"EXPERIMENTS ON HAEMOLYTIC ANAEMIAS, AND ON HAEMOLYTIC ICTERUS."

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T H E S I S

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C O N T E N T S.

INTRODUCTION.

The work presented in this thesis consists of a series of researches carried on over a period of several years, begun with the object of throwing light on and explaining some of the phenomena met with in cases of anaemia in the human subject, and more especially with reference to one type of anaemia, Pernicious Anaemia.

It would serve no useful purpose here to go into the history of this disease, and the contests which have been waged with regard to its etiology, since the main theories which have been held are referred to in every text-book. It must be pointed out, however, that much of the controversy has been with regard to the interpretation of the changes found in the bone marrow and other organs post-mortem. So far as the bone marrow is concerned, the question is whether the appearances are to be referred to a primary defect or lesion of that organ, or whether they are simply secondary, dependent on a process of blood destruction going on in some part of the body, whether it be in the liver and spleen, or in the intestinal tract, or in the general circulation.

From the work of many observers, and, it is hoped, from my own, evidence has accumulated which shows that within a week or ten days a blood picture and changes in the marrow and other organs, similar in almost every respect to those of human Pernicious Anaemia, can be reproduced experimentally in animals. Thus the idea that Pernicious Anaemia is a primary disease of the marrow has steadily lost ground, being superseded by the view that the essential condition is a

process of blood destruction or haemolysis going on in the body, the other changes being all secondary. The probability that death occurs in Pernicious Anaemia as a result of the bone marrow giving out functionally, because it is unable to keep up the continued over-regeneration of red corpuscles to replace lost ones, does not of course infer a primary marrow fault, but a secondary marrow failure.

With the haemolytic view of Pernicious anaemia in mind, I have investigated the anaemia produced in animals by a variety of blood destroying agents, obtaining in this way a very complete view of what may be termed "Haemolytic Anaemias." It must be recognised at once, a point brought out strongly by my experiments, that the various substances now known to cause blood destruction in the animal body almost all act differently from one another. Thus the advantage of studying the action of a number of these blood poisons, in order to correlate the results, must be self-evident. These substances, as regards their mode of action, may be broadly divided into three groups. First of all, there are those whose action consists almost entirely in haemolysing the blood in the peripheral circulation. Second, there are those whose action is essentially a destructive one on the bone marrow as an organ. Lastly, there is a group in which the mode of action is so far not quite clear, such as for example Toluylendiamin. Examples of all these types of blood destroying agents have been used in my experiments. The characteristics of the anaemia induced, and the interpretations to be put on the various phenomena observed, are gone into fully in the different parts of this work, so that no further reference to them need be made here. It suffices to say that experiments have been carried out with the following blood

destroying agents: 1. a haemolytic immune-serum, 2. the haemolysin derived from streptococci, 3. saponin, 4. toluylendiamin, 5. cobra-lecithid.

In the animals injected with the haemolytic serum, interesting changes of a degenerative nature were in some instances found in the heart muscle and in the liver. These have been included in a separate small chapter in this thesis.

To give as complete a view as possible of the phenomena of anaemia in general, a different type of anaemia was induced in rabbits by bleeding, in order that a comparison might be made between the anaemia following blood destruction, and that after haemorrhage.

From the study of haemolytic anaemias, it was but a step further to go on and investigate the difficult but closely allied subject of "Haemolytic Icterus". On this subject I have carried out an extensive research, and the conclusions arrived at are to my mind of such interest as to warrant me placing this part of my work in the forefront of this thesis.

I wish to make mention that the research on the anaemia produced by the haemolytic serum was carried out in collaboration with Professor Muir, but all the details of the experiments (blood counts, histology, etc.,) were my own personal work. Then in the section dealing with the anaemia produced by the haemolysin obtained from streptococci, the haemolysin used was all prepared for my experiments by Dr. J.W.McLeod.

In conclusion, I wish to take the opportunity of thanking Professor Robert Muir, Professor of Pathology in this University, and Professor L. Aschoff, Professor of Pathology in the University of

Freiburg-i-B, Germany, for all their assistance and help of every kind to me in the researches included in my thesis.

EXPERIMENTS ON HAEMOLYTIC ICTERUS.

The term "Haemolytic Icterus" has been in use since the time of Virchow(1854),who from his observations,believed that bile could be formed from the blood itself,and so introduced the term. His observations were made on old blood extravasations,etc.,in which he demonstrated a change from haematin into a golden-yellow pigment (haematoidin),which microscopically and chemically gave the same reactions as bilirubin. Later on by the work of Jaffé,Hoppe-Seyler Salkowski,etc.,the identity of haematoidin and bilirubin was established.

The term "An-hepatogenous" Icterus was proposed subsequently by Quincke(1884) for the same condition,to indicate that the jaundice arose apart from an action of the liver.

The most important experimental work on the question up till now is unquestionably that of Minkowski and Naunyn,which appeared in 1886,and which since then has always been quoted,and the conclusions stated there accepted,in all text-books dealing with the subject. It seems curious,however,that so far as can be found from a wide search through the literature,their experiments seem never to have been repeated,and their results remain uncontrolled. This is especially remarkable when the fewness of their experiments is considered. As their work will be gone into fully later on,it is enough to state here that they found that by poisoning birds,especially geese,with arseniuretted hydrogen(AsH_3),no jaundice arose if the liver of the bird had been removed completely,whereas in normal geese jaundice

always resulted from the inhalation of the gas. They concluded from this that jaundice could not occur without the liver, and that in consequence no truly haemolytic icterus apart from the liver was possible.

Stadelmann (1881) used toluylendiamin to induce jaundice by blood destruction, and concluded that the result of the blood destruction was the production of a thickened bile, rich in colouring matter, which led to obstruction in the smaller bile ducts. On his view, then the jaundice so produced is essentially obstructive, and arises in the liver.

Afannassiew (1883) continued experiments on the same lines, using AsH_3 and toluylendiamin to produce haemolysis and jaundice. He supported Stadelmann's conclusions, and made the important observation that jaundice ensues after poisoning with AsH_3 or toluylendiamin much earlier (within the first twenty-four hours) than where the common bile duct is ligatured. He explained this by stating that, following ligation of the bile duct, considerable dilatation of the gall bladder and large ducts can occur before the obstruction makes itself felt, whereas the obstruction in the smaller bile ducts in the other case is rapid and affects all of them at once. 1895-?

Next came the theory of Minkowski (1905), who attempted to explain the occurrence of haemolytic jaundice, not by a mechanical obstruction but by a functional disturbance of the liver cells themselves, induced by the poison. The liver cells damaged by the poison, on his view, do not send their secretion through the bile duct as they do normally, but send it backwards into the blood stream, perhaps by way of the lymphatics. In support of this theory he made comparisons with functional disturbances in other organs, e.g.

the excretion of albumen by the kidney cells in nephritis. He pointed out an analogy between these two conditions, jaundice and albuminuria, in that both may occur in infectious diseases, intoxications and in diseases of the circulatory system. To the aberrant backward flow of bile which occurs, according to his theory, he applied the name Paranedesis.

Liebermeister(1893), Pick(1892), Browicz(1900), etc., all supported Minkowski's views, and the latter author even goes the length of fitting in the theory to explain most cases of obstructive jaundice as well.

Eppinger(1902-3), with the introduction of his staining method, by which the finer structure of the bile capillaries could be studied, advanced our knowledge considerably. He found that in obstructive jaundice the bile capillaries widen markedly, and the pressure being chiefly exerted on the small intercellular capillaries, which end blindly, these blind ends lengthen, and finally rupture into the perivascular lymph spaces which surround the blood capillaries. Thus he states that the bile reaches the circulation through the lymphatics. He would explain cases of haemolytic jaundice on very similar lines, and bases his conclusions on his findings in a case of acute phosphorus poisoning. In this case he found the bile capillaries also widely dilated, and filled with almost black coloured masses completely blocking the lumen. He called these masses of bile "Gallenthromben", on the analogy with ordinary blood vessel thrombi. He found dilatation of the capillaries behind these thrombi, and rupture through the perivascular lymph spaces. Eppinger states that he was only able to make these observations

in human cases, and could not find them in dogs after toluylendiamin poisoning. This is important in connection with my own results to be described later. Eppinger claims to have found the same changes in cases of cyanotic icterus (Gallenthromben, etc.,), and states that they are the essential cause of jaundice.

These two opposing theories of Minkowski and Eppinger have received much attention in recent yéars. While one author holds that a disturbed function of the liver cells causes an aberrant flow of the bile into the blood stream instead of along the bile ducts, the other view is that the essential cause is the formation of "Gallenthromben", which by causing obstruction lead to dilatation of the bile ducts and rupture into the perivascular lymph spaces.

With regard to Eppinger's theory, it has been strongly upheld by Abramow and Semoilowitz (1904), Jagić (1906), etc., as far as obstructive jaundice goes only. In the case of other forms of jaundice, recent experiments by Ogata (1912) with icterogen are of great interest. He could find in many cases absolutely no widening of the bile capillaries. These experiments with icterogen, first carried out by Goldmann (1912), are of great interest in connection with the pathology of jaundice. Goldmann states that obstruction of the bile ducts obviously plays no part in the process, and no Gallenthromben are certainly found.

Thus up till now the pathology of haemolytic and toxic jaundice is very far from clear. Cases of jaundice are constantly occurring clinically which follow a haemolysis or are associated with a toxic process, and in which the microscopic changes are quite anomalous. Such cases of jaundice occur, for example, after the transfusion of

blood,in severe sepsis, in the course of cases of paroxysmal haemoglobinuria, and in cases of cardiac disease, especially with backward pressure. Since the experiments of Minkowski and Naunyn on geese have never been controlled,and it is of prime importance to decide definitely the rôle of the liver in the production of haemolytic jaundice,the experiments have been carefully repeated. Experiments on dogs have also been carried out with toluylendiamin to decide other points of importance.

EXPERIMENTS ON GEESE.

The experiments to control those published by Minkowski and Naunyn were carried out in geese,since,as these authors pointed out, these birds are in several ways especially suitable for the purpose. Other animals,such as dogs,cannot survive extirpation of the liver for more than half an hour. Then the broad sternum of geese makes the operative technique easier than in other fowls where the sternum is narrow. Further,the anastomosis between the portal vein and the vena renalis advehens (Jacobson's vein) is so good in fowls that practically no signs of back pressure occur in the abdominal organs after the portal vein has been tied. A further point is that geese drink water freely after the operation,and secrete urine right up to their death. The bile of birds,too,contains biliverdin,which is very readily recognisable in urine and in sections of tissue.

The operation consists in complete removal of the liver,as far as possible,but for anatomical reasons an absolute and total extirpation is impossible,as Minkowski admits in his description of the operative technique. He says; "Es blieb hierbei in der Regel noch ein kleines

Stück vom rechten Leberlappen in der unmittelbaren Umgebung, resp. hinter der Vena Cava zürück. Dieses wurde vorsichtig zerdruckt, die Gewebstücken möglichst vollständig entfernt, und auf diese Weise eine Fortdauer der Leberfunction mit Sicherheit ausgeschlossen." No mention is made of the small lump of liver tissue left behind the vena cava having been subjected to microscopic examination, and in a courteous personal communication from Professor Minkowski he states that none was made. This omission is important, for careful examination shows that it is impossible to completely destroy the function of this small piece of liver tissue, as its relations to the vein wall are so intimate, even although attempts are made to scrape it away or squeeze it with the fingers. The small piece of liver tissue remaining after the operation is shown in Fig. I. The operation was carried out in an exactly similar manner to what Minkowski described. The operation is not easy, and takes, as a rule, thirty-five to forty-five minutes to perform. The rectum was always at the same time tied a short distance above the cloaca to prevent admixture of fæces with the urine.

