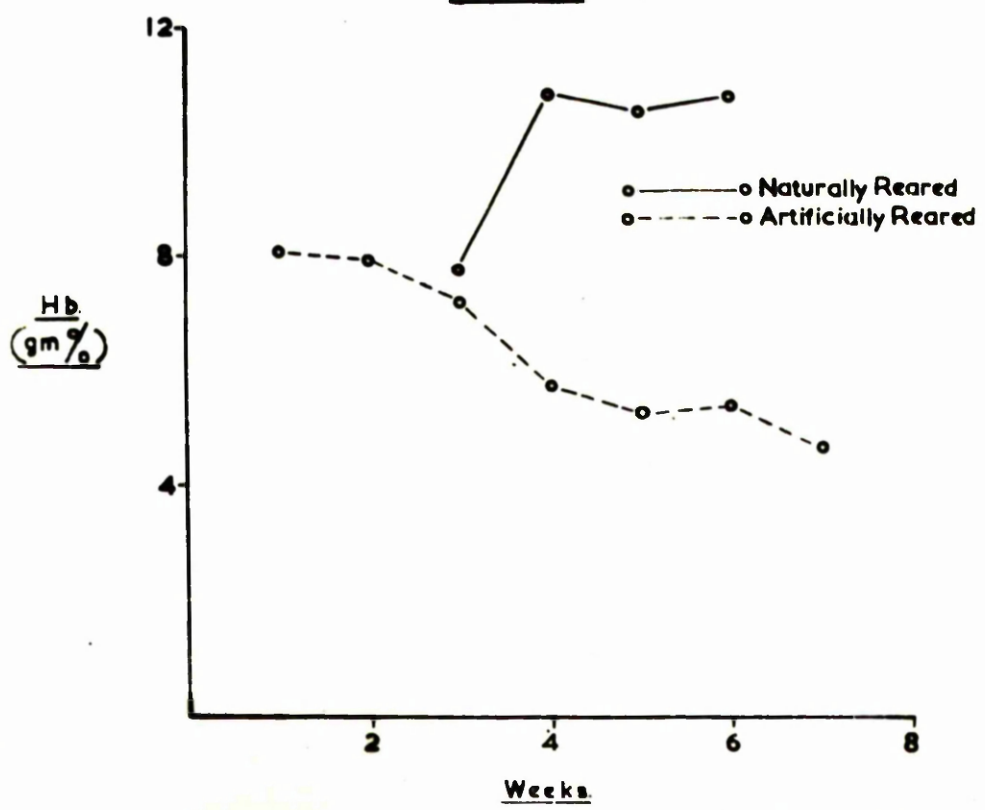

12
8
4
Hb.
(gm%)
Naturally Reared
Artificially Reared
2
4
6
8
Weeks.

TABLE 3 D. Individual Mean Weekly P.C.Vs (ml per 100ml).

Week	Artificially Reared (Iron-Poor)				Naturally Reared				Art. Reared.
	Piglet Number				Piglet Number				Pig No.
	K1	K2	K3	K4	K7	K8	K9	K10	K6
1	27	25	30	23	-	-	-	-	26
2	27	25	30	25	-	-	-	-	-
3	22.5	23	28	25	32	24	29	29	40
4	24	16.5	27	16.5	40	33	38	39	-
5	23	13.5	23.5	16	43	48	41	-	39
6	23	11	28	16	-	39	38	37	-
7	13	12	22.5	-	-	-	-	-	40
8	-	-	21	-	-	-	-	-	-
9	18	-	22	-	-	-	-	-	-
10	20	-	22	-	-	-	-	-	-
11	21.5	-	26.5	-	-	-	-	-	-
12	15	-	24	-	-	-	-	-	-
13	24	-	28	-	-	-	-	-	-

TABLE 3E Group Mean P.C.V (ml per 100ml).

Week	Artificially-reared Group	Naturally - reared Group
1	26.25 ± 2.98	-
2	26.75 ± 2.36	-
3	24.62 ± 2.49	28.50 ± 3.31
4	21.0 ± 5.33	37.50 ± 3.10
5	19.0 ± 5.02	44.0 ± 3.60
6	19.50 ± 7.50	38.0 ± 1.0
7	15.83 ± 5.79	-

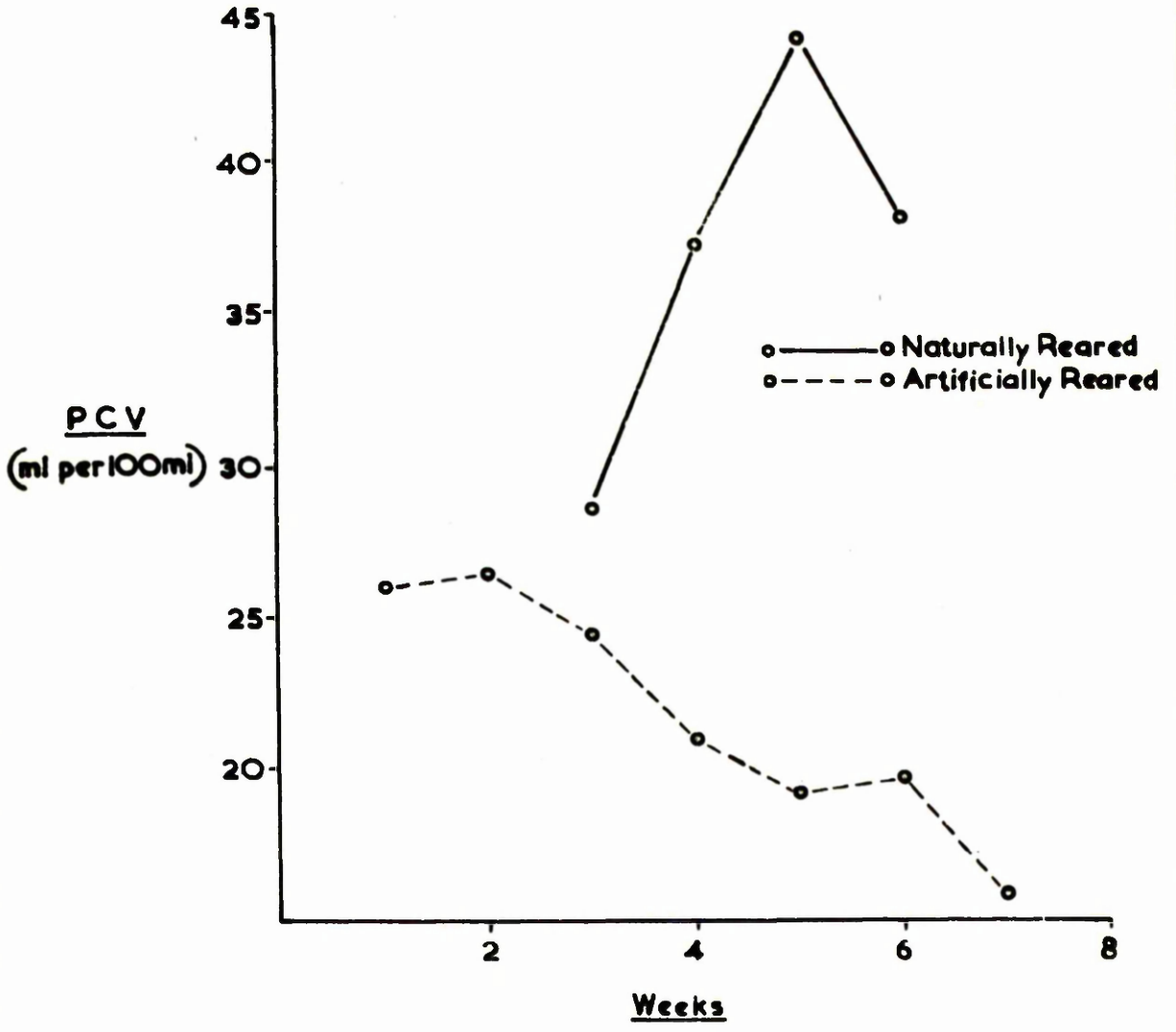
Figure 3B illustrates Table 3 E.

At each week (except the third week) in which the volume of packed red cells was recorded, and the sample means evaluated, there was a highly significant difference between the means of the group samples ($P < 0.01$).

It is likely that until the fourth week any interference with normal erythropoiesis was not more apparent in one group than the other, but after this period the gap widened presumably because the artificially reared pigs continued short of iron whereas those on the sow were able to supplement their diet.

The lone piglet reared artificially but which received iron supplements showed normal values for the P.C.V (& Hb) and thus would not appear to have suffered from iron deficiency. At the third week the haematocrit values for those on the sow were lower than for this single piglet but subsequently there seemed little difference.

FIG 3B


45
40
35
30
25
20
PCV
(ml per 100ml)
2
4
6
8
Weeks
Naturally Reared
Artificially Reared

Erythrocyte Counts.

Figures for the mean weekly erythrocyte counts are given in Table 3 F and for the group samples in 3 G.

TABLE 3 F Mean weekly erythrocyte counts (RBCs x 10^6 per c.mm) for the individual pigs.

Week	Artificially Reared (Fe-ive)				Naturally Reared				Art. Reared (Fe+ive)
	Piglet No.				Piglet No.				Piglet No.
	K1	K2	K3	K4	K7	K8	K9	K10	K6
1	3.8	3.8	4.9	3.8	-	-	-	-	5.2
2	5.4	4.8	5.0	4.0	-	-	-	-	-
3	5.2	3.9	4.3	3.3	4.2	2.8	3.9	4.2	8.1
4	4.5	3.9	5.0	4.2	6.2	5.1	5.9	5.8	-
5	4.2	3.2	4.7	3.8	6.7	5.2	5.5	-	5.3
6	5.6	2.3	6.2	3.6	-	-	-	-	-
7	4.7	3.6	5.8	-	-	-	-	-	8.05
8	-	-	3.9	-	-	-	-	-	-
9	4.4	-	4.9	-	-	-	-	-	-
10	6.7	-	5.9	-	-	-	-	-	-
11	4.3	-	6.1	-	-	-	-	-	-
12	5.1	-	6.2	-	-	-	-	-	-
13	5.2	-	6.8	-	-	-	-	-	-

TABLE 3 G Group mean erythrocyte counts.(RBCs x 10^6 c.mm).

Week	Artificially-Reared (Fe-ive)Group	Naturally-Reared Group
1	4.07 ± 0.55	-
2	4.80 ± 0.58	-
3	4.17 ± 1.14	3.77 ± 0.66
4	4.40 ± 0.46	5.75 ± 0.46
5	3.97 ± 0.63	5.80 ± 0.79
6	4.42 ± 1.80	-
7	4.70 ± 1.10	-

At the 5% level of probability there existed a significant difference between the group sample means for the fifth week.

Red Cell Indices.

Mean Corpuscular Volume.

TABLE 3 H. Group mean M.C.Vs & S.Ds. (μ^3).

Week	Artificially-Reared(Fe-ive)Group	Naturally-Reared Group
1	64.60 ± 4.30	-
2	56.10 ± 6.05	-
3	59.85 ± 13.20	76.37 ± 6.96
4	45.40 ± 6.99	66.02 ± 1.65
5	46.30 ± 5.68	76.96 ± 14.20
6	44.57 ± 2.79	-
7	33.20 ± 5.54	-

A reduction in the volume of the red cells of the piglets reared on the iron-poor diet can be seen to have occurred gradually over the experimental period.

At week three the difference between the group means was only significant ($P < 0.05 > 0.01$) but at the fourth and fifth weeks the differences were highly significant. ($P < 0.01$).

The Mean Corpuscular Haemoglobin Concentration.

The mean concentrations of corpuscular haemoglobin expressed as group means are recorded in Table 3 I.

TABLE 3 I. M.C.H.C. Group means (%).

Week	Artificially-Reared Group (Fe-ive)	Naturally-Reared Group
1	30.85 ± 1.60	-
2	29.57 ± 0.65	-
3	29.20 ± 2.82	27.0 ± 1.25
4	29.18 ± 1.75	28.80 ± 0.65
5	28.50 ± 1.96	24.03 ± 2.66
6	27.92 ± 1.10	28.40 ± 0.43
7	30.13 ± 6.47	-

The greatest difference in the group means for the M.C.H.Cs appeared to be at week five. At no other week, however, did the difference between the group means appear to be significant.

The belief expressed earlier that the M.C.H.C does not readily reflect the degree of iron deficiency anaemia in pigs still appeared justified. The lowest value recorded being that of the group reared on the sow at the fifth week, at which time these piglets were unlikely to be suffering from any appreciable deficiency of iron (q.v. Hb level). The S.D. at this week was lrgely due to a low value for one pig.

The Mean Corpusculer Haemoglobin.

The Table 3 J gives the M.C.H for each group at the critical weeks of the experiment as obtained from the individual values for Hb and R.B.Cs.

TABLE 3 J. Group Mean M.C.H.indices. (γ γ).

Week	Artificially - Reared Group (Fe-ive)	Naturally - Reared Group.
1	19.90 ± 1.91	-
2	16.60 ± 1.55	-
3	17.47 ± 2.73	21.82 ± 2.96
4	13.31 ± 2.51	18.77 ± 0.60
5	13.17 ± 1.58	19.02 ± 1.40
6	12.67 ± 1.15	-
7	9.80 ± 0.87	-

The M.C.H indices for the groups which received iron, in Experiments 1 & 2, were around 20 γ.γ. in this experiment the indices for the group reared naturally

remained about this level throughout. The other group, however, showed a drop in the weight of Hb in the average cell to a level which was about 5 - 10 micro-micrograms lower and which corresponded with those noted in the other experiments for the group which received inadequate iron.

Reticulocyte counts.

The mean reticulocyte counts for the groups are given in Table 3 K ; where only one count was available at any week, this is recorded without a deviation.

TABLE 3 K. Group Mean and Individual Reticulocyte counts (% of erythrocytes).

Week	Artificially-Reared Group (Fe-ive)	Naturally - Reared Group
3	6.32 ± 2.19	7.70 ± 1.70
4	7.30 ± 0.42	6.07 ± 2.34
5	7.54 ± 1.39	3.40 ± 1.04
6	7.90	-
7	7.0 ± 0.28	-
8	6.40	-
10	6.70	-
11	6.10 ± 0.22	-

Though the difference in the reticulocyte counts between the groups, at week 5 was significant ($P < 0.01$) it is not necessarily important. All the counts for the group reared on the semi-synthetic diet do seem somewhat high however. This trend will be considered in the light of the other experiments.

Since the pigs of the naturally-reared group all showed an increase of Hb between the third and fourth week it is somewhat surprising that this was not reflected in grossly increased reticulocyte counts at week 3. When the individual counts for this group were examined however it was apparent that only K 10 failed to show a reticulocytosis of more than 7% at week 3 or 4 and by the fifth week the counts were down to between 2 & 4 % of the red cells.

Cell Morphology.

At the third week the red cells of both the naturally reared and the main artificially reared group showed little morphological difference and exhibited microcytosis, with usually a minority of the cells fully haemoglobinised and from half to most of the cells of the pessary type, with an occasional target cell. Smears of the blood from K 10 at this time showed only a few cells with ring staining. After this the difference between the cells of the individuals of these groups became marked. K7 to 10 improved noticably in the succeeding weeks whereas those on the iron-poor diet deteriorated (vide illustrations).

At the fourth week smears from K 4 contained numerous white cells and the total white cell count at this week was 24.2 x 10^3. Large numbers of platelets were present too in smears of the blood from K2 and K4 at the fourth week. Cartwright et al (1944) noted an increase in the platelets in iron deficiency.

Howell -Jolly bodies and normoblasts were seen in many smears.

Osmotic Fragility of the Red-Cells.

Conforming with the method of expressing these results outlined in Experiment 2 the figures quoted in Table 3L and 3M indicate the concentration

TABLE 3L Osmotic fragility (% x concentration of salt).

Week	Artificially - Reared				Naturally - Reared			
	Piglet Number				Piglet Number			
	K1	K2	K3	K4	K7	K8	K9	K10
2	0.65/ 0.30	0.65/ 0.35	0.70/ 0.35	0.55/ 0.30	-	-	-	-
4	0.65/ 0.35	0.65/ 0.35 0.60/ 0.30	0.70/ 0.35 0.55/ 0.25	0.60/ 0.30 0.55/ 0.25	0.75/ 0.40	0.80/ 0.45	0.65/ 0.40	0.70/ 0.35
5	0.60/ 0.30	0.55/ 0.25	0.55/ 0.25	0.50/ 0.30	0.75/ 0.45	0.75/ 0.45	0.80/ 0.45	-
6	0.60/ 0.30	0.50/ 0.25	0.65/ 0.35	0.55/ 0.30	-	-	-	-
9	0.65/ 0.25	-	0.60/ 0.30	-	-	-	-	-
11	0.55/ 0.25	-	0.65/ 0.25	-	-	-	-	-
12	0.55/ 0.25	-	0.60/ 0.25	-	-	-	-	-
13	0.55/ 0.25	-	0.06/ 0.25	-	-	-	-	-

of salt at which the start/completion of haemolysis was observed. At the fourth week two estimations were made of the fragility of the cells from some pigs.

While it would be impossible to draw any firm conclusions from the figures for the comparative fragility of the red cells of the groups it might be inferred that the anaemic pigs showed a decreased fragility, as has been noted in iron-deficiency anaemia in man (Whitby & Britton 1957).

White Cells.

Total and differential counts are given in table 3 M & 3 N.

TABLE 3 M. Total White Cell Counts (x 10^3 per c.mm.)

Week	Artificially Reared (Iron-Poor)	Naturally Reared
1	8.90 ± 1.51	- -
2	10.55 ± 0.78	- -
3	10.47 ± 1.06	14.47 ± 3.49
4	13.02 ± 4.60	12.35 ± 0.81
5	12.02 ± 1.14	10.33 ± 0.81
6	12.97 ± 2.82	- -
7	14.35 ± 6.82	- -

TABLE 3 M. Mean Differential White Cell Count (%) for Artificially Reared Group.

Type of Cell	Weeks				
	1	2	4	5	7
Band	2.0 ± 1.0	1.0	2.0 ± 1.41	2.25 ± 1.04	1.0
Poly.	93.5 ± 7.38	35.5 ± 2.23	59.5 ± 6.40	38.0 ± 20.5	19.5 ± 6.40
Lymph	33.3 ± 10.1	60.0 ± 1.41	37.0 ± 9.89	55.5 ± 16.80	76.5 ± 5.0
Monos	2.33 ± 2.31	3.5 ± 0.70	1.5	4.12 ± 1.54	3.0 ± 2.82

One differential white cell count was made on blood from the piglets reared naturally with the following group mean results at the fifth week:- Polymorphonuclear leucos. 17.66 ± 4.06%: Lymphocytes .....79.0 ± 5.29%: Eosinophils .....0.6% Monocytes .....1.3 ± 0.70%:

At week 4 the mean percentage of polymorphs. and lymphocytes for the artificially reared group appeared to show a reversal of the ratio seen at other weeks. An excess of neutrophils can be expected for a week or so after birth,(Gardiner et al 1953) so that the values at week 1 were not considered unusual; other figures seem quite normal.

Serum - Iron Estimations.

Values obtained for the iron contents of the sera of the pigs are recorded in Table 3 0.

TABLE 3 0. Serum - iron estimations (gm%).

Week	Artificially - Reared Group				Naturally - Reared Group			
	Piglet Number				Piglet Number			
	K1	K2	K3	K4	K7	K8	K9	K10
3	-	72.8	-	44.8	361.6	556.0	194.8	53.6
6	83.6	106.8	94.0	64.0	-	-	-	-
7	-	72.8	-	-	-	-	-	-
9	62.0	-	90.0	-	-	-	-	-

It is of interest to observe the difference between the pigs of each group and with the exception of the value for K10 the reasonable correlation within the groups. Secondly it should be noted that while the Hb values showed no significant difference between the groups by week 3, the difference in the serum-iron levels was remarkable.

The mean value and S.D. for all the estimation in each group are :-

Artificially - reared group 76.66 ± 19.30 µgm%(S.E. ± 6.43)

Naturally-reared group 291.50 ± 216.8 " (S.E. ±103.4)

A wide range of deviation in the serum-iron content of human blood is well

recognised apparently in relation to time of day, meals or age (Whitby & Britton 1957). Thus Wintrobe (1956) gave a standard deviation of ± 32.8 for a mean of 104.7 µgm per cent.

Red - Cell Distribution Curve.

Diameter measurements were made of the red cells from two of the piglets reared on the semi - synthetic diet, with low content of iron (K1 & K4), one piglet on the diet with a good content of iron (K6) and one reared naturally(K7).

The curves of red cell distribution are given in the accompanying figures, 3C and 3 D, and these indicate the extent of microcytoses occurring at the weeks other than the first, for the piglets reared on the iron - poor diet.

The extent of the microcytoses of the R.B.Cs of K1 & K4, compared with those of K7 at the fourth week are given in Table 3 P.

FIG 3c

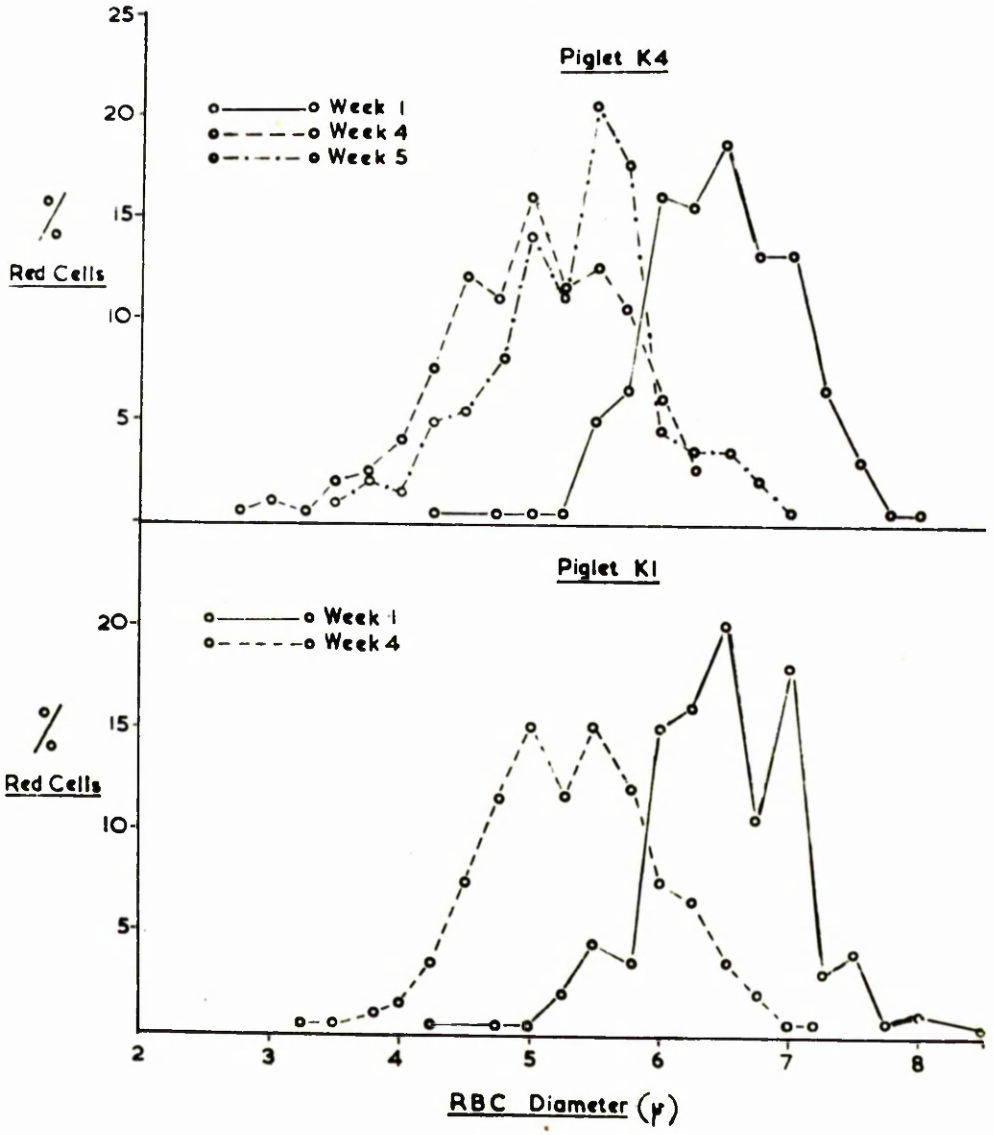

Piglet K4
Week 1
Week 4
Week 5
% Red Cells
25
20
15
10
5
Piglet K1
Week 1
Week 4
% Red Cells
20
15
10
5
2
3
4
5
6
7
8
RBC Diameter (μ)

FIG 3D

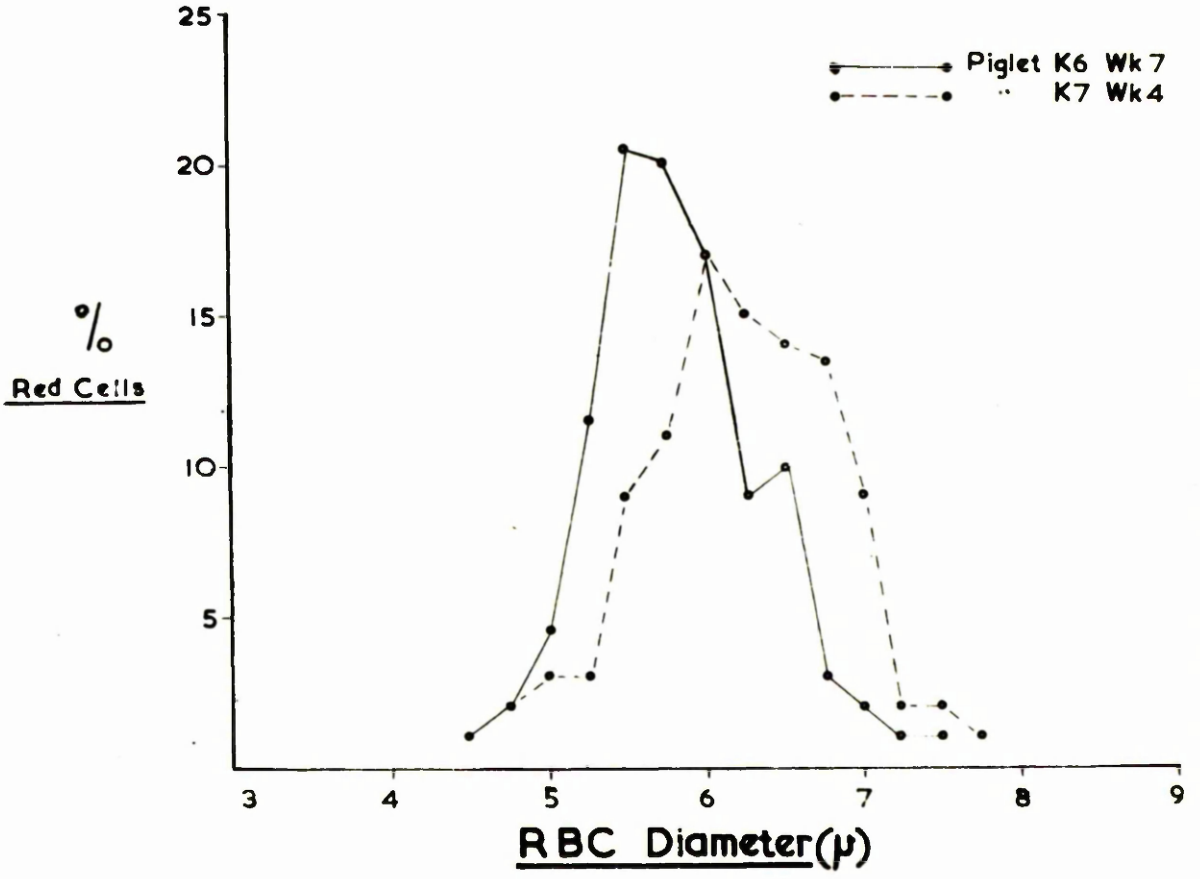

25
20
15
10
5
%
Red Cells
Piglet K6 Wk 7
K7 Wk 4
3
4
5
6
7
8
9
RBC Diameter(μ)

TABLE 3 P. Percentage of microcytosis at week 4.

Mid-point of Class Interval (0.25/u)	K7 % cells	K1 % cells	K4 % cells
6.0	17.0	7.5	6.0
5.75	11.0	12.0	10.5
5.5	9.0	15.0	12.5
5.25	3.0	11.5	11.5
5.0	3.0	15.0	16.0
4.75	0	11.5	11.0
4.5	1.0	7.5	12.0
4.25	0	3.5	7.5
4.0	0	1.5	4.0
3.75	0	1.0	2.5
3.5	0	0.5	2.0
3.25	0	0.5	0.5
3.0	0	0	1.0
Total 1	44.0	87.0	97.0

Percentage of microcytosis :-

K 1 = 87.0 - 44.0 = 43.0%

K 6 = 97.0 - 44.0 = 53.0%

PATHOLOGICAL DETAILS.

Two piglets died, both of which belonged to the group on the iron-poor semi-synthetic diet. Their numbers were K2 & K4.

Piglet K2 died when 7 weeks of age. The last Hb value noted prior to death was 3.2 gm%. Autopsy showed evidence of acute pleurisy of the left side of the thorax. No other abnormalities were noted. The unusual occurrence of two cases of unilateral pleurisy in two experiments (piglet number 16, Experiment 2 & K2, Experiment 3) was considered due to the method of withdrawing blood. Frequent withdrawals may have caused trauma, or predisposed to infection.

A diagnosis of chronic paratyphoid - type ulceration was made with piglet $K4_3$ which died when $6\frac{1}{2}$ weeks old. Sections of the liver showed centrolobular fatty change progressing to centrolobular necrosis. Pneumonia was not present in either pig.

The iron contents of the liver and spleen of K2 were evaluated viz:-

Spleen - 49.1 mgm/100 gm dry tissue

Liver - (7.41 " " " " "
(1.99 " " " wet "

CLINICAL SIGNS AND MORTALITY.

Few clinical signs were noted in the group reared on the sow. When about 3 weeks of age each piglet was noticed to have diarrhoea which cleared within a few days. Later the piglets were observed to have an occasional short cough. A combination of these signs, together with the knowledge that the sow had been in contact frequently with pigs affected with pneumonia, created the suspicion that they were due to infection from the sow with so-called virus pneumonia (Betts 1952). Neverthless these piglets gained weight rapidly and outstripped the artificially reared group which although unaffected with pneumonia had intermittent bouts of scouring and the two which died K2 & K4, within the two weeks prior to death, excreted almost continuous soft pale foetid stools. It may be reasoned that the anaemia was not the only reasnn for the necrotic enteric condition, since the other two pigs K1 & K3 were also anaemic. However, at the time of onset of the diarrhoea the Hb levels of K2 & K4 appeared to drop. It is not possible to say catagorically that the anaemia produced favourable conditions for secondary organisms to cause the enteric condition or whether this latter trouble influenced the severity of the anaemia. Since it is recognised that pigs reared artificially are liable to have digestive disorders (Bellis 1957) it might be more reasonable to hypothesise that the anaemia was not directly associated with the bowel ulceration, but may have influenced the failure of the pigs to recover from the enteritis.

Only one pig on the semi-synthetic diet made a weight gain which compared favourably with any of those on the sow, The piglet reared artificially but

with iron in the diet also failed to reach a satisfactory weight, being only 11 lbs. when five weeks old.

The group mean weights were:-

Naturally - reared : at 3 weeks 12.1 lbs at 6 wks 28.5 lbs.

Artificially - " : " 3 " 6.7 lbs. " " " 12.8 lbs.
(without iron)

ARTIFICIAL REARING EXPERIMENT 4.

Introduction.

The influence of cold environment on the health and survival of piglets is well recognised (Shanks 1942: Inglis & Robertson 1949: Lamont 1951: Lucas 1954) and its association with changes leading to anaemia has not been overlooked (Naftalin & Howie 1949). It was decided therefore to test the influence of cold environment on the course and severity of iron-deficiency.