In the first experiments the liver was first removed and the goose thereafter poisoned with AsH_3, but the animals then stood the poisoning so badly that in all the later experiments the inhalation of AsH_3 immediately preceded the operation. It is certain that active functionating of the liver during the short period which elapsed between the poisoning and the tying off of the liver vessels at the operation had nothing to do with the weak jaundice obtained in several experiments, since the jaundice did not occur until several hours after the operation, and continued to increase until the death

of the bird.

The AsH_3 by means of which the birds were poisoned, was obtained by Minkowski and Naunyn by adding Fowler's solution to a flask in which hydrogen was being generated by the action of diluted H_2SO_4 on zinc. At first Fowler's solution (containing 1% of arsenic) was used in the experiments, but later on it was found that much more constant results were obtained by using a 20% solution of arsenic, prepared by dissolving the arsenic with the help of $KHCO_3$ (potassium bicarbonate) according to the following equation:-

$$As_2O_3 + 2KHCO_3 = 2KAsO_2 + 2CO_2 + H_2O.$$

Here the arsenic exists as potassium metarsenite, and it was found that 5 c.c. of this solution added to the flask in which hydrogen was being generated, could produce, as a rule, in normal geese severe jaundice after inhalation for about five minutes. The goose was enclosed in a large almost air-tight chest, with entrance and exit openings for the gas, and owing to the poisonous nature of the AsH_3 the experiment was always made in the neighbourhood of a large open window.

The experiments themselves will now be briefly described.

EXPERIMENT 1. Goose 7 (large male).

At 3.50 p.m. inhalation of AsH_3 for four minutes; 5 c.c. of twenty per cent solution of arsenic used. The animal on being removed from the chest appeared to be distinctly poisoned. At 4.15 p.m. the operation to remove the liver was commenced, and was finished at 5 p.m. A very small stump of liver tissue remained attached to the vena cava. The bird stood up after the operation, and appeared to be quite strong. It drank water freely throughout the evening

At 9 p.m. (four hours after the operation) 5 c.c. of clear yellow urine without a trace of bile were obtained. At 9.45 p.m. about 10 c. c. of distinctly green urine. Next morning four test tubes full of definitely green urine were obtained, giving a very positive Gmelin test for bile. A further 10 c.c. of even greener urine were passed just before the bird died at 8.40 a.m.,having lived about sixteen hours after the removal of the liver.

The stump of liver tissue left behind was very carefully examined after the death of the bird. The small part of the stump outside ligature was completely necrotic, but behind the vena cava a small piece of liver tissue weighing 0.68 grm., and about the size of a pea,was not obviously necrosed. The microscopic appearances ofthis tissue will be referred to again.

Distinct evidences of the icterus induced were found microscopically in the kidneys, quite similar in nature to those described by Ulrich(1912) for the human subject, and by Susuki(1912) in his monograph on the "Morphologie der Nierensekretion" for animals. Some of the smaller descending tubulesfof Henle's loops were found plugged with dark green coloured casts.

The spleen showed considerable phagocytosis of cells, many erythrocytes being contained within cells of the pulp and in endothelial cells. Much pigment giving the iron reaction was found both intra- and extra-cellularly.

EXPERIMENT 11. Goose 9(large male).

At 3.45 p.m. inhaled AsH_3 for four minutes; 5 c.c. of twenty per cent arsenic used. The operation lasted from 4.15 to 5.5 p.m.,

being rather more difficult than usual owing to the goose being fat. A very small stump of liver tissue was left behind the vena cava. At 11 c.m. (five and three-quarters after the operation) 20 c.c. of dark brown urine were obtained. Half an hour later 15 c.c. of similarly coloured urine were passed. Next morning at 8 a.m. the goose was found dead. The bird had probably lived eight to ten hours after the operation, since 20 c.c. of dark brown urine had been voided during the night. In the urine the bands of oxy-haemoglobin were readily detected spectroscopically after diluting down sufficiently with water. The tests for bile showed its presence but only in traces. The Gmelin test was uncertain, and Huppert's test gave only a weakly positive green coloration.
The stump of liver tissue behind the vena cava was found penetrated throughout by haemorrhage, and no evidence of it having functionated could be discovered.
In the kidneys no trace of icterus and no haemoglobin casts could be seen. The cortical tubules gave a weak iron reaction, chiefly diffuse, but in a few cells granules of haemosiderin were present.
In the spleen there was very marked phagocytosis of red cells by pulp and endothelial cells, and much pigment giving the iron reaction.

EXPERIMENT 111. Goose 10 (large female).
At 3 p.m. inhalation of AsH_3 for three and a half minutes; 5 c.c. of twenty per cent solution used as before. Operation from 3.15 to 4 p.m. The bird appeared quite strong after the operation, and drank water freely. At 6 p.m. (two hours after the operation)

a large amount of clear yellow urine was passed, free from bile or haemoglobin. At 8 o'clock a quantity of dark brown urine containing haemoglobin was obtained. The bird died at 9 p.m., five hours after the operation. After death a quantity of dark brown urine was found in the rectum below the ligature. No bile was at any time present in the urine.

The small stump of liver tissue left behind was penetrated by haemorrhage as in the previous experiment.

In the kidneys only a few haemoglobin casts were found, and the iron reaction was negative.

In the spleen a similar marked phagocytosis of red cells, and much intra- and extra-cellular iron pigment was found.

EXPERIMENT IV. Goose 11 (large male).

At 3 p.m. inhalation of AsH_3 for three minutes; 5c.c. of twenty per cent solution used. Operation commenced at once, and was completed at 4 p.m. Especial care was taken in this experiment to attempt to completely destroy the function of the small stump of liver tissue by squeezing it with pressure-forceps and with the fingers. The goose was quite strong after the operation, and drank water freely. Between 4 and 8 p.m. about 100 c.c. of quite normal urine free from bile and haemoglobin, were obtained. At 8 p.m. urine of a distinctly green colour, and containing undoubted bile was passed. Between 8 and 9 o'clock two further samples were obtained, both of distinct green colour. At 9.15 p.m. the bird died suddenly, five and a quarter hours after the operation.

The histology of the stump of liver tissue left will be again referred to.

In the kidneys no trace of icterus was recognisable.

In the spleen, phagocytosis of red cells, but not so marked as in previous experiments. Much iron pigment both inside and outside cells.

EXPERIMENT V. Goose 13 (moderate-sized male).

At 3 p.m. inhalation of AsH_3 for four minutes; 7 c.c. of twenty per cent of arsenic used. Immediately after the poisoning both liver and spleen were removed. The goose was quite strong after the operation, which was finished at 3.50 p.m. At 9 p.m. the goose was found dead, but quite warm. About 5 c.c. of deep brownish-red urine had been passed. On standing, this separated into a chocolate coloured precipitate covered by a bright red fluid containing haemoglobin. From the cloaca a further small amount of deep red urine was obtained post-mortem.

In the kidneys, casts of haemoglobin were found.

Two control geese were poisoned with AsH_3 without removal of the liver, and the results of these two experiments are of great importance for the conclusions to be drawn from this research.

EXPERIMENT VI. Goose 8 (large male)

On 18th February, at 10 a.m., the rectum was tied. At 10.30 a.m. inhalation of AsH_3 for five minutes, 5 c.c. of Fowler's solution

being used. No urine was obtained through out the day. Next morning a small amount of thick urine was obtained, containing no bile. A copious flow of urine was induced by putting water into the stomach through a tube, but this sample also contained no bile.

On 19th February, at 3 p.m. the goose inhaled AsH_3 again for four minutes, five c.c. of Fowler's solution being used. Urine obtained the same evening was free from bile. It became clear that the goose was not being poisoned strongly enough. It is interesting to note here that the goose lived fifty hours after the tying of the rectum without showing any bile in the urine, and hence the possibility of icterus from resorption of bile from the intestine seems quite out of the question.

On 20th February, at 11.35 a.m., the twenty per cent solution of arsenic was used for the first time, 5 c.c. being employed, and the gas inhaled for five minutes. After removal from the chest the bird showed undoubted symptoms of poisoning. Between 12 noon and 3 p.m. the goose drank water freely, and passed large quantities of urine. This was at first clear and yellowish, but soon became distinctly green. Later, the urine was intensely green, and contained very abundant bile. In the evening haemoglobinuria set in, and a mixture of bile and of haemoglobin could be detected on testing the urine. Next morning, 21st February, there was practically pure haemoglobinuria, with only a trace of bile. But late in the day the haemoglobinuria passed off and was replaced by biliuria, just as marked as before. On 22nd February the goose was killed by bleeding, and in the blood serum biliverdin was readily recognised.

EXPERIMENT VII. Goose 12 (young male).

On 24th May, at 3 p.m., the rectum was tied. Soon after, AsH_3 was inhaled for four minutes, 5 c.c. of the twenty per cent solution being used. The urine obtained throughout the day was clear and free from bile. On 25th May, at 10 a.m., the goose again inhaled AsH_3 for five minutes, 7 c.c. of the twenty per cent solution being employed. Urine obtained between 12 noon and 2 p.m. was quite clear At 4 p.m. haemoglobinuria set in and was soon very marked. On 25th May the urine still contained haemoglobin in abundance, but had a slightly greenish tinge. About 4 p.m. the haemoglobinuria passed off and was replaced by a marked icterus, the urine becoming intensely green in colour. Marked biliuria continued until the goose was killed at 4 p.m. on 27th May.

Since the experiments of Minkowski and Naunyn appeared so long ago, and it is essential that the reader should be able to compare their findings with those just described, their most important experiments will be shortly given here.

EXPERIMENT 1. Duck.

Poisoned at 10 a.m. At 4 p.m. definite icterus. The urine contained also a little haemoglobin. At 4.30 p.m. operation begun to remove the liver; finished at 5.30 p.m. Soon after, marked haemoglobinuria ensued. The amount of bile in the urine was less than before the operation, and remained so until the bird was killed at 10.30 p.m., five hours after the operation. In 35 c.c. blood no trace of bile

could be recognised.

A control goose poisoned at the same time,and whose liver was not removed,developed a marked icterus,which continued until the bird was killed on the next day. A green coloured alcoholic extract obtained from the blood contained biliverdin.

EXPERIMENT 11. Large goose.

In this bird all the liver vessels were tied,and the liver tissue destroyed by crushing,but the organ itself was left "in situ". Operation finished at 10 a.m. At 11.30 a.m. the goose inhaled AsH_3 for three minutes,along with a control bird. The control bird passed green coloured urine at 12 noon,and by 2 p.m. had a marked icterus. In contrast to this,the operated goose showed at 12 o'clock a marked haemoglobinuria. At 2.30 p.m. this latter goose was killed,four and a half hours after the operation,and three hours after the poisoning. After the operation the urine constantly contained bile, but in small amount,and this disappeared when the haemoglobinuria set in. In the blood,after death,no bile could be recognised.

EXPERIMENT 111. Goose.

Inhaled AsH_3 at 9.45 a.m. for four and a half minutes. The urine at 11.30 a.m. contained bile in small amount. At 12 o'clock traces of haemoglobim and distinct bile were present. At 1 p.m. the bile and haemoglobin were both present much more abundantly in the urine. At this hour the liver was removed. The bird died at 2.30 p.m.,an hour after the operation. Shortly before death urine containing haemoglobin,but no bile,was passed.

EXPERIMENT 1V. Goose.

Inhaled AsH_3 at 9.45 a.m. for four minutes. At 12 o'clock the urine was greenish, and at 1.30 p.m. distinctly green, and gave a positive reaction for biliverdin. At 2 p.m. the operation to remove the liver commenced, and by 3 p.m. was finished. After the operation the urine was more strongly bile-containing than in previous cases, but the sample was more concentrated than usual. At 7 p.m. the goose was killed, four hours after the operation. In 130 c.c. blood no bile could be recognised.

DISCUSSION OF RESULTS.

In discussing the experiments it will be at once perceived that the number of positive experiments from which Minkowski and Naunyn drew their important conclusions is a small one. Of four experiments, in one the liver was not removed, but only functionally destroyed by crushing. The first bird was killed five hours after the operation, the second four and a half hours after the crushing and three and a half hours after the poisoning, the third bird died an hour after the liver was removed, and the fourth was killed four hours after the operation. It must be noticed also that in Experiments 111. and 1V. the birds had marked jaundice before the operation to remove the liver was begun. My own experiments number five, but it will be observed that in these the birds lived on an average longer than in the experiments of Minkowski and Naunyn, nearly, sixteen, eight, five, five and a quarter, and five hours after the operation. Also, in my experiments, the poisoning and the removal of the liver followed one another so closely that the whole experiment

may be said practically to have been conducted on a bird without a liver. In two experiments of Minkowski and Naunyn (11.and 1V.) bile persisted in the urine after extirpation of the liver,but it is a question whether this was just a continued excretion of bile formed before the liver was destroyed,or whether it depended on other circumstances which now require mention. Minkowski and Naunyn put down this continued presence of biliverdin in the urine to a resorption of bile from the bowel. They state; "Es ist nicht statthaft,hieraus auf die Fortdauer der Gallenbildung nach der Beseitigung der Leber zu schliessen,vielmehr muss nach unserer Ansicht dieser geringfügige Gallenfarbstoffgehalt des Urins,sofern er nicht darin seine Erklärung findet,dass die Entleberung unvollständig war,auf eine Resorption von Gallenfarbstoff aus dem Darme bezogen werden." Such resorption from the bowel is,to my view,quite negatived by the results of Experiment Vl. of my series,which lived for fifty hours,after tying of the rectum,without developing a trace of jaundice. The other part of the sentence quoted,however,which concerns the possibility of incomplete extirpation of the liver,seems to be much more important. Allusion has already been made to the small piece of liver tissue which must remain behind the vena cava,and careful microscopical examination has shown me that it is impossible to be sure that this tiny piece of tissue remains inactive. How much bile could be produced by such a small amount of liver tissue,however actively it functionated,is of course difficult to say,and certainly the amount must be scanty. Still,the possibility of continued function of this stump cannot be entirely passed over. Under a later heading,the probable action of the spleen,

marrow,etc.,in accounting for this weak jaundice after liver extirpation and poisoning,will be fully entered into. A further point in connection with the conclusions drawn by Minkowski and Naunyn is that they do not appear to have taken notice sufficiently of the close relationships between icterus and haemoglobinuria. Making use again of Experiments Vl. and Vll. in my series,it is seen that in Experiment Vl. a severe icterus was followed by a period of haemoglobinuria,and this was in turn again replaced by a period of jaundice. In Experiment Vll. also,after severe poisoning,haemoglobinuria appeared at first,to be subsequently followed by a marked biliuria. Thus it seems to be probable that severe poisoning in birds brings on haemoglobinuria,whereas icterus ensues when the poisoning is not so severe or so rapid in action. It was found in the experiments made by injecting a haemolytic serum into animals to produce anaemia (Muir and McNee,1912),which have been dealt with in another part of this thesis,that haemoglobinuria only occurs when the haemolysis has been severe and rapid enough to produce haemoglobinaemia of sufficient intensity. The term "threshold value" of the kidneys has been applied by American authors (Pearce, Austin, Krumbhaar, Eisenbrey,1912),to designate the point at which,haemoglobinaemia being intense enough,the kidneys excrete the haemoglobin. At any rate,it must be pointed out that where in Experiment 1. of Minkowski and Naunyn the bird had icterus,and only a trace of haemoglobin in the urine before the operation,and pure haemoglobinuria after the operation,the authors have not considered sufficiently the close relations of icterus and haemoglobinuria. The operation was carried out six hours after the poisoning,just the time when the

action of the poison had reached its maximum. To conclude definitely therefore, that the conversion of biliuria into haemoglobinuria was a result of the liver extirpation is not, in our view, admissible. Apart from these considerations, which only have to do with the explanation of various points in the experiments, I have as a result of my work arrived at a similar conclusion to that of Minkowski and Naunyn, namely, without the liver no marked icterus can arise in the goose. I have put it no marked icterus, - the previous authors, no icterus at all.

But does this conclusion in any way solve the problem of haemolytic icterus? I think not, for the question at once arises as to what happens in the goose liver during the production of the icterus. What elements in the liver have to do with the process? Is it the liver cells alone, or other cells enclosed within the liver tissue? For this part of my research the histological appearances found in the control geese, after inhalation of AsH_3, are of the greatest importance.

Before, however, the question of what elements are concerned in the production of icterus in the livers of geese could be approached, a study was made of the histology of the normal goose liver. The livers of six normal geese, which had been removed before the poisoning (as was done in the early but abortive experiments), and of two normal pigeons, were examined. The chief interest centres on the so-called cells of v. Kupffer (1899), the "Kupfferschen Sternzellen" of German authors, which in birds are very readily recognisable as endothelial cells lining the blood capillaries. In their number and size these cells are quite different in birds from what is found in man, dogs, or rabbits. It was found that normally these Kupffer

cells, especially those nearer the periphery of the liver lobules, show a very marked diffuse iron reaction. (see Fig.3). In these cells red corpuscles, or their nuclear remnants (the erythrocytes are of course nucleated in birds), are found normally enclosed, but pigment was never seen in their protoplasm. Thus the blood corpuscle-containing cells described by Minkowski and Naunyn (1886) as the result of poisoning with AsH_3, and which were subsequently by various authors (Löwit, (1889), etc.) identified with the cells first described by v. Kupffer, normally in the goose liver enclose erythrocytes. The intense iron reaction in these Kupffer cells in the normal liver of geese proves the existence in them of a very special iron metabolism, which has apparently up till now escaped recognition. It seems clear that in the goose red corpuscles are, under normal circumstances, taken up by these cells, and the iron portion of the haemoglobin molecule is split off and retained, at first at least, in the cell protoplasm. What happens to the iron-free pigment portion, whether it becomes the biliverdin of the bile directly, or only after further change, must as yet remain unanswered. One thing at any rate is certain, that this iron-free pigment portion is at once shed out from the Kupffer cells, for in the normal liver no trace of pigment was ever seen in these cells.

With these observations on the normal histology and physiology of the goose liver, we can turn much better prepared to consider the structure of the liver in icterus. The appearances obtained in Experiments VI. and VII. are identical, and of great interest. The changes here again affect essentially the Kupffer cells, and are well shown in Fig. 4, and Fig. 5. These cells are obviously much increased

above the normal in number,and in many places block completely the widened blood capillaries. The central venules of the liver lobules and the branches of the hepatic vein are also filled up with these cells,which are readily identified by the beautiful reaction for iron which they give. In the blood of the right side of the heart,and in the capillaries of the lungs,large numbers of these cells are found. This shows that not only do these cells multiply in the liver capillaries,but they become detached,enter the circulating blood by the inferior vena cava,and finally stick in the capillaries of the lungs. (see Fig.6). This extraordinary desquamation of endothelial cells of the liver capillaries and their coming to rest in the lung capillaries has a parallel in the observations of Aschoff and Kiyono (1913) in animals injected with carmine. It is of course well known that the endothelial cells of the liver and the endothelial cells of the spleen and bone marrow all take up readily injected stains. Aschoff and Kiyono showed that many of these endothelial cells containing carmine become detached,circulate in the blood and form a part of the large mononuclear cells of the blood. They also found numbers of the cells containing carmine in the lung capillaries,and most of them die there. These changes are quite analogous with what I have found in my own experiments.

The contents of the Kupffer cells in my experiments,whether they remain attached to the capillary wall or whether they circulate free in the blood,is the same,namely,large numbers of red blood corpuscles in all stages of degeneration. Some of these red corpuscles stain quite normally,but frequently simply the oval nucleus surrounded by a clear halo remains. Large quantities of yellow and yellowish-

green pigment are also found in the cells, obviously the product of red cell degeneration. Some of this pigment was of so marked a green colour as to suggest biliverdin, but this could not be proved chemically. The cell protoplasm has a diffuse iron reaction, just as is found normally, but haemosiderin is present as well in the form of large and small droplets or granules, coloured deep blue with potassium ferrocyanide and dilute HCl, and black with ammonium sulphide. Droplets of haemosiderin are also found free in the capillaries and in the branches of the hepatic veins. These are obviously either derived from broken-down endothelial cells, or perhaps are set free in the circulating blood.

In the liver cells themselves haemosiderin was also present, but in comparatively small amount, and only in the form of small granules. Bile itself was only found in scanty bile capillaries, and more frequently in the bile ducts in the form of dark green masses.

The explanation of these changes must now be considered. The goose's liver seems to be undoubtedly a double organ, consisting of the specific liver cells, and of the cells of v. Kupffer, which, because of their identical characters with the endothelial cells of the spleen and the cells of the splenic pulp, may be almost described as a splenic tissue enclosed within the liver. At any rate, by viewing the question in this way a much better idea is given of the close relationship between spleen and liver in geese. The Kupffer cells seem to have as a special function, to do with iron metabolism. The iron portion of the haemoglobin of the blood corpuscles is removed, and held firmly in these cells. What happens to the iron-free pigment portion of the haemoglobin is for haemolytic icterus the

important question. This question requires to be answered for normal conditions as well, where the cells have a similar function in iron metabolism. The changes induced by the AsH_3 are of course merely a great exaggeration of the normal. Either this iron-free portion of haemoglobin must be extruded from the Kupffer cells as the finished product biliverdin, or else as some intermediate compound which is so far unknown. If the iron-free portion is extruded as biliverdin, then, since so many of these cells overflow into the blood stream, biliverdin must be set free in the blood. If this occurs, then is produced a true haemolytic icterus, with which only these endothelial cells, and not the liver cells are concerned. If the iron-free portion is set free as some intermediate product, then this intermediate body may perhaps have to pass through the liver cells before it becomes true biliverdin. Such a possibility cannot be dismissed, and if this is what happens, then there can be no true haemolytic icterus without the intervention of the liver cells. In the latter case the question, of course, would still remain how the bile, produced by the liver cells in excess, finds its way back into the blood. The views if Stadelmann, Minkowski, Eppinger, etc., on the question have been already referred to. The work of Ogata, however, has shown that in cases of toxic icterus stasis of the bile in the finer bile capillaries does not exist, for in many such cases with marked jaundice, no widening of the bile capillaries, which would speak for obstruction, can be demonstrated at all.