The experiment was conducted during October and November when the weather was undoubtedly cold. The environmental temperatures varied from 39 - 53 °F for the group kept in the cold environment and 63 - 67 °F for that maintained under warm conditions. The group held in a warm environment were kept in cages in a warm loose box as in earlier experiments. Those kept at the lower temperature were housed individually in large galvanised wire rabbit cages placed in the feeding passage of a byre, where draughts and low temperatures were usual. Table 4 A gives the allocation of the piglets.

The sow had been housed on concrete and fed a commercially prepared diet for over one year.

The group known as the control group received a diet containing 27.01 mgm iron per 100 gm diet. The experimental group was maintained on a diet containing merely 2.42 mgm iron per 100 gm. The composition of the diets has been described earlier.

TABLE 4 A. Allocation of Piglets in Experiment 4.

Piglet No.	Sex.	Iron cont. of diet (mgm/100gm)	Environmental Temperature
0	F	2.42	Warm
1	M	2.42	Warm
2	M	27.01	Warm
3	M	27.01	Warm
4	M	27.01	Cold
5	M	2.42	Cold
6	M	27.01	Cold
7	F	2.42	Cold

Haematological Results.

Haemoglobin.

Table 4 B gives the individual Hb readings for the pigs of both groups, in the respective environments, and Table 4 C the group mean values.

TABLE 4 B. Haemoglobin values for the individual piglets (gm%).

Group	Piglet No.	Week 1	2	3	4	5	6	7	8	9	10
Control	2	7.8	6.3 6.1	5.5 6.3	5.9 5.9	7.8 9.7	9.8 10.0	9.3	- -	- -	- -
(Warm)	3	-	6.2 6.8	6.3 5.7	5.3 5.5	6.8 6.0	6.5 8.3	7.3	-	-	-
Control	4	-	6.6 6.8	6.5 6.8	5.1 5.9	7.2 7.6	10.4	9.6	10.5	-	-
(Cold)	6	-	6.1 6.8	5.5 6.8	5.7 6.2	6.6 7.6	8.7	8.9	9.8	-	-
Exp.	0	7.8	6.9 7.6	6.6	6.1 6.8	9.1 9.6	8.7	8.7	-	-	8.3
(Warm)	1	7.8	5.5 5.9	5.3	6.8 5.7	7.0 8.0	8.7	7.8	8.1	-	8.7
Exp.	5	-	6.7 6.3	5.7 6.3	5.5 6.8	8.3 8.4	8.7	8.1	7.6	-	9.6
(Cold)	7		6.6 6.8	6.1	5.1 6.0	5.5 5.3	6.5	6.9	8.5		8.9

TABLE 4 C. Group Mean Hb. values (gm%).

Group	Week	Warm Environment		Cold Environment	
		Mean	S.E.	Mean	S.E.
Control	1	-	-	-	-
	2	6.25 ± 0.31	± 0.15	6.57 ± 0.33	± 0.16
	3	5.95 ± 0.41	± 0.20	6.40 -	-
	4	5.65 ± 0.30	± 0.15	5.72 ± 0.46	± 0.23
	5	7.57 ± 1.59	± 0.79	7.25 ± 0.47	± 0.23
	6	8.65 ± 1.62	± 0.81	9.55 ± 1.20	± 0.85
	7	8.30 ± 1.41	± 1.0	9.25 ± 0.49	± 0.34
	8	-		10.15 ± 0.49	± 0.34
Experimental	1	7.80 -	-	- -	-
	2	6.47 ± 0.95	± 0.47	6.60 ± 0.21	± 0.10
	3	5.95 ± 0.91	± 0.65	6.03 ± 0.30	± 0.17
	4	6.35 ± 0.54	± 0.27	5.85 ± 0.73	± 0.36
	5	8.42 ± 1.16	± 0.58	6.87 ± 1.70	± 0.85
	6	8.70 ± -	-	7.60 ± 1.55	± 1.09
	7	8.25 ± 0.64	± 0.45	7.50 ± 0.84	± 0.59
	8	8.10 ± -	-	8.05 ± 0.64	± 0.45
	10	8.50 ± 0.28	± 0.19	9.25 ± 0.50	± 0.35

By inspection a significant difference did not appear to exist either between the means of the control groups reared in the different environments, or between the mean Hb values for the two experimental groups.

Comparing now the means for the two groups reared in the warm environment: at no week was the difference between the group means of significance ($P < 0.1 > 0.05$), nor between the two lots of pigs kept in the cold environment. Also at no time did a significant difference (i.e. $P = \text{or} < 0.05$) exist between the means for the combined control and combined experimental groups, discounting environmental temperature.

The Hb levels recorded for all pigs were low until week five when some improvement occurred. None of the piglets appeared to start their experimental life with Hb levels which could be considered within normal limits, and even in those on inadequate iron levels, slight recovery occurred. These matters will be considered in the discussion.

Haematocrit Values. The P.C.Vs for the individual pigs (or the mean values where more than one was obtained) for each week are recorded in table 4 D, and table 4 E gives the group mean values.

No significant difference could be shown either between the group means of the two control groups or between the means for the two experimental groups. Thirdly the differences between the control groups and the experimental group reared in the warm environment was without significance. While similar analysis of the control and experimental groups reared in the cold was equally unrewarding. As with the Hb findings the haematocrit values for all pigs were low. Some recovery occurred after the fourth week.

TABLE 4 D. Mean weekly P.C.V (ml per 100 ml) for the individual piglets.

Group	Piglet No.	Week 1	2	3	4	5	6	7	8	9	10
Control	2	24	20.5	20.5	20.5	32	35.5	32	-	-	-
(Warm)	3	22	20.5	19	21.5	27.5	29	-	-	-	-
Control	4	-	22	23	20	28.5	36	35	39	-	-
(Cold)	6	-	22.5	21.5	21	24.5	30	32	35	-	-
Exp.	0	25	23.7	22	23	32	28	28	-	-	29
(Warm)	1	24	18.5	18	19	25.5	30	28	28	-	29
Exp.	5	-	22.5	22.5	22	30	31	31	26	-	34
(Cold)	7	-	21.5	20	19	19.5	25	25	30	-	31

TABLE 4 E. Group mean haematocrit values (ml per 100 ml).

Week	Warm Environment		Cold Environment	
	Control Group	Exp. Group.	Control Group	Exp. Group.
1	-	24.50 ± 0.70	- -	-
2	21.25 ± 1.06	21.20 ± 3.67	22.25 ± 0.35	22.0 ± 0.70
3	20.50 ± 0.0	20.0 ± 2.82	22.25 ± 1.06	21.25 ± 1.76
4	19.75 ± 1.06	21.0 ± 2.82	20.50 ± 0.70	20.50 ± 2.12
5	26.75 ± 7.40	28.75 ± 4.59	26.50 ± 2.82	24.75 ± 7.40
6	31.50 ± 5.65	29.0 ± 1.41	33.0 ± 4.24	28.0 ± 4.24
7	3 0.50 ± 2.12	28.0 ± 0.0	33.50 ± 2.12	28.0 ± 4.24
8	-	-	37.0 ± 2.40	28.0 ± 2.82
9	-	-	-	-
10	-	29.0 ± 0.0	-	32.5 ± 2.20

Erythrocyte Counts.

The accompanying tables(4 F and 4 G)give the mean weekly red cell counts for the individual pigs for the groups.

TABLE 4 F Mean weekly R.B.Cs ($\times 10^6$ per cmm) for the control piglets.

Group	Piglet No.	Week 1	2	3	4	5	6	7	8	9	10
Control	2	3.6	3.7	3.6	4.3	5.2	7.2	6.7	-	-	-
(Warm)	3	-	4.0	4.7	4.2	4.3	5.5	5.0	-	-	-
Control	4	-	3.8	3.7	4.9	5.9	6.9	6.9	7.2	-	-
(Cold)	6	-	4.6	3.4	4.6	5.2	6.6	6.5	6.9		
Exp.	0	4.5	4.7	3.5	4.3	5.8	5.1	6.8	-	-	6.8
(Warm)	1	3.7	3.3	2.8	3.7	3.9	4.7	5.4	5.9	-	7.5
Exp.	5	-	4.4	4.0	4.1	6.4	6.8	6.3	6.2	-	7.7
(Cold)	7	-	4.1	4.2	3.8	4.2	4.8	5.3	6.5	-	5.4

TABLE 4 G. Group mean R.B.Cs (x 10^6 per c.mm).

Week	Warm Environment		Cold Environment	
	Control Group	Exp. Group	Control Group	Exp. Group
1	-	4.10 ± 0.57	-	-
2	3.85 ± 0.22	4.00 ± 0.99	4.20 ± 0.56	4.25 ± 0.22
3	4.15 ± 0.78	3.15 ± 0.50	3.55 ± 0.22	4.10 ± 0.14
4	4.25 ± 0.10	4.00 ± 0.42	4.75 ± 0.22	3.95 ± 0.22
5	4.75 ± 0.64	4.85 ± 1.34	5.60 ± 0.42	5.30 ± 1.55
6	6.35 ± 1.20	4.90 ± 0.28	6.75 ± 0.22	5.80 ± 1.40
7	5.85 ± 1.20	6.10 ± 0.98	6.70 ± 0.28	5.80 ± 0.70
8	-	-	7.05 ± 0.22	6.35 ± 0.22
10	-	7.15 ± 0.50	-	6.55 ± 1.62

The differences between the means of the following at all weeks were without significance ($P > 0.1$) :-

1. The groups kept in warm environment.
2. The groups kept in cold environment.
3. The control group samples.
4. The experimental group samples.

Red Cell Indices.

The Mean Corpuscular Volume.

Table 4 H gives the mean figures for the M.C.V for the control and experimental groups under the different environmental states.

TABLE 4 H: Group mean values for the M.C.Vs. (μ^3).

Week	Warm Environment		Cold Environment.	
	Control Group	Exp. Group	Control Group	Expo Group
1		60.15 ± 6.58	-	- -
2	56.07 ± 7.35	53.57 ± 6.73	53.75 ± 7.51	52.30 ± 6.97
3	50.22 ± 8.47	63.50 ± 0.98	63.07 ± 9.56	52.83 ± 4.82
4	42.17 ± 7.30	54.10 ± 9.92	44.02 ± 4.34	53.82 ± 10.12
5	56.35 ± 8.44	60.80 ± 12.60	47.27 ± 2.17	46.75 ± 0.96
6	49.72 ± 1.46	59.35 ± 6.29	48.75 ± 4.74	48.75 ± 4.50
7	52.85 ± 7.28	46.45 ± 7.56	49.95 ± 1.06	48.15 ± 1.48
8	-	-	52.40 ± 2.40	44.0 ± 2.96
10	-	40.60 ± 2.82	-	50.75 ± 9.35

Inspection of these indices did not indicate any consistent trend and the data did not show obvious significant change to be present according to the treatment. Most values seemed low and approached those for the experimental group of Experiment 1.

Mean Corpuscular Haemoglobin Concentration.

The mean group values for the M.C.H.C are set out in Table 4 I.

All the values appeared low and while no analysis of significance was made, by inspection it would appear that significant differences between the sample means did not exist.

TABLE 4 I. Group mean value for M.C.H.C.(%).

Week	Warm Environment		Cold Environment	
	Control Group	Exp. Group	Control Group	Exp. Group
1	-	31.85 ± 0.86	-	-
2	29.87 ± 1.10	30.70 ± 1.16	29.62 ± 2.37	30.00 ± 1.84
3	29.0 ± 0.68	29.65 ± 0.50	28.72 ± 0.60	28.56 ± 2.30
4	28.60 ± 1.0	30.55 ± 4.90	27.92 ± 1.48	28.50 ± 2.12
5	28.62 ± 2.0	29.25 ± 0.69	27.45 ± 2.10	27.75 ± 1.36
6	27.45 ± 1.05	30.0 ± 1.41	28.90 ± 0.14	27.0 ± 1.41
7	27.05 ± 2.75	29.40 ± 2.26	27.60 ± 0.28	26.80 ± 1.13
8	-	-	27.45 ± 0.78	28.75 ± 0.64
10	-	29.30 ± 0.98	-	28.45 ± 0.36

The Mean Corpuscular Haemoglobin.

The values for M.C.H given in table 4 J were obtained from the group mean figures for Hb & R.B.Cs. All the M.C.H. indices were low and except those for the experimental group, in the warmer conditions, were like those recorded in previous experiments for the pigs maintained on low-iron rations.

TABLE 4 J: M.C.H. Group mean indices (yy).

Week	Warm Environment		Cold Environment	
	Control Group	Exp. Group.	Control Group	Exp. Group.
1	-	19.02	-	-
2	16.20	16.17	15.64	15.52
3	14.36	18.88	18.02	14.70
4	13.29	15.87	12.04	14.81
5	15.93	17.38	12.95	12.69
6	13.62	17.75	14.0	13.10
7	14.23	13.52	13.80	12.93
8	-	-	14.39	12.67
10	-	11.88	-	14.12

Reticulocyte Counts.

Table 4 K gives the mean reticulocyte count as a percentage of the R.B.Cs for the individual pigs.

TABLE 4 K.Mean reticulocyte counts for the individual pigs.(% of erythrocytes).

	Warm Environment Piglets				Cold Environment Piglets			
Week	With Iron		Without Iron		With Iron		Without Iron	
	2	3	0	1	4	6	5	7
1	5.3	-	5.0	4.5	-	-	-	-
2	5.8	-	4.3	5.2	-	5.2	-	4.8
3	6.5	3.2	-	-	5.0	6. 6	6.1	5.8
4	5.1	5.1	5.1	5.3	5.2	5.8	6.4	5.7
5	6.5	-	5.8	4.1	-	-	-	-
6	5.8	4.8	-	-	2.8	7.2	4.6	-
7	6.1	7.4	4.9	5.4	2.2	5.6	4.4	5.2
8	-	-	-	4.2	4.2	4.2	4.5	5.2
10	-	-	2.0	2.1	-	-	1.9	2.3

The reticulocyte counts recorded did not appear to be unusual or different from those recorded in other experiments for piglets which received iron or were reared on an iron-deficient diet, and no significance was attached to them.

Osmotic Fragility of the Red Cells.

The osmotic fragility of the red cells is expressed as the concentration of saline required to initiate or complete haemolysis of the R.B.Cs from the individual pigs. No marked difference appeared to exist between the pigs of the various group. The % concentrations of salt required to effect haemolysis did not vary from those indicated for the iron-deficient piglets of experiment 3, which appeared to show decreased fragility.

TABLE 4 I. Osmotic fragility of the R.B.Cs (% conc. of salt).

Week	Warm Environment Piglets				Cold Environment Piglets			
	With Iron		Without Iron		With Iron		Without Iron	
	2	3	0	1	4	6	5	7
2	0.65/ 0.35	0.65/ 0.30	-	-	0.65/ 0.30	-	0.60/ 0.30	-
3	0.60/ 0.30	0.55/ 0.30	-	0.60/ 0.30	0.66/ 0.30	0.60/ 0.30	"	0.55/ 0.30
4	"	0.60/ 0.30	0.60/ 0.30	"	0.60/ 0.30	"	0.55/ 0.30	"
5	"	0.55/ 0.20	"	-	0.50/ 0.20	0.60/ 0.25	0.55/ 0.25	0.55/ 0.25
6	"	0.55/ 0.25	"	0.60/ 0.30	0.60/ 0.30	0.65/ 0.30	0.60/ 0.30	0.60/ 0.30

Red - Cell Diameters.

Red cells from piglets 1 (experimental group: warm environment) and 2 (control group: warm environment) were measured. Figures 4 A & 4 B record the values graphically at weeks 1, 3, & 7. Both pigs showed some degree of microcytosis which was more marked in piglet 1 which was fed the iron-poor diet. Because of this the percentage of microcytosis was not calculated.

White Cells.

TABLE 4 M. Total White Cell Counts for the Individual Pigs ($x10^3$ per c.mm).

Group	Piglet No.	Weeks 1	2	3	4	5	6	7	8	9
Control Warm	2	4.7	9.8	9.8	10.2	11.4	17.8	16.4	-	-
	3	-	11.8	15.4	10.5	10.5	13.4	14.5	-	-
Control Cold	4	-	9.2	9.2	10.7	11.8	22.8	19.2	16.0	-
	6	-	9.3	10.2	9.0	9.5	15.1	13.2	19.2	-
Exp. Warm	0	7.5	9.4	11.8	9.2	14.9	14.6	12.3	-	-
	1	6.5	9.4	9.0	8.1	8.2	7.9	9.6	-	-
Exp. Cold	5	-	9.7	8.6	8.4	13.0	13.4	14.9	-	9.6
	7	-	11.2	9.1	9.7	14.6	17.4	10.2	8.6	19.7

Most of the piglets showed an increase in the white cells between weeks five and eight which appeared to coincide with an increase in Hb and it might be reasoned that these phenomena suggested rising resistance to some "infection". Differential white cell counts were made at several weeks for all pigs but no unusual ratios were noticed.

FIG. 4A

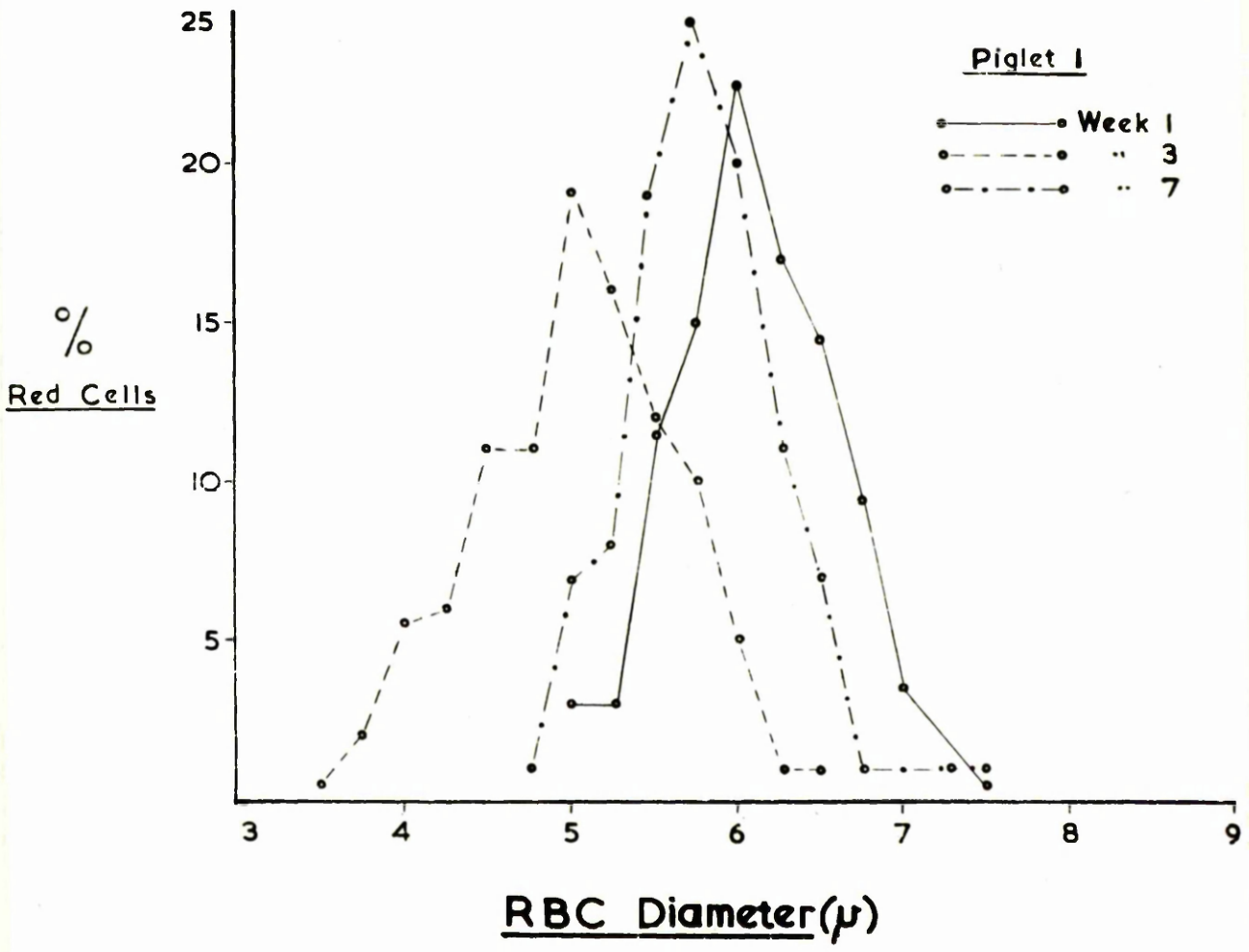
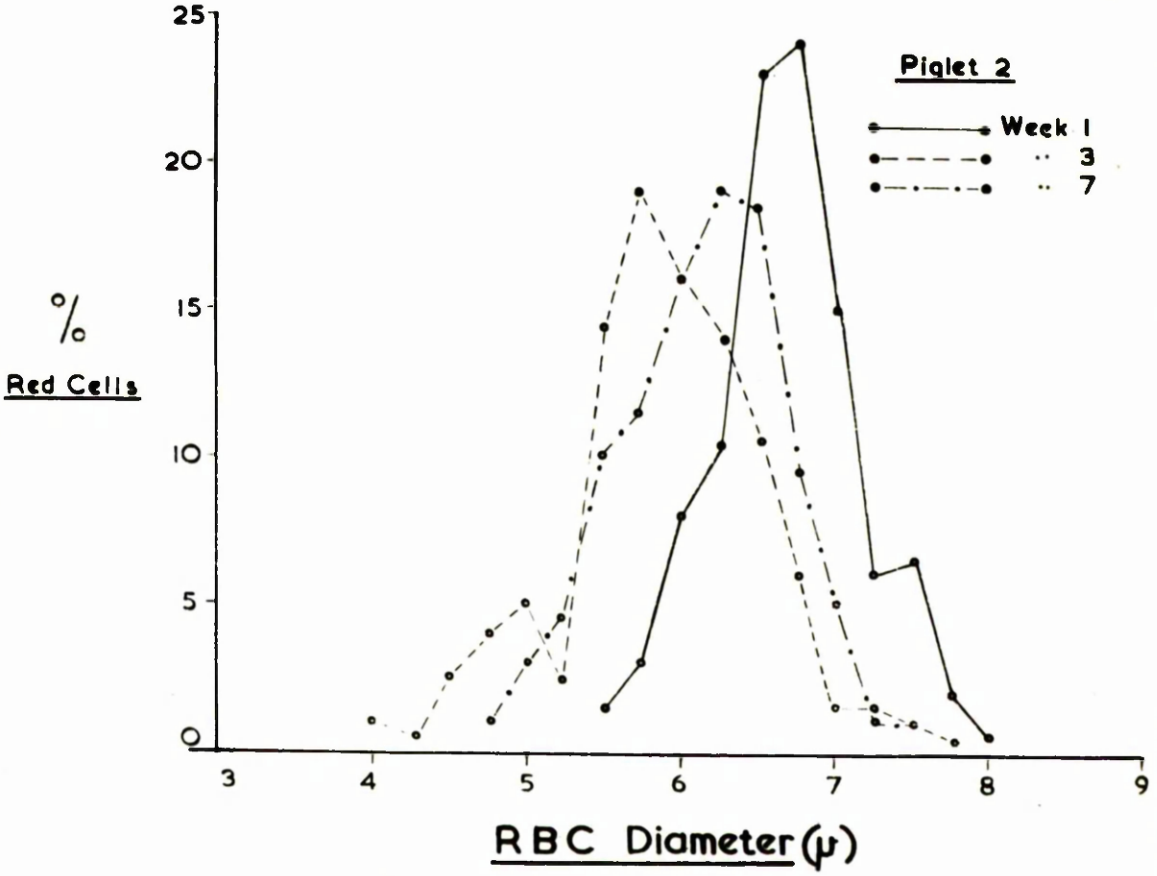

25
20
15
10
5
%
Red Cells
Piglet I
Week 1
" 3
" 7
3
4
5
6
7
8
9
RBC Diameter(μ)

FIG. 4B


Piglet 2
Week 1
3
7
%
Red Cells
25
20
15
10
5
0
3
4
5
6
7
8
9
RBC Diameter (μ)

CLINICAL SIGNS & MORTALITY.

It had been observed that piglets reared in individual cages did not start feeding as readily as those housed with companions. The weight gains however, of those held in the individual cages in the cold environment did not reflect any initial setback. The average body weights at three chronological intervals are given in Table 4 N.

TABLE 4 N. Group mean body weights (lbs) at weeks 2, 4 & 6.

	Warm Environment		Cold Environment	
Week	Control Group	Exp. Group	Control Group	Exp. Group
2	6.0	6.5	6.5	6.0
4	12.0	13.0	10.75	9.25
6	17.12	21.0	17.0	15.0

It is doubtful if much interpretation can be placed on the results of the body weight increases because of the small number of piglets in each group. One piglet (No.7) in the experimental group kept in the cold environment failed to gain weight as readily as the other piglets. Indeed none of the piglets grew well and all tended to be excessively hairy though no other signs of disease were apparent and none died. The conjunctivae of all were pink and no

cough or other indications of lung or heart disease were noticed.

It should be emphasised that these pigs did not grow as rapidly as expected and did not achieve good body weights at six weeks.

Nevertheless while it would seem that some inherent defect may have existed either in the sample of piglets or in the diet neither the temperatures to which these pigs were subjected nor the lack of dietary iron appeared to influence the change.

DISCUSSION.

The results in this experiment do not allow of strict interpretation because of the small group numbers and since no large differences developed in the various blood parameters of the groups.

The Hb values recorded during the first week of life seemed low. They continued to fall and were not checked even in the pigs kept at a suitable environmental temperature and fed iron. Only after several weeks did some recovery occur. Why this fall took place is somewhat enigmatic. Two main possibilities exist . First, a deficiency of some substance existed, more essential or severe than the iron deficiency. Cartwright et al (1944) have claimed that pyrodoxine deficiency in pigs causes an anaemia with features similar to those of iron deficiency, namely a hypochromic microcytic anaemia with marked anisocytosis. These workers noted that in pyrodoxine deficiency the piglets showed impaired growth, curled hair and diarrhoea.

In retrospect it seems possible that this may have been a factor since the yeast used had been first used for Experiment 1 some ten months or so earlier. Other deficiencies such as the protein deficiency described by Köhler (1956) as a cause of anaemia in pigs seem less likely.

The second main cause it may be hypothesised was some inherent defect in the litter, congenital or inherited, which might have been some pre- or post - natal infection which was not apparent.

The low environmental temperature did not influence significantly the anaemia but then perhaps the conditions were not sufficiently cold or wet.

Interpretation of the part played by such factors would be almost impossible when an unknown one existed.

While normally a commendable object to use litter mates for experimental purposes, in this case since some other factor appears to have been present it would seem to have been unfortunate.

This experiment suffered also by having too many treatments for too few pigs. A simpler experiment to confirm the influence of dietary iron on anaemia would have been of more value.

ARTIFICIAL REARING EXPERIMENT 5.

INTRODUCTION.

This was the fifth and final experiment performed using semi-synthetic diets and artificial methods of rearing. The object of this experiment was to confirm the previous observations on iron-deficiency anaemia, namely that a microcytic hypochromic anaemia could be produced on diets low in iron but without the clinical or autopsical features generally attributed to this deficiency. In order to make the results more conclusive larger groups of pigs were used. One other deviation from the methods used in previous experiments was that the piglets were housed on a concrete floor in two completely separate pens one for each group and not in cages. A third change adopted was that both the control and the experimental groups were held on the iron-poor diet initially and only during the fourth week of life were the piglets comprising the control group allowed iron. This variation was introduced to show how an iron-deficiency anaemia could be produced and corrected.

The galvanised metal metabolic cages already described had been found completely satisfactory for housing piglets and appeared to have advantages over other methods, for example by allowing excretions, water and spilled food to drop through the floor the pigs were kept drier than on a more solid floor: also the heat from the infra-red bulb was contained and thus the pigs were freed, almost completely from draughts and chills. It was considered, however, that since the cages were made of metal, albeit galvanised, a possible objection to experiments necessitating the production of iron deficient states could be

raised on these grounds. Despite this consideration Wintrobe et al (1953) had described the use of a similar cage for comparable experiments with pigs.

The pens used in this experiment occupied one half of a loose-box made as draught-free as possible. Each pen measured approximately 6 x 6 feet. Two sides of each were formed by the smooth concrete walls of the loose-box and the others by wooden planking to form solid divisions three feet high. Inside each pen was a small wooden "kennel" 2 ft. wide and 2 ft. high at the sides by a yard long with a ridge roof 3 ft. high from which hung an infra-red bulb. The floor of this was made of wood. The temperature of the loose-box was maintained usually between 60 - 70° F.

The fall of the floor of the pens prevented food or faeces being washed from one pen into the other during the daily cleaning. It was found necessary to put a limited amount of sawdust down in the pens to absorb moisture from the spilled water or gruel as well as this cleansing process.

The food and feeding were as previously described. The ration which was fed to all the pigs during the period of the experiment had on four separate analysis an average iron content of 3.65 mg per 100 gm of food. The control pigs were allowed to become iron deficient, and only at the beginning of the fourth week did they receive iron. This was administered from this time till the termination of the experiment at the rate of 0.5 gm. reduced iron per pig twice each week. The experimental lot never received supplementary iron. In order to ensure that no deficiency of copper had arisen during the experimental period copper as $CuSO_4$ - 0.5 mg per 2 lbs. body weight - was administered to

all the pigs per os for five consecutive days during the eighth week. By following the haematological changes and the difference in biopsy and autopsy samples of liver copper and iron it was hoped to clarify this point.

Piglets for this experiment were purchased from three sources and were the offspring of seven sows, which had farrowed within four days of one another. All were put on to the experimental diet within two to six days of birth, each group consisted of 11 piglets, six piglets died in the initial few days, which was a rather high mortality. The final distribution of the twenty-two piglets left on the experiment is given in table 5 A.

It became obvious early in the experiment that some infection was present, which was later identified as a mycotic one. The results must be interpreted in the light of this complication and it will be discussed more fully under clinical signs and discussion.

TABLE 5 A. Source, sex, weight and distribution of the pigs used in Experiment 5.

CONTROL GROUP (IRON)				EXPERIMENTAL GROUP (IRON-POOR)			
No.	SOURCE	Weight (lbs)	Sex	No.	Source	Weight (lbs)	Sex
1	H5873	3¼	M	7	H5873	3½	M
2	"	3½	M	8	"	3¼	F
3	"	3	M	9	"	3	F
4	"	3¾	F	10	"	2	F
5	"	2¾	F	13	H4853	3½	M
11	H4853	2½	M	14	Mc L	3½	M
12	"	2	M	17	B	3½	F
15	H7472	4	F	18	B	3½	F
16	"	3¾	F	21	Mc L.	6	M
19	Mc L.	6	M	22	"	6	M
25	"	5½	F	24	"	6½	F

HAEMATOLOGICAL RESULTS.

Haemoglobin.

The tables following give the values of Hb for the individual pigs and the mean value for the groups.

In considering these results it should be recalled that iron was supplied to the control group, only from the fourth week onward.

TABLE 5 D.Hb. Values for the Piglets comprising the Control Group (gm%).