I have carried out experiments in dogs which were poisoned with toluylendiamin, and in which a marked jaundice was produced, and in other dogs where the common bile duct was tied to bring about

obstructive jaundice. The appearances of the bile duct are well shown in Fig.2. In the one case (obstructive) the dilatation of the bile ducts is very marked,whereas in the haemolytic icterus induced by toluylendiamin the widening is minimal in degree.

From the findings in my experiments the conclusion has been arrived at that it is not the removal of the liver cells themselves,but the removal at the same time of the tissue similar in function to the spleen but enclosed in the liver, i.e. the cells of v. Kupffer,that leads to the production of no jaundice,or only a slight one,when the liver has been removed.

For the occurrence of jaundice after poisoning normal birds,and for the presence of a weak jaundice after the removal of the liver in some of the experiments,the action of the spleen and bone marrow cannot of course be ignored. These tissues normally show the same reaction for iron in endothelial cells to what is found in the liver. In Experiments VI. and VII. the endothelial cells of the spleen were packed with red corpuscles in all stages of disintegration. These cells give a marked haemosiderin reaction,and pigment masses exactly similar to those in the Kupffer cells of the liver were present in abundance.

The question may be asked,how it is that the activity of the spleen and bone marrow gives rise to no,or merely a very weak,icterus when a goose is poisoned after removal of the liver. It is a fact that the spleen of birds,at least of all those observed by me,is extremely small in comparison with the spleen of higher animals. When compared with the relatively enormous capillary system of the liver it is evident that the spleen in birds can have comparatively

little to do with the breaking down of haemoglobin. The bone marrow in birds is also extremely fatty. When one thinks that even the relatively huge liver of the goose must functionate for some time -about four hours on the average- before sufficient bile can be present in the blood to lead to its appearance in the urine, it is in no way extraordinary that following removal of the liver, the spleen and bone marrow, because of their small size, should be unable in the six or eight hours which the goose lives to produce sufficient bile to be recognisable as such in the blood or urine. At any rate, the negative findings of Minkowski and Naunyn in the blood of their animals can be readily explained by the shortness of the time of the experiment. The question of haemolytic icterus could be much more easily solved if liver extirpation experiments could be carried out in animals such as dogs, etc., where the spleen is of such size as to be of marked importance for iron metabolism, and where the bone marrow is a very considerable organ. Here these two organs could probably produce a haemolytic icterus on the basis of the above theory, owing to their greater bulk.

CONCLUSIONS.

Although the above experiments give no positive proof of the existence of a true haemolytic icterus apart from the liver cells, yet from a repetition of the experiments of Minkowski and Naunyn, the following conclusions can be drawn:-

1. There is no doubt at all that after removal of the liver, in geese poisoned with AsH_3, no <u>marked</u> icterus occurs.

The weak icterus which occurred in some of my experiments after

removal of the liver,must depend either on the functional activity of the spleen and bone marrow,or on continued activity of a small piece of liver tissue left behind the vena cava.

2. The reason why no marked icterus follows extirpation of the liver is not that the liver cells have been removed,but depends upon the removal of the tissue enclosed within the liver,namely,the endothelial cells of v. Kupffer. These cells have,at any rate,to do with the first phase in the production of bile,since they split off and retain the iron part of haemoglobin molecule,and set free the pigment portion.

3. Neither from the experiments of Minkowski and Naunyn,nor from my own,may a definite conclusion be drawn that a true haemolytic icterus is impossible. On the contrary,the histological appearances,especially the proliferation and desquamation of the Kupffer cells,their circulation in the blood stream,and their destruction there,speak strongly in favour of the occurrence of an icterus quite apart from any action of the liver cells. The argument that when the liver is removed,the homologous endothelial cells in the spleen and bone marrow do not take up the work,is met by the fact of the extreme smallness of these latter organs in birds,and the short duration of the experiment.

4. An important question is how far these conclusions,arrived at by experiments on geese,can be applied to human pathology. My experiments certainly show that the structure of the liver in birds is different from that in higher animals. Quite apart from the relative difference in size of the organ in birds,there is in them a very special iron metabolism in the liver,with which not the liver cells,

but the endothelial cells lining the vascular capillaries, have to do.

EXPERIMENTS ON DOGS WITH TOLUYLENDIAMIN.

To compare in the light of these experiments on geese the appearances produced in haemolytic icterus in the higher animals, dogs were poisoned with toluylendiamin (Stadelmann, Afanassiew, etc.) to bring about jaundice.

Experiment 1. Dog 2, weight 22 kilos.
On 16th, 20th, 22nd, 25th, and 29th November, the dog received respectively 0.4, 0.7, 1.0 grms. toluylendiamin per os, and 0.75, and 1.0 grms. subcutaneously. From 17th November until the animal was killed on 6th December, nineteen days in all, the animal had constantly jaundice and biliuria, the yellowness of the conjunctivae and the amount of bile varying with the dose of the drug given. The dog lost weight, and at death weighed only 18 kilos.
Post-mortem. Only the liver showed the jaundice microscopically being yellowish brown in colour, very greasy, and obviously very fatty. Spleen firm, and dark blue in colour. The liver and spleen both gave a slight haemosiderin reaction, the kidneys none. Bone marrow very red throughout the whole shaft of the femur.
Microscopically, the liver showed a marked icterus, especially seen in the central part of the lobules. Bile was present mainly enclosed in the Kupffer cells, both as dark green droplets and as rods, probably identical with the Gallenthromben described by Eppinger. A few of these dark green rods were found lying free in the bile capillaries, but most were inside the endothelial cells. Bile was also present in the liver cells themselves, especially

near the centre of the lobules,in the form of very fine green granules. In films made by breaking up small pieces of liver tissue, and stained by Giemsa's stain,the so-called Gallenthromben of Eppinger stood out very clearly as dark green rods,some showing distinct bifurcation. The fine green granules of bile in the liver cells themselves were also well seen by this method. In sections of liver stained for iron,the Kupffer cells,which are not nearly so numerous as in geese,practically all gave a marked diffuse haemosiderin reaction, Many red blood corpuscles in all stages of disintegration were seen inside the endothelial cells, while in some of them bile,disintegrating red corpuscles,and a diffuse haemosiderin reaction of the protoplasm were present together.

In sections stained by Eppinger's method,to show the bile capillaries,hardly a trace of widening of the capillaries could be made out, For comparison the common bile duct of another dog was ligatured,and an obstructeive icterus induced. The two appearances are seen side by side in Fig.2,and have already been referred to.

The spleen showed marked congestion,all the sinuses being filled with erythrocytes. Marked phagocytosis of red corpuscles by large cells was present,and a large amount of pigment,partly intracellular but chiefly extra-cellular,in the form of irregular golden-brown masses,all giving a haemosiderin reaction,was seen. In some of the large cells disintegrating red corpuscles and haemosiderin were present together,but no bile was observed.

The lymph glands showed marked phagocytosis of red corpuscles

by large cells,in the loose central parts of the glands. In certain glands,especially those lying along the spinal column,the phagocytosis of red cells was quite as marked as in the spleen. These cells gave again a definite iron reaction.

Films of bone marrow showed fairly numerous large pale-staining cells (Giemsa's stain) containing droplets of green bile,and brownish pigment and remnants of red corpuscles were also found in these cells. The reaction for iron was not applied here.

The kidneys gave no haemosiderin reaction,the only changes noticed being the presence of fine granules of bile in cells of the convoluted tubules of the cortex.

Experiment 11. Small dog,weight 9,950 grms.
1 grm. of toluylendiamin was injected subcutaneously; next day there was very marked jaundice,the urine containing abundant bile but no haemoglobin. The conjunctivae became deep yellow in colour, and the animal was ill and refused all food. Twenty-nine hours after the injection the dog was killed.
Post-mortem. All the organs were tinged a deep yellow. Microscopically the liver showed a very striking picture. It was extremely congested,and all the capillaries were stuffed full of erythrocytes. Very little bile was found in the whole section, and none at all inside liver cells. In scanty and irregular areas a few narrow green rods were seen in the bile capillaries,in sections stained with carmalum. The scarcity of bile pigment was really very noteworthy,considering the extreme degree of jaundice.

In the endothelial Kupffer cells numerous red corpuscles were seen enclosed, but they stained quite normally, and showed no signs of disintegration. In sections stained for iron very little was found. In a few endothelial cells, however, a positive result was obtained, the haemosiderin forming a single droplet about the size of a red corpuscle.

In films of the liver pulp, stained by Giemsa's method, no bile was found intracellularly, but small Gallenthromben were definitely recognised. By this method, too, numerous nucleated red corpuscles (normablasts) were seen to have been circulating in the blood of the liver, an evidence of the anaemia which tolylendiamin induces.

The spleen sinuses were all stuffed with red corpuscles, which however stained quite normally. There was considerable phagocytosis of erythrocytes, as in the previous experiment.

The lymph glands showed also very considerable phagocytosis in the sinuses. There was a considerable amount of pigment, chiefly in the form of extra-cellular masses, and giving the iron reaction.

Films of bone marrow stained by Giemsa's stain showed no real abnormality. Pigment was present in a few cells, but this may be quite physiological.

The kidneys showed really an extraordinary excretion of bile. The tubules were in many areas filled up with large casts of golden-yellow bile. In the secretory portion of the organ, the cells were stained a deep and diffuse brown by the bile.

Experiment 111. Small dog, weight 3,500 grms.

0.75 grm. of tolylendiamin was injected subcutaneously, and the

animal was killed fourteen and a half hours after the injection. Before death a quantity of urine of light brown colour, giving a positive test for bile, was obtained. No icterus was present in the conjunctivae.

Post-mortem. Urine taken from the bladder after death was darker brown in colour, and gave a marked reaction for bile. The tissues showed practically no trace of jaundice. On centrifugalising the blood obtained at death, marked haemoglobinaemia was found to be present.

The liver was bright red in colour, and microscopically showed extreme congestion, the vascular capillaries being filled with red corpuscles. A few large endothelial cells gave a distinct iron reaction, and in them erythrocytes and masses of pigment were found.

The spleen showed comparatively little phagocytosis of red cells.

The lymph glands, on the contrary, gave evidence of much phagocytosis.

The kidneys had no trace of jaundice, and no abnormal appearances were seen in them.

CONCLUSIONS.

The characters of a haemolytic icterus induced by toluylendiamin in dogs are not in any essential feature different from what we have observed in geese poisoned by AsH_3. The chief point of difference is that the structure of the dog's liver is different from that of birds. In dogs the endothelial Kupffer cells are much less numerous than in fowls, and normally give no iron reaction. Thus in dogs, as far as phagocytosis goes, the liver does not seem

to be so directly associated with the iron metabolism as in birds. It is likely, as has been suggested already, that the spleen in higher animals has taken on this function. But in the icterus induced the endothelial cells of the dog's liver show quite similar changes to those found in geese, namely, phagocytosis of red corpuscles, disintegration of them, and the appearance of a haemosiderin reaction in the cell protoplasm. The cells being much less numerous, these findings are not so prominent and readily recognisable. In the spleen the changes seen in dogs and geese during the icterus are also similar, but it has been sufficiently emphasised already how much larger an organ the spleen is, comparatively speaking, in the higher animals than in birds. In the lymphatic glands of dogs the changes are also very marked, and of a similar nature to those found in the spleen. In the geese it was generally a matter of extreme difficulty to find lymphatic glands at all, and hence no observations on them have been described.

Taking all these points into consideration, it seems quite probable that, with modifications depending on the different importance of the spleen, all that has been suggested in connection with the mode of origin of haemolytic icterus as a result of the experiments on geese, can be applied to higher animals, and to man.

Experiments on dogs by Whipple and Hooper (1913), which have only recently been published, support the conclusions arrived at in my experiments very considerably. After injecting haemoglobin into the blood stream of dogs in which the liver had been completely excluded from the circulation, they were able to prove that part of the free haemoglobin was rapidly changed into bile. They made

experiments in addition on dogs in which the blood was only allowed to circulate in the head and thorax, the abdominal circulation being completely stopped by certain surgical procedures, such as clamping the aorta. The dogs were kept alive for about two hours by artificial respiration, and in three out of four such experiments, haemoglobin injected was partly changed into bile. To recognise bile in the serum they used the Huppert test. Although they give no histological details, they suggest that the endothelial cells of the blood vessels, bone marrow, and muscles, may have to do with this alteration of the haemoglobin. Their experiments, when looked at in connection with the histological changes described in my own work, are of great interest and importance.

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DESCRIPTION OF FIGURES.

Fig. 1. To show the small piece of liver tissue which must remain in connection with the wall of the inferior vena cava, after extirpation of the liver. A., Vena cava; B. and C., Site of the ligature round the stump. (x 8)

Fig. 2. Liver stained by Eppinger's method to show the bile capillaries. A.,Toluylendiamin icterus (pog 1),with minimal dilatation of the capillaries. B.,After ligature of the common bile duct a number of days previously,showing the marked widening which occurs in obstructive icterus.

(x 1000)

Fig. 3. Normal goose liver,stained for iron and counter-stained with carmalum. Shows endothelial cells,all giving a marked iron reaction,most numerous at the periphery of the lobule.

(x 80)

Fig. 4. Goose liver after AsH_3 poisoning (Experiment VII.). Shows endothelial cells,all giving a marked iron reaction. A branch of the hepatic vein is cut in section,and is seen filled with desquamated endothelial cells. Counter-stained with carmalum. (x 80)

Fig. 5. Similar to Fig. 4,but under high magnification. The contents of the endothelial cells are seen to be haemosiderin (diffuse and in the form of droplets),erythrocytes in all stages of disintegration,and masses of pigment of yellowish and distinctly green colour. Scanty granules of haemosiderin are present near the margin of some liver cells.

(x 1000)

Fig. 6. Section of lung (Experiment VII.) stained for iron,and counter-stained with carmalum. Numerous desquamated endothelial cells from the liver are seen caught in the lung capillaries. The contents of the cells are similar to what is seen in sections of liver. (x 1000)

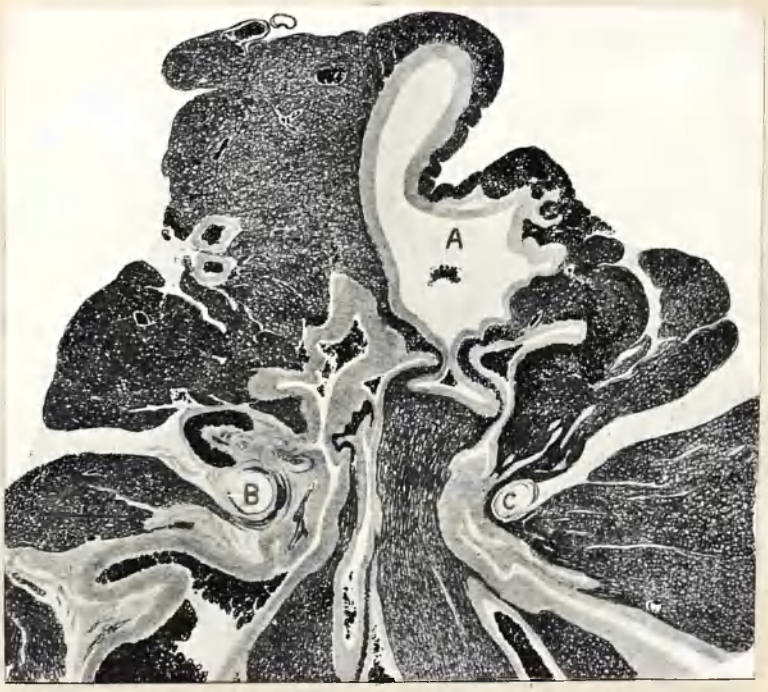
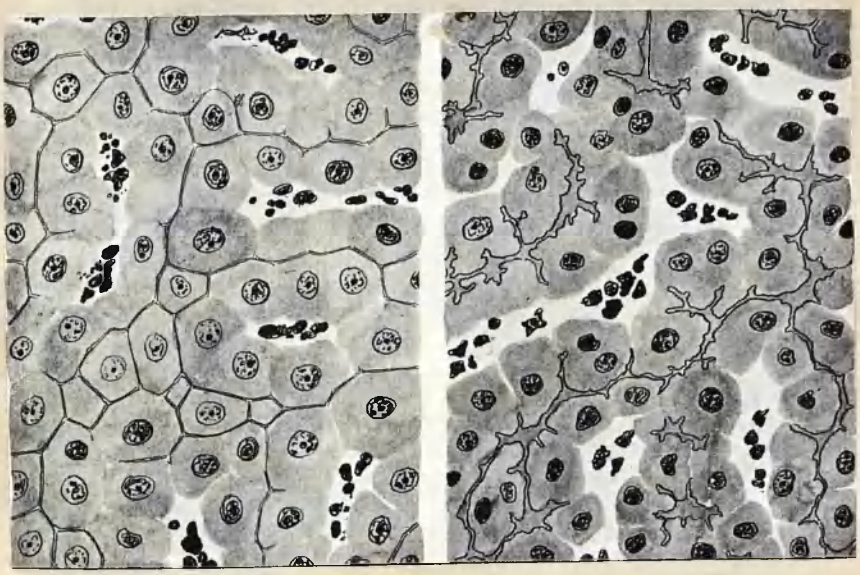


Fig I.



A B

Fig II.

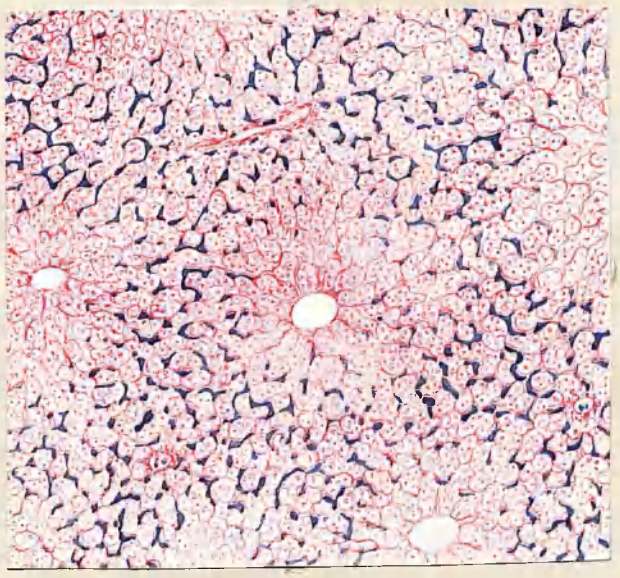
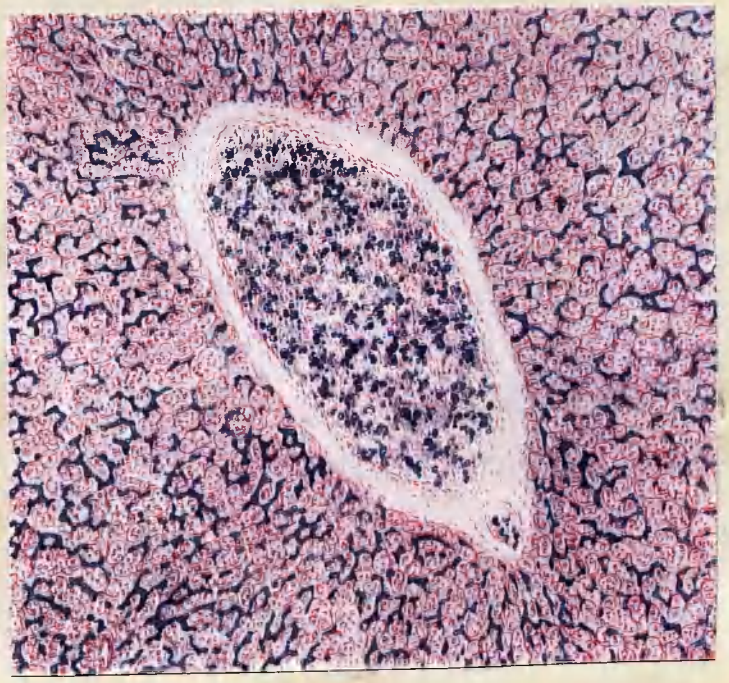


fig 3.



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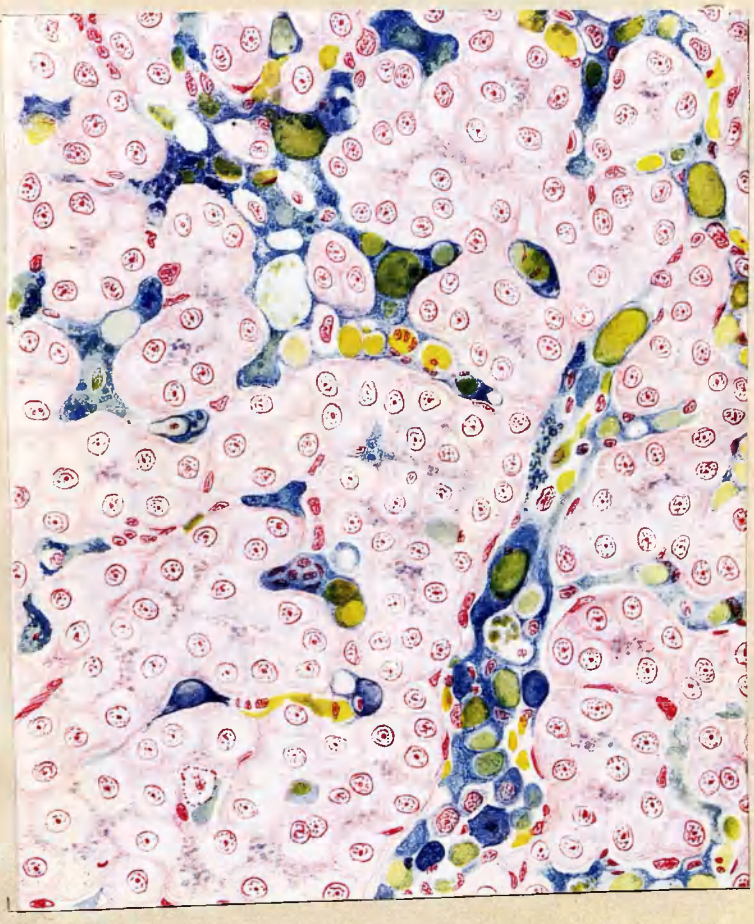

Fig 5.