Week	Piglet Number										
	1	2	3	4	5	11	12	15	16	19	25
1	10.0	10.3	9.5	10.6	9.1	11.5	11.7	10.4	10.8	-	-
						9.8	9.8				
2	10.6	10.2	9.5	10.9	8.9	9.9	11.5	8.9	9.7	6.2	7.7
	10.2	9.7	8.6	9.7	8.3	8.8	8.4	9.8	9.8	7.0	8.6
	8.0	7.5	7.7	8.4	8.0					5.6	6.9
3	8.3	6.4	7.3	7.8	5.6	6.4	6.7	8.0	7.3	4.6	6.0
	7.1	6.2	6.3	6.9	5.4	6.3	7.1		6.2	5.6	6.9
									6.2		
4	5.7	5.4	5.7	5.7	4.3	5.7	6.0	-	5.4	5.7	5.8
	5.5	5.6	5.6	6.2	4.6	5.4	5.3			6.2	6.5
5	5.6	5.6	5.2	6.8	4.2	5.4	6.5		5.4	6.0	6.5
	6.4	5.9	5.9	6.2	5.9	5.6	5.4		6.3	6.7	6.5
	6.2	6.5	6.5	6.5	6.5	6.0	6.5		6.2	5.2	7.6
						7.1	6.9			6.3	6.7
6	6.9	6.5	5.8	6.3	6.3	6.7	5.8	-	5.8	6.5	7.1
	6.5	6.7	5.7	7.3	6.5	6.3			8.0	6.5	7.8
	6.2	8.0	7.3	6.9	6.2				8.0	6.5	6.7
7	8.4		8.9	8.4	9.3	8.8			7.1	7.2	7.3
			8.9	9.5	8.6	8.0			8.7	7.7	7.7
8	8.2		6.7	8.6	8.0	7.6			7.7	6.3	8.6
	8.7		6.5	7.8	8.7				6.7	8.0	6.9
9	10.0		6.9	7.1	8.7	7.4			5.9		6.7

TABLE 5 C.Hb. Values for the Piglets comprising the Experimental Group (gm%).

WEEK	PIGLET NUMBER										
	7	8	9	10	13	14	17	18	21	22	24
1	10.6	9.6	8.6	7.5	9.8 8.2	-	11.5	7.8	-	-	-
2	8.7 7.5	6.9 7.8	7.7 7.5	7.1 8.2	9.3 7.8	6.7 7.3	9.8 9.5	6.5 5.8	8.2 8.4	4.7 5.8	6.0 5.8
3	8.5 6.5	6.9 4.8	6.4 5.8	6.6 5.0	5.8 5.7	7.1 6.0	7.6 7.3	5.3 5.6	7.7 6.0	8.9 5.0	5.8 4.5
4	5.8 5.8	4.9 6.5	5.1 4.4		5.4 4.6	5.4	6.9 6.9	4.2 6.2	6.4 6.2	6.6 5.0	5.0 5.0
5	5.1 4.1 4.3 5.0	5.8	-	-	5.1 4.6 5.0	5.2 4.8 4.4 4.3	6.0 6.1 5.1 5.4	4.6 3.1	6.5 5.5 6.7 5.2	4.6 4.0 3.1 3.9	4.8 3.8 4.8
6	4.5 4.6				4.1	4.5 3.9	5.6 4.6		6.1 5.0	4.5	4.8 4.8
7	4.9 4.2				4.7 4.8 3.9	4.7 4.7	6.2 4.7		6.3 5.0		
8	4.2 4.1				5.4	4.7 4.3	5.6 4.7		5.6 4.6		
9					6.5	3.7	4.7		4.6		

TABLE 5 D. Group Mean Values for Hb. (gm%).

WEEK	CONTROL GROUP		EXPERIMENTAL GROUP	
	MEAN	S.E.	MEAN	S.E.
1	10.30 ± 0.80	± 0.24	9.20 ± 1.40	± 0.50
2	8.07 ± 1.40	± 0.26	7.40 ± 1.32	± 0.28
3	6.57 ± 0.88	± 0.19	6.30 ± 1.16	± 0.25
4	5.59 ± 0.50	± 0.11	5.59 ± 0.84	± 0.19
5.	6.20 ± 0.64	± 0.11	4.85 ± 0.88	± 0.16
6	6.69 ± 0.67	± 0.13	4.75 ± 0.42	± 0.12
7	8.27 ± 0.71	± 0.17	4.91 ± 0.72	± 0.21
8	7.60 ± 0.84	± 0.22	4.80 ± 0.59	± 0.19
9	7.52 ± 1.37	± 0.51	4.87 ± 1.17	± 0.58

A highly significant difference in the means of the two groups was established from the fifth week till the experiment terminated ($P < 0.01$). Figure 5 A aids in the appreciation of the variations which occurred in the group means.

Haematocrit Values.

The volumes of packed red-cells are given in Tables 5 E and F. Where more than one reading was obtained the mean is given though usually only one value was obtained for each pig per week.

FIG. 5A.

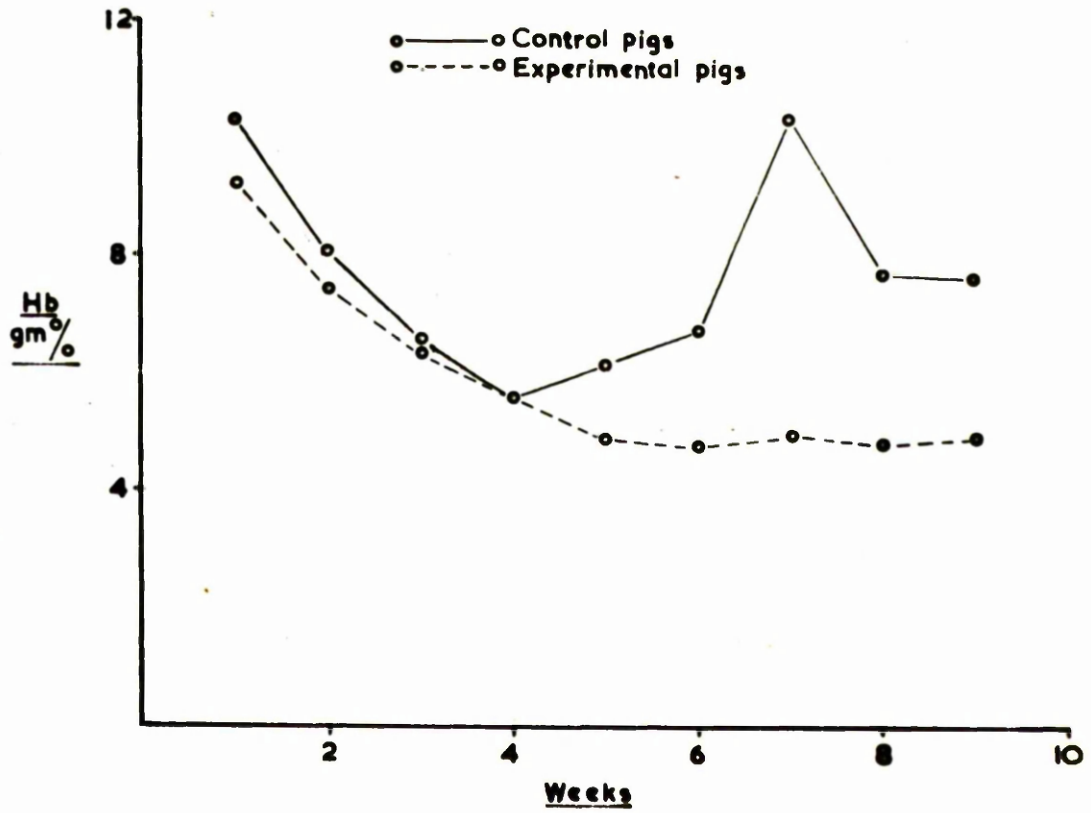


TABLE 5 B. Haematocrit Values for the Piglets comprising each Group(ml per 100ml).

GROUP	WEEK	PIGLET NUMBER										
		1	2	3	4	5	11	12	15	16	19	25
CONTROL	2	39	35	31	34	29	36	43	30	35	25	32
	3	29	24	28	-	21.5	27	28	-	24.5	22	26.5
	4	21	20	19	19	14	26	21	-	19	22.5	21
	5	21	24	20	28	16	21	27	-	25	28	36
	6	22	29	24	29	25	26	23	-	34	25	30
	7	32	-	32	31	31	32	-	-	35	28	28
	8	30	-	25	32	28	-	-	-	28	26	31
	9	33	-	21	28	28	28	-	-	21	-	23
		PIGLET NUMBER										
		7	8	9	10	13	14	17	18	21	22	24
TREATMENT	2	32	23	28	-	28	28	35	31	29	22	22
	3	31	23	23	30	21	26	25	18	28	19	21
	4	22	19	16	-	17.5	22	26	18	25	20	21
	5	17.5	19	-	-	19	19	24	17	22	17.5	19
	6	18	-	-	-	-	18	22	-	23	19	20
	7	19	-	-	-	20.5	18	23	-	24	-	-
	8	17	-	-	-	-	19	23	-	23	-	-
	9	-	-	-	-	23	14	18	-	19	-	-

TABLE 5 F. Group Mean Haematocrit Values (ml per 100ml).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
2	34.0 ± 4.81	27.80 ± 4.37
3	25.61 ± 2.72	24.09 ± 4.41
4	20.25 ± 3.04	20.65 ± 3.20
5	24.60 ± 5.58	17.33 ± 2.27
6	26.70 ± 3.71	20.0 ± 2.01
7	31.12 ± 2.29	20.90 ± 2.55
8	28.57 ± 2.57	20.50 ± 3.0
9	26.0 ± 4.47	18.50 ± 3.68

From week 5 on, a highly significant difference existed between the group means ($P < 0.01$). Figure 5 B expresses table 5 F as a curve for each group.

Erythrocyte Counts.

The individual and group mean red-cell counts are recorded in Tables 5 G and 5 H.

FIG 5B

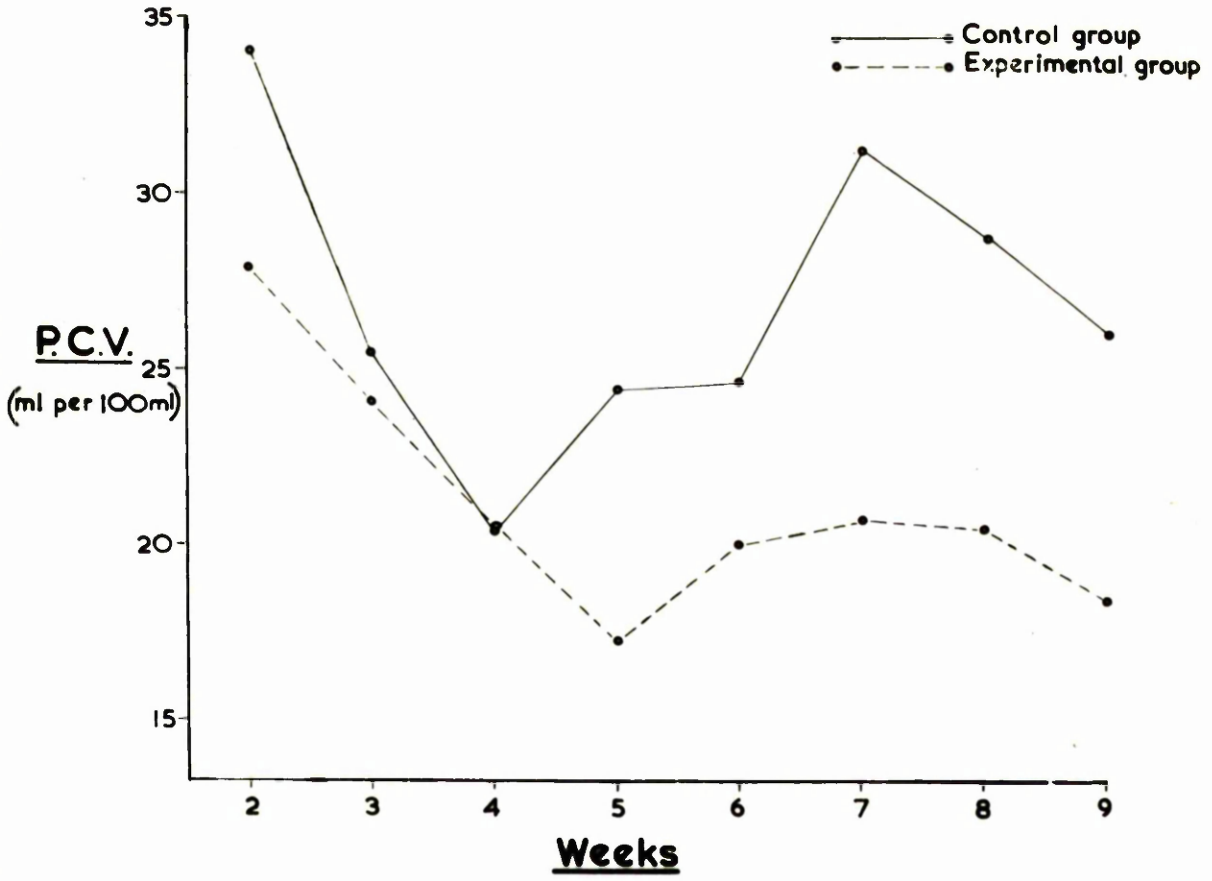

Control group
Experimental group
P.C.V.
(ml per 100ml)
35
30
25
20
15
2
3
4
5
6
7
8
9
Weeks

TABLE 5 G. Erythrocyte Counts for the Individual Pigs ($x10^6$ per c.mm).

GROUP.		PIGLET NUMBER										
		1	2	3	4	5	11	12	15	16	19	25
CONTROL	2	6.5	6.7	4.7	4.9	4.4	5.6	5.0	4.8	4.6	4.2	5.0
	3	4.2	4.3	4.5	4.5	3.9	4.7	4.7	-	4.2	4.6	4.5
	4	4.0	5.6	3.8	5.0	2.9	4.6	4.4	-	4.2	5.6	4.5
	5	4.0	5.2	4.5	4.9	3.0	4.1	5.0	-	4.5	5.0	4.9
	6.	4.0	4.7	3.9	5.2	4.1	4.5	3.9	-	5.6	5.0	5.1
	7	5.1	-	6.1	4.8	4.8	5.5	-	-	6.4	4.9	5.6
	8	5.1	-	4.5	5.6	5.5	-	-	-	5.2	5.0	5.5
	9	5.3	-	4.4	4.5	4.5	5.0	0	0	4.0	-	4.7
		PIGLET NUMBER										
		7	8	9	10	13	14	17	18	21	22	24
EXPERIMENTAL	1	-	-	-	-	5.6	-	-	-	-	-	-
	2	5.1	3.6	4.1	-	5.1	5.1	5.5	4.9	5.1	3.0	3.4
	3	4.7	4.0	3.9	3.9	4.0	5.3	4.5	3.8	5.0	4.2	4.1
	4	3.8	4.0	3.1	-	3.6	4.6	4.9	3.2	5.2	4.5	4.0
	5	3.7	4.0	-	-	4.1	3.7	4.7	3.7	4.7	3.8	3.9
	6	3.9	-	-	-	-	3.9	4.2	-	4.6	3.8	3.7
	7	4.6	-	-	-	4.3	4.6	5.3	-	5.5	-	-
	8	4.2	-	-	-	-	4.1	4.9	-	4.9	-	-
	9	-	-	-	-	4.3	3.4	3.1	-	3.5	-	-

TABLE 5 H. Group Mean Erythrocyte Counts. ($x10^6$ per c.mm).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
2	5.12 ± 0.81	4.49 ± 0.88
3	4.41 ± 0.24	4.30 ± 0.49
4	4.46 ± 0.82	4.09 ± 0.70
5	4.51 ± 0.66	4.03 ± 0.40
6	4.60 ± 0.61	4.01 ± 0.33
7	5.40 ± 0.60	4.86 ± 0.51
8	5.20 ± 0.38	4.52 ± 0.43
9	4.62 ± 0.42	3.57 ± 0.53

While it may be shown that a significant difference existed between the group mean counts (Table 5 H) at certain weeks (viz. weeks 6 & 8, $P<0.05>0.01$) if allowance be made for the minimum error of ± 5% inherent in the erythrocyte count (Whitby & Britton 1957; Biggs & Macmillan 1948) by approximating each group mean by 0.1×10^6 the differences in the means became not significant except at the ninth week at which week significance was established ($P< 0.05 > 0.01$).

The Red Cell Indices.

The Mean Corpuscular Volume.

TABLE 5 I. M.C.V Group Mean Indices (μ 3).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
2	66.76 ± 9.48	62.61 ± 5.82
3	58.20 ± 5.64	53.65 ± 5.90
4	44.74 ± 5.40	50.81 ± 4.01
5	52.31 ± 4.10	47.84 ± 2.06
6	58.09 ± 3.67	49.75 ± 3.19
7	58.14 ± 5.27	44.03 ± 6.06
8	54.65 ± 3.19	45.12 ± 3.16
9	55.95 ± 6.41	51.70 ± 7.35

A microcytosis was present in the control group by the fourth week but appeared to be corrected coincident with the administration of iron (Table 5 I) whereas the M.C.V indices of the experimental group failed to show a similar rise at least till the ninth week, when a slight improvement may have occurred.

Analysis of these data shows that the two means differed significantly at week 5 ($P < 0.05 = 0.01$) and highly significantly at weeks 6, 7, & 8. ($P < 0.01$).

The Mean Corpuscular Haemoglobin Concentration.

TABLE 5 J. MCHC Group Mean Indices (%).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
1	-	-
2	27.64 ± 0.74	26.21 ± 3.08
3	24.98 ± 2.26	30.47 ± 5.83
4	28.46 ± 1.68	26.86 ± 4.24
5	25.05 ± 1.07	25.74 ± 2.22
6	25.46 ± 1.83	25.10 ± 0.99
7	26.71 ± 1.48	24.76 + - 3.87
8	26.98 ± 1.35	24.50 ± 0.23
9	29.04 ± 2.66	26.32 ± 1.64

Most group M.C.H.C values were low, and both groups showed a gradual decrease in the mean Hb concentration till the fifth and sixth weeks were reached (Table 5 J) thereafter the experimental group continued to show a low M.C.H.C while the indices for the controls increased, indicative of the therapeutic value of the iron administered.

Significance in the difference between the two group means was present at week 8 only ($P < 0.05 = 0.01$).

The Mean Corpuscular Haemoglobin.

The indices given in table 5 K for the M.C.H. are derived from the group mean values for Hb and the R.B.Cs.

The indices for the experimental group can be seen to have fallen gradually till very low levels were reached at the seventh and eighth week. In contrast the control piglets showed an initial fall in the M.C.H. indices, which paralleled that noted for the experimental group and after the administration of iron, some recovery.

TABLE 5 K. Mean Weekly M.C.H. Indices for the groups ($\bar{y}$ $\bar{y}$).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
1	-	16.4
2	15.7	16.4
3	14.8	14.6
4	12.5	13.6
5	13.5	12.3
6	14.5	11.8
7	15.3	10.1
8	14.6	10.8
9	16.2	13.6

Cell Morphology.

The red cells of all pigs showed a decrease in size and in stainable Hb until by the third week in many of the pigs a high proportion of the cells were mere rings of eosinophilic cytoplasm. These hypochromic microcytic cells continued in abundance in smears from the experimental pigs throughout the period of the experiment. In the control group, in contrast, after the fourth week many of the cells stained homogeneously though the cells remained small. This improvemnat continued and by the sixth week little abnormality could be noted in smears from most of the control pigs, though in some a percentage of cells showed ring-staining as if some deterioration in the condition had occurred, presumably because of the impairment of health due to the mycotic infection.

Osmotic Fragility of Erythrocytes.

As has been stated in relation to tables giving the osmotic fragility of R.B.Cs in the previous experiments, the figures quoted (Table 5L) are the per cent concentration of saline required to initiate and to complete haemolysis, i.e. minimum to maximum resistance. No indication of increased resistance of the red cells of the experimental group was evident in this experiment.

TABLE 5 I. The Osmotic Fragility of the R.B.Cs of some Control & Experimental Pigs on certain weeks. (start/complete haemolysis).

WEEK	CONTROL PIGLETS.						
	1	3	4	12	16	19	25
5	0.60/ 0.30	0.65/ 0.30	0.55/ 0.30	0.55/ 0.30	-	0.55/ 0.30	0.60/ 0.30
7	"	-	0.60/ 0.25	-	0.55/ 0.25	"	0.55/ 0.30

WEEK	EXPERIMENTAL PIGLETS							
	7	9	13	14	18	21	22	24
3	0.60/ 0.30	0.60/ 0.30	-	-	0.60/ 0.30	0.55/ 0.30	0.60/ 0.30	0.60/ 0.30
4	-	-	0.60/ 0.30	-	-	-	-	-
5	-	-	-	0.50/ 0.30	0.50/ 0.30	-	-	0.55/ 0.30
7	-	-	0.60/ 0.25	0.55/ 0.25	-	0.55/ 0.30	-	-

Serum - Iron Estimations.

Serum - iron level are given in Tables 5 M & 5 N with the means for the group in Tables 5 O.

TABLE 5 M Serum - Iron Estimations for the Control Piglets. (μ gm%).

WEEK	PIGLET NUMBER										
	1	2	3	4	5	11	12	15	16	19	25
2	209.6	146.1	94.0	154.6	168.0	188.8	198.9	206.4	152.8	136.8	113.7
3	117.8	91.2	176.8	86.4	120.5	121.9	101.2	-	95.6	67.7	50.6
4	116.8	112.0	129.0	65.0	160.0	82.6	62.0	-	82.6	82.1	88.0
5	250.4	104.5	106.1	192.5	171.5	226.4	122.4	-	186.0	174.4	238.9
6	75.7	224.0	216.0	66.1	81.0	81.0	84.0	-	85.8	122.6	63.4
7	309.6	-	162.1	283.2 152.5	288.0	160.0	-	-	346.1	352.0	271.4 249.0
8	222.4	-	143.4	108.8	190.4	248.0	-	-	147.7	201.6	133.3
9	178.1	-	96.0	166.4	219.2	130.1	-	-	185.6	313.6	93.8

TABLE 5 N. Serum - Iron Estimations for the Experimental piglets (µgm %).

WEEK	PIGLET NUMBER										
	7	8	9	10	13	14	17	18	21	22	24
1	-	-	-	-	114.2	-	-	-	-	-	-
2	188.8	139.7	132.4	-	97.0	129.7	412.7	137.6	101.3	126.4	152.0
3	68.2	82.6	77.8	106.5	184.0	80.8	-	85.2	58.1	90.1	-
4	113.6	52.0	134.9 125.3	-	105.0	102.4	126.4	70.8	50.6	-	156.8
5	68.2	94.4			91.7	22.08	115.6	82.6	-	-	127.4
6	73.0	-	-	-	-	74.6	99.2	-	112	101.8	122.6
7	84.6	-	-	-	125.8	82.1	-	-	171.7	-	-
8	108.8	-	-	-	187.7	69.8	157.3	-	241.0 101.3	-	-
9	81.6	-	-	-	154.1	66.1	-	-	93.8	-	-

TABLE 5 0. Serum Iron. Group Means S.Ds. (μ gm %).

WEEK	CONTROL GROUP	EXPERIMENTAL GROUP
2	160.8 ± 37.9	161.7 ± 91.7
3	102.9 ± 34.6	92.5 ± 36.8
4	98.0 ± 30.8	100.8 ± 36.4
5	177.3 ± 53.0	85.7 ± 34.1
6	109.9 ± 60.2	97.2 ± 19.9
7	271.5 ± 83.0	116.0 ± 12.8
8	184.4 ± 77.3	138.9 ± 15.8
9	172.8 ± 71.8	98.9 ± 38.5

It can be seen that at weeks five and seven only, were the differences in the sample means, highly significant ($P < 0.01$). No significance could be shown at week eight, and at the ninth week the probability of these two means arising from the same population sample was less than 10% but greater than 5%.

Measurement of Red Cell Diameters.

The red cells of one control piglet (number 4) and one experimental piglet (number 14) only were measured. These were chosen at random and measurements were made at weeks 1 and 6 in both cases, but only in the case of the experimental piglet were cells measured at the third week. It was thus hoped to show the microcytosis of the R.B.Cs from the experimental pig compared with those from a pig which received iron in the control group. The curves

are given in Figures 5 C & D, from which it is apparent that a microcytosis was present and that at the sixth week the extent of this was less for the control pig than for the experimental one.

Table 5 F gives the percentage difference for each 0.25 μ class interval under 6 μ. The total difference seemed to be about 11.5%.

TABLE 5 F. Percentage of Microcytosis at week 6.

MID-POINT of CLASS Intervals (0.25 μ).	CONTROL PIGLET No.4 (%)	EXPERIMENTAL PIGLET No.14 (%)
6.0	11	13
5.75	10	11.5
5.5	9	12
5.25	7	7
5.0	5	8
4.75	3.5	6.5
4.5	3.5	1
4.25	0.5	0.5
4.0	0.5	1.5
3.75	0	0.5
Total	50.0	61.5

Percentage of Microcytosis : -

61.5 - 50.0 = 11.5%

FIG. 5C

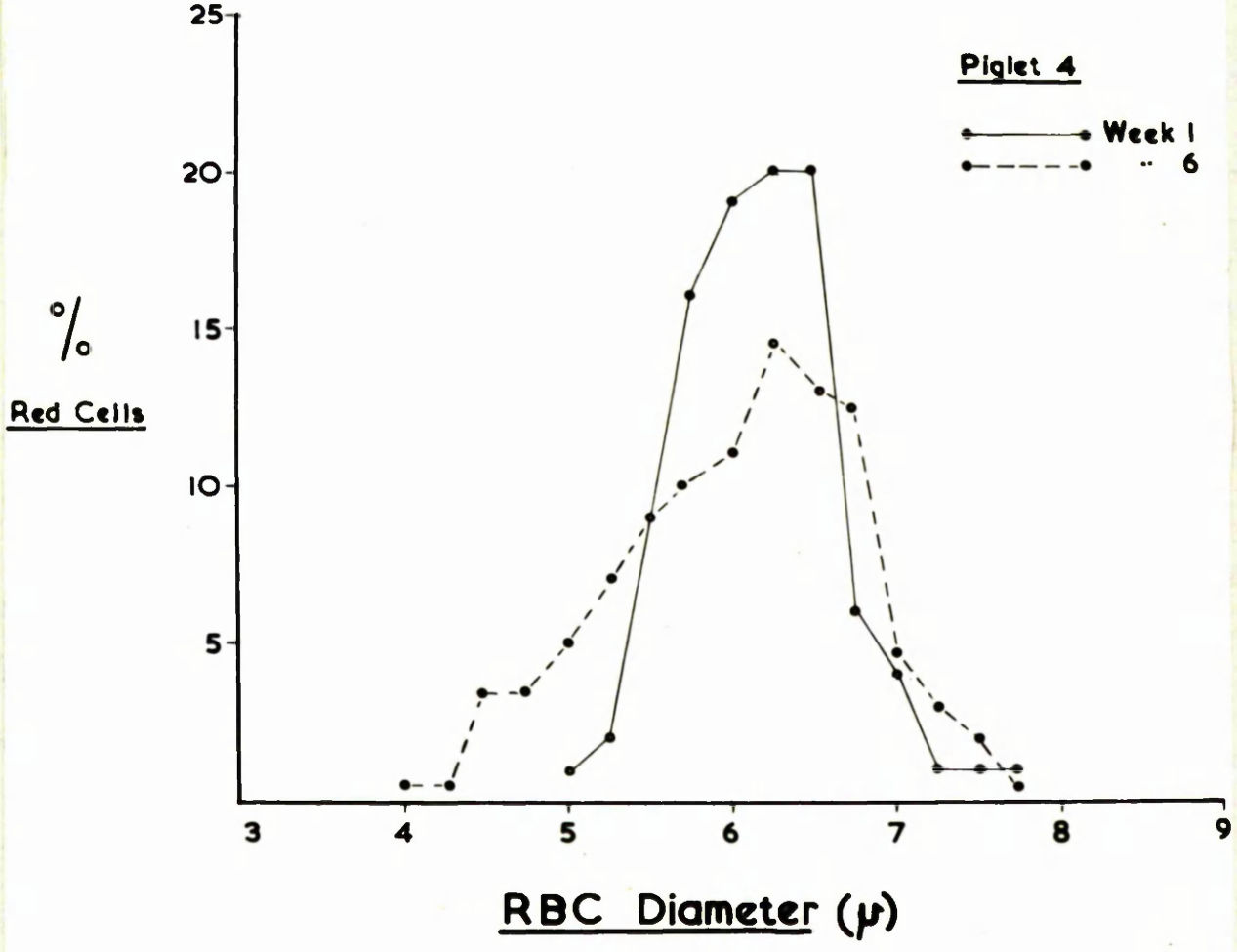
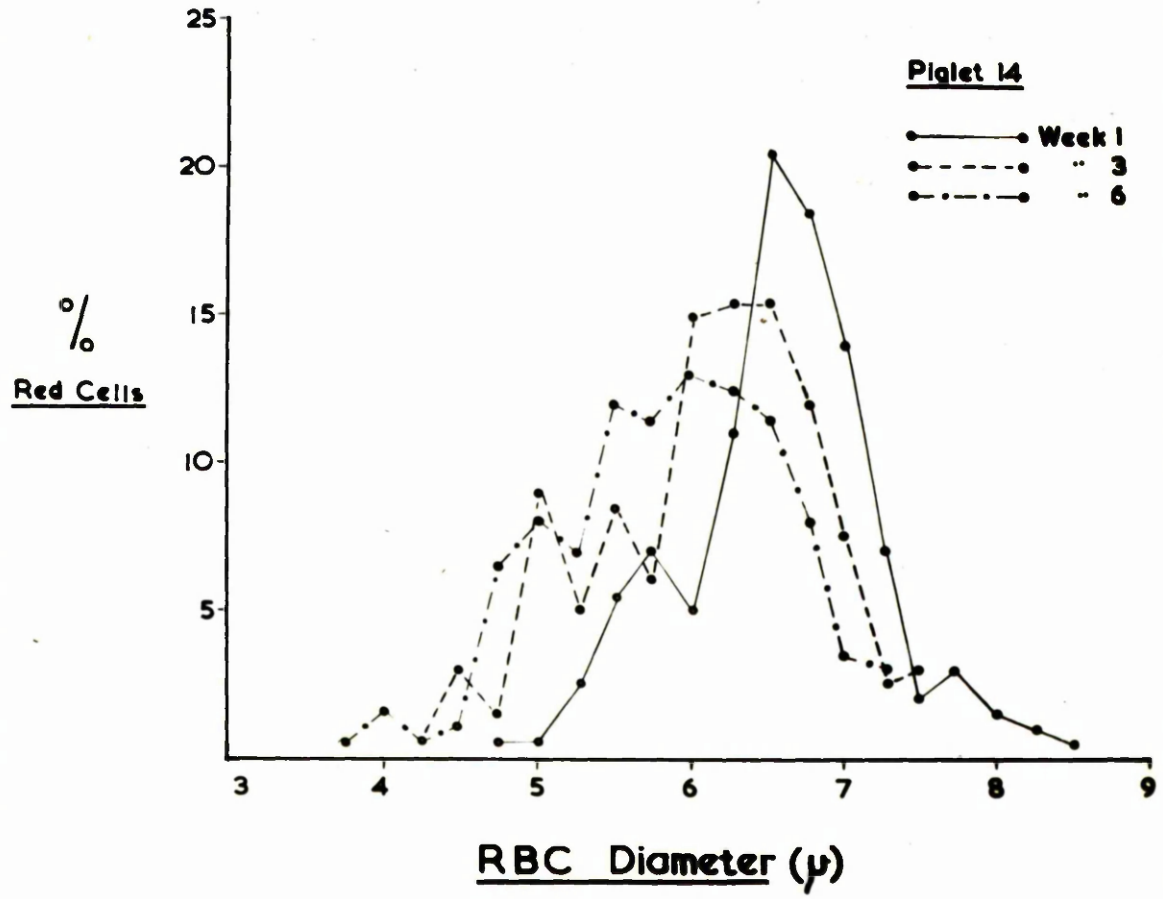


FIG 5D


Piglet 14
Week 1
" 3
" 6
%
Red Cells
25
20
15
10
5
3
4
5
6
7
8
9
RBC Diameter (μ)

White Cells.

Total white cell counts were made for all pigs mainlyat weeks two, three and four. The counts are given in the accompanying table. (Table 5 Q).

TABLE 5 Q. Total W.B.C counts for all pigs (x 10^3 per c.mm).

GROUP	PIGLET NO.	WEEK		
		2	3	4
CONTROL	1	15.8	9.6	8.6
	2	10.7	8.6	10.1
	3	9.2	9.8	14.3
	4	9.6	11.0	10.2
	5	7.1	6.2	8.0
	11	8.8	8.2	9.6
	12	7.8	10.0	13.0
	15	16.0	-	-
	16	9.6	12.0	11.1
	19	12.0	13.0	12.3
	25	10.2	9.7	9.8
EXPERIMENTAL	7	8.7	12.6	9.0
	8	7.3	9.4	10.2
	9	12.8	8.0	6.5
	10	-	20.1	-
	13	9.4	10.0	16.8
	14	8.4	24.8	18.2
	17	15.4	8.6	11.4
	18	7.8	6.2	18.0
	21	7.8	16.5	12.8
	22	9.6	8.4	10.1
	24	4.7	6.2	8.6

Only 1 piglet showed a white cell count greater than 20.0 thousands per c.mm which was the upper limit given by Wintrobe (1956) for the pig.

Parenchymal Iron & Copper Content.

The contents of iron and copper in both biopsy and autopsy samples of the liver (and of the spleen in the case of post-mortem) specimens are given in tables 5 R for the controls and 5 S for the other pigs.

TABLE 5 R. Parenchymal Iron and Copper: Control Piglets.

PIGLET No.	SPECIMEN	WEIGHT gm	MOISTURE %	CONTENT of METAL	
				Iron mgm 100gm (wet tissue)	Copper p.p.m (wet tissue)
1	Liver:Autopsy	213	71.5	4.02	10.2
	Spleen "	9.75	75.0	10.8	-
2	Liver "	127	76.5	11.2	15.1
	Spleen "	4.0	-	45.6	-
3	Liver "	125	72.0	3.04	9.0
	Spleen "	5.5	75.5	16.8	-
4	Liver "	210	71.5	3.08	8.45
	Spleen "	10.5	75.0	11.2	-
5	Liver "	121	74.5	5.2	5.82
	Spleen "	4.0	-	20.5	-
11	Liver "	207	73.3	2.94	10.45
	Spleen "	9.0	70.2	10.8	-
12	Liver "	112	79.0	10.5	8.0
	Spleen "	7.75	81.5	29.8	-
16	Liver Biopsy	-	52.5	11.35	9.9
	" Autopsy	317	73.6	5.15	5.15
	Spleen "	17.5	79.5	17.1	-
19	Liver "	247	73.0	10.75	5.78
	Spleen "	12.0	78.8	24.0	-
25	Liver Biopsy	-	59.0	6.0	10.6
	" Autopsy	360	74.2	4.3	4.8
	Spleen "	22.0	79.0	10.8	-

TABLE 5 S. Parenchymal Iron & Copper: Experimental Piglets.

PIGLET No.	SPECIMEN	WEIGHT (gm)	MOISTURE (%)	CONTENT OF METAL	
				IRON mgm/100gm (wet tissue)	COPPER ppm. (wet tissue)
7	Liver Autopsy	168	75.0	1.47	9.55
	Spleen "	10.0	72.3	7.9	-
8	Liver "	78	80.0	6.48	35.0
	Spleen "	3.0	-	14.2	-
13	Liver "	310	74.0	2.47	4.05
	Spleen "	24.0	79.5	7.42	-
14	Liver Biopsy	-	45.0	3.01	30.6
	" Autopsy	183	76.0	2.06	13.8
	Spleen "	15.0	74.0	7.68	-
17	Liver "	189	76.0	2.82	2.34
	Spleen "	13.5	79.0	11.8	-
18	Liver "	85	79.5	3.81	23.7
	Spleen "	2.0	-	6.9	-
21	Liver Biopsy	-	67.5	2.2	7.35
	" Autopsy	300	74.5	2.32	4.65
	Spleen "	30.0	79.3	6.62	-
22	Liver "	154	81	5.98	34.0
	Spleen "	7.75	80.5	12.7	-
24	Liver "	151	79.5	4.95	24.6
	Spleen "	8.0	80.5	16.8	-

The means liver iron content for each group sample was :-

Controls group 6.02 ± 3.42 mgm per 100 gm. wet tissue

Experimental " 3.59 ± 1.81 " " " " " " "

The difference between the means for these parameters is only just outside the 10% level of probability.

CLINICAL SIGNS.

During the first few days on the experiment it became obvious that the pigs, of the experimental group, were not thriving as expected. They were in addition suffering more with diarrhoea than piglets in the previous experiments, and after about a week diarrhoea was noticed in the control group. Within eleven days six of the piglets died, leaving twenty - two on the experiment. Reasons were sought for this disasterous start to the experiment and it was considered that the promiscuous mixing of pigs from so many sources may have introduced an enteric infection. Second the daily cleaning process, which had to be thorough because of the fluid consistency of the faeces was making the concrete cold and damp and thus reducing their resistance. Third, some mycotic infection may have been introduced with the sawdust which was used during the first few days and which had been reintroduced to make conditions more comfortable for the piglets.

The position became so serious that limited counter-measures were adopted. The pens were scrubbed daily with a pine - tar disinfectant, and all dishes steeped in the same solution for 24 hours . Systemic aqueous penicillin (100,000 I.U., B.I.D) was given initially, replaced later by the procaine salt and an oral preparation of streptomycin to those seriously ill. Eventually as all pigs became affected either mildly or more seriously the streptomycin was added to the drinking water. These antibiotics did not seem to have any beneficial effect, and no pig received treatment for longer than seven days.

The clinical signs shown, were failure to make satisfactory weight gains, even loss of weight, dejected appearance, pale fluid stools, and white coating on the dorsum of the tongue of some pigs. Vomiting was not a feature, nor was a

febrile temperature ever recorded. During the fourth and fifth week several pigs were noticed sneezing and had an obvious serious nasal discharge.

No improvement was observed after iron was given to the control group, and several pigs died and are listed below.

GROUP	PIG NUMBER	WEEK OF DEATH
Control	15	3rd
"	12	6th
"	2	7 th
Experimental	10	3rd
"	9	4th
"	8	5th
"	18	"
"	22	6th
"	24	7th

From the failure to respond to antibiotics, and because of the white coating of the tongue which could not be removed by washing or even by gentle scraping, it was considered that a mycotic infection might be present. This was later confirmed. Mc Crea and Tribe (1956) quoting a personal communication from Osborne (1955) recorded thrush in an artificially reared piglet, soon after this observation was made. Naturally under the conditions which prevailed the body weights,

were not satisfactory in either group, and at week 8 the mean body weights for the group samples were : -

	3 weeks.	8 weeks.
Control Group	4.66 lbs.	10.92 lbs.
Experimental Group	5.07 lbs.	9.37 lbs.

The heaviest control pig weighed 16.0 lbs. at week 8, while 14.0 lbs. was the best weight achieved by an experimental pig. In spite of this serious complication, which may have affected the haematological results and probably prevented a marked response when iron was administered to the controls, the experimental results are capable of interpretation and on analysis gave significant differences in the sample means.

PATHOLOGICAL DETAILS.

Details of the gross and microscopic findings were recorded for each pig and these data have been briefly summarised in this section. No piglet was allowed to survive after the termination of the experiment, all being destroyed during the ninth week.

Of the twenty - two piglets used in this experiment nineteen had lesions of the oesophagus at autopsy and ten of these piglets were from the control group. The lesions were raised verrucose pseudo-membranes, usually yellow in colour and most frequent at the cardiac end of the oesophagus. In the most severe cases they extended almost the whole length of the gullet and nearly occluded the lumen. In thirteen of the pigs similar lesions were present in the stomach around the cardia.

Histological examination of the affected areas showed a marked hyperkeratosis, and an inflammatory cell infiltrate with vesiculation. Some sections also showed basophilic masses in the upper layers, and numerous filaments probably fungal filaments.

In the other organs there were no consistent changes. In the majority the lungs, livers, spleens and kidneys wereall normal, even on histological examination. In one piglet (19) fatty change was present in the liver, and the sections of this organfrom several pigs reveal haemopoietic centres, more frequently recorded in the experimental ones. Similar centres are present in the spleen and kidney sections occasionally.

No abnormal liver or heart enlargement was noted, though pleurisy and

pericarditis werepresent in one pig (3) and peritonitis in three pigs. The latter inflammatory change seemed to arise from abscesses in the wall of the stomach.

A purulent exudate was present in the turbinates of one pig.

A difference in the appearance of the femoral marrow of the pigs from each group was obvious. Those from piglets which received no iron were regularly of a dark red colour whereas the control group piglets had pale pink marrows. The difference was also clearly evident on the sections of the marrows. Though in both, the marrows seemed very active the sections show a lack or even complete absence of fat cells in the experimental piglets in contrast to the more numerous fat cells in those given iron.

DISCUSSION.

The main aims as set out in the introduction to this experiment were achieved. An iron deficiency anaemia was produced and then corrected by the inclusion of iron in the diet, as judged by the haematological parameters. Though the experimental results were nearly prejudiced by the coincidental mycotic infection.

It proved difficult to keep piglets clean and warm when housed on concrete and fed artificially. Though sawdust was used as bedding this tended to become damp and may have been a source of the infecting fungus. The promiscuous mixing of experimental animals from various farms had also the serious disadvantage that some piglets possibly introduced the infection into the group.

It was unfortunate that, presumably as a result of the infection and the subsequent diarrhoea, the response of the control group to oral iron while evident was undoubtedly slow and rather poor.

The reticulocyte response, and a return to normal of the blood parameters of most pigs was evident following the ingestion of the additional iron. It was reassuring to note that the administration of copper failed to produce a measurable erythropoietic response in either the control or experimental group pigs. It was also of interest to see that the parenchymal copper was at considerably higher levels in the latter presumably because they were unable to utilise it fully for erythropoiesis since iron was lacking. Lahey et al (1952) had noted the increase of liver copper in iron deficient pigs.

The response to the administration of iron orally, was slow, and was not reflected in increased levels of Hb or serum iron until the seventh week. Control piglet number 12, which died when six weeks old with enteritis, had not shown any improvement in the anaemia from which it was suffering, despite the administration of five 0.5 gm. doses of reduced iron. The various blood parameters were below normal levels including the serum iron. Despite this the response may have been initiated since the sections showed great activity in the marrow with few fat spaces, and a reasonable quantity of iron was found in the liver sample on autopsy.

A higher level of iron was found on biopsy than at autopsy in the livers of the control piglets, and a slightly greater level in one experimental piglet. Similarly the copper content was higher, as estimated on biopsy samples, than after the administration of copper. It would seem unlikely that this could be explained on a greater blood content of the sample from the organ and no other reasonable explanation comes readily to mind.

The pathology of the individual was studied in some detail but in general it may be said that few lesions were evident that were not attributed to the mycotic infection. There were no flagrant changes of the liver and no increase in size.

EXPERIMENTS ON PIGLETS REARED NATURALLY.

GENERAL INTRODUCTION.

In order to confirm the main findings from the five experiments performed on piglets reared artificially on semi - synthetic diets it was decided to carry out observations on pigs which were suckling. It was hoped that nutritional iron deficiency anaemia might be produced in some pigs and prevented, by the use of iron, in others. Any difficulties which might arise with regard to obtaining and estimating the parameters of the numerous blood samples would be offset partly by studying the clinical signs produced by the lack of iron. For this purpose three experiments were conducted on a farm where large white pigs were reared commercially. These premises were chosen for three main reasons. First the management was such that the piglets were kept in extremely clean warm conditions (and thus satisfying important conditions for the production of uncomplicated nutritional iron-deficiency);second, the piglets were readily accessible for handling, and last the size of the herd provided a constant flow of experimental animals.

Since the system of husbandry has considerable bearing on this type of experiment and the production of nutritional anaemia some details of management are given.

On these premises, the sows were allowed access to grass during the summer months, but were kept, at other times, in a large shed. Parturition occurred in a series of brick and concrete farrowing pens, each approximately 7'8" long by 6'2" broad divided as the conventional farrowing crate into a stall for the sow with a creep for the piglets on either side. Each sow and litter was kept in

these for 17 - 21 days before removing to a more spacious pen or weaning the young on to early weaning pellets.

The temperature in the house was kept around 65 °F and infra-red or electric globes were suspended in each compartment for the piglets. Bedding in the pens consisting of wood-chips and sawdust, was removed daily, to provide a high standard of cleanliness.

The sows were fed, in earthenware troughs, sow and weaner meal plus water; no other source of water was provided. Four samples of this meal gave an average iron content of 21.95 mg per 100 gm. From about one week after birth the piglets were allowed small quantities of a pellet food. The amount was increased as the piglets grew till about a half-pound was allowed daily for an average litter of around ten piglets at the third week.

The two most constant disease problems evident among the pigs on this farm were diarrhoea apparently associated with the supplementary feeding supplied to the young pigs and particularly troublesome when the pigs were 4 - 8 weeks old, and so-called virus pneumonia which was evident both clinically and on autopsy.

The piggery was run as a commercial enterprise and while the facilities granted were given unstintingly, any procedures adopted had to interfere as little as possible with the normal management of the piggery. In these experiments several iron preparations were examined, to compare the response to and the economic value of each. This was of interest to pig breeders and veterinary surgeons and also consiliatory to the owner of the premises.

NATURAL REARING EXPERIMENT 1.

INTRODUCTION.

Twenty-four piglets were used in this experiment. Fifteen were selected from 3 litters, to receive the three iron supplements to be tested. No bias was exerted in the selection of the pigs, which were from each litter. Each iron supplement was given to certain individual pigs in each of the three litters, and they were allowed to remain with their respective mothers. These three litters were born within 96 hours. The next litter to be farrowed, was given no supplementary iron. It was considered inadvisable to mix the pigs on iron-supplements with those which received none because of the possibility that the former might obtain some iron from the excreta of the latter.

As far as possible in a commercially run piggery the four litters were contemporaries and were thus exposed to the same environmental conditions, infections, etc.

The day of farrowing was taken as the start of the experiment for each litter.

The distribution was as given in the following table : -

Group	Number in Group	Iron Supplement	Total Amount of Iron Administered.
A	5	Iron-Dextran	100 mgm
B	4	Iron-Carbohyd.	" "
C	6	Reduced Iron	3 gm
D	9	Nil	-

The iron-dextran complex was a commercial preparation with an iron content of 50 mg. per 1 ml.* The pharmacology of this haematinic has been described (Martin et al 1955) and its use in piglets in preventing anaemia recorded, (Brownlie 1955: Birk - Sørensen & Christensen 1956: Kernkamp 1957). It was administered intramuscularly in a dose of 2 ml. to each of the five piglets when 3 days old.

The same method and dose was used for the iron-carbohydrate complex.** Reduced iron was in use in this as in many piggeries as a haematinic. This was given orally in three doses, one at the third, and the others at the tenth and seventeenth day after birth. The metallic iron content of Ferro redactum is not less than 80% calculated as Fe., (British Veterinary Codex 1953).

These preparations were chosen as they are used commonly to prevent anaemia, and the methods of administration were either recommended or in general use.

HAEMATOLOGICAL RESULTS.

Any value recorded without a S.D. indicates that only one reading was available.

Most of the blood samples were obtained from the ear veins of the pigs in this experiment. On some occasions however, permission was obtained to collect larger samples by the anterior vena - cava route, and then full haematological examinations were made.

* Imferon (Bengers Lab. Ltd.,)

** Ferrovet (Crookes Labs. Ltd.).

Haemoglobin.

TABLE H. 1 A. Hb Values for the Individual Pigs.

Group	Piglet No.	Week			
		1	2	3	4
	22	11.9	11.9	10.6	-
A	24	10.5	9.9	9.4	9.3
Iron-	2	13.3	10.5	15.3	-
Dextran	3	12.9	10.7	11.7	-
	1	12.3	12.8	11.3	10.9
	16	10.5	10.8	-	-
B	15	10.9	10.5	10.5	-
Iron-	14	9.9	11.2	-	-
Carbohyd.	21	11.1	9.5	10.5	10.1
	6	12.1	10.7	10.7	-
C	10	-	-	11.9	-
Reduced	26	9.7	-	10.6	11.9
Iron	20	9.7	11.9	-	-
	17	9.3	9.5	-	-
	19	8.7	9.4	10.6	10.3

Cont'n of TABLE H 1 A.

Group	Piglet No.	Weeks.			
		1	2	3	4
	1	7.6	6.9	6.0	5.9
	2	6.0	6.0	5.3	6.2
	3	6.2	5.4	5.1	-
D	4	8.0	7.1	5.7	6.2
No	5	7.6	6.7	6.0	6.1
Iron	6	6.7	-	4.7	6.7
	7	8.0	6.2	7.1	-
	8	7.6	5.7	5.3	-
	9	7.1	7.1	4.3	5.9

These values are given as group means in Table H 1 B and are depicted graphically in Fig. H 1 A.

TABLE H 1 B. Group Mean Hb Values (gm per 100 ml).

Week	Group							
	A		B		C		D	
	Iron - Dextran		Iron - Carbohyd		Reduced Iron		No Iron	
	Mean	S.E.	Mean	S.E.	Mean	S.E.	Mean	S. E.
1	12.18 ± 1.08	±0.49	10.60 ±0.52	±0.26	9.90 ±1.29	±0.58	7.20 ±0.74	±0.25
2	11.16 ± 1.17	±0.53	10.50 ±0.72	±0.36	10.37 ±1.17	±0.58	6.40 ±0.64	±0.23
3	11.66 ± 2.21	±1.00	10.50	-	10.95 ±0.63	±0.31	5.50 ±1.78	±0.59
4	10.10 ± 1.13	±0.81	10.10	-	11.10 ±1.13	±0.81	6.16 ±0.29	±0.12

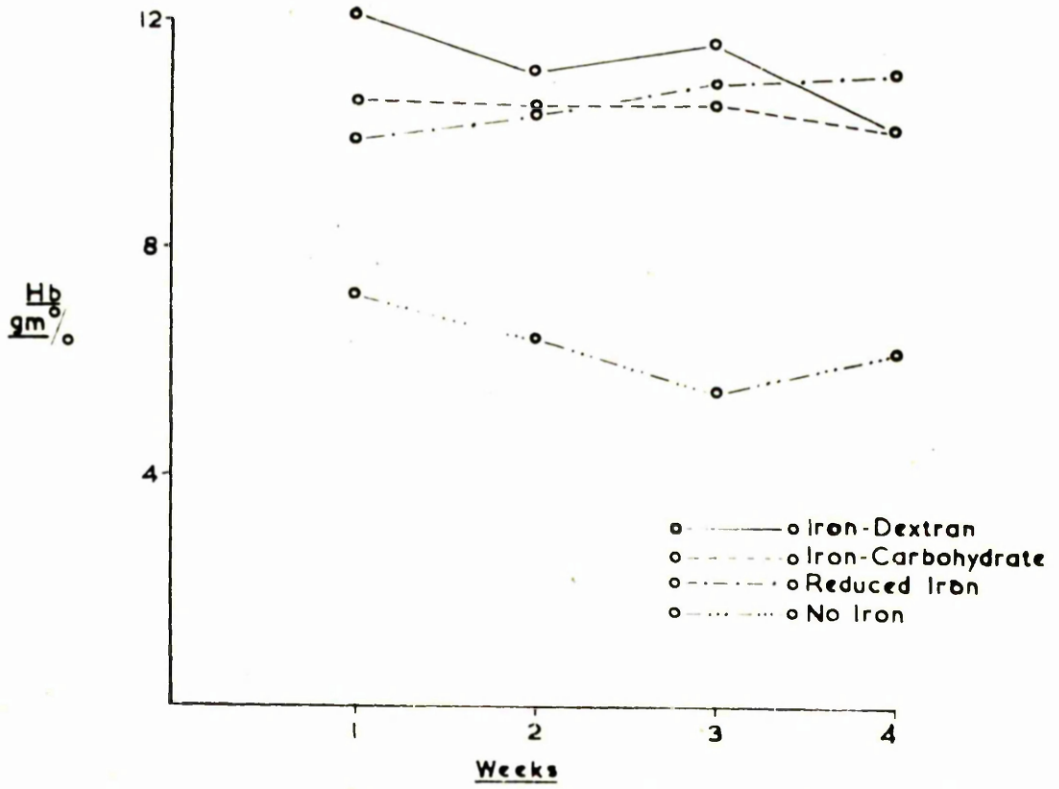
Pooling the results for all the pigs which received iron gave the following mean values for Hb (gm. per 100 ml).

Week 1 : 10.90 ± 1.40 S.E. ± 0.37 Week 2 : 10.70 ± 1.03 S.E. ± 0.28

" 3 : 11.10 ± 1.51 " " ± 0.45 " 4 : 10.50 ± 0.96 " " ± 0.43

These values when compared with the means for Group D, showed very marked differences, which were in fact, highly significant ($P < 0.01$) at all weeks.

FIG. H. IA


12
8
4
Hb
gm%
1
2
3
4
Weeks
Iron-Dextran
Iron-Carbohydrate
Reduced Iron
No Iron

The differences between the means of the group samples on the three iron supplements at each week were not significant, except for a significant difference ($P < 0.05 > 0.01$) between groups A & D and also A & C for the first week: a result which might indicate a quicker haematological response to iron-dextran. In this respect it is of interest to see the curves given in Fig. H 1 A.

Haematocrit Values.

In the table H 1 C. P.C.Vs are recorded as single readings when only one value was obtained or as means of the group when from 4.- 6 were available.

TABLE H 1 C. Group Mean and Individual P.C.Vs (ml per 100 ml).

Week	GROUPS.			
	A Iron-Dextran	B Iron-Carbohyd	C Reduced Iron	D No Iron
1	37.0	-	27.5	-
2	34.37 ± 3.89	28.0	34.50 ± 7.77	24.0
3	36.90 ± 6.58	26.0	34.50 ± 3.68	19.0
4	-	-	-	20.0

While no analysis for significance of these results can be made there did appear to be a marked difference between the results recorded for the samples of groups A & C and the individuals from group D which were bled. The three values given under group D were obtained from different pigs (numbers 6,8 & 9) which were representative of the group according to their Hb values.

Emphasis was given to recording the parameters of Groups A & C so that the value of the haematinics could be appreciated.

Erythrocyte Counts & Red Cell Indices.

The tables following record some other parameters, for group mean or individual values. The values recorded in these tables do not differ from those obtained in the earlier experiments using semi-synthetic diets.

TABLE H 1 D. Group Mean and Individual Erythrocyte Counts ($x10^6$ per c.mm)

Week	Group			
	A Iron-Dextran	B Iron-Carbohyd.	C Reduced Iron	D No Iron
2	5.32 ± 0.60	5.10	5.15 ± 1.48	4.7
3	6.12 ± 0.97	6.00	5.92 ± 0.89	3.6
4	-	-	-	4.1

TABLE H 1 E. Group Mean & Individual M.C.Vs (μ^3).

Week	Group			
	A Iron-Dextran	B Iron-Carbohyd.	C Reduced Iron	D No Iron
2	65.02 ± 5.45	54.9	67.60 ± 4.38	51.0
3	59.80 ± 3.84	60.0	58.92 ± 8.31	52.7
4	-	-	-	48.7

TABLE H 1 F. Group Mean and Individual MCHCs.(%).

Week	GROUPS.			
	A Iron-Dextran	B Iron-Carbohyd	C Reduced Iron	D No Iron
1	31.6	-	35.2	-
2	32.92 ± 1.12	34.6	31.20 ± 2.12	29.5
3	31.74 ± 1.46	29.1	31.87 ± 1.15	27.8
4	-	-		33.5

TABLE H 1 G. Group Mean and Individual MCHs. (yy).

Week	GROUPS.			
	A Iron-Dextran	B Iron-Carbohyd	C Reduced Iron	D No Iron
2	21.25 ± 1.13	18.60 -	21.10 ± 2.82	15.10 -
3	18.94 ± 0.94	17.50 -	18.70 ± 2.67	14.70 -
4	- -	-	- -	16.30

Reticulocytes.

The reticulocyte counts are of interest since it would seem that the counts for the anaemic pigs were higher than for those which received iron. However the results are too few to allow of interpretation.

TABLE H 1. H. Group Mean and Individual Reticulocyte Counts. (% of R.B.Cs).

Week	GROUPS			
	A Iron-Dextran	B Iron-Carbohyd.	C Reduced Iron	D No Iron
1	5.5	-	5.4	-
2	0.7 ± 0.8	-	1.45 ± 0.64	7.7
3	1.5 ± 0.5	0.5	4.15 ± 4.17	-
4	-	-	-	7.1

Serum Iron Estimations.

Some serum irons were estimated and are given in table H 1 J. The few values recorded follow the levels noted in previous experiments.

TABLE H 1 J. Group Mean & Individual Values of Serum Iron (µg per 100ml).

Week	GROUPS			
	A Iron-Dextran	B Iron-Carbohyd.	C Reduced Iron	D No Iron
1	149.9	-	-	-
2	207.4 ± 84.9	190.9	153.4 ± 41.9	81.4
3	109.9 ± 34.4	64.8	140.7 ± 69.7	-
4	-	-	-	102.5

Red Cell Distribution.

The diameters of the red cells of one piglet only were measured. This animal, number 22, was from the group given the iron-dextran complex, and the lowest Hb value recorded for it was 10.6 gm. It would be reasonable thus to assume that the measurements recorded might be those of a normally haemoglobinised piglet of the same age. It is of interest to note an increase in the number of smaller cells between the measurements made at the second and third week, (Fig. H 1 B).

Thus the percentage of cells measuring 6μ or less for this pig on two weeks were : -

Week 2 : 17.5% Week 3 79%

Undoubtedly the peak of the diameter curve shifted to the left. Whether this can be considered a normal deviation must be considered in the light of other results.

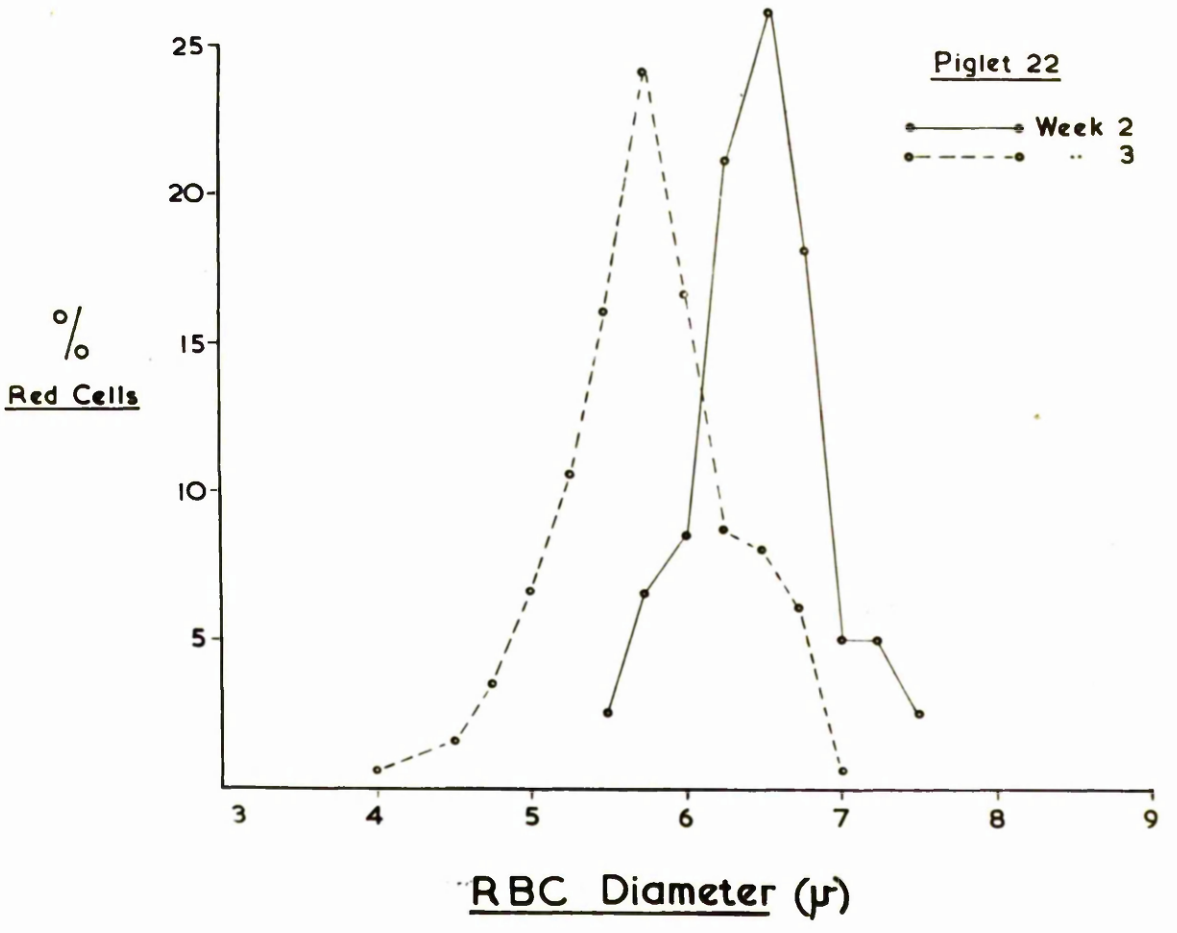
The M.C.V. of the red cells of this pig showed a slight decrease from 60.3 cμ at the second week to 56.6 cμ at week three.

Red Cell Morphology.

The red cells of the piglets supplied with iron appeared of uniform size and stained evenly. In contrast those of the anaemic piglets showed microcytosis and numerous pessary forms at the third week.

These changes were illustrated in the accompanying photographs, for Exp. 3

FIG. HIB


25
20
15
10
5
%
Red Cells
Piglet 22
Week 2
3
3
4
5
6
7
8
9
RBC Diameter (μ)

CLINICAL SIGNS.

At no time during the course of the experiment were diarrhoea, respiratory distress, cardiac embarrassment, excessive hair growth or unthriftiness noted in any group, or indeed in any individual. Pallor of the skin and conjunctivae was detected however in anaemic piglets. No distress was evident when the piglets were forcibly exercised.

The body weights reached at the third week were all good but, particularly so for the piglets which received no iron. These are given in Table II 1 K. Whether the pigs in this group would have gained more weight had they been given iron is a matter for conjecture. Nevertheless these pigs, despite an obvious anaemia, showed no unthriftiness and made excellent weight gains. No obvious management differences apart from those described, existed between the four litters to account for the greater weight increase of the anaemic litter, in which there were nine piglets compared with nine, ten and ten in the three other litters.

TABLE II 1 K. Group Mean Body Weights (lbs) at 3 weeks of age.

	Group			
	A Iron Dextran	B Iron Carbohyd	C Reduced Iron	D No Iron
Group Mean	12.87 ± 0.62	11.12 ± 0.76	10.40 ± 1.84	15.88 ± 2.14
Range	12 - 13.5	10.5 - 12	8.5 - 13	12.5 - 20

PATHOLOGICAL DETAILS.

No pigs died during the experiment. Three pigs were killed to allow of gross and histological examination.

One piglet which received reduced iron was destroyed when two weeks old. One anaemic piglet and another given iron-dextran were destroyed during their third week of life.

None of these piglets showed any gross pathological changes of the liver, spleen or other organs except some small areas of consolidation principally in the apical and cardiac lobes of the lung. Histology confirmed the presence of mild broncho-pneumonia. There were no significant bacteriological findings.

Numerous fat cells are evident in the sections of tibial marrow from the two pigs which received iron, in contrast to the hyperactivity and replacement of the fat cells in the marrow of the anaemic piglet.

Iron and copper estimations were made of the liver and spleen from the anaemic piglet, and the following results obtained : -

Liver 2 .07 mg iron per 100 gm. fresh tissue.

Total in liver 3 mg iron.

Liver 67.5 p.p.m copper in fresh tissue

Spleen 12.2 mg iron per 100 gm. fresh tissue.

DISCUSSION.

It had been the object of the experiment to produce anaemia, to prevent it in other pigs and to study the effects clinically. These objects were achieved, though the paucity of recordings for the haematological parameters except Hb allowed little statistical interpretation to be placed on the results. Neverthless, the anaemia which occurred in the iron deficient piglets appeared to be microcytic and hypochromic, though as noted previously the M.C.H.C. did not reflect this significantly.

It has been mentioned already that despite the comparatively low Hb. levels in the litter which received no iron, they gained weight rapidly and were without serious evidence of disease, such as has been described as occurring in piglet anaemia.

Probably the most important result was the lack of symptomatic evidence of anaemia or illness in these piglets even with Hb levels as low as they were in number 9 at the third week, when 4.3 gms % Hb were recorded, and also that no mortality, coincident with these low Hb values, occurred.