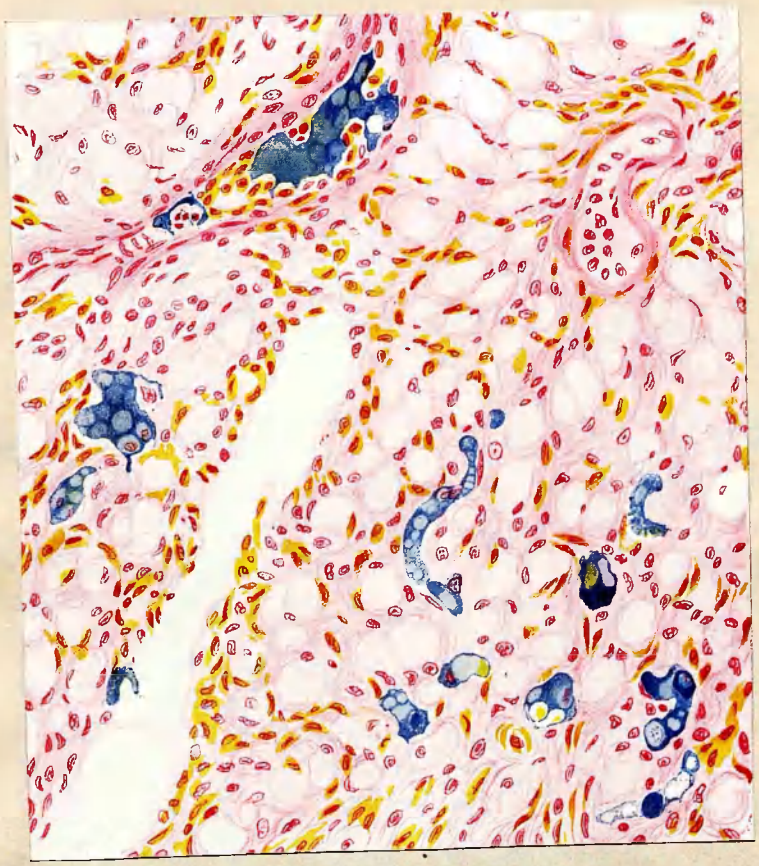

Fig 6.

A.

THE ANAEMIA PRODUCED BY THE INJECTION OF A HAEMOLYTIC SERUM.

The first observation on the action of a haemolytic serum is said to be that of Belfante and Carbone, who showed that when an animal was injected with the blood from another species, its blood became highly toxic to animals of that species. Our exact knowledge of the nature of haemolysins may, however, be said to date from the work of Bordet (1898), who showed that there was an essential similarity in the constitution and mode of action of haemolytic and bacteriolytic sera. This was shortly followed by the well-known papers on the subject by Ehrlich and Morgenroth (1899). The literature on the subject of haemolysis is now immense, but the part dealing with the action of haemolytic sera in the living body is comparatively small, as compared with that on haemolysis "in vitro".

Cantacuzène (1900) first studied the action of a haemolytic serum on rabbits, and his results showed that whereas a fairly large dose caused a very rapid blood destruction and a resulting anaemia, the administration of small doses might raise both the number of red corpuscles, and the percentage of haemoglobin, above the normal.

Gruber (1901) used a haemolytic serum while studying certain questions with regard to complement, and believed he had demonstrated by its use the pre-existence of complement in the plasma.

Levaditi (1902), in a paper on the same subject refers to the method of blood destruction.

Since that time haemolytic sera have been used by various workers

to produce anaemia, but there is no good detailed account of the resulting morphological changes in the blood, whilst information on many other points is still wanting. I have accordingly carried out a series of experiments on rabbits, and in the subsequent pages an account will be given of the important changes met with in the blood, bone marrow, and other organs of the body.

The serum used in the experiments was obtained by injecting on several occasions a young goat with rabbit's corpuscles, which had been washed free from serum. The subcutaneous method of injection was used, and the corpuscles corresponding to 15 to 20 c.c. of blood were injected on five occasions. The haemolytic dose of the serum obtained was in a test-tube experiment 0.01 c.c. for 1 c.c. of a five per cent suspension of rabbit's corpuscles, along with the fresh serum of a normal rabbit as complement.

As will be seen from the results of the experiments, the serum used is in the rabbit's body a very active haemolytic agent. It was found that the mode of injection affected the result very much, since, on the one hand, the intravenous injection of 1 c.c. may cause the destruction of the greater part of the animal's blood; while, on the other hand, the intraperitoneal injection of 5 c.c. or more may have a much less effect. The blood destruction in the latter case is also a much more gradual process, as in no such experiment was haemoglobinuria observed with doses of 5 c.c., although this was of common occurrence after the injection of much smaller amounts by the intravenous method. This latter method was found the more convenient, and was followed almost exclusively in the experiments.

Another point is that the individual susceptibility of rabbits

varies enormously,so much so that it is impossible to gauge the degree of resulting anaemia from the dose of serum injected. This will be readily appreciated on looking at the protocols of the experiments. It should also be mentioned that the intravenous injection is frequently followed by toxic effects,which may sometimes lead to a rapidly fatal result. In many cases,within two or three minutes after the injection of 1 c.c. of serum the animal shows symptoms of asthenia,falling over on its side in an extended position and the respirations becoming much accelerated; in some cases death results,but in most others a rapid recovery follows. Again, in some instances symptoms occur almost instantaneously after the injection; clonic convulsions appear,and death occurs within a very short time,apparently as a result of respiratory failure. It was found that these acute toxic effects may be to a large extent obviated by injecting the serum in smaller amounts,with short intervals of time between.

With regard to these initial toxic effects,marked variations have been met with in different animals,some of them dying in a comparatively short time even when very small doses were used. As a rule an animal which has survived the first injection stands a second dose without serious spmptoms,and later on a certain degree of immunity is established. It is also noteworthy that when the initial effects have passed off without fatal result,the animals survive well,and do not lose weight. The subsequent phenomena depend upon the degree of blood destruction; either an acute and fatal anaemia follows,or an anaemia which is followed by rapid regeneration.

Comparatively few lesions could be found in other organs, which could be put down to a specific action of the haemolytic serum. Changes were, however, found in the heart muscle in several of the experiments, and in one case there were irregular areas of necrosis in the liver. These changes are gone into fully in a subsequent part of this thesis. Our results differ in this respect from those of Dudgeon, Panton, and Ross (1909), who employed sera obtained by immunising rabbits against guinea-pig's and cat's corpuscles. In their experiments death occurred at varying intervals up to several days, and was found to be associated with severe necrotic and fatty changes in the organs, especially in the liver and kidneys, and with haemorrhages.

EXPERIMENTS.

In all about twenty experiments with the haemolytic serum have been carried out. Reference will not be made to all of these here, since an account of about half that number is sufficient to illustrate all the effects of the serum. The experiments fall naturally into two groups. The first group comprises the more acute cases, where death either occurred, or probably would have occurred, as the result of the anaemia; in the second group recovery had taken place, and the animals were killed at varying intervals to study the regeneration of the blood.

The individual experiments will now be described.

EXPERIMENT 1.

Rabbit 3. weight 1360 grms.

Day.	Red Corpuscles.
0[1]	7,200,000
1	3,400,000
2	460,000

[1]1.5 c.c. of haemolytic serum injected intravenously.

In this experiment there occurred a very rapid and intense anaemia, although the total amount of serum injected did not exceed 1.5 c.c.. On the day after the injection the animal passed 80 c.c. of porter-coloured urine, which contained a large amount of haemoglobin, and on the second day 77 c.c. of urine of dark port-wine colour, also very rich in haemoglobin. The blood films showed slight variation in the size of the corpuscles, and there were many partially decolorised corpuscles, "shadows" also being present. The ground of the films stained a distinct reddish colour with eosin, apparently owing to the haemoglobin in the plasma. The test made in the ordinary way gave marked haemoglobinaemia. The number of corpuscles fell to a very low level, and on the second day it was difficult to enumerate them satisfactorily owing to the number of partially decolorised corpuscles which were present. There was distinct although not great leucocytosis. The animal was killed on the second day, as it appeared to be dying from the profound anaemia.

Post-mortem. The cortex of the kidney had a deep brown, almost black, colour, owing to the amount of blood pigment which it contain..

The spleen also was almost black in colour, greatly congested, and somewhat increased in size. The liver was unaltered in appearance. All three organs gave a very marked haemosiderin reaction, which, however, was greatest in the kidneys and in the spleen. The bladder contained about 10 c.c. of dark brown urine. On microscopic examination, the cells in the splenic pulp showed active phagocytosis of red corpuscles and clumps of granules of haemosiderin; many of the cells also gave a diffuse iron reaction. The bone marrow of the femur was soft, red, and friable. On microscopic examination it was found to be unusually rich in cells, the amount of fat being diminished; the proportion of the different kinds of cells was, however, practically normal.

EXPERIMENT 11.

Rabbit 8. weight 1590 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1]	6,060,000	7,000	74	1.0
1	2,160,000	11,000	40	1.5

[1] 1 c.c. of haemolytic serum injected intravenously.

In this experiment a very intense anaemia was produced by the injection of only 1 c.c. of serum; the animal was accordingly an example of very great susceptibility to the poison. On the day after the injection there was marked haemoglobinuria, and the urine gave the spectrum not only of haemoglobin, but also of acid haematin. Stained films of the blood on this day showed no change in the size or form of the corpuscles, but a considerable number of "shadows" or

imperfectly decolorised corpuscles were present. As the blood destruction was apparently very rapid the animal was killed nineteen hours after the injection. The time of coagulation of the blood was much delayed,no clot occurring for twenty minutes. Marked haemoglobinaemia was present,as shown by the ordinary test.

Post-mortem. The kidneys,as in the previous experiment,showed a dark brown cortex,but the colour was not uniform,being arranged in broad bands in the lines of the vessels. The spleen was not enlarged,but was almost black in colour. The liver was normal in appearance. There was a marked haemosiderin reaction in the kidneys,spleen,and liver,but especially in the two former. Microscopic examination of the spleen showed,as in the previous experiment,much phagocytosis of red corpuscles,and a considerable amount of haemosiderin in granules. The marrow contained a large proportion of fat and blood,the cellular elements of the marrow proper being comparatively few. In the sections some of the red corpuscles seemed to be undergoing lysis. Some of the myelocytes showed chromatorrhexis of the nuclei. The haemosiderin in the kidneys and liver gave only a diffuse iron reaction. Haemoglobin casts were present in the convoluted tubules of the kidneys in enormous numbers,and there were also cloudy swelling and some vacuolation of the cells of these tubules. In the liver there were found,on microscopic examination,some irregular areas of recent necrosis.This lesion will be referred to later on.

EXPERIMENT.111.

Rabbit 5. weight 2126 grms.

Day.	Red Corpuscles.	Leucocytes.
0[1.]	5,900,000	6,000
1[2.]	5,280,000	10,000
2[3.]	3,520,000	10,000
3	1,440,000	7,000

[1.] 1.5 c.c.of haemolytic serum injected intravenously.