A few white cell counts were made but since they were infrequent and were within normal limits have not been recorded.

NATURAL REARING EXPERIMENT 2.

INTRODUCTION.

This, the second, experiment was undertaken to compare the value of three iron preparations in preventing anaemia; to study principally the Hb and clinical signs of pigs kept replete and others kept short of iron.

To eliminate the possibility of one litter outstripping another as in the first experiment, the four groups were made up from individuals belonging to three litters born within four days. One possible disadvantage of this arrangement was the probability of the piglets to be kept iron deficient obtaining iron from the excreta of the dosed piglets.

The group individuals were thus randomly distributed within three litters though each litter was left with its sow. The iron supplements used and number in each group were :-

Iron - dextran complex	:	6 piglets
Reduced iron	:	9 "
Anti - anaemia paste	:	8 "
No iron	:	7 "

The iron - dextran complex and reduced iron were administered as in the first experiment. The paste was given by mouth at the same periods as the reduced iron and the total amount of iron administered to each pig which received the paste was about 1 gm iron.

HAEMATOLOGICAL RESULTS.

Haemoglobin.

TABLE H 2 A. Group Mean Hb. Value (± S.D.) gm per 100 ml.

WEEK	IRON-DEXTRAN	REDUCED IRON	IRON PASTE	NO IRON
2	11.70 ± 2.15	11.52 ± 0.75	11.53 ± 2.02	9,82 ± 3.08
3	12.15 ± 2.73	12.47 ± 1.44	12.55 ± 2.37	9.34 ± 2.77

Comparing the means of the samples showed that the differences were without significance ($P > 0.1$) except for the mean of the group which received iron paste compared with that for the pigs which were given no iron at the third week. At this week the probability of obtaining such a result by chance was just greater than 5%.

Since no significant difference can be shown between the mean Hb value of the groups, detailed analysis of the other haematological results will not be given. In summarising the data obtained the piglets to which iron was administered have been considered as one group. The figures quoted have been obtained from three to five values each from individual pigs. Where only one value was obtained no S.D. is quoted, as in the group which was given no iron.

TABLE H 2 B. Haematological Values * (Group Means & S.D. ♣)

WEEK 2							
GROUP	PCV	RBC	MCV	MCHC	MCH	RETICS	SERUM-IRON
Iron	40.6 ±2.60	5.12 ±0.64	80.3 ±8.46	31.92 ±1.91	25.42 ±1.79	3.53 ±0.3	397.4 ±169.9
No Iron	35.50 ±16.3	5.5 ±1.83	63.1 ±8.48	31.25 ±4.59	19.55 ±0.22	2.0 -	144.4 ± 93.81

WEEK 3							
GROUP	PCV	RBC	MCV	MCHC	MCH	RETICS	SERUM-IRON
Iron	35.60 ±2.31	6.1 ±0.43	58.43 ± .58	33.60 ±1.03	19.70 ±0.55	3.60 ±1.02	255.0 ±241.8
No Iron	25	3.1	80.6	28.4	22.9	3.6	78.4

* The measurements used were as has been mentioned in other experiments.

♣ Where no S.D. is given one reading only was available.

Samples of blood were not obtained after the third week because of the pressure on accomodation and since it was considered that no more useful information would be obtained by continuing the experiment.

It would seem from the results obtained that each iron preparation was satisfactory in maintaining the Hb levels. Also the pigs given no iron did not show a very marked fall of Hb; the explanation was probably that they obtained some iron from the faeces of the other piglets - indicating the difficulty of mixing the groups, even those receiving iron.

Other Data.

Clinical signs of unthriftiness or illness were not noted in any group. One pig which received iron paste died, and two pigs, one given no iron and the other iron-dextran were killed for analysis when three weeks old. No pathological findings of importance were noted. The bone-marrow sections from the piglet given iron-dextran showed the presence of numerous fat spaces in contrast to the difficulty in seeing any fat cells in the marrow of the one given no iron.

Analysis of the liver gave the following iron contents:-

Iron-dextran pig	11.70 mg per 100 gm. fresh tissue
No iron	2.82 " " " " " "

Despite the reasonable Hb levels shown by the piglets, as a group, given no iron, individuals certainly appeared to be somewhat iron-deficient as indicated by the low liver iron and bone-marrow picture of the one killed. This pig had a Hb level of 7.1gm% when destroyed, but had been noted as 5.5gm% the previous week. This was the lowest Hb recorded by any pig in this group and the range of Hb over the two weeks for the pigs in the group was 5.5 to 13.9gm% which might indicate that some pigs found and ingested more iron than others.

All pigs gained weight satisfactorily, and the mean weights at the end of the experiment are given for comparison.

The mean body weights (lbs) of the groups, at the third week were:-

WEEK	IRON-DEXTRAN	REDUCED IRON	IRON PASTE	NO IRON
3	12.3 ± 3.28	14.0 ± 3.24	11.91 ± 4.35	13.25 ± 2.01

NATURAL REARING EXPERIMENT 3.

INTRODUCTION.

Husbandry methods were as have been described in the introduction to this section.

Three litters were used: two were born on the same day and the individual pigs were divided up into two groups keeping the body weights as even as possible. One group was given reduced iron and the other an iron paste by mouth. The dose and quantity of iron has been described in the previous experiment. Each sow was allowed to nurse her own litter.

A third litter born four days later was allowed no supplementary iron, though small quantities of creep-feed pellets were allowed to all litters.

Because of limited accommodation and to prevent serious interference with the management of the piggery it was necessary to remove both litters given iron from the farrowing pens and to wean them during their fourth week. The anaemic litter was allowed to remain in the farrowing pen till five weeks old, when they were weaned also.

The groups comprised of eight pigs for those which received iron paste and six for the other two. Apart from the Hb the other blood values were obtained from three pigs only in each group.

HAEMATOLOGICAL RESULTS.

Haemoglobin.

The individual and group mean results are given in Tables H3A & 3 B.

TABLE H3 A. Individual Hb Values (gm per 100 ml).

PIGLET NO.	GROUP	WEEK							
		24hrs.	1	2	3	4	5	6	7
1		-	6.9	6.7	6.1	8.1	7.7	8.9	10.1
2		-	7.9	7.9	6.6	8.1	7.5	9.3	11.5
3		-	7.9	-	-	-	-	-	-
4	Iron	-	8.3	7.7	5.6	7.5	6.2	7.9	10.3
5	Paste	-	7.7	7.7	5.8	7.3	6.7	8.1	9.9
22		-	9.1	7.5	7.1	12.1	9.9	11.3	12.7
23		-	9.3	10.6	10.5	11.3	10.7	10.3	11.1
24		-	9.5	9.3	9.2	11.9	8.5	9.6	11.5
6		-	9.3	8.3	9.9	11.3	8.7	9.3	9.5
7		-	7.7	7.5	7.3	9.1	7.7	8.3	9.7
8	Reduced	-	7.5	10.8	8.1	9.1	9.5	9.7	11.7
27	Iron	-	10.1	10.8	11.7	13.1	10.7	11.9	11.7
28		-	10.3	12.0	12.3	11.9	10.3	10.3	10.7
29		-	9.5	9.5	10.9	13.5	12.5	11.7	11.5

Continuation of TABLE H 3 A.

PIGLET NO	GROUP	WEEK							
		24hrs	1	2	3	4	5	6	7
11		11.5	8.0	6.3	6.7	3.7	5.7	-	9.1
12		9.3	6.9	5.3	4.3	3.4	6.9	-	-
13	No	10.9	7.3	5.3	5.1	3.4	6.7	8.7	-
14	Iron	9.1	7.3	4.9	5.3	4.5	6.8	6.2	-
15		8.7	6.2	4.7	3.7	-	-	-	-
16		11.3	7.4	5.8	6.1	3.7	-	-	-

TABLE H 3 B. Group Mean Hb Values (gm per 100 ml).

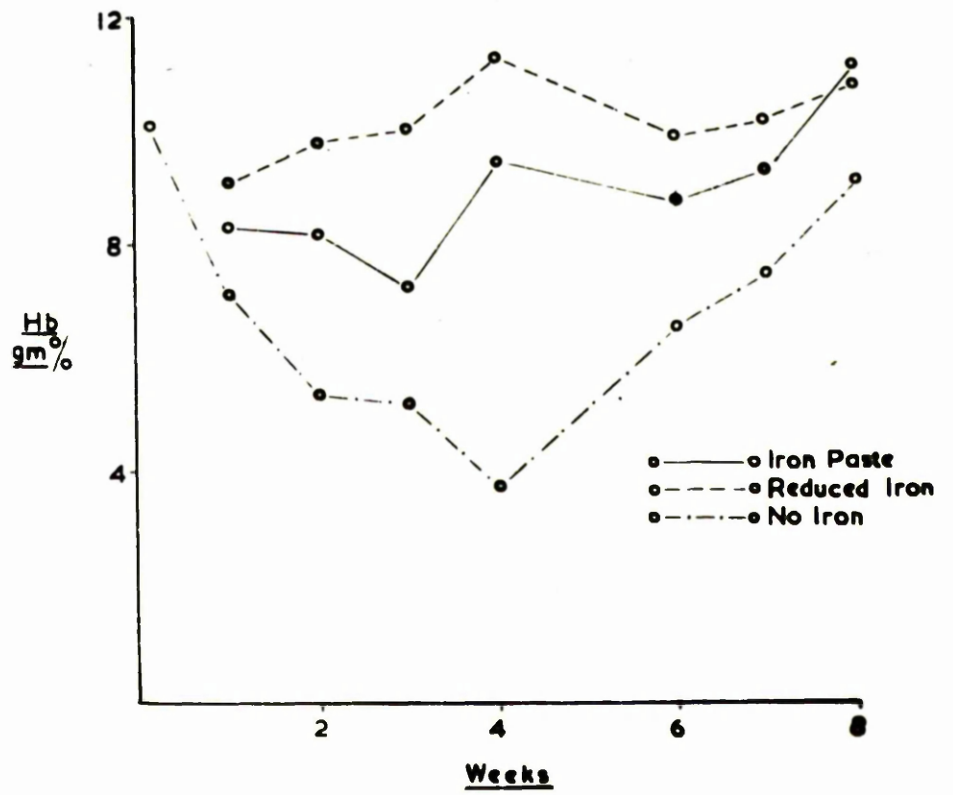
WEEK	IRON PASTE	REDUCED IRON	NO IRON
24 hours	-	-	10.13 ± 1.23
1	8.32 ± 0.90	9.06 ± 1.19	7.18 ± 0.79
2	8.20 ± 0.50	9.81 ± 1.70	5.38 ± 0.58
3	7.27 ± 1.86	10.03 ± 1.99	5.20 ± 1.10
4	9.40 ± 2.17	11.33 ± 1.90	3.74 ± 0.45
6	8.17 ± 1.64	9.90 ± 1.67	6.54 ± 0.55
7	9.34 ± 1.20	10.20 ± 1.40	7.45 ± 1.76
8	11.01 ± 0.99	10.80 ± 1.00	9.10 ± -

The difference between the means of the two iron groups was without significance at any week except week three when a significant difference was present ($P < 0.05 > 0.01$).

In contrast even at week 1 the difference between the mean of the group on reduced iron and that without iron was highly significant ($P<0.01$) and continued so at least to week four.

Comparing the group means of the other group on iron (viz. iron paste) with the severely anaemic lot showed a highly significant difference ($P < 0.01$) at the second and fourth week and significance at weeks 1 & 3 ($P< 0.05 > 0.01$). Though the group on iron paste appeared to have rather low Hb levels throughout. Fig. H 3 A depicts the fluctuations and differences at each week in the mean Hb results for the groups.

FIG. H 3A



Haematocrit Values.

TABLE H3 C Group Mean Values (ml per 100 ml).

WEEK	IRON PASTE	REDUCED IRON	NO IRON
1	-	-	24.75 ± 3.17
2	27.83 ± 3.61	38.75 ± 1.45	19.0 ± 2.82
3	24.50 ± 6.30	39.50 ± 3.53	16.0 ± 2.15
4	29.0 ± 7.0	37.50 ± 0.70	14.0 ± 1.41
6	25.66 ± 4.04	34.0 ± 1.41	25.5 ± 3.53
7	32.66 ± 4.73	40.0 ± 2.82	-

The differences between the means for the groups given iron were not significant ($P > 0.1$) except at week two.

Between the means of the 'Iron Paste' group and the 'No Iron' group significance never existed at the 5% level but between the other iron group and the latter the difference in the means was highly significant ($P<0.01$) at the third and fourth week.

Erythrocyte Counts.

TABLE H 3 D. Group Mean R.B.Cs ($\times 10^6$ per c.m.m.)

WEEK	IRON PASTE	REDUCED IRON	NO IRON
1	-	-	4.05 ± 0.92
2	4.40 ± 0.40	4.70 ± 0.56	3.60 ± 0.28
3	3.95 ± 0.22	5.85 ± 0.10	3.22 ± 0.20
4	5.60 ± 0.50	6.10 ± 0.14	3.65 ± 0.36
6	5.06 ± 0.40	5.80 ± 0.28	4.60 ± 0.31
7	6.10 ± 0.45	6.50 ± 0.14	-

At the fourth week, while no difference ($P > 0.1$) existed between the values recorded for both groups given iron, each differed significantly from the non-iron group ($P < 0.05 > 0.01$).

Red Cell Indices.

TABLE H 3 E. Group Mean Values for M.C.Vs (c μ).

WEEK	IRON PASTE	REDUCED IRON	NO IRON
1	-	-	57.4
2	63.10 ± 3.83	83.15 ± 12.2	51.90 ± 2.68
3	70.7 -	67.65 ± 6.99	51.33 ± 4.60
4	51.43 ± 8.90	61.45 ± 4.09	38.40 -
6	50.43 ± 4.87	58.60 ± 0.42	57.10 ± 5.23
7	53.93 ± 10.90	61.55 ± 5.72	-

TABLE H 3 F. Group Mean Values for M.C.H.C. (gm%).

WEEK	IRON PASTE	REDUCED IRON	NO IRON
1	-	-	29.60
2	29.63 ± 0.64	29.40 ± 8.82	30.70 ± 0.98
3	31.70 -	30.40 ± 1.69	31.13 ± 4.43
4	30.43 ± 1.64	33.35 ± 2.90	24.60 -
6	27.73 ± 1.33	30.90 ± 2.12	24.30 ± 0.56
7	26.16 ± 1.0	27.70 ± 0.84	-

TABLE H 3 G. Group Mean M.C.H. (y y).

WEEK	IRON PASTE	REDUCED IRON	NO IRON
1	-	-	18.60 ± 2.26
2	18.56 ± 1.18	23.10 ± 0.56	16.0 ± 0.70
3	18.80 ± 5.08	20.50 ± 0.98	15.25 ± 3.50
4	15.73 ± 3.36	20.45 ± 0.92	9.75 ± 0.36
6	13.96 ± 1.52	18.10 ± 1.41	13.90 ± 0.65
7	14.03 ± 2.43	17.0 ± 1.70	-

Of the red cell indices the M.C.Vs showed a significant difference between the means of the group given the paste and the anaemic ones at week 2 only.

The values for the Hb concentrations expressed as group means showed on analysis that the chance of the iron deprived pigs and those from each of the other groups coming from the same population with such a parameter at week six was less than 5% but greater than 1%. At no other week was any difference apparent.

The average M.C.H. figures for the 'No Iron' group differed significantly ($P < 0.05 > 0.01$) from the group given reduced iron at week 2 and even more so at week 4 ($P < 0.01$). At no week was the difference between the former and the 'Iron Paste' group significant however ($P < 0.1 > 0.05$).

TABLE H 3 H.* Individual & Group Mean Serum Iron Levels (µg per 100 ml).

WEEK	IRON PASTE	REDUCED IRON	NO IRON.
1	-	-	111.7 ± 18.85
2	81.2 ± 55.17	192 ± 24.72	151.40 ± 72.85
3	98.0 ± 10.18	118.4 -	139.8 ± 43.51
4	198.9 ± 77.53	205.8 -	76.3 -
6	191.4 ± 92.78	169.3± 77.36	112.9 ± 20.0
7	121.7 ± 27.44	154.1± 55.86	154.4 -

* Individual results are those without a S.D.

The group means were calculated from the serum iron estimations for two or three pigs. The lowest individual value was that for piglet 11 at week 4, which was 76.3 µg 100 ml.

Clinical Signs.

The body weights of the pigs under experiment were recorded at most weeks and these are given in the accompanying table (H 3 I).

It is interesting to note the flattening of the growth curves for both iron groups between the third and fourth weeks; presumably due to a check received during weaning which had taken place a few days earlier.

The anaemic group also can be seen to have made no weight gains between the fourth and sixth weeks, though initially the weight gains were as good as those of the pigs in the other groups. At the fifth week when unfortunately the

farm could not be visited, these piglets became affected with a severe diarrhoea, and within three days three pigs had died, only one of which was being bled. The last Hb recorded for this pig, was 3.7 gms.

TABLE H 3 I. Group Mean Body Weights (lbs).

WEEK	IRON PASTE	REDUCED IRON	NO IRON
24 hrs.	-	-	4.0
1	4.63	5.0	7.08
2	7.57	8.45	10.45
3	11.42	12.29	11.0
4	11.35	12.50	13.25
6	15.82	16.21	13.25
7	27.55	31.62	17.33
8	-	30.60	-

The clinical features of this illness were, apart from the severe diarrhoea, extreme thirst and dehydration with marked loss of weight, though the appetite seemed to be maintained. Corrective treatment was applied by the owner, who also removed the litter from the sow immediately.

Whether this can be considered as the clinical signs to be expected in severe terminal anaemia must remain a matter for conjecture. The following points should be considered however. The pigs with the lowest Hb levels at

the previous sampling did not necessarily die. Outbreaks of diarrhoea among one or more litters were not uncommon, though in this instance appeared abnormally severe. The possibility that these pigs may have received an infection which in their depleted state they were unable to resist might be the explanation.

No difference in the total serum proteins or the A/G ratio was noted in any of the pigs in which estimations were made.

These values at week 2 were : -

GROUP	PIGLET NUMBER	TOTAL SERUM PROTEIN (gm.per 100ml.)	ALB/GLOB RATIO
Iron Paste	4	5.5	0.8
" "	5	5.0	1.4
" "	24	5.2	0.8
Reduced	27	5.6	1.2
Iron	28	5.3	0.8
No Iron	11	5.4	1.0
" "	12	5.0	0.8
" "	15	5.5	0.8

PATHOLOGICAL DETAILS.

Two piglets were destroyed when anaemic. One (number 15) when three weeks old and the other (number 12)at the sixth week. The Hb values were respectively 3.7 and 6.9 gm per 100 ml.

No macroscopic abnormality of the heart, liver, lungs or other organ was noted in either pig. In piglet 15fat spaces were irregularly present in an obviously active marrow.

The iron content of the liver and spleen was estimated in these piglets. In one several samples were selected from different areas of the liver to find out if the metal content varied throughout the parenchyma. Variation in the iron deposits might have explained the differences noted, in other experiments, between biopsy and autopsy samples. No appreciable differences were found on analysis of the samples, however, and the readings obtained were as follows:-

Piglet 15.

Right	lateral	lobe	2.53	mg	per	100	gm. fresh	liver.
"	central	"	2.35	"	"	"	"	"
Left	lateral	"	2.40	"	"	"	"	"
"	central	"	2.35	"	"	"	"	"
Mixed	sample	(1)	2.54	"	"	"	"	"
"	"	(2)	2.22	"	"	"	"	"

Piglet 12

Liver	sample		2.08	"	"	"	"	"

These estimates are consistent with the contents of iron recorded in the majority of anaemic piglets in other experiments.

The spleen samples contained 9.9 mg & 14.5 mg per 100 gm fresh tissue for number 15, and 12 respectively.

DISCUSSION.

The most important point arising from the results obtained was the illness and mortality of the pigs which were very anaemic. Despite this the autopsical picture in two survivors did not reveal any of the changes so frequently described. Examination of the dead ones was unsatisfactory because of post-mortem change, though there was obviously no gross hepatic necrosis, hepatomegally or serious effusion.

Another feature of interest was the poor response of the pigs given iron particularly those given the paste. On further consideration of the individual results it did appear as if one litter, which contributed five individuals to this group and three to the other iron group, was mainly concerned in the poor haematologic response and it was noted by the pigman that they received a check, when about 7 - 10 days old, though no explanation of this was apparent. While no ready answer can be given to the question of the association between the anaemia and the morbidity and mortality experienced, it seems probable that some additional factor complicated this experiment. It may be hypothesised that this stress, which may have been an enteric infection, threw too great a strain on the precarious state of these severely anaemic piglets with disastrous consequences.

THE INFLUENCE OF INFECTION ON ANAEMIA.

INTRODUCTION.

Infection seldom if ever occurs without concomitant metabolic changes of varying profundity. Fluid and electrolyte balance may be affected, the pattern of serum proteins altered, basal metabolic rate increased and marked change result in the function of many organs and endocrine systems. It is thus, hardly surprising that the functional capacity of blood or more precisely the erythron may be altered.

Anaemia may develop in association with infections in several ways. Haemorrhage or haemolysis may occur or an anaemia already affecting the host may be exacerbated. Though by "anaemia of infection" is generally meant that anaemia which develops during the course of an infection (Cartwright & Wintrobe 1955). Gasser (1949) for example has reported an 'aplastic' reaction of the bone marrow as one manifestation of several sorts of infections and intoxications.

Experimentally it has been shown by Daft et al (1937) that subcutaneous injection of turpentine with resultant abscess formation interfered with Hb production in anaemia and non-anaemic dogs. Hb was considered to be part of a large protein pool, liable to be called on in emergencies (Whipple & Madden 1944).

Sterile turpentine abscesses were used by Wintrobe et al (1947) to show that, in pigs the uptake of iron was interfered with in the presence of inflammation and that the anaemia of infection was caused by impaired Hb

production. Since iron therapy is ineffective in raising the Hb level in the anaemia of chronic infection it appears that iron is not the limiting factor in the production of the anaemia (Greenberg et al 1947 1 & 11).

Some workers however, considered that the anaemia of infection was relieved by the administration of iron parenterally (Sinclair & Duthie 1949) whereas others disagree (Totteman 1949; Cartwright & Wintrobe 1952).

Whether some of the known or as yet undescribed diseases of pigs are liable to increase an iron deficiency anaemia is a matter for conjecture.

It was decided as a result of these consideration to see if a nutritional iron deficiency anaemia in piglets could be exacerbated by sub-cutaneous abscess formation and prevented or reduced by the administration of parenteral iron.

In order to investigate this problem it was planned to allow a pig to farrow and rear her piglets under clean conditions and the piglets were to be divided into groups, when they were at the critical stage of anaemia, i.e. when about 3 weeks old. One group was to receive parenteral iron, another subcutaneous turpentine, a third both and the control group neither treatment.

METHOD.

A gilt in late pregnancy was purchased and allowed to farrow and raise her litter under conditions designed to create nutritional iron-deficiency anaemia.

For this purpose a loose - box measuring approximately 12 x 12 feet was cleaned, and a wooden creep built in one corner in which hung a light bulb. Three walls of the loose-box were of brick and concrete, and the fourth a wooden partition. The floor was paved with granite setts. All metal projections were either removed or suitably covered. An earthenware trough was used to feed the sow, and oat straw allowed as bedding. The gilt was fed twice daily with a wet sow and weaner meal.

By using oil of turpentine (Ol. Terebinth.), subcutaneously in the piglets it was intended to produce a condition analagous to an acute infection, as had been described by Whipple & Madden (1944) and Wintrobe et al (1947). Thus permutations of iron and turpentine were given to the piglets farrowed by this gilt, as in Table 1.

The iron preparation used was an iron-dextran complex.* This haematinic was chosen since it could be given intra-muscularly and thus the possibility of those piglets not injected obtaining iron from the others was reduced.

Eight live pigs were farrowed by this gilt and these were allowed to suck the dam. It was intended to wait until they became markedly anaemic before administering the iron or the turpentine. Three weeks were thus allowed to elapse before any interference, apart from the withdrawal of blood samples

* Imferon (Bengers Lab.,Ltd.)

was attempted. The turpentine and iron-dextran were given at the same time; when the piglets were three weeks old, and the turpentine treatment repeated one week later.

The total amounts of iron and turpentine administered are included in table 1.

TABLE 1. Amounts of Iron and Turpentine Administered to the Individual Piglets.

PIGLET NUMBER	IRON INTRA-MUSC.DOSE mg.	TURPENTINE sub.-cut dose ml.
1	25	-
2	-	-
3	100	-
4	-	-
5	-	3
6	-	4
7	25	3
8	100	4

HAEMATOLOGICAL RESULTS.

The piglets were bled at frequent intervals from their ear veins and Hb only estimated. Samples were obtained from the anterior vena-cava, three times prior to the administration of the treatments and a full haematological examination made. Subsequent Hb levels did not justify continuing the full haematological examinations.

Haemoglobin.

The first Hb samples, taken up to the eighth day were lower than those subsequently recorded; though as a group mean value (8.41 gm%) not exceptionally low at the eighth day. Howeve , they failed to decline as was anticipated. By the third week when it appeared that a dramatic fall was unlikely to occur, it was decided to continue as planned to see if the formation of abscesses would affect adversely the Hb values. As can be seen from Table 2 no deleterious effects were apparent.

Haematocrit Values.

The volumes of packed cells which at the fourth day, appeared to be rather low, rapidly returned to more normal values. (Table 3).

Erythrocyte Counts.

The initial low values for the two parameters already mentioned were similarly present for the R.B.Cs initially (Table 3).

Red Cell Indices. (Table 4).

The values for the M.C.H.C & M.C.V are within what can be considered normal limits. It is interesting to see the fluctations in the M.C.H.Cs even

in pigs without low Hb values, for example piglet 6.

Reticulocytes.

At the 3 periods at which reticulocyte counts were made no unusual percentages were recorded. (Table 5).

Serum Iron Estimations.

This parameter was estimated at the eighth week only and the following values, none of which is low, were obtained.

Piglet Number	Serum Iron (μ g per 100 ml).
1	318.9
2	163.6
3	224.5
4	-
5	183.4
6	206.9
7	284.2
8	380.0

White Cell Counts.

Except for piglet 1 when 8 days old which had a total white cell of 18,200 per c.mm all other counts were within the normal range, but all were made prior to the injection of the turpentine for reasons given earlier.

The differential white cell counts made only when the piglets were four days old, showed a group mean count of, 65% polymorph. leucocytes, 30% lymphocytes, 4% monocytes, 0.5% basophil leucocytes and 0.5% eosinophil leucocytes.

Serum Proteins.

The total serum proteins and the albumin to globulin ratios were estimated when the pigs were eight days old. Examinations at later periods were not made since it was unlikely that useful information could be gained. In most of the sera the albumin content seemed low. Those recorded were :-

PIGLET NO.	TOTAL SERUM PROTEINS (gm per 100 ml)	A/G RATIO.
1	6.0	0.6
2	6.5	0.6
3	6.5	0.8
4	6.4	0.5
5	5.75	0.7
6	6.3	0.5
7	6.4	0.5
8	6.3	0.4

CLINICAL SIGNS.

It is apparent from the blood attributes that no anaemia devloped in any of the piglets even those deprived of iron. It is therefore not surprising that no abnormal signs were recorded in these piglets.

The subcutaneous turpentine produced necrosis of the skin and underlying panniculus adiposus but no quantity of pus ever accumulated or was discharged. The lesion was rather that of a dry necrotic area which eventually sloughed.

The piglets grew well (see Table 6), though they received no special supplementary feeding but were seen to partake of that supplied to the sow. The body weights did not appear to be affected by either the administration of the haematinic or the subcutaneous turpentine.

DISCUSSION.

As to achieving its object this experiment was a failure, since none of the pigs became even slightly anaemic. Several useful and interesting points arose however and for this reason the experiment has been reproduced here.

First of all it does illustrate the difficulty which can be experienced in attempting to reproduce an iron-deficiency anaemia, and when, as in this case, care was taken to exclude the major sources of iron it does cause one to hypothesise on the influence of pre-natal stores of iron.

It is interesting also to see that supplementary iron given parenterally did not influence the body weights achieved. The failure of the subcutaneous turpentine to result in gross abscess formation was notable.

The blood parameters recorded are useful as a comparison with those obtained

in other experiments. It may be noted that wide fluctuations in the Hbs occurred even at short intervals in the same pig. THis may be due partly to the method of taking the samples namely from the ear. It had been found previously that provided a good flow of blood was obtained from the pricked vessel the Hb recorded did not differ significantly from a sample obtained aththe same time from the anterior vena - cava . Another explanation may be that given by Wintrobe et al (1947) of shifts in plasma volume. These workers stated that "in growing pigs the Hb per 100 ml. of blood shows wide and rapid variations".

TABLE 2. Haemoglobin (gm.per 100 ml).

PIG-LET NO.	DAYS.													
	4	8	13	15	18	19	20	22	23	25	26	28	32	39
1	7.5	7.7	10.7	11.1	10.7	10.7	13.3	11.9	11.5	12.3	12.5	11.5	13.1	11.9
2	7.9	8.3	10.4	10.9	11.7	10.7	11.5	10.3	10.7	10.5	10.7	9.9	11.5	10.5
3	8.5	8.1	10.0	10.3	11.5	10.7	11.2	11.5	11.5	11.9	11.7	9.9	11.7	10.7
4	10.1	-	11.5	11.3	10.7	10.7	11.0	10.5	10.5	11.3	10.7	9.9	10.3	10.7
5	9.3	8.1	10.7	10.3	11.3	10.7	10.5	9.5	10.1	11.1	9.9	9.1	10.9	11.1
6	10.3	10.5	12.3	11.5	11.9	9.9	11.1	11.1	11.7	12.1	10.7	9.9	11.5	10.1
7	-	8.9	11.2	10.9	11.5	10.1	11.1	10.7	11.7	11.5	11.5	10.7	12.3	11.9
8	7.3	7.3	10.3	9.5	9.9	8.3	10.0	9.7	10.3	10.9	11.9	9.9	11.5	11.7

TABLE 3. Haematocrit Values (ml per 100 ml) and Red Cell Counts ($x10^6$ per c.mm).

Piglet No.	HAEMATOCRIT VALUE (ml/100ml)				ERYTHROCYTE COUNT ($x10^6$/c.mm).			
	Days				Days			
	4	8	15	32	4	8	15	32
1	21	30	35.0	-	4.1	5.2	5.5	-
2	23	30	36	-	4.3	5.4	6.1	-
3	26	30	37	-	3.5	5.4	5.6	-
4	34	-	37	34	5.2	-	5.9	5.9
5	29	32	35	-	4.7	5.3	5.2	-
6	34	36	37	-	5.6	5.9	8.4	-
7	-	31	37	38	-	5.5	5.9	6.0
8	22	29	32	-	4.0	4.9	4.6	-

TABLE 4. Red Cell Indices.

Piglet No.	M.C.H.C. (%)				M.C.V. (c μ).			
	Days				Days			
	4	8	15	32	4	8	15	32
1	35.7	25.6	32.7	-	52.5	57.6	63.6	-
2	31.6	27.6	30.2	-	58.1	55.5	59	-
3	32.6	27.0	27.8	-	74.2	55.5	66	-
4	29.7	-	30.5	30.2	65.2	-	62.7	57.6
5	32.0	25.3	29.4	-	61.7	60.3	67.3	-
6	30.2	29.1	31.0	-	60.7	61.0	58.7	-
7	-	28.7	29.4	32.3	-	56.1	62.7	63.3
8	33.1	25.1	29.6	-	55.0	59.1	69.5	-

TABLE 5 Reticulocytes (% of erythrocytes).

Piglet No.	Days.		
	4	8	15
1	-	5.4	6.2
2	4.6	4.0	5.6
3	-	4.8	5.0
4	2.8	-	3.8
5	-	5.1	5.8
6	-	3.9	6.0
7	-	5.8	6.4
8	-	4.2	5.8

TABLE 6. Body Weight (lbs).