[2.] 1.0 : : :

[3.] 1.0 : : :

In this experiment haemoglobinuria was present on the second day after the injection,and on the day following the animal passed 140 c.c. of dark red urine,there being evidently an extremely rapid blood destruction going on. The spectrum of haemoglobin was still faintly visible when the urine was diluted 1 : 30. On the day following the injection the blood showed comparatively little change,one or two normoblasts,however,being present. On the second day there was very marked anaemia,numerous "shadows" were present in the films,and there were also some basophil megalocytes. The animal was found dead on the morning of the fourth day of the experiment. The bladder contained post-mortem a quantity of dark chocolate-coloured urine ,giving a distinct spectrum of haemoglobin.

Post-mortem. The appearance of the kidneys,liver,and spleen,was practically the same as in Experiment 1,although the cortex of the kidney was not of so dark brown a colour. The microscopic appearances were also similar. The spleen showed much phagocytosis of

red corpuscles, and also contained much granular haemosiderin. The bone marrow was found to be partly depleted of cells: the red corpuscles in the vascular spaces appeared to be partially lysed, and some of the myelocytes showed chromatorrhexis. The kidneys showed some cloudy swelling, and contained some haemoglobin casts.

EXPERIMENT IV.

Rabbit 15. weight 2170 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1]	7,200,000	5,600	85	1.0
1	4,620,000	11,000	62	1.1
2	2,080,000	5,300	34	1.4

[1] 1 c.c. of haemolytic serum injected intravenously.

In this experiment the injection of 1 c.c. of serum, in two equal parts at an interval of an hour, led to a very rapid blood destruction and marked anaemia, as is shown in the table. Careful examinations of the blood showed that haemoglobinaemia first appeared two and three-quarter hours after the first injection, and gradually increased till the end of the experiment, when it was very marked. Severe haemoglobinuria occurred on the day after the injection, the spectrum being still readily detected in a dilution of 1 : 25. On the following day haemoglobin was also present in abundance in the urine. The changes in the blood corresponded to those in the other acute cases. Nucleated red cells were very numerous, and a prominent feature also was the number of "shadows" present. The alterations in the organs (deposits of haemosiderin,

and so forth), were of similar nature to those already recorded. The animal was found dead on the third day after the injection.

EXPERIMENT V.

Rabbit 23. weight 1445 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1]	5,370,000	6,000	60	1.0
1[2]	2,680,000	11,200	35	1.19
2	1,730,000	9,000	22	1.15

[1] 1 c.c. of haemolytic serum injected intravenously.

[2] 1 c.c. : : :

In this experiment a rapidly progressive anaemia was induced. The animal was killed on the second day after the injection, in order to estimate its blood volume. Because of the washing out of the organs necessary for the blood volume estimation, the histological appearances were, of course, much disturbed.

EXPERIMENT VI.

Rabbit 24. weight 1474 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1]	5,620,000	5,300	63	1.0
1[2]	3,480,000	12,100	52	1.36
2	1,040,000	9,200	18	1.6

[1] 1 c.c. of haemolytic serum injected intravenously.

[2] 1 c.c. : : :

This was a remarkably acute case, and attention may be drawn to

the high Colour Index obtained on the second day. Haemoglobinuria was very marked, and the blood became very thin and watery. In blood films "shadows" were extremely numerous. The animal died on the evening of the second day after the experiment was commenced, and post-mortem exactly similar changes to those already described were met with in the organs.

In the experiments now to be described the injection of the serum produced a much less acute effect, more than one injection being necessary to bring about a marked degree of anaemia. In none of these cases did a fatal result follow the injection of the serum, but the animals were killed at varying stages of recovery from the anaemia. It was in these experiments that the most marked morphological changes were met with in the blood and blood forming organs.

EXPERIMENT V11.

Rabbit 4. weight 1600 grms.

Day.	Red Corpuscles.	Leucocytes.
0 1.	7,100,000	8,000
1 2.	4,850,000	10,000
2	3,250,000	14,000
3	2,950,000	11,000
4 3.	3,360,000	12,500
5	1,520,000	32,000
6	1,280,000	20,000
7 4.	1,680,000	11,000
8	2,000,000	15,000

1. 1.5 c.c. of haemolytic serum injected intravenously.
2. 0.75 : : :
3. 1.0 : : :
4. 0.75 : : :

Note. The leucocyte counts on days 5 and 6 include the erythroblasts, which were present in large numbers (see under).

On the second day after the injection, basophil megalocytes and nucleated red corpuscles were present in the blood; the former gradually increased in number, and on the fourth day after the injection formed a large proportion of the red corpuscles present, - in fact, on the fifth day they appeared almost as numerous as the ordinary red corpuscles. This condition continued, but the basophil reaction became much less marked in many of the corpuscles, so that on the day when the animal was killed all stages in staining reaction were seen. A few red corpuscles showed punctate basophilia, but this condition was never common. The nucleated red corpuscles also increased in number, the maximum being reached on the fifth day after the injection, when they numbered 9500 per c.mm., by far the largest number met with in the experiments; there were also on this day nearly 1000 free nuclei of erythroblasts. The nucleated red corpuscles were numerous till the day of death, when they numbered 5000 per c.mm.; for the most part they were of the normoblast type, but there were also a considerable portion of megaloblasts, especially in the last three days. The protoplasm of the megaloblasts had a basophil reaction, and most of them showed a punctate condition. As the anaemia became more marked small red corpuscles measuring

down to 3μ appeared, but these were not so numerous as in other experiments. The number of leucocytes was increased throughout the experiment; in a series of differential counts the proportion of the different classes of white cells vary considerably, but there was an absolute increase of lymphocytes as well as of polymorphs. The polymorphs showed a maximum of 58 per cent on the fourth day, a large percentage for the rabbit. The blood-platelets throughout the experiment showed a considerable increase. There was no haemoglobinuria at any time, and the animal was killed when blood regeneration appeared to be progressing rapidly.

Post-mortem. The various organs were practically normal in appearance; a haemosiderin reaction was present in the liver, spleen, and kidneys, but was comparatively slight in each. Microscopic examination showed a slight iron reaction in these organs in the usual situations. The spleen showed a considerable amount of phagocytosis of red corpuscles, and there was a considerable amount of haemosiderin in cells throughout the pulp. The bone marrow was very red and soft and on microscopic examination there was found to be a very marked erythroblastic reaction. Nucleated red corpuscles were present in enormous numbers,- megaloblasts, normoblasts, and intermediate forms,- and ~~by~~ mitotic figures occurred in all of these (see Fig. ?). The relations of these cells will be entered into later on. There was also an absolute increase of the finely granular myelocytes, although their relative proportion remained still below the normal. This fact might be taken to indicate that the serum had some effect on cells of the granular series as well, but this is not certain, since we know that in every erythroblastic reaction there is always an

accompanying increase of the leucoblastic tissues.

EXPERIMENT V111.

Rabbit 7. weight 1590 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1.]	6,760,000	13,000	82	1.0
1[2.]	4,590,000	15,000	73	1.31
2[3.]	4,480,000	14,000	55	1.0
3[4.]	3,840,000	8,000	52	1.11
4	2,240,000	23,000	42	1.5
5	1,250,000	14,500	31	2.05
6	1,240,000	27,500	32	2.14
7	1,896,000	21,000	33	1.43
8[5.]	1,960,000	10,000	40	1.7
9	2,360,000	14,300	40	1.4
10	2,430,000	9,000	43	1.46
11	3,274,000	9,100	44	1.1
12	3,180,000	8,800	44	1.14
13	4,340,000	7,500	60	1.14
14	4,780,000	4,500	55	0.95
15	5,800,000	5,000	63	0.9
16	6,110,000	8,000	70	0.95
17	5,750,000	6,500	70	1.0

[1.] 1 c.c. of haemolytic serum injected intravenously.

[2.] 1 c.c. : : :

[3.] 1 c.c. : : :

[4.] 5 c.c. : : intraperitoneally.

[5.] 4 c.c. : : :

In this experiment there was no haemoglobinuria, although three intravenous and two intraperitoneal injections were made. The second of the latter injections on the eighth day caused no further drop in the number of red corpuscles, there being apparently some immunity established. As in previous experiments, some basophil corpuscles appeared on the second day after the injection; on this day there were also a few erythroblasts. The basophil corpuscles increased in number on subsequent days, and some of them reached a large size. There was some punctate basophilia on the sixth and following days. On the eighth day the same changes were found, but there was also many microcytes and a few poikilocytes, the former being intensely eosinophil. Nucleated red corpuscles, both normoblasts and megaloblasts, occurred throughout the experiment, but were always scanty. Recovery was very rapid, and by the thirteenth day the red corpuscles were nearly all well shaped and of uniform size; the great majority were, however, over-sized; only a few basophil corpuscles were then present.

Post-mortem. The organs were practically normal in appearance, the spleen and kidneys gave a well-marked haemosiderin reaction; the liver gave a very slight reaction. Microscopic examination of the marrow showed a well-marked erythroblastic reaction, but this was not so marked as in the previous experiment. The spleen also showed less phagocytosis, and contained fewer haemosiderin granules. The cells of the liver and kidneys gave a diffuse iron reaction.

EXPERIMENT IX.

Rabbit 10. weight 1660 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1.]	4,570,000	3,500	59	1.0
1[2.]	3,520,000	8,400	55	1.2
2	2,390,000	6,000	38	1.26
3	2,024,000	7,500	25	0.96
4	1,360,000	6,600	23	1.34
5	1,420,000	8,000	24	1.3
6	1,250,000	8,500	18	1.15
7	1,270,000	9,000	20	1.23
8[3.]	1,500,000	9,700	20	1.02
9	1,120,000	12,500	19	1.32
10	1,150,000	10,600	22	1.54
11	1,250,000	7,500	20	1.34
12	1,770,000	7,000	22	1.0
13	1,700,000	4,000	24	1.07
14	1,690,000	4,000	28	1.23
15	1,736,000	4,700	30	1.31
16	2,190,000	4,000	38	1.38
17	2,630,000	4,000	40	1.23
18	2,870,000	4,700	40	1.07
19	3,280,000	4,800	42	1.0
20	3,240,000	4,000	45	1.07
21	3,400,000	3,800	50	1.11

1. 1 c.c. of haemolytic serum injected intravenously.
2. 1 c.c. : : :
3. 0.75 c.c. : : :

In this experiment the animal was allowed to live for about ten weeks, in order to determine whether any change occurred in the disposition of the haemosiderin. During the first two days of the experiment there was no haemoglobinuria, but on the third day the animal passed a large amount of urine which had a distinct reddish colour and gave the spectrum of haemoglobin. On this day there was also distinct haemoglobinaemia. On the fourth day the urine was very dark in colour, but did not give any spectrum of haemoglobin.

The following are the chief blood changes. On the second day "shadows" of red corpuscles were found in the films, and there were also a few basophil megalocytes. On the third day the basophil corpuscles increased in number, and afterwards formed a prominent feature in the blood picture. In the later stages, as in previous experiments, the basophil reaction became less marked, so that variations in the staining reaction were present. By the ninth day (after the last injection) the corpuscles showed remarkable variation in size, measuring from 2 to 9.5μ, and there was also a definite tendency to poikilocytosis. Nucleated red corpuscles occurred from the fourth day onward, but were always very scanty. Both normoblasts and megaloblasts were present. At the end of the experiment the corpuscles were again comparatively uniform in size, though basophil megalocytes were still present. Enumeration of corpuscles was not made after the eighteenth day. The animal was killed on the seventy-second day, having apparently regained perfect health.

Post-mortem. The spleen and kidneys gave a marked haemosiderin reaction; the liver also, although to a less extent. There was

practically no phagocytosis of red corpuscles in the spleen, but it contained numerous haemosiderin granules, enclosed chiefly within endothelial and connective tissue cells. The abdominal lymph glands contained a considerable amount of orange-coloured pigment, but this gave no iron reaction. The bone marrow contained a considerable proportion of fat, and the number of cells present was really below normal limits. There were still, however, well marked islets of nucleated red corpuscles.

EXPERIMENT X.

Rabbit 21. weight 2296 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1]	5,960,000	4,400	70	1.0
1[2]	5,180,000	11,200	60	1.0
2	3,010,000	20,000	44	1.2
3	960,000	9,000	20	1.7
4	900,000	13,100	16	1.52
5	1,320,000	16,500	18	1.19
6	1,470,000	11,200	20	1.19
7	2,180,000	4,000	30	1.19
8	2,400,000	4,600	38	1.34
9	2,450,000	4,700	40	1.4
10	2,980,000	6,000	48	1.4
11	3,370,000	4,700	54	1.4

1. 1.25 c.c. of haemolytic serum injected intravenously.

2. 1.0 c.c. : : :

On the day of the first injection haemoglobinuria was found to be present four hours, and again six hours, after the injection. An examination for haemoglobinaemia was negative on these two occasions. On the next day there was no haemoglobinuria, and it did not reappear. On the second day, however, the urine was very dark in colour, and gave the reaction for bile pigment. On the third day bile was present again in considerable amount, and it is to be noted that this is the only experiment in which biliuria followed the injection of the serum. On this day too slight haemoglobinaemia was present, and also lipaemia. On the fourth day bile had disappeared from the urine. The changes in the blood corresponded to those in the previous experiment, basophil megalocytes appearing on the second day of the experiment, and thereafter gradually increasing in number; at a later period they lost to a considerable extent their basophil reaction. Erythroblasts of both types occurred in the blood from the fourth day onwards, but were never very numerous.

Post-mortem. The organs were practically healthy in appearance. The kidneys, spleen, and liver gave a distinct haemosiderin reaction, which was most marked in the case of the two first. The microscopic appearances with regard to the haemosiderin corresponded with those in previous experiments, the amount of granules in the spleen, however, being specially great. The bone marrow showed an extremely marked erythroblastic reaction, the proportion of fat being diminished, and there being large areas of erythroblasts. Megaloblasts and normoblasts with all transitions were present. As in Experiment VII, there was an absolute, though not relative, increase of leucoblastic tissue. The lymphatic glands, as in the previous experiment,

contained a large amount of yellow pigment which did not give the iron reaction.

EXPERIMENT XI.

Rabbit 25. weight 1460 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0 [1.]	5,960,000	5,300	82	1.0
1 [2.]	5,900,000	15,600	80	1.0
2 [3.]	3,820,000	27,000	58	1.09
3	3,200,000	29,000	50	1.12
4	1,690,000	27,500	38	1.7
5	1,170,000	40,000	22	1.36
6	1,860,000	19,400	34	1.3
7	2,640,000	27,000	48	1.3
8	3,190,000	13,100	52	1.18
9	3,830,000	9,000	60	1.12
10	4,730,000	8,000	66	1.0
11	4,840,000	6,000	68	1.0

1. 1 c.c. of haemolytic serum injected intravenously.
2. 1 c.c. : : :
3. 1 c.c. : : :

The high leucocyte counts are partly due to the presence of erythroblasts.

In this experiment the anaemia was rather less acute. The point of greatest interest in the blood was the marked erythroblastic reaction, which appeared about the second day and continued through

the greater part of the experiment. Many of the nucleated red cells exhibited beautiful punctate basophilia of their protoplasm.

Post-mortem. The changes found in this case were exactly similar to those of the previous experiment.

From the above protocols it will be observed that in all cases marked anaemia followed the injection of the serum, and in six of the experiments there was also haemoglobinuria. The severity of the anaemia, and the rapidity of its course, varied much in different cases. In several experiments a single injection was sufficient to destroy fully two-thirds of the animal's corpuscles. In other experiments more than one injection was required to bring about marked anaemia. It was evident in some of the longer and more chronic experiments that a certain degree of immunity to the serum had been developed. It is also very noteworthy that in one experiment a fall in the number of corpuscles of five million per c.mm. took place within five days, without any haemoglobin appearing in the urine. An important point observed, too, is that as a rule the full effect of the injection, as evidenced by the fall in the red cell count, is not reached until about the third day after an injection.

THE RESULTS OF THE EXPERIMENTS.

The most important results are concerned with the morphological changes produced in the peripheral blood, and in the bone marrow. These will now be discussed in greater detail.

Changes in the blood. When the blood destruction has been rapid as in the six first experiments, stromata or "shadows" of red corpuscles may be seen in considerable numbers on the day following an injection. Corpuscles which have lost part of their haemoglobin, so that only a narrow ring at the periphery stains pink with eosin, are also found (see Fig.1.). These changes, which soon disappear, constitute, along with the fall in the number of red corpuscles and drop in the haemoglobin percentage, the direct evidence of blood destruction. The chief and most interesting alterations, however, are dependent on the process of blood regeneration, and the first of these is the appearance of basophil or polychromatophil megalocytes in the circulation (see Figs.1 and 2). A few of these may be seen on the day following the first injection, but it is usually on the second day that they begin to appear in any number. Thereafter they become more and more numerous. These basophil megalocytes are undoubtedly young and immature corpuscles, and when erythroblasts some of them correspond with the former cells in size and in staining reaction of the protoplasm. At first there is a marked distinction between these immature megalocytes and the old red corpuscles, the bluish staining of the former contrasting with the pure eosinophil reaction of the latter. It must be noted however, that not all the newly formed megalocytes are basophil in staining reaction.

Whenever erythroblasts appear in the circulation the protoplasm of many of them has a pure eosin staining, and the megalocytes derived from these will have the same reaction. Therefore it must be that two kinds of young corpuscles enter the blood stream; first, corpuscles of normal or somewhat above normal size and with pure eosinophil reaction, and second, the larger basophil megalocytes. The former will be distinguishable only with difficulty from the pre-existing corpuscles, while the latter are readily identified. It is the size of the basophil megalocytes which is so noteworthy in the anaemia produced by the serum. In none of the other experimental anaemias to be described were such large cells met with, although smaller cells of the same type were frequent. Thus we would lay especial stress on the fact that in the anaemia induced by the serum the blood picture is more definitely megalocytic, and more closely resembles the condition seen in pernicious anaemia in man, than in any of our other experiments. As the anaemia progresses a number of the old erythrocytes apparently diminish in size, so that corpuscles of 3 to 5μ in diameter (microcytes), which stain intensely with eosin, are present. The result of all these changes is that about the end of a week, when the anaemia is very marked, and regeneration going on rapidly, great variations in the size of the corpuscles are seen (see Fig.5). Careful measurements in fresh unstained films at this time showed that the corpuscles varied in diameter from 3 to 9.5μ in diameter. Corpuscles of all degrees of basophilia are met with at this stage. As the animal recovers there is a comparatively rapid change in the blood picture. The basophil reaction in the erythrocytes passes off, and at the same time the variations

in size gradually disappear, so that before the blood count has returned to normal the cells may be nearly uniform in size (see Fig.6). These changes in size and in staining reaction, which may occur in a few days are indeed very remarkable. One can imagine the megalocytes undergoing contraction, but it is less likely that the microcytes increase in size; yet either this occurs or they must be rapidly destroyed.

Punctate basophilia was a fairly common, but very variable feature of the anaemia (see Fig.3.). As a rule it appeared later on in the experiment and subsequent to the occurrence of the diffuse basophilia. It seems certain, in these experiments at least, that it is simply a further stage of polychromatophilia, due to the basophil substance becoming condensed into foci, and not a degenerative change in any way.

Marked poikilocytosis was never met with. When the anaemia was severe, some variation in shape was present along with the great variation in size already referred to. But the change was comparatively slight as compared with what is found in pernicious anaemia in man. It is probable that a time element plays a part in the development of poikilocytosis, and our experiments were of course of very brief duration.

Nucleated red corpuscles were found in the blood in all the experiments, but in very varying numbers. Those which appear first are all normoblasts (see Fig.3). Later, generally about the fourth or fifth day, megaloblasts also appear (see Fig.4); the protoplasm of these is frequently basophil, or else shows marked punctate basophilia. The number of erythroblasts present bore no relation to the rapidity

of blood regeneration. In one experiment the number of nucleated reds in the blood was found on one occasion to be 3,500 per c.mm., but as a rule they were much less numerous than this.

The Colour Index in practically all the experiments in which the haemoglobin was estimated, became definitely raised during the height of the anaemia. The highest Index met with was 2.14 on the sixth day of Experiment VIII.; other Indices of 1.5 to 1.7 were met with more than once. As a rule the highest Index corresponded very closely to the greatest degrees of anaemia.

The Leucocytes were as a rule increased during the experiment, but to a very varying degree. The differential count of the white cells seems to vary considerably in the normal rabbit. Where differential counts were made however, in experiments where there was a considerable leucocytosis, there appeared to be a distinctly increased percentage of lymphocytes. In a few experiments distinct pyknotic change was seen in some of the leucocytes in the blood films, which shows that the serum used had some leucotoxic action as well as its haemolytic effects.

The blood platelets were always markedly increased in number during the experiment. In one case where a count was made by Aynaud's method, they were found to number about 1,200,000 per c.mm.

The rate of regeneration of the blood was extremely rapid. Thus in Experiment VIII. there was for three days an average daily increase of 870,000 per c.mm.; in Experiment X. for seven days an average increase of 350,000; and in Experiment XI. for five days an average increase of 700,000. Cantacuzène observed the same thing in his experiments, and Ritz, (as we have also seen in our own work)

first pointed out that the blood regeneration was not nearly so rapid after haemorrhage as after blood destruction. The probable reason for this is th t after blood destruction the haemoglobin derivatives are available for purposes of blood regeneration.

Changes in the bone marrow. These are of two chief types. In the acute cases some evidence of a direct toxic action of the serum on the bone marrow is met with. But the toxic changes are really extremely slight, when compared with what occurs as a result of the action of some blood poisons, e.g. Saponin. The evidences found of a direct destructive effect on the marrow are chiefly the presence of karyorrhexis and other degenerative changes in some of the myelocytes. The total number of cells in the marrow also in such cases appears to be somewhat diminished below the normal. These changes are only met with in the acute experiments of short duration.

In the more chronic cases, where recovery was going on when the animal was killed, the changes found are all of a reactive or regenerative nature. In sections of marrow there is found to be a great increase in the cellular elements, with a corresponding marked diminution in size of the fat spaces. This increase of cells is seen to be due to the presence of large areas of erythroblasts, in which many mititic figures are noticeable. These groups of cells are made up of elements of very varying size and development. By means of the Oxydase reaction, carried out after the method described by Shaw Dunn, it became evident at once that these cells were in no way connected with the myelocyte series, since the reaction was in all of them absolutely negative. The possibility arose however, that some

of them might be lymphocytes, which they resemble closely when stained by certain methods, and especially when dry