Piglet No.	DAYS				
	8	15	25	32	39
1	3.5	6.0	10.5	14.25	13.25
2	6.5	11.0	16.75	20.25	24.0
3	5.5	9.25	16.25	21.25	24.0
4	5.5	10.25	15.25	18.0	19.5
5	5.0	9.0	13.25	15.0	18.75
6	5.75	9.75	15.75	19.5	21.5
7	4.0	-	12.0	15.0	17.0
8	5.25	7.75	10.75	13.0	15.25

FARM SURVEY.

INTRODUCTION.

Several farms were visited in order to obtain some information on the levels of Hb achieved by piglets on different farms and therefore under varying systems of husbandry. Such a survey would also provide details of counter-measures used to prevent anaemia.

The farms were visited without prior notification of the reason. Any available litter about two to four weeks of age was selected, and samples were obtained from the ear veins. Usually only Hb was determined, but sometimes fuller haematological examinations were made and occasionally a pig was purchased for autopsical examination.

RESULTS.

Farm C.

On these premises two systems of pig husbandry were practiced. Sows were allowed to farrow in insulated wooden arks placed either in the middle of large grass paddocks or on concrete runs approximately 8 x 16 feet. It was usual to prevent the piglets from leaving the ark for the first two weeks or so by placing a wooden board across the entrance; creep feeding was introduced about the third week. Litters reared on concrete were dosed with an iron paste during the second week of life; those in arks on the grass paddocks received no iron supplement.

The lowest Hb recorded was 7.8 gm/100 ml (Table 3) and this for a piglet during the first week of life. One implication from the figures recorded was that, as would be expected, the piglets given no supplementary iron showed the lower Hb levels of the two groups. These seemed to be corrected readily as the

piglets grew older and were able presumably to supplement their diet.

Another interesting feature was the difference in the Hb values not only between pigs in different litters but between those in the same litter. The high Hb values noted at the second day after birth declined in litter A by the sixth day (Table 5).

The red cell counts were usually about 5 million per c.mm. though during the initial week or so after birth lower values were often recorded for both the erythrocyte numbers and packed cell volume.

The M.C.H.C. levels were variable and no constant correlation appeared to exist simply between the Hb and M.C.H.C. parameters. Levels of below 30% of corpuscular Hb content were not always associated with the lower values of Hb calculated as gm. per 100 ml. It is interesting to note in Table 4 the close proximity of the M.C.H.C. values for the three piglets and yet the discrepancy between this parameter and the Hbs and the P.C.Vs . A proportional alteration of the Hb and the P.C.V. results in a constant M.C.H.C. value.

The M.C.V. also showed wide variations, and the group mean volume of the cells varied from about 60 to 80 c.μ. The normal range of the M.C.V for man was given as 78 - 94 c μ by Whitby & Britton (1957). Except in litter 1790 which had rather low Hb values during the first two weeks or so with erythrocyte counts about the normal range, the mean M.C.H values were usually above 20 γ γ.

Several workers have noted a rapid decline in the Hb levels of piglets

shortly after birth (Hamilton et al 1930; Jesperson & Olsen 1939). Fraser (1938) held this fall to be more striking in pigs than that in other animals. Bearing this consideration in mind Hb samples were taken from piglets shortly after birth and repeated at frequent intervals. The results are given in Table 6.

From these values it is apparent that no consistent alteration occurred in the Hb levels of the blood from the piglets in this litter. In some of the experiments on piglets reared artificially a drop in Hb occurred between the first and second week, even in those supplied with iron. It was pointed out by Wintrobe et al (1947) that the piglet is subject to marked changes in plasma volume and it may be that alteration in the fluid balance in neonatal pigs may increase the tendency for change to occur in the Hb level at this time. In infants there is a considerable reduction in Hb during the first twelve days of life (Whitby & Britton, 1957) as the circulation adjusts itself to meet the requirements of the richer respiratory oxygenation. Croft & Moe (1933) pointed out that while in most instances there was a marked fall in Hb during the first week there was marked variation between piglets and some did not show this drop by the seventh day after birth.

TABLES OF BLOOD PARAMETERS FOR LITTERS ON FARM C.

TABLE 1. Blood Parameters for 4 Piglets of litter 1205 with access to concrete.

PARAMETER	PIGLET No.	DAYS.						
		7	9	14	16	21	23	25
	118	9.6	9.7	11.0	11.1	14.4	15.0	-
Hb.	119	9.4	-	11.0	-	13.7	15.0	-
(gm%)	120	9.4	9.5	11.1	10.4	14.0	15.3	13.1
	121	8.9	-	-	-	14.4	14.0	-
	Mean	9.32	9.60	11.03	10.75	14.12	14.82	-
	S.D.	±0.30	±0.14	±0.07	±0.50	±0.35	±0.56	-
	118	35	33	42	44	42	42	-
P.C.V	119	33	-	41	-	41	41	-
ml/	120	31	39	43	37	41	41	38
100ml	121	30	-	-	-	43	41	-
	Mean	32.35	36.0	42.0	40.5	41.75	41.15	-
	S.D.	±2.22	±4.24	±1.0	±4.94	±1.0	±0.5	-
	118	4.5	4.2	5.6	5.9	4.6	4.9	-
R.B.Cs	119	4.0	-	5.0	-	5.3	5.1	-
($x10^6$ per	120	4.7	4.5	5.6	4.6	5.4	5.3	5.2
c.mm)	121	4.5	-	-	-	5.1	5.3	-
	Mean	4.42	4.35	5.40	5.25	5.10	5.15	-
	S.D.	±0.30	±0.22	±0.34	±0.92	±0.35	±0.17	-

Continuation of Table 1.

PARAMETER	PIGLET No.	DAYS.						
		7	9	14	16	21	25	25
	118	4.6	9.6	6.4	8.9	6.6	-	-
Retics.	119	9.8	-	7.6	-	7.1	4.1	-
(% of	120	7.8	8.4	8.8	9.5	8.9	4.8	-
R.B.Cs)	121	9.6	-	-	-	8.7	5.6	-
	Mean	7.95	9.0	7.6	9.2	7.82	4.85	
	S.D.	±2.40	±0.84	±1.2	±0.42	±1.14	±0.74	
	118	15.4	8.2	11.8	7.4	11.0	12.1	
W.B.Cs	119	13.9	-	9.4	-	15.4	14.6	
($x10^3$	120	13.6	9.2	10.8	8.2	9.8	10.2	13.5
per c.mm)	121	14.4	-	-	-	8.7	9.6	
	Mean	14.32	8.70	10.66	7.80	11.22	11.62	
	S.D.	±0.78	±0.70	±1.20	±0.56	±2.93	±2.25	
	Polys.	53.7	46.5	39.0	-	48.0		
Mean	Lymphs	41.2	46.0	63.0	-	49.0		
different	Monos	0.5	0.25	0.25				
white cell	Basos	0.0	0.0	0.0	-	0.0		
counts.	Monos	4.2	2.0	1.25	-	2.25		

Continuation of Table 1.

PARAMETER	PIGLET No.	DAYS.						
		7	9	14	16	21	23	25
M.C.H.C (%)	118	27.4	24.3	26.1	25.2	34.2	35.7	-
	119	27.8	-	26.8	-	33.4	36.5	-
	120	30.3	29.3	25.8	29.1	34.1	37.3	34.4
	121	27.6	-	-	-	33.4	34.1	-
	Mean	28.28	26.80	32.90	27.15	33.77	35.90	-
	S.D.	±1.36	±3.53	±0.31	±2.75	±0.43	±1.36	-
M.C.V. (c. μ)	118	77.7	86.6	75	74.4	91.3	85.7	-
	119	82.5	-	82.0	-	77.3	80.4	-
	120	65.9	78.5	76.7	80.4	75.9	77.3	73
	121	66.6	-	-	-	84.3	77.3	-
	Mean	63.17	82.55	77.90	77.40	82.20	80.17	-
	S.D.	±8.23	±5.72	±2.96	±4.24	±7.08	±3.96	-
M.C.H. (y.y.)	118	21.3	23	19.6	18.8	31.3	30.6	-
	119	23.5	-	22.0	-	25.8	29.4	-
	120	20.0	21.1	19.8	22.6	25.9	28.8	25.1
	121	19.7	-	-	-	28.2	26.4	-
	Mean	21.12	22.05	20.46	20.7	27.8	28.8	-
	S.D.	±1.72	±1.34	±1.33	±2.68	±2.58	± 1.76	

TABLE 2. Blood Parameters for 3 Individuals of Litter S.P. with access to Concrete run.

PARAMETER	PIGLETS	DAYS.		
		12	18	29
Hb (gm %)	LT	10.1	9.9	11.7
	LB	10.1	10.9	11.7
	RT	9.4	10.3	12.2
	RB	9.4	10.3	10.9
	Mean	9.7	10.3	11.6
	SD	±0.4	±0.4	±0.5
P.C.V (ml/100ml)	LT	35	32	37
	LB	35	34	37
	RT	32	32	39
	RB	34	33	34
	Mean	34	32.7	36.7
	SD	± 1.4	±1.0	±2.0
R.B.C (10^6/cmm)	LT	5.0	4.0	5.2
	LB	4.2	5.1	6.5
	RT	3.5	4.7	6.0
	RB	3.9	4.8	5.7
	Mean	4.15	4.65	5.85
	SD	±0.6	±0.4	±0.5

Continuation of Table 2.

PARAMETERS.	PIGLETS.	DAYS.		
		12	18	29
M.C.H.C (%)	LT	28.8	30.9	31.6
	LB	28.8	32.0	31.6
	RT	29.3	32.1	31.2
	RB	27.6	31.2	32.0
	Mean	28.6	31.5	31.6
	SD	±0.7	±0.5	±0.5
M.C.V. (σ/μ)	LT	70	80.0	71.1
	LB	83.3	86.6	56.9
	RT	91.4	68.0	65
	RB	87.1	68.7	59.6
	Mean	82.9	70.8	63.1
	SD	±9.2	±6.1	±6.1
M.C.H (y.y.)	LT	20.2	24.7	22.5
	LB	26.4	21.3	18.0
	RT	26.8	21.9	20.3
	RB	24.1	21.4	19.1
	Mean	24.3	22.3	19.9
	SD	±3.0	±1.6	±1.9

Continuation of Table 2.

PARAMETERS.	PIGLET.	DAYS.		
		12	18	29
W.B.C ($\times 10^3$ per cmm)	LT	7.5	9.9	14.6
	LB	12.6	10.2	12.3
	RT	10.2	7.2	9.6
	RB	6.7	6.6	9.0
	Mean	9.2	8.4	11.3
	SD	±2 6	±1.8	±2.5
Mean Differential W.B.C Cell Counts	Poly	45.0 ±11.7		
	Lym	50.25 ±20.9		
	Monos	4.0 ± 2.9		

TABLE 3. Parameters for 4 Individuals of litter 1790 Housed in ark with grass run.

PARAMETER	PIGLET No. 1790	DAYS. 6	13	15	28
Hb (gm %)	RB	8.5	9.6	10.2	12.1
	RT	7.8	8.8	9.6	11.3
	DN	8.8	9.1	9.5	11.5
	LT	8.5	8.4	8.9	11.1
	MEAN	8.4	8.97	9.55	11.50
	SD	± 0.42	±0.5	±0.52	±0.43
P.C.V. (ml/100ml)	RB	27.5	37.0	36	41
	RT	25.0	30	36	39
	DN	28.0	33	35	39
	LT	27.0	30	31	40
	Mean	26.87	32.5	34.5	39.75
	SD "	±1.31	±3.3	±2.34	±1.0
R.B.C (x 10^b per c mm)	RB	4.7	5.0	6.7	7.5
	RT	4.1	4.7	3.0	6.0
	DN	4.6	4.9	5.1	6.0
	LT	4.0	4.2	4.8	5.9
	Mean	4.35	4.70	5.40	6.35
	SD	±0.34	± 0.35	± 1.51	±0.76

Continuation of Table 3.

PARAMETER	PIGLET No. 1790	DAYS. 6	13	15	28
M.C.H.C (%)	RB	30.9	25.9	28.3	30.2
	RT	31.0	29.0	27.2	28.9
	DN	31.2	27.5	27.1	29.0
	LT	31.4	28.0	28.7	27.7
	Mean	31.12	27.6	27.82	28.95
	SD	±0.22	±1.29	±0.79	±1.01
M.C.V (c μ).	RB	58.5	74	53.7	54.6
	RT	60.9	63	72.0	62.9
	DN	60.8	67.3	69.0	65.0
	LT	67.5	71.1	64.5	67.4
	Mean	61.92	68.85	64.8	62.47
	SD	±3.8	±4.7	±8.0	±5.5
M.C.H (y.y.)	RB	18.1	19.2	15.2	16.1
	RT	19.0	18.7	19.2	18.8
	DN	19.1	18.5	18.6	19.1
	LT	21.2	20.0	18.5	18.8
	Mean	19.35	19.10	17.87	18.20
	SD	±1.31	±0.66	±3.94	±1.40

Continuation of Table 3.

PARAMETER	PIGLET No. 1790	DAYS.			
		6	13	15	28
Ratios (% of R.B C)	RB	8.4			
	RT	8.0			
	DN	4.7			
	Mean	7.03			
	SD	±2.02			
Mean Differential White cell Counts.	Poly	44.5	34.5	-	34.0
	Lyms	48.0	63.5	-	64.7
	Eosin	2.0	0.5	-	0.75
	Monos	0.75	1.5	-	0.5

TABLE 4. Parameters for 3 Individuals of litter 6336 in Ark with grass run.

PARAMETER.	PIGLET	DAYS.		
	6336	2	4	17
Hb (gm%)	RB	9.9	-	-
	RT	11.3	10.3	12.8
	LT	10.5	11.7	13.8
	Mean	10.5	11.0	13.3
	SD	±0.7	±0.9	±0.7
P.C.V (ml/100ml)	RB	28	-	-
	RT	32	30	36
	LT	30	36	50
	Mean	30.0	33.0	43
	SD	±2.0	±4.2	±9.8
R.B.C ($\times 10^6$/cmm)	RB	3.8	-	-
	RT	4.0	4.1	5.2
	LT	5.1	4.9	8.6
	Mean	4.3	4.5	6.9
	SD	±0.7	±0.5	±2.4

Continuation of Table 4.

PARAMETER	PIGLET	DAYS.		
	6336	2	4	17
M.C.H.C. (%)	RB	35.3	-	-
	RT	35.3	34.3	35.5
	LT	35.0	32.5	27.6
	Mean	35.2	33.4	31.5
	SD	±0.1	±1.2	±5.5
M.C.V (c μ)	RB	73.6	-	-
	RT	80.0	73.1	69.2
	LT	58.8	73.4	58.1
	Mean	70.8	73.2	63.6
	SD	±10.9	±0.2	±7.8
M.C.H (yy)	RB	26.0	-	-
	RT	28.2	25.1	24.6
	LT	20.5	23.8	16.0
	Mean	24.9	24.4	20.3
	SD	±3.96	±0.92	±6.07
W.B.C ($x\ 10^3$ per cmm).	RB	12	-	-
	RT	13.2	9.2	10.9
	LT	9.1	13.4	19.8
	Mean	11.43	11.30	15.35
	SD	±2.10	±2.96	±6.29

TABLE 5. Parameters for 3 Individuals of litter A in ark with grass run.

PARAMETER	PIGLETS	DAYS.				
		2	6	10	13	27
Hb (gm%).	RB	11.7	10	9.3	10.3	13.3
	RT	10.3	9.6	8.4	-	13.3
	LT	16.3	9.6	9.0	9.7	-
	Mean	12.76	9.73	8.9	10.0	13.3
	SD	±3.14	±0.23	±0.45	-	±0.0
P.C.V (ml/100ml)	RB	34	28	28	32	40
	RT	31	26	31	-	27
	LT	31	28	24	30	-
	Mean	32	27.3	28.0	31.0	32.3
	SD	±1.73	±1.22	±3.60	±1.41	±9.19
R.B.C ($\times 10^6$ per c mm)	RB	4.6	4.0	4.1	4.5	5.3
	RT	4.5	4.8	4.3	-	5.8
	LT	5.5	4.1	4.0	4.0	-
	Mean	4.86	4.30	4.13	4.25	5.55
	SD	±0.54	±0.43	±0.15	±0.36	±0.36

Continuation of Table 5.

PARAMETER	PIGLETS	DAYS.				
		2	6	10	13	27
M.C.H.C. (%)	RB	34.4	35.7	32.0	32.1	33.2
	RT	33.2	36.9	27.0	-	32.4
	LT	52.5	34.2	37.5	32.3	-
	Mean	40.0	35.6	32.16	32.2	32.8
	SD	±10.8	±4.3	±5.24	±0.26	±0.56
M.C.V (c/u)	RB	73.9	70.0	70.7	71.1	75.5
	RT	68.8	54.1	72.1	-	70.6
	LT	56.3	68.2	60.0	75.0	-
	Mean	66.3	64.1	67.6	73.0	73.0
	SD	±8.4	±8.7	±6.61	±2.7	±3.4
M.C.H (yy)	RB	25.4	25.0	22.6	22.8	25.1
	RT	22.8	20.0	19.5	-	22.9
	LT	29.6	23.4	22.5	24.2	-
[illegible]	Mean	25.93	22.80	21.53	23.5	24.0
	SD	±3.4	±2.5	±1.7	±0.98	±1.55

Continuation of Table 5.

PARAMETER	PIGLETS	DAYS.				
		2	6	10	13	27
W.B.C ($\times 10^3$ per c.mm)	RB	9.4	8.9	10.7	10.9	11.8
	RT	13.9	12.4	14.2	-	11.8
	LT	8.7	9.8	16.5	17.2	-
	Mean	10.66	10.36	13.8	14.05	11.8
	SD	$\pm$2.8	$\pm$1.81	$\pm$2.91	$\pm$4.4	$\pm$0.0
Mean Different White Cell Counts	Poly	61.33				
	Lyms	34.6				
	Monos	4.0				

TABLE 6. Haemoglobin values for litter 1205 for six days post-partum.

Parameter	Piglet	Hours post-partum								
		2	7	15	28	34	51	105	124	136
Hb (gm%)	1	9.6	11.6	8.7	10.0	9.7	9.7	10.0	8.7	8.6
	2	9.1	9.7	10.0	10.3	10.2	10.7	9.4	8,7	9.1
	3	14.0	11.6	10.6	12.3	9.9	11.3	10.7	10.2	9.6
	4	12.7	11.3	-	10.7	10.6	10.7	11.2	12.0	10.9
	5	8.9	9.7	8.7	8.3	8.5	10.7	9.7	9.3	8.0
	6	7.7	11.0	-	10.3	8.8	9.1	9.0	910	8.2
	Mean	10.3	10.8	9.5	10.3	9.6	10.4	10.0	9.6	9.0
	± SD	±2.4	±0.8	±0.9	±1.2	±0.8	±0.6	±0.8	±1.2	±1.0

Continuation Table 6.

Blood Attributes for Litter 1205 When Six Days Old.

PIGLET	P.C.V (ml/100ml)	R.B.C ($x10^6$/c.mm)	M.C.H.C (%)	M.C.V (cμ)	M.C.H (yy)	W.B.C ($x10^3$/c.mm)
1	30	4.3	28.6	69.7	20.0	11.0
2	29	4.3	31.3	67.4	21.2	7.5
3	34	5.7	28.2	56.1	16.8	12.0
4	31	4.2	35.1	73.8	25.9	12.1
5	34	5.1	23.5	66.6	15.6	9.1
6	28	4.5	29.2	62.2	18.2	10.4
Mean	31	4.6	29.3	65.9	19.6	10.3
$\pm$ S.D.	$\pm$2.5	$\pm$0.5	$\pm$5.1	$\pm$6.1	$\pm$3.6	$\pm$1.7

FARM C/N.

This was a medium - sized piggery with two Danish type pig-houses in one of which breeding was undertaken. Pigs were of the large White Breed, and while no serious losses were encountered before weaning, after this time pneumonia was troublesome.

Piglets were allowed a creep in which hung a lamp in each breeding pen, and weaning did not take place until eight weeks. Supplementary feeding was supplied to the piglets from the age of three weeks. When three days old each piglet was given 100 mg of an iron-dextran* intra-muscularly. The piglets from two litters were bled from the ear veins; one thirteen and the other twenty-five days old. The Hb attributes are recorded in table number 7.

It is interesting to note the difference in the Hb levels between the litters, and the variation which occurred within the litters, despite the relatively massive iron dose.

* 'Imferon' (Bengers Lab. Ltd.,)

TABLE 7. Hb Levels of the Individuals in two Litters on Farm C/N.

Litter A. 25 days old.

PIGLET	Hb (gm%)	BODY WEIGHT (lbs).
1	8.7	14.5
2	6.9	19.2
3	8.9	17.7
4	8.7	16.0
5	9.6	12.5
6	9.3	13.5
7	9.1	16.5
8	8.9	16.7
Mean ± S.D.	8.5 ± 0.8	15.8

Litter B. 13 days old.

1	11.0	8.2
2	10.2	7.5
3	12.2	4.0
4	12.4	9.0
5	10.2	6.2
6	10.4	6.0
7	9.3	8.5
8	10.4	7.0
9	9.3	7.7
Mean ± S.D.	10.6 ±1.1	7.1

FARM H/G.

This herd of Large White pigs was kept in two Danish - type houses, in one end of which breeding sows were allowed to farrow. Wooden kennels in which electric bulbs hung were provided for the piglets. When about one week old small amounts of solid food were supplied to the piglets and earth was placed in the pen at the third day and thereafter twice weekly for three weeks. Diarrhoea was frequent in the piglets of most litters during the first few weeks after birth, all the piglets in litter A being affected when bled, it was usual to find one or more piglets in occasional litters which because of their thinness merited the description 'runts'. These unthrifty piglets showed no response to iron therapy; nor did the diarrhoea affecting other piglets. Pneumonia was known to occur in the older piglets.

Table 8 gives the Hb values for the piglets in three litters. There was a wide variation in the Hb values recorded though most of the piglets had low values. For instance in litter A the lowest values was 3.2 gm% and the highest 9.2 gm %. The difference in this parameter possibly reflects the ability or desire of each piglet to ingest and absorb sufficient iron from the soil to meet its particular needs. Since the ingestion of the haematinic material was entirely voluntary wide variations in the amount consumed and thus in the Hb (and other parameters) from pig to pig might be expected. A few piglets from two of these litters were selected for autopsy mainly because they were the smallest or thinnest piglets in the litter. These piglets were blood sampled six days after the first Hb values (Table 8) then destroyed. The haematology,

serum protein values and parenchymal iron and copper values have been recorded in Table 9. Except in one piglet (B5) little change occurred in the Hb values. The serum iron levels corresponded in remarkable manner with the Hb levels. The P.C.Vs, M.C.H.Cs (which were all below 27%) and the M.C.Vs all seemed rather low and it would seem as if those piglets were suffering from a mild degree of anaemia which was microcytic and hypochromic. The liver iron estimations could be taken as indicating a rather borderline intake of iron.

One piglet on autopsy showed some degree of pneumonia and two had diffusely pale yellow fatty livers. Unfortunately the post-mortem reports on them were not available. It was however recognised that dystrophic changes of the liver of unknown cause did occur periodically in the piglets in this herd.

TABLE 8. Hb. Level of the Individuals in three Litters on Farm H/G.

PIGLET	Hb (gm%).		
	Litter A. 19 days old.	Litter B. 24 days old	Litter C. 14 days old.
1	5.3	6.1	5.9
2	7.2	4.2	6.0
3	7.5	7.3	5.4
4	3.2	6.0	5.4
5	7.6	5.6	8.4
6	9.2	6.8	6.6
7	8.9	5.5	6.6
8	5.8	5.3	6.2
9	6.2	7.8	7.0
10	8.9	5.4	-
Mean ± SD	6.9 ± 1.8	6.0 ± 1.1	6.3 ± 0.9

TABLE 9. Blood Values for Piglets in Three Litters on Farm H/G.

PARAMETERS.	IDENTIFICATION OF PIGLETS (LITTER & NO).				
	A2	A3	A7	B5	B6
Hb (gm%)	7.1	7.5	8.7	6.5	6.9
R.B.C ($x10^6$/cmm)	5.7	5.1	6.6	5.0	4.1
P.C.V (ml/100ml)	27	28	31	27	26
M.C.H.C (%)	26.2	26.7	26.2	24.2	26.5
M.C.V (c μ)	47.3	54.9	46.9	54.0	63.4
Serum Iron μ g %	124.7	149.3	204.2	91.7	124.2
W.B.C($x10^3$/cmm)	9.2	14.4	5.0	7.6	11.4
Total Protein (gm%)	6.4	4.2	3.7	4.6	3.8
A/G ration	1.9	0.7	1.1	1.1	0.7
Albumin (gm%)	4.2	1.7	1.9	2.4	1.6
Globulin (gm%)	2.2	2.5	1.8	2.2	2.2
Liver Iron mg/100 gm fresh tissue	2.58	3.28	2.96	2.74	3.38
Spleen Iron mg/100 gm fresh tissue	9.9	11.6	18.7	11.8	12.0
Liver Copper p.p.m/ fresh tissue	36.5	42.1	25.9	69.0	64.0

FARM K.

This was a small farm concentrating mainly on pig breeding. The majority of those bred were generally sold when weaned. About 12 to 15 sows were kept, & farrowed in a converted cow-byre which tended to be cold and draughty but by the use of wooden kennels and lamps the piglets were maintained in reasonably warm conditions. Sows and piglets were swill fed. Piglets received no iron supplements but weather permitting were allowed access to soil and grass in a field from about 10 days onwards after birth, and thus had ample opportunity to pick up soil and faeces from their mother.

The Hb values for five litters are given in Table 10. Litters 1, 2 and 5 had been allowed outside for several hours daily for the last 3 or 4 days. Litter 3 and 4 had not been outside and all were excreting soft yellow faeces.

Metastrongylus apri, and trichostrongyle worm infestations, pneumonia and diarrhoea of unknown and probably varied aetiology were known to occur in the pigs. Agglutination titres to leptospira canicola and icterohaemorrhagiae were present in most of the adult pigs.

The Hb values were not extremely low even in those without direct access to soil and probably reflected to the standard of cleanliness of thepiggery and the rather slow growth rate made by the majority of the piglets on these premises.

TABLE 10. Hb. Values for Piglets in Five Litters on Farm K.

PIGLET	Hb (gm%).				
	Litter 1 16 days	Litter 2 16 days	Litter 3 10 days	Litter 4 10 days	Litter 5 14 days
1	9.5	7.9	7.3	7.7	9.5
2	7.9	7.7	9.5	8.6	8.4
3	8.7	7.3	6.9	8.4	8.7
4	8.9	7.9	7.1	9.5	9.8
5	11.1	7.5	6.9	10.4	9.1
6	-	9.5	6.4	10.2	10.2
7	-	11.1	7.5	9.1	9.5
Mean ± SD	9.2 ± 1.1	8.4 ±1.3	7.3 ±1.0	9.1 ±0.9	9.1 ±0.6

FARM K/H.

This was a small piggery with about a dozen Large White sows, which were farrowed in farrowing crates and after about ten days moved to a pen in the same buildings. Despite the existence of electric lamps in each creep the environmental temperature was definitely low. The owner held the idea that cold made the piglets hardy. Diarrhoea of an orange colour and semi-fluid consistency had been troublesome and affected almost all litters for a period, but oxytetracycline therapy had reduced the incidence just before the farm was visited for the purpose of this survey.

All piglets were dosed, at least once a week till they were eating solid food, with a liquid iron preparation of unknown composition.

Two litters were available at this visit and were bled from the ear veins. Both were just under five weeks old and had been eating solid food for about one week. One litter (1) had been allowed out to grass on several occasions.

The Hb values (Table 11) all reflect normal erythropoiesis and need no comment.

TABLE 11. Hb. Values for Piglets in 2 Litters on Farm K/H.

PIGLET	Hb (gm %).	
	Litter 1	Litter 2
1	11.7	11.1
2	10.5	11.7
3	10.7	11.1
4	10.5	12.1
5	11.9	12.5
6	9.3	11.1
7	12.1	10.9
8	10.9	10.3
9	11.7	10.9
10	10.9	10.7
11	9.9	
Mean	10.9	11.2
± SD	±0.8	±0.6

FARM O'H.

A large piggery with a self-contained herd mainly of large White breed which fed only swill to the pigs. Prior to this visit there had been a history of trouble with the litters extending over several months, but which had cleared spontaneously. It is apposite to remark that this condition became apparent in about 80% of litters and which when they were about 10 - 14 days old lost weight rapidly and most of the piglets died within a week. Occasional ones in the litter remained unaffected and grew apparently normally. Diarrhoea was not a feature of the illness. Those few which survived remained extremely thin and wasted and recovery was slow. The autopsical picture was mainly one of extreme fatty change of the liver; no specific aetiology for this condition was discovered.

Both litters bled had lost several members, according to the owner by being laid on by the sow. Each piglet received one proprietary haematinic pill when three days old. Litter 1 had been showing evidence of diarrhoea for about three days; neither lot had received solid food.

The Hb values (Table 12) were consistent with a mild anaemia.

TABLE 12. Hb. Values for Piglets in 2 Litters on Farm O'H.

PIGLETS	Hb gm %.	
	Litter 1 10 days	Litter 2 14 days.
1	6.2	7.6
2	7.1	7.8
3	7.7	8.2
4	5.8	-
5	8.2	-
6	7.3	-
Mean + - SD	7.0 ± 0.9	7.8 ±0.3

FARM McL.

This was a general farm with a large herd of Large White and Cross Landrace and Wessex pigs. Three litters of the appropriate age were available when the farm was visited, and samples from the ear veins of these individuals were taken, and are recorded in Table 13.

All the litters had been dosed with reduced iron at the third, tenth, and eighteenth days after birth. The piggery where the sows and piglets were kept, was rather cold, windows and doors were frequently kept open to provide a "fresh" atmosphere. No protective kennels were provided for the piglets and reliance was placed on electric lamps and a bed of straw.

One litter (2) was not gaining weight satisfactorily and while no diarrhoea or respiratory distress was noticeable the piglets looked rather thin and hairy. Because of this they had been given 100 mg. of an iron carbohydrate preparation intra-muscularly when three weeks old. It is noteworthy that despite iron therapy the Hb levels were low when sampled. These piglets seemed so interesting that three of the poorest were purchased a few days later and additional haematological information obtained, before they were autopsied.(Table 14).

No pathological features were present in piglet A but in the liver of B one small area about 2 cm. in diameter, in appearance similar to that seen in C, was present. Piglet C had a liver which had a mottled appearance caused by dilated sinusoids and areas of cell regeneration.

One might interpret the results in Table 14 as describing a hypochromic

slightly microcytic anaemia and since the serum iron levels were rather low, they might indicate an iron deficiency, from which the piglets were starting to recover. The enigma, however, lies in the amount of iron administered, for which there is only the attendant's word, and the iron contents of the livers. Were these in fact cases of toxic or aplastic dyshaemopoiesis?

Smears of the blood from these pigs showed large numbers of small pessary-form erythrocytes with a minority of normal sized fully haemoglobinised cells.

Piglet C which had the lowest Hb., P.C.V and R.B.C count, serum iron, total serum protein values and a dystrophic liver had in contrast the greatest amount of iron per 100 gm in its liver.

Since iron deficiency was a probable factor in the aetiology of the condition the attendant was further questioned on the possibility of iron not being supplied to this litter but he was adamant that all had been dosed three times and it seems unlikely that all three doses would have been missed.

TABLE 13 Hb Values for Piglets in 3 Litters on Farm Mc L.

PIGLET	Hb (gm %)		
	Litter 1	Litter 2	Litter 3
	21 days	28 days	21 days.
1	11.1	3.7	8.8
2	10.0	5.2	5.8
3	7.5	5.0	8.0
4	8.7	6.5	9.3
5	8.0	8.0	7.1
6	-	6.5	9.8
7	-	7.1	8.7
8	-	5.8	10.0
9	-	-	8.4
Mean	9.0	5.9	8.4
± SD	±1.4	± 1.3	± 1.3

TABLE 14. Haematology, Blood Biochemistry and Parenchymal Iron and Copper of 3 Piglets from Litter 2 on Farm Mc.L.

PARAMETER	PIGLET	DAYS.		
		27	30	34
Hb (gm%)	A	6.7	-	-
	B	6.4	8.4	7.3
	C	5.1	6.3	-
PCV (ml/100ml)	A	25	-	-
	B	25	30	29
	C	19	26.5	-
RBC ($x10^6$/cmm)	A	5.8	-	-
	B	4.6	5.3	5.8
	C	3.8	4.8	-
M.C.H.C.%	A	26.8	-	-
	B	25.4	25.2	25.1
	C	26.8	24.5	-
M.C.V (cu μ).	A	43.1	-	-
	B	54.3	56.6	50.0
	C	50.0	55.2	-
M.C.H ($\gamma\ \gamma$).	A	11.5	-	-
	B	13.9	15.8	12.5
	C	13.4	13.5	-
W.B.C ($x10^3$/cmm)	A	14.0	-	-
	B	15.6	13.0	18.2
	C	12.1	10.2	-

Continuation of Table 14.

PARAMETER.	PIGLET	DAYS.	
		30	34
Serum Iron µ gm%.	A	98.0	-
	B	89.0	141.6
	C	68.0	-
Total Protein gm.	A	4.9	-
	B	-	5.0
	C	3.9	-
A/G	A	1.9	-
	B	-	1.3
	C	2.0	-
Albumin gm%	A	3.2	-
	B	-	2.8
	C	2.6	-
Globulin gm%	A	1.7	-
	B	-	2.2
	C	1.3	-
Liver Iron mg/100 gm fresh tissue	A	6.5	-
	B	-	8.7
	C	9.8	-
Spleen Iron mg/100gm fresh tissue	A	18.3	-
	B	-	12.0
	C	9.2	-
Liver copper ppm/fresh tissue	A	31.5	-
	B	-	12.6
	C	18.8	-

FARM B.

This was a small-holding developed for the breeding and rearing of pigs. One large Scandinavian type house was used for breeding Large White and Cross Landrace pigs. Wooden kennels were provided for the piglets and while the temperature inside the house was cold these areas were reasonably warm.

Iron was supplied to the piglets as a proprietary iron paste smeared on the tongue when they were eleven and eighteen days old. Creep feeding was introduced when the pigs were four weeks old.

Three litters were available and Hb estimations were made on the piglets from them. The two oldest litters (numbered 1 & 3) had been on solid food for 1 week and 10 days respectively but litter number 2 had received no supplementary feeding. The piglets in this latter litter were apparently thriving well though they had been noticed coughing occasionally and had slight diarrhoea.

From the Hb values (Table 15) it can be seen that all the piglets in litter number 2 had low values and since they had been dosed with iron they were made the subject of further study.

The dependence of erythropoiesis in pigs on copper as well as iron has been described (Lahey et al 1952; Gubler et al 1956; Wintrobe et al 1953). It has been claimed that in pigs copper is essential for the proper absorbtion, mobilisation and utilisation of iron (Wintrobe et al 1953). With this in mind it was decided to try the value of copper with and without iron despite the few pigs available. Two piglets were given iron and copper, two

iron only, one copper only and the sixth pig neither metal. Iron was administered intra-muscularly as an iron-dextran complex preparation, at approximately 50 mg per 5 lbs. body weight. The total amount given is detailed in Table 16. Those pigs given copper received it daily in the form of copper sulphate and again the total dose is given in the table.

All the pigs except the control pig showed a rise in Hb of at least 1 gm in the seven days between each sample. Apart from the control pig in which only slight change in the Hb took place the smallest response was made by the piglet given copper only. The P.C.Vs almost doubled approximately in all pigs given Cu or Fe. The red cell indices are interesting since the mean cell volume increased considerably but the M.C.H.Cs fell despite the improvement in the Hb per 100 ml.

The serum iron levels showed a certain variability. The control, that given copper only and one pig which got both iron and copper (no.3) all were estimated slightly lower than the initial reading.

Turning to weight gains there again was marked variation, the least gain was made by the control piglet and the best, a gain of 7 lbs. was made by one given only iron. The increases in weight were :-

1.	0.25 lbs.	4.	7.0 lbs.
2.	2.75 lbs.	5.	1.5 lbs.
3.	2.75 lbs.	6.	2.0 lbs.

It would appear from this experience that copper was not deficient in these piglets and iron alone seemed to be responsible for the anaemia.

TABLE 15. Hb. Values for Piglets in 3 Litters on Farm B.

PIGLET	Hb (gm%).		
	Litter 1 44 day	Litter 2 32 day	Litter 3 38 day
1	6.6	5.0	7.5
2	7.5	5.0	8.4
3	7.7	6.2	8.0
4	6.9	5.0	9.7
5	5.4	5.4	10.2
6	8.2	5.2	8.7
7	5.8		10.4
8	7.8		11.3
9	6.0		
10	7.1		
Mean ± S.D.	6.9 ±0.93	5.13 ±0.16	9.27 ±1.32

TABLE 16. Haematology & Blood Biochemistry of Piglets Before and After Treatment on Farm B.

PARAMETER.	DAY	PIGLETS AND TREATMENT.					
		1 Fe nil ou nil	2 Fe 150mg ou 3.5mg	3 FE 100mg ou 2.5mg	4 FE 150 mg ou nil	5 Fe 150 mg ou nil	6 FE nil ou 3.5mg
Hb gm%	1 8	3.9 3.2	3.9 8.4	4.9 6.9	4.7 8.0	4.3 7.7	5.1 6.0
PCV ml/100ml	1 8	13 15	15 34	17 29	17 29	17 34	18 30
RBC ($x10^6$/cmm)	1 8	3.5 2.9	3.7 4.1	4.0 4.0	6.2 4.0	3.7 4.3	4.5 5.2
MCHC (%)	1 8	30 21.3	26 24.7	28.8 23.6	27.6 27.5	25.2 22.6	28.0 20.0
MCV (c μ)	1 8	37.1 51.7	40.5 82.9	42.5 72.5	27.4 72.5	45.9 79.0	40.4 57.6
MCH ($\gamma\gamma$)	1 8	11.1 11.0	10.5 20.4	12.2 17.2	7.5 20.0	11.6 17.9	11.3 11.5
W.B.C ($x10^3$/cm)	1 8	9.1 8.0	8.6 8.1	13.2 10.1	8.0 10.2	10.2 8.4	10.1 9.6
Serum Iron (μ g%)	1 8	65 58.1	55.4 113.6	90.4 82.1	67.7 138.1	53.3 77.3	84.8 72.6
Body Weight	1 8	10.25 10.5	15.25 18.0	10.25 13.0	16.0 23.0	16.5 18.0	16.0 18.0

EXPERIMENTS WITH NON-RADIOACTIVE ('IMFERON') AND ^{59}Fe-Containing IRON-DEXTRAN COMPLEXES. *

INTRODUCTION.

The original object of these investigations was to obtain information on the possibility of transfer of iron-dextran complexes from the sow to the litter, either *in utero* across the placenta, or after farrowing *via* the colostrum and milk.

The secretion of the iron-dextran complexes into colostrum and milk was to be studied by the intra-muscular injection of the ^{59}Fe-labelled preparation into a sow just before, or at the time of farrowing. However, only a small amount of labelled material was available at the time of the first experiment, and it was decided to inject the sow with ordinary 'Imferon' on the grounds that if any appreciable quantity was being secreted into the milk or colostrum and was being absorbed by the suckling piglets it would be possible to follow this by ordinary iron analyses on the milk and the piglets. In the first experiment (Experiment A) about 3,000 mg. of the non-radioactive iron-dextran complex was given intramuscularly to a sow immediately after parturition. Samples of colostrum were collected and the iron content examined. When this experiment was carried out there was nothing in the results to suggest any significant transfer of 'Imferon' from the sow to the suckling piglets.

An experiment was carried out on one of the piglets from this litter with a small amount of ^{59}Fe-labelled iron-dextran (Experiment B). 3 ml. of this material was given to the piglet by stomach tube and it was found to be absorb-

* Kindly supplied by Bengers Lab., Ltd.,

ed from the gut quite readily. This result was interesting and seemed to warrant further investigation for two reasons (a) It suggested that oral administration of iron-dextran complexes might be of value and (b) It seemed that the absorption of such complexes might parallel the absorption of antibody molecules from colostrum in the new-born pig. To obtain information on this point an experiment was carried out with the next batch of labelled iron-dextran on a new born litter of pigs (ExperimentC). It is known that absorption of antibody from the gut only goes on appreciably during the first 24 - 48 hrs of life. Therefore labelled Fe - dextran was administered to piglets of the litter at different times during the first few days oflife. One piglet from this litter was given an intramuscular injection of the preparation to compare the efficiency of the absorption from the gut with that of muscle.

A number of piglets having a weight range 1.8 - 4.5 Kg. were given 'Imferon' by intramuscular injection to study the absorption from muscle in this species. The results are given under Experiment D.

In all the following experiments the symbol Fe^{*} is used to indicate the iron of the labelled Fe-dextran complexes.

EXPERIMENT 5.

Absorption from the gut in the new-born pig of an *Fe-dextran preparation.

*Fe-dextran preparations were estimated to contain 20 μC ^{59}Fe and 250mg Fe per 5 ml. of solution prior to the test.

The piglets used were 6 hours old at the time of the experiment and had been allowed to suckle normally 5.0 ml of the above preparation (i.e.150 mg. *Fe) were administered by stomach tube and blood samples were collected from the anterior vena cava at intervals up to 20 hours after dosing. Radioactivity determinations were carried out on the blood samples and on a standard prepared from the original *Fe-dextran using the N550 scintillation counter (Ecko Electronics Ltd). The amount of *Fe/ml. blood at the various times was then calculated. The results are shown in Fig. 1. *Fe activity appears in the blood quite rapidly, rises to a maximum at about 6 hours, and thereafter declines. No blood volume determination was carried out on the particular pig, but a pig of the same size from the same litter had a blood volume of 80 ml. Taking this as a reasonable estimate forthe pig under experiment, at the time when the blood level of *Fe was maximal (ca 300 μg/ml), about 18% of the *Fe of the administered dose was circulating. The total per cent absorbed was probably in excess of this. Radioactivity measurements carried out on the plasma of the 20 hour bleeding as well as on the whole blood showed that at this time more than 95% of the *Fe absorbed was still circulating in the plasma.

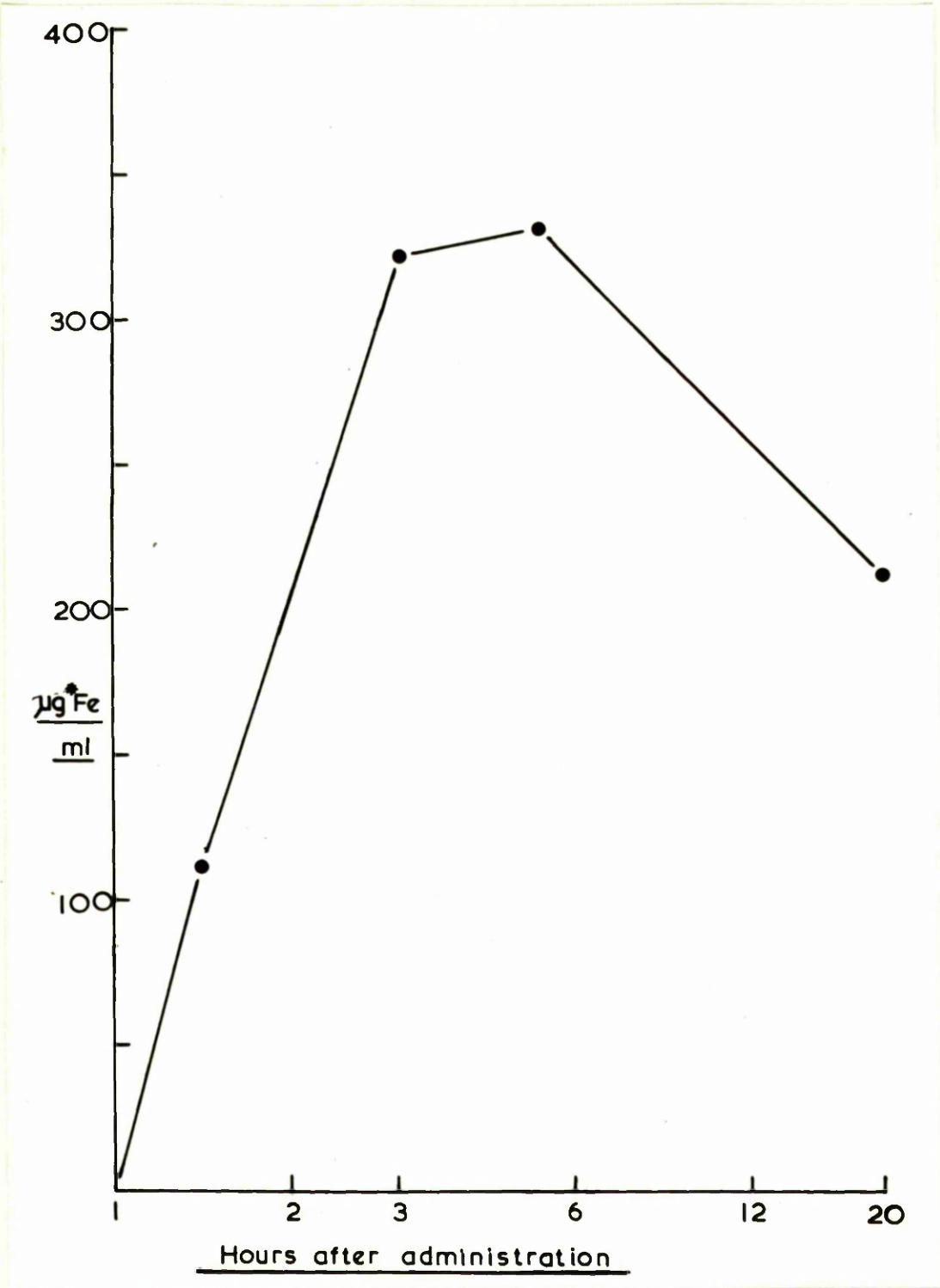

400
300
200
100
µg Fe
ml
1
2
3
6
12
20
Hours after administration

FIGURE 1.

EXPERIMENT C.

Absorption from the Gut of an *Fe-dextran preparation in young piglets of different ages.

The object of this experiment was to confirm the result of Experiment B and to find out if the absorption from the gut of *Fe-dextran paralleled the absorption of antibody by the new-born piglet. *Fe-dextran was given by stomach tube to piglets ranging from 3 hours to 36 hours old from one litter. Blood samples were withdrawn from time to time for radioactivity determinations and when it was considered that absorption had probably ceased the piglets were killed for analyses. Five minutes before killing each piglet was injected with ^{32}P - labelled red cells. This enabled a blood volume determination to be carried out and also made it possible in the analysis of the tissues to determine how much *Fe was due to blood contamination of the tissue. ^{59}Fe determinations were carried out using the scintillation counter and ^{32}P was determined with the M6 liquid gieger counter. Each sample was analysed in both counters along with the appropriate standards. With the range of activities used the ^{32}P gave no significant count in the scintillation counter, however, a small correction was sometimes necessary to the ^{32}P counts to allow for the presence of ^{59}Fe in the sample.

The *Fe-dextran preparation continued 20 μC/5 ml. and 250 mg Fe/5 ml nine days before use.

All piglets received 5.0 ml (i.e. 250 mg. *Fe) by stomach tube except piglet No.10 which was given 2.0 ml of the preparation intramuscularly. Piglets 1 and 2 were dosed when 3 hours old, piglets 3 and 4 at 12 hours old, piglets

5 and 6 at 25 hours old and 7 and 8 at 36 hours old. All piglets were allowed to suckle normally during the experiment except piglet No. 1 which was taken away from the sow at birth and not allowed to suckle for 15 hours. The reason for treating piglet No.1 in this way was to see if the presence of colostrum in the gut was necessary for the absorption of Fe-dextran. (There is evidence now that in the new born calf a factor in the colostral whey is necessary for the absorption of antibody globulins and that if colostrum is withheld an antibody globulin preparation introduced into the gut is not absorbed). There does in fact appear to be a parallel here in the absorption of Fe-dextran by the young pig for no activity appeared in the blood of No.1 up to 20 hours, when it died, whereas piglet No. 2 given the same dose at the same age showed a good absorption. The level of *Fe in the blood of the various piglets at different times after administration is shown in Fig. 2.

In piglets 2, 3 and 4 appreciable amounts of *Fe appeared in the blood within a few hours of administration of the *Fe - dextran.

In 5, 6, 7 and 8 very little *Fe appeared in the blood until about 24 hours after dosing. At first it was assumed that little or no absorption was taking place in these animals, however, from 24 hours onwards the activity in the blood continued to increase and in case this might represent a delayed absorption these pigs were kept alive for a longer period than 2 and 3. On consideration of all the results it now appears that the rise in blood level of * Fe in piglets, 5, 6, 7, and 8 after 24 hours was due to the incorporations of *Fe into the red cells and not to a delayed absorption from the gut.

This being the case it seems a little odd that during the first 24 hours after dosing, when *Fe must have been entering the pig, little activity appeared in the blood. It must be concluded that during t is period the activity in the blood is determined by two processes (a) absorption from the gut into the blood stream and (b) withdrawal from the blood stream by the tissues. In the rapidly growing pig (b) would appear to be able to keep pace with (a) so that the net *Fe content of the blood during the absorption stage remained low. It may be hypothesised that up to 6 - 12 hours the demand for iron may be slight and any excess is deposited in the liver mainly (vide nos. 2 & 4) but once active erythropoiesis begins the iron becomes incorporated in the erythrocytes(nos 5 - 8). The two phases in blood *Fe level are seen in the case of pig 10 which received the intramuscular injection. The distribution of *Fe between cells and plasma at the time of killing is shown in Table 1.

Analysis of Tissue.

Five minutes before killing each pig was injected with a sample of ^{32}P - labelled red cells as described earlier. The pigs were killed by anaesthetization and sectioning the main arteries in the neck. They were allowed to bleed out as completely as possible. After death, the alimentary tract, liver and spleen were removed and put in separate beakers. The remainder of the carcase was then divided into muscle and bone as carefully as possible. Each tissue was treated with and H_2SO_4 ; HNO_3 ; $HClO_4$ digestion mixture and heated carefully until a pipettable digest was obtained. Aliquots were then measured out for radioactivity determinations with the scintillation

and M6 counters. From the scintillation the amount of blood in the sample was calculated and, knowing the *Fe content of the blood at the time of death, the *Fe content of the sample due to blood contamination was determined.

The results of the tissue analyses for *Fe on the various pigs are shown in Tables 2 - 8. The results obtained on piglets 2 and 3 (Tables 2 and 3) are not dissimilar. The total *Fe absorbed represented about 12% of the administered dose and most of this was found in the various tissues. In both piglets there was an appreciable amount of *Fe left in the alimentary canal at the time of death and it may be that given time some of this would have been absorbed. Piglet No.4 died early on in the experiment.

Tables 4 and 5 show the results on piglets 5 and 6 which were given *Fe-dextran at 25 hours old. The total amount of *Fe absorbed differed quite markedly in these two pigs. In both cases the amount of *Fe remaining in the gut was quite small so that unlike piglets 2 and 3 it can be assumed here that the *Fe found in the piglets represented the limit of absorption under the conditions of the experiment.

It can be seen also from Tables 4 & 5 that most of the absorbed *Fe was now circulating in the blood (cf.Table 1). There was one anomaly in the results of piglet 6 which occurred again in those of piglet 8. It will be observed in the analytical figures for muscle in these two animals that the *Fe due to blood contamination exceeded the total *Fe present. The most likely cause of this anomaly was an overestimate of the blood content of the muscle. On consideration of how this might arise it seems most likely that

during the injection of ^{32}P labelled cells a small quantity may have gone into the tissue instead of into the blood stream (the piglets were all injected into an ear vein which occasionally presents some difficulty). The ears were of course included with muscle in the tissue analysis and quite a small amount of the injection material remaining at the injection site could lead to a fairly substantial error in the estimate of the blood volume content of the tissue which included the injection site.

The results obtained with piglets 7 and 8 are shown in Tables 6 and 7. The results are comparable to those of piglets 5 and 6 in that at death most of the $^{*}Fe$ was circulating in the red cells. Here again the amount remaining in the gut was quite small.

Piglet No.10 (Table 8) was given an injection of 2.0 ml. of the $^{*}Fe$ - dextran preparation intramuscularly to compare the efficiency of absorption from muscle with that from the gut. It is obvious from the results that with this particular preparation the intramuscular injection is very much to be preferred. It is interesting to note, however, that in the 100 hours elapsing between injection and the death of the pig, about 40% of the $^{*}Fe$ had become lost from the animal. The finding of 2.81 mg $^{*}Fe$ in the gut would suggest the gut as a significant route of excretion.

Summary of Experiments B & C.

When $^{*}Fe$-dextran preparations are administered to new born piglets by stomach tube absorption from the gut takes place. The phenomenon would appear to have something in common with the absorption of antibody by the new born

piglet, although in Experiment C a stage was not reached where little or no absorption was taking place. (It would have been better in Experiment C to dose some of the pigs at about 1 week, however, during the experiment due to the delayed appearance of *Fe in the blood of piglets 5 and upwards it was thought that the stage of poor absorption had been reached).

With the particular *Fe-dextran used in Experiment C, oral administration compares very unfavourably with intramuscular injection as a method for getting Fe into the pig. By comparison of the amount administered and *Fe absorption curves in Experiment B with those of piglet 2 in Experiment C it would appear that the material used in Experiment B was being absorbed more efficiently than that used in Experiment C.

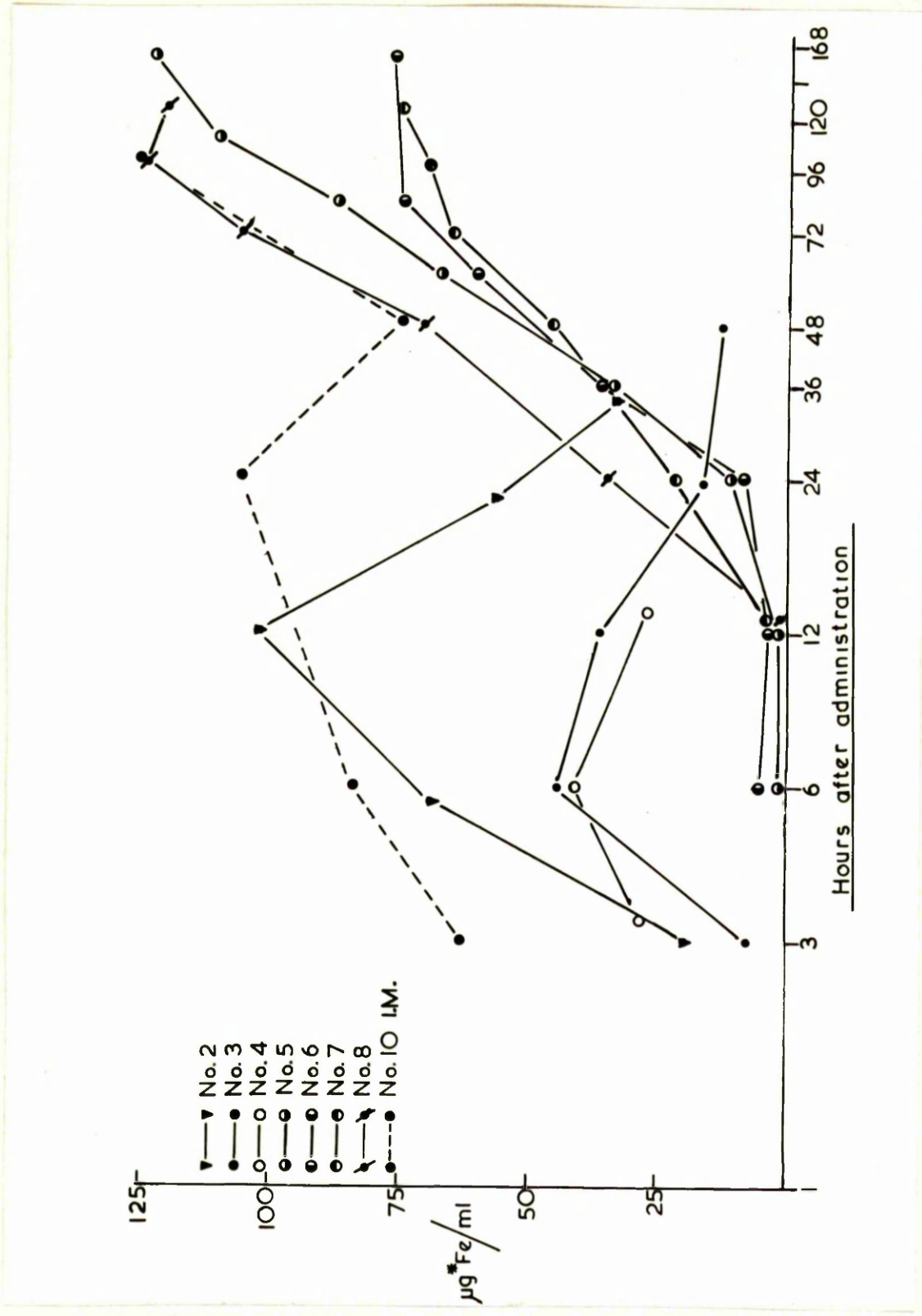

No. 2
No. 3
No. 4
No. 5
No. 6
No. 7
No. 8
No. 10 I.M.
125
100
75
50
25
μg *Fe/ml
3
6
12
24
36
48
72
96
120
168
Hours after administration

Figure 2.

TABLE 1. Distribution of Fe in the Blood at Time of Killing.

PIGLET NO.	% of BLOOD Fe in CELLS.
2	41
3	60
5	98
6	97
7	97
8	98
10	98

TABLE 2. Tissue Analyses of Piglet No.2.

Dosage: 5.0 ml. (250 mg Fe) by stomach tube when 3 hrs old killed and analysed 34 hours later.

TISSUE	TOTAL Fe FOUND IN TISSUE (mg)	Fe due to blood contamination (mg)	Net Fe content OF TISSUE. (mg)
Spleen	0.073	0.006	0.067
Liver	11.89	0.077	11.81
Muscle	7.41	0.516	6.89
Bone	9.92	0.403	9.52
Blood	3.61	-	3.61
		Total Fe absorbed	31.90
Alim. Canal	46.9	0.044	46.86

TABLE 3. Tissue Analyses of Piglet No.3.

Dosage: 5.0 ml.(250 mg Fe) by stomach tube when 12 hours old; killed and analysed 36 hours later.

TISSUE	TOTAL Fe Found IN TISSUE	Fe due to BLOOD CONTAMINATION.	NET Fe CONTENT OF TISSUE.
	(mg)	(mg)	(mg)
Spleen	0.123	0.011	0.112
Liver	14.04	0.138	13.90
Muscle	7.23	0.525	6.71
Bone	8.55	0.136	8.41
Blood	1.44	-	1.44
		Total Fe absorbed	30.57
Alim. Canal	81.97	0.028	81.94

TABLE 4. Tissue Analyses of Piglet No.5.

Dosage : 5.0 ml. (250 mg. *Fe) by stomach tube when 25 hrs. old killed and analysed 173 hours later.

TISSUE	*TOTAL Fe FOUND IN TISSUE	*Fe DUE TO BLOOD CONTAMINATION	*NET Fe CONTENT OF TISSUE.
	(mg)	(mg)	(mg)
Spleen	0.113	0.052	0.061
Liver	2.44	0.655	1.78
Muscle	3.91	3.83	0.08
Bone	5.02	2.63	2.39
Blood	25.03	-	25.03
		*Total Fe absorbed	29.34
Alim.Canal	1.52	0.317	1.20

TABLE 5. Tissue Analyses of Piglet No. 6.*

Dosage: 5.0 ml. (250 mg. Fe) by stomach tube when 25 hrs. old; killed and analysed 173 hours later.

TISSUE	TOTAL Fe FOUND IN TISSUE*	Fe DUE TO BLOOD CONTAMINATION*	NET Fe CONTENT OF TISSUE.*
	(mg)	(mg)	(mg)
Spleen	0.111	.094	0.017
Liver	0.686	0.448	0.238
Muscle	2.66	3.66	‡
Bone	1.94	1.48	0.46
Blood	13.47	-	13.47
		Total Fe absorbed*	14.19
Ali . Canal	0.493	0.132	0.361

‡ See text for Discussion.

TABLE 6. Tissue Analyses of Piglet No.7.

Dosage : 5.0 ml. (250 mg. *Fe) by stomach tube when 36 hrs. old; killed and analysed 163 hours later.

TISSUE	TOTAL *Fe Found IN TISSUE	*Fe DUE TO BLOOD CONTAMINATION	NET *Fe CONTENT OF TISSUE.
	(mg)	(mg)	(mg)
Spleen	0.059	0.033	0.026
Liver	0.397	0.250	0.147
Muscle	2.09	1.23	0.86
Bone	1.62	1.16	0.46
Blood	11.13	-	11.13
		*Total Fe absorbed	12.62
Alim. Canal	0.426	0.132	0.292

TABLE 7. Tissue Analyses of Piglet No.8.

Dosage : 5.0 ml. (250 mg *Fe) by stomach tube when 36 hrs. old; Killed and analysed 162 hours later.

TISSUE	TOTAL *Fe FOUND IN TISSUE	*Fe DUE TO BLOOD CONTAMINATION	NET *Fe CONTENT OF TISSUE
	(mg)	(mg)	(mg)
Spleen	0.099	0.069	0.030
Liver	1.24	0.620	0.620
Muscle	5.63	6.31	‡
Bone	3.81	2.74	1.07
Blood	21.05	-	21.06
		Total *Fe absorbed	22.78
Alim. Canal	1.34	0.276	1.06

‡ See text for Discussion.

TABLE 8. Tissue Analyses of Piglet No.10.

Dosage: 2 ml. (100mg * Fe) intramuscularly when 90 hours old; killed and analysed 100 hours later.

TISSUE	TOTAL * Fe FOUND IN TISSUE	* Fe DUE TO BLOOD CONTAMINATION	* NET Fe CONTENT OF TISSUE
	(mg)	(mg)	(mg)
Spleen	0.287	0.088	0.199
Liver	13.33	1.07	12.26
Bone	14.19	3.19	11.00
Muscle	18.85	8.36	10.49
Injection site	3.92	0.31	3.61
Blood	22.19	-	22.19
		* Total Fe in piglet	59.75
Alimentary Canal	3.16	0.35	2.81

EXPERIMENT D.

A number of piglets of weight range 1.8 kg - 4.5 kg. were injected intramuscularly with "Imferon" (non-radioactive) at a dose rate of 50 mg Fe/kg. Blood samples were collected at various time intervals after injection and analysed for serum iron. The results shown in Fig. 3.

The maximum serum Fe level reached occurred 6 - 8 hours after injection. This appears to be a good deal earlier than the peak serum level in rabbits (Martin et al 1955) and might be interpreted as a faster absorption from muscle in the pig than in the rabbit. However, the serum Fe level represents the resultant of the two processes - absorption from muscle and withdrawal from blood for utilization and it may be that in the rapidly growing piglet the demand for Fe is very great. A more rapid withdrawal from the circulation would have the effect of causing the peak serum level to occur earlier.

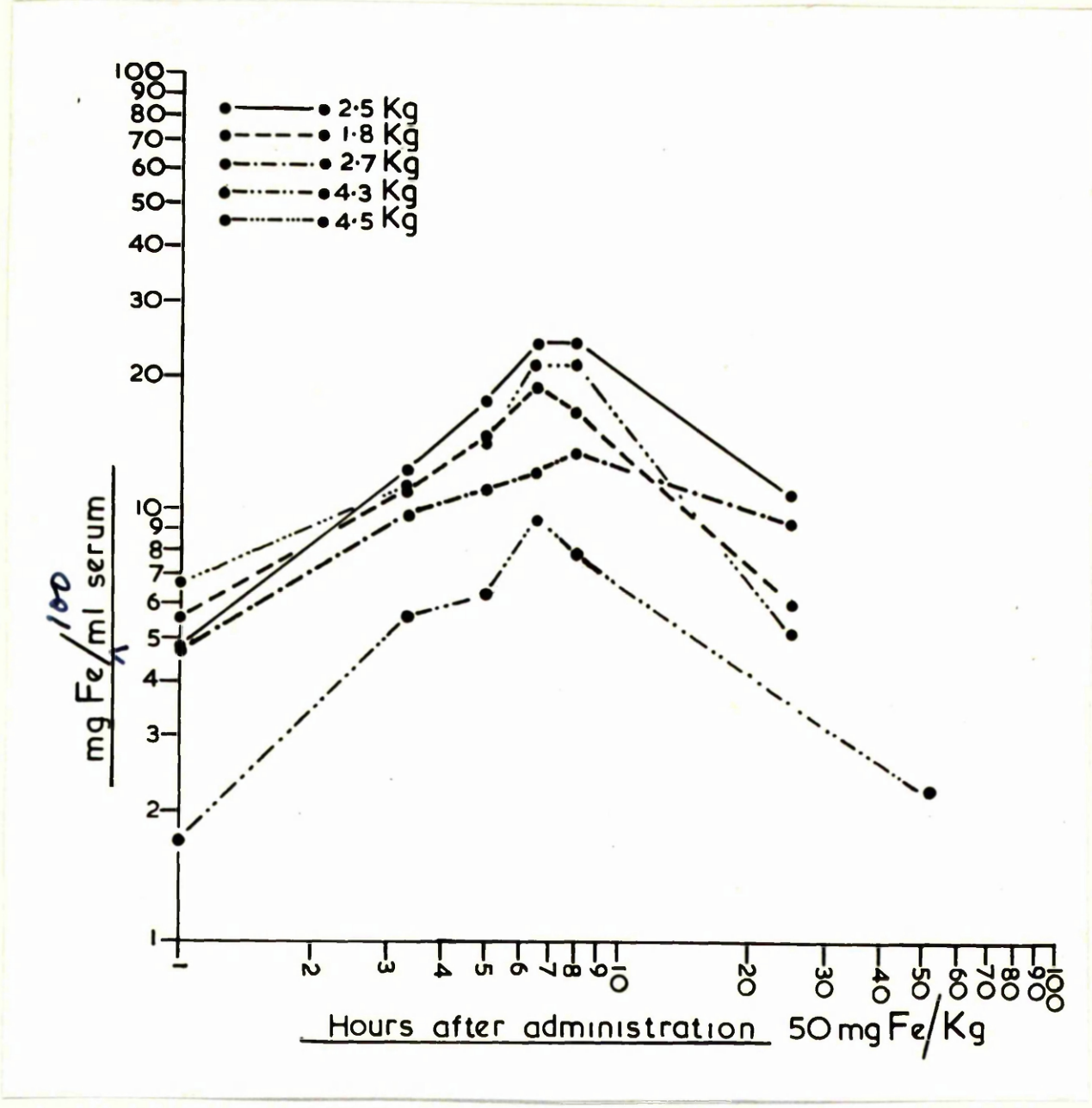

2·5 Kg
1·8 Kg
2·7 Kg
4·3 Kg
4·5 Kg
mg Fe/100 ml serum
Hours after administration 50 mg Fe/Kg

FIGURE 3.

EXPERIMENT E.

Since one piglet (number 1 Experiment C) deprived of colostrum failed to show any absorption of *Fe it was decided to give further consideration to this point. Two possibilities might have accounted for this phenomenon. First, as already stated colostrum may be necessary for the absorption of iron-dextran from the intestine or secondly the dose of *Fe may have been regurgitated unobserved, and thus an insignificant amount was left in the alimentary canal.

To elucidate this point six piglets were removed at birth from the sow. Three were allowed to suck the sow for 10 minutes before dosing per os with 'Imferon', and thereafter were put back with sow. The other three were not allowed to suck or eat any food for 12 hours after birth. All six piglets were bled at time 0 and then given iron-dextran (non-radioactive) by stomach tube.

Treatment of the individual pigs is detailed below:-

PIGLET NO.	WEIGHT (lbs)	TIME DEPRIVED OF MILK	AMOUNT OF ORAL Fe - Dextran (mg FE)
1	3.25	0 hours	112.5
2	3.5		125.0
3	3.0		150.0
4	3.0	12 hours	150.0
5.	3.5		125.0
6	3.25		112.6

All the piglets were bled at 0, 3, 6, 12, 24, and 48 hours after dosing and the serum iron levels estimated. The values are given in Table 9.

The piglets deprived of colostrum for 12 hours were able to absorb iron as readily as those given access to the sow before and after dosing. There also did not appear to be a significant difference between the groups in the height to which the iron content of the sera rose when dosage is considered.

The samples taken before dosing showed interestingly high contents of iron.

From the results it may be concluded that the probable explanation of the failure of piglet 1 in Experiment C to absorb the iron was that it vomited shortly after dosing with the *Fe-dextran.

TABLE 9. Experiment E Piglet: Serum Iron Levels (mg/100 ml).

PIGLETS.	HOURS AFTER DOSING.					
	0	3	6	12	24	48
1	0.619	8.812	0.871	15.192	9.871	1.494
2	0.732	7.307	18.707	21.346	6.976	0.899
3	0.740	23.782	45.440	49.120	13.717	2.429
4	0.337	20.448	13.785	40.960	14.871	2.899
5	0.921	14.615	9.184	7.200	2.328	0.371
6	0.892	10.192	7.648	6.624	3.589	0.880

DISCUSSION & CONCLUSIONS.

It is obvious on perusal of the literature on piglet anaemia that many contradictions exist on the clinical, pathological and haematological aspects of this condition. Much of the confusion may have arisen because of concomitant disease or unsatisfactory husbandry conditions, complicating or precipitating an iron-deficiency. Indeed it seems doubtful if iron deficiency has been present in every instance. It is impossible however, at this time to indicate all conditions which may as a result of damage or impairment to the erythron, at whatever stage, cause anaemia in piglets. Thus for the important reasons that known and unknown diseases might be more readily eliminated and husbandry methods and diet controlled the choice of artificial rearing techniques would seem reasonable in a study on iron-deficiency anaemia.

The use of artificial rearing methods together with the recent advances in knowledge on the nutrition of the piglet allows stricter control of experimental conditions than was possible in the past. The difficulty of reducing the iron content of the diet to negligible amounts yet retaining all other essential ingredients was not overcome in these experiments. Thus the piglets used, even in the control groups, seldom made as rapid weight increases as modern pig rearers desire. Nevertheless a severe microcytic hypochromic anaemia was produced in many pigs.

In collecting the data on the various blood parameters a balance had to be struck between the number of piglets used and the volume of work. In retrospect it would seem as if about 6 pigs in each group was the ideal

number in these experiments with the facilities available. With this number the differences in most parameters was sufficiently great to allow of statistical analysis, though some of the data might have been improved had consideration been given to Poisson's distribution.

Hb levels for piglets under 8 weeks which did not appear to be iron-deficient were often from 9 - 12 gm%. In anaemic animals values as low as 5 gm % were readily produced but lower values were somewhat difficult to achieve, though in some cases levels between 3 & 4 gm % of Hb were recorded. It has been pointed out (Tasker 1949) that in humans with chronic nutritional anaemia the blood volume (in particular the red-cell volume) decreases and therefore as the anaemia becomes more severe the Hb level reads progressively too high. In the most severely anaemic cases the error may be as high as 50%. The values recorded for the piglets with very low levels may indeed have been too high.

In the cases of severe iron-deficiency most of the other blood parameters were markedly altered. The volume of packed red cells fell to below 20 ml per 100 ml., which was about half the normal level, and though the erythrocyte counts failed to reflect the milder anaemias, as the severity increased a drop in R.B.C count, from 5 down to 3 million per c.mm. was evident. The M.C.V dropped early in the course of the anaemia and this decreased volume was apparent in the fall in the haematocrit value. Values below 40 c.c. were sometimes recorded for the former parameter. Alterations in the Hb concentration of the average red corpuscle were seldom marked or obvious, and often

seemed within the normal variation. Wintrobe et al (1943) have given the normal value as 33% for pigs, with a drop to 28% in anaemic ones (Wintrobe et al 1953). However, in this present work no regular levels with a distinct difference were apparent. In this respect I must agree with Miller (1958) who obtained a relatively constant value for both anaemic and normal piglets.

The description given by Parsons (1938) of the three stages of iron-deficiency anaemia in man seems to agree with the course of the disease in the pig.

Morphological changes were notable and marked and seemed to correlate closely with the severity of the anaemia. Provided care was taken over the preparation of the smears, and these were made without undue delay, crenation and mis-shapen erythrocytes were unusual. Blood smears from apparently normal young piglets contain a surprising number of large basophilic erythrocytes, and these should not be taken as indicative of anaemia. Mild cases of iron-deficiency exhibit microcytosis and numbers of pale red cells. As the anaemia progressed large numbers of 'pessary forms' which were merely rings of stainable Hb with clear central areas becameeapparent. Target cells were noticed also and anisocytosis, particularly microcytosis, was always marked, in contrast to the rarity of poikilocytosis which was only present in the most extreme anaemias.

Reticulocytes were not regularly increased in anaemia nor could significance be attached to the presence of Howell-Jolly bodies. Normoblasts were seen frequently.

Wide discrepancies were noted within groups on serum-iron estimations; a feature well known in humans. Though a broad division seemed to occur about the 100-120 μ g region below which most anaemic pigs fell. One thus has difficulty in agreeing with Köhler (1957) who has stated that the best indication of iron-deficiency is a serum - iron estimation. It is recognised also that in anaemia of infection a persistently low level of serum-iron often occurs (Wilkinson 1955).

On the question of clinical signs associated with iron-deficiency it would seem necessary to disagree with most workers. Even with low levels of Hb (circa 4 gm%) few clinical signs which could be attributed directly to iron-deficiency were noticed. Certainly tachycardia, and dyspnoea were never observed and diarrhoea, dullness and long hair were present irregularly and did not occur more in the anaemic piglets than in the non-anaemic without some other reason existing. However, it is possible and would be reasonable to expect, that a critical level of anaemia exists below which any additional stress is liable to result in severe illness or death, as seemed to occur in the third experiment using natural rearing methods. Such stress conditions (e.g. cold or infections) might well occur under field conditions but against this it did seem difficult to prevent piglets from ingesting sufficient iron to counter the more severe forms of anaemia. Many modern rapidly maturing piglets, in the first three weeks of life, do have levels of Hb below 9 gm % but only uncommonly were levels lower than 7 gm % recorded in the farm survey. It would seem possible that some other

condition, or complex of conditions, as yet unelucidated might render piglets anaemic or aggravate an existing iron-deficiency. It has been maintained that anaemic pigs are more susceptible to certain infections (Craig, 1930; Sheffy 1958).

Wintrobe et al (1947) have shown how "infection" can impair Hb synthesis, and the value of iron in relieving the anaemia of infection has been for long in dispute (Sinclair & Duthie, 1949; Cartwright & Wintrobe 1952).

On the question of the much vaunted value of iron for maintaining or increasing weight gains it is difficult to give a catagorical answer. It may be that there is a compensatory mechanism on the part of the body which slows down growth and thus expansion of blood volume when the oxygen carrying capacity of the blood is reduced. Yet it was clear that piglets in many of these experiments could grow rapidly and achieve good weight increases despite low Hb levels.

Boutler's work with rats (1957) appeared to demonstrate that iron enzymes are not inviolate, as had been thought and from this he suggests that clinical symptoms may arise even in the absence of a marked deficiency of Hb in humans. Subjective signs may be more readily appreciated than objective ones if mild in character so that it is possible that the pigs suffered some impairment which went unrecognised.

Despite the low Hb levels obtained in these experiments the autopsical picture described by some authors, notably McGowan and Crichton (1924 ‡‡) was never apparent. The serous effusions, with later adhesions, the pneumonia,

oedema of the lungs, hepatic changes, and the apparently characteristic "fairy-rings" were missing. Cases of liver dystrophy unassociated with iron-deficiency were seen; but only in one piglet, one of those in the farm survey series, did the changes coincide with an anaemia which had all the characteristics of iron-deficiency, and this despite the administration of oral and later intra-muscular iron. It thus seems a reasonable hypothesis that the lesions of iron-deficiency so often described as characteristic are indeed those resulting from a complex of diseases. Confusion at least with so-called virus pneumonia has certainly occurred (Lamont et al 1950). As knowledge about pig diseases increases it may be possible to divide the complex and thus arrive at a clearer picture of nutritional anaemia. If this work does no more than point to the need for a more critical appraisal of iron-deficiency then it will have been of value, but it probably goes a little further and allows at least an initial description of the anaemia in piglets uncomplicated by the many diseases or environmental factors which confuse or alter the clinical and pathological picture.

With regard to the marrow changes it is difficult to see the rationale behind McGowan's (1924) description of an aplastic state of the bone-marrow in iron-deficiency and the conclusions of Howie et al (1947) seems much more valid, that the syndrome described should not be attributed to iron-deficiency. It was obvious in the marrow sections examined in this study and also in the gross appearence of the femoral marrow that great activity of the marrow occurred and under severe iron shortage all the fat spaces disappeared to compensate for the increased time of maturation required by the erythrocytes.

Estimations of liver iron frequently gave lower values for the anaemic than the non-anaemic pigs and figures for the former of 2 - 3 mg per 100 gm wet tissue were common, though surprisingly high values were occasionally recorded even in deficient pigs. The volume of blood contained in the pieces of liver used would hardly explain such discrepancies and it may be that the deposition of iron in the liver is not uniform though no evidence for this belief was found.

In conclusion it would seem as if much of the recorded information on nutritional anaemia of piglets requires reappraisal or perhaps discarding.

A fresh approach, unhampered by early dogmas, in a search for diseases as yet unknown or partially known, would assist in elucidating many of the current problems in pig rearing and reduce the tremendous pre-weaning losses in this industry.

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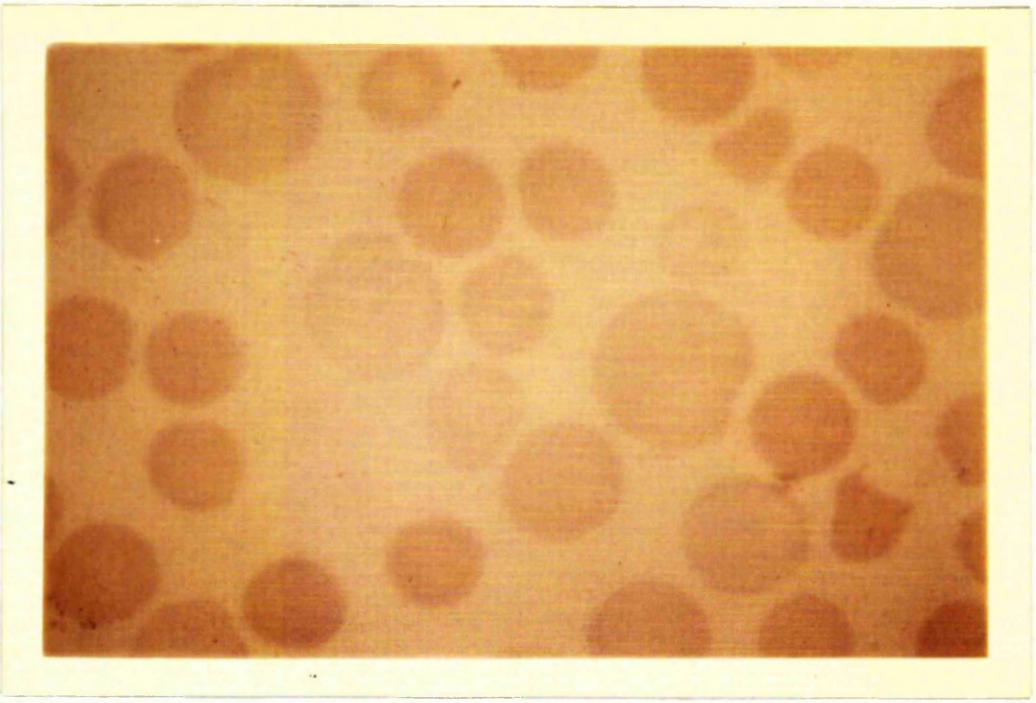
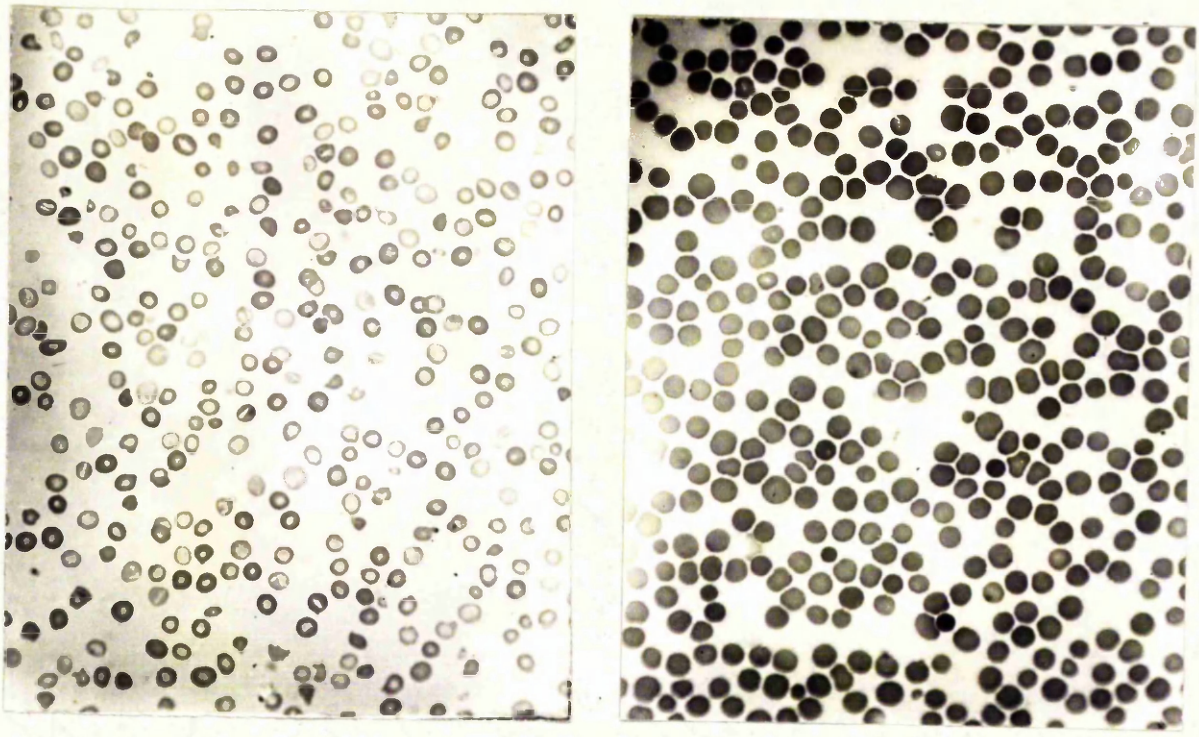


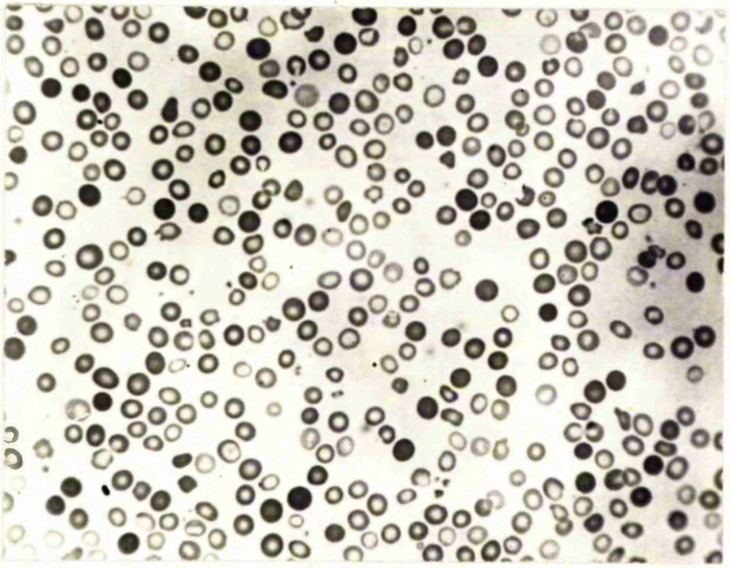
EXPERIMENT 2 PIGLET 4.

Smear showing the polychromasia normally present in blood films from young piglets. Note the large basophilic cells.



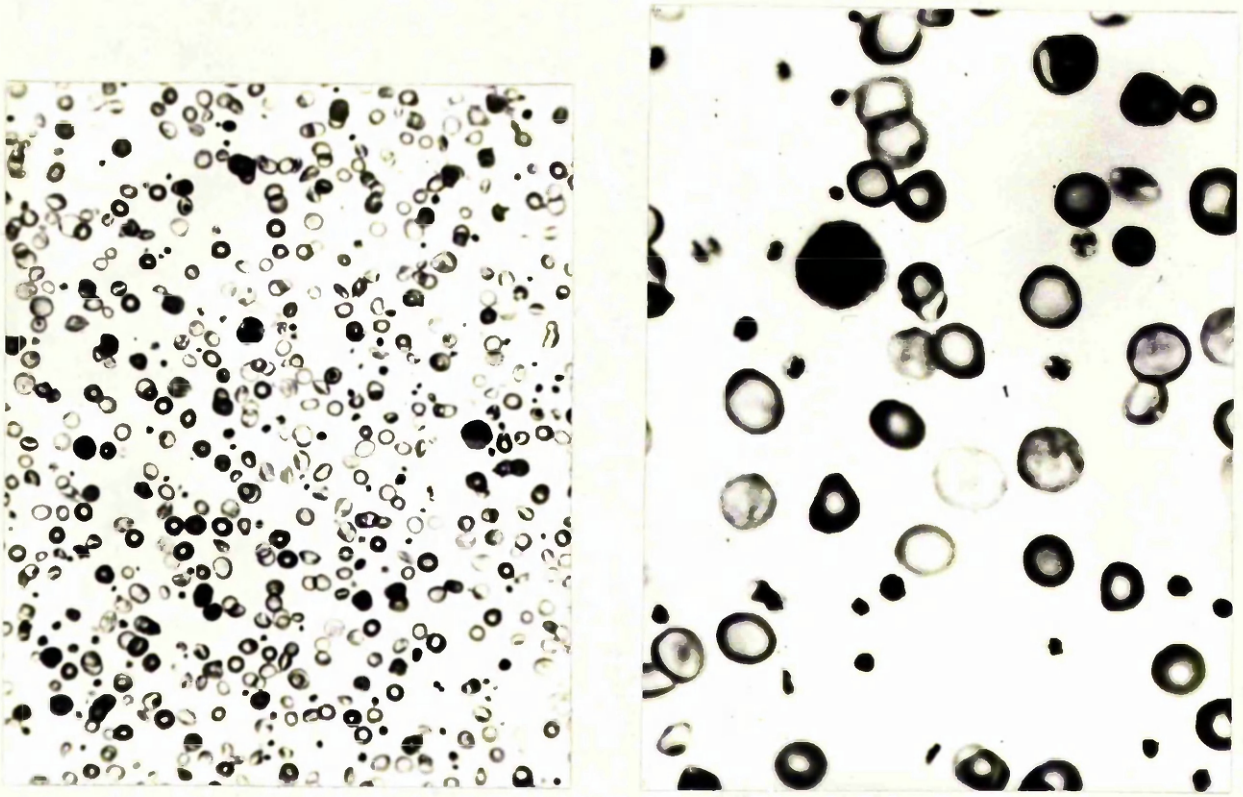
EXPERIMENT 2. PIGLETS 11 & 12. x470

Red cell changes exhibited by an iron - deficient piglet (No.12 Left) compared with those of a control piglet (No.11 Right).



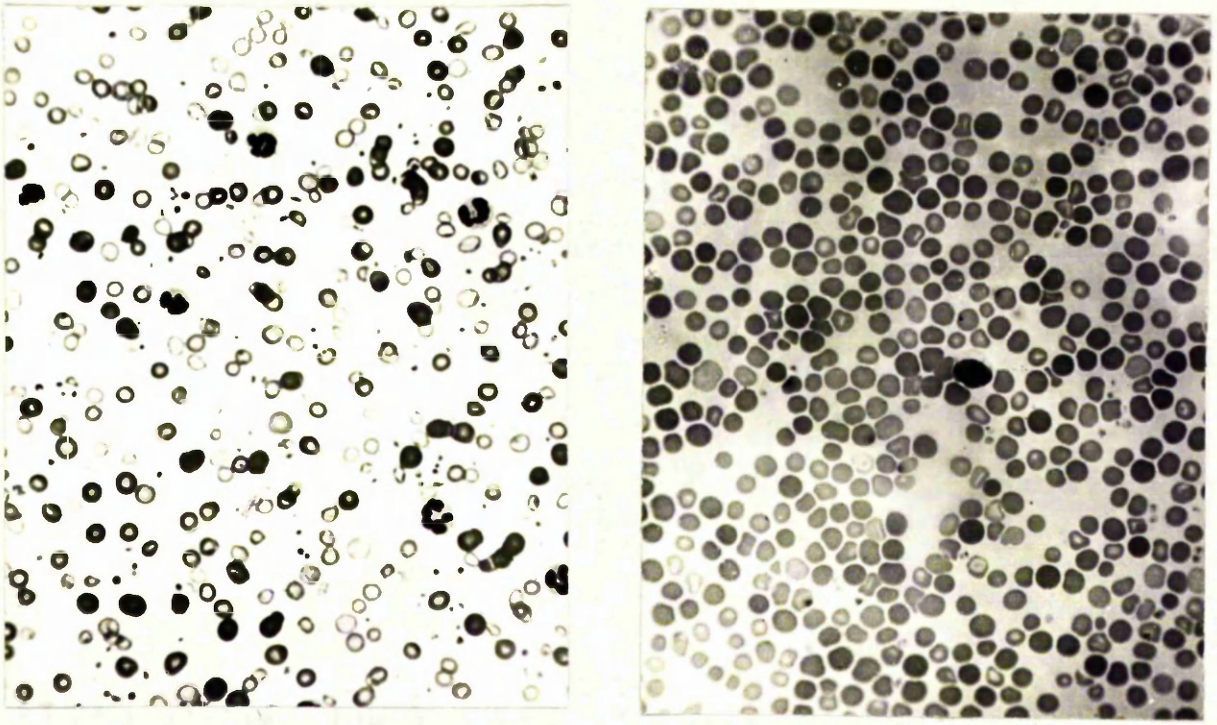
EXPERIMENT 2. PIGLET J 2. x 470

Blood smear from an iron - deficient piglet at the fourth week showing the marked morphological changes of the erythrocytes.



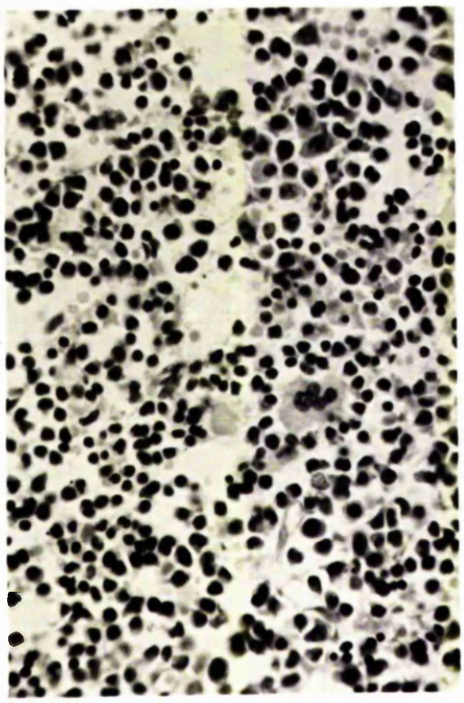
EXPERIMENT 3. PIGLET K2.

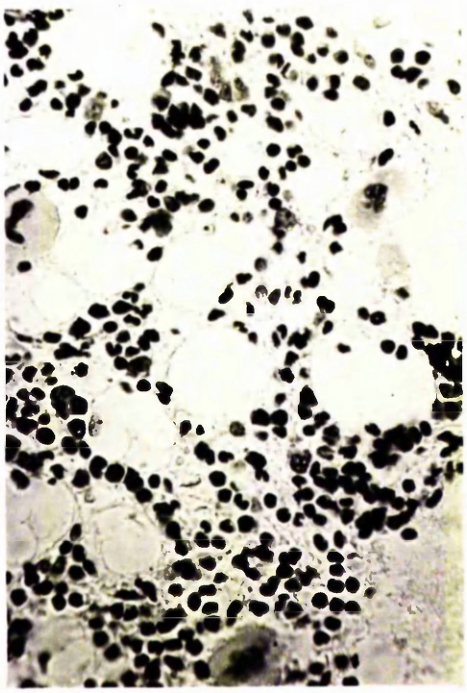
Blood smear from a case of severe iron - deficiency anaemia, showing the microcytic cells deficient in Hb.



EXPERIMENT 3. PIGLETS K4 & K9. x 470

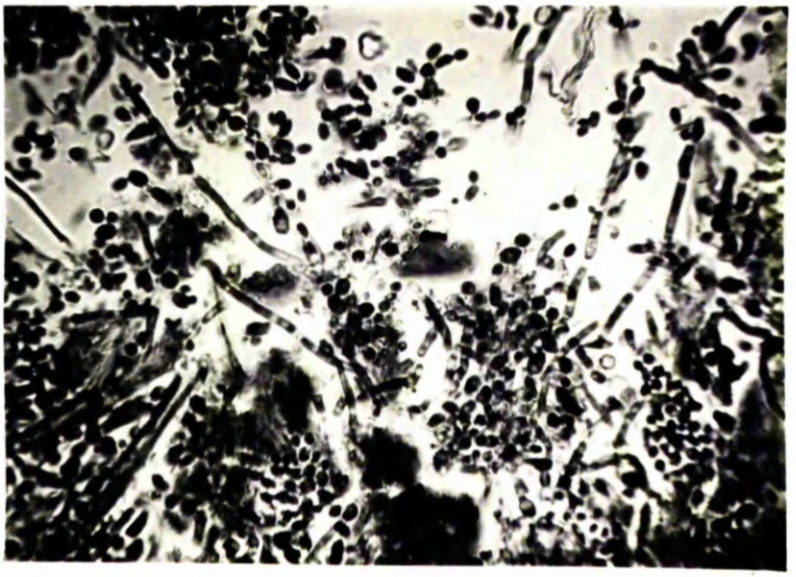
Changes in the erythrocytes of an iron - deficient piglet (K4 Left) and one recovering (K 9 Right).





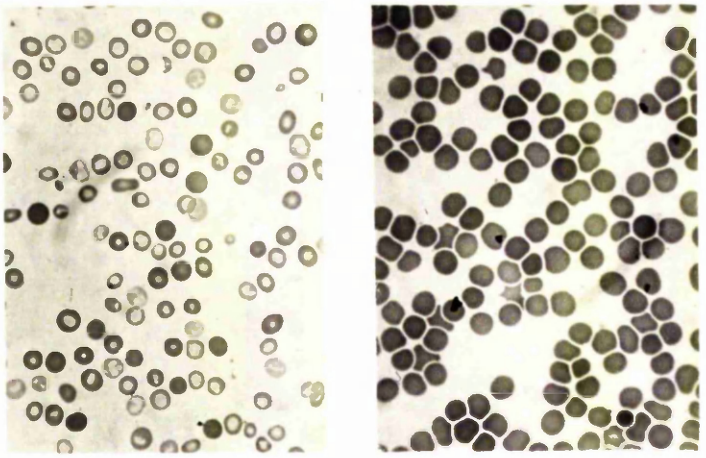
EXPERIMENT 5. PIGLETS 17 & 19. x 420.

Bone-marrow sections from an iron - deficient piglet (No. 17 Left) and one supplied with iron showing numerous fat cells. (No. 19 Right).



EXPERIMENT 5. PIGLET 18. x 550

Mycelia in a pseudo - membrane on the tongue.



NATURAL REARING EXPERIMENT 3.

Smears of red cells from a piglet which received no supplementary iron (No. 11) and one given reduced iron (No. 28).

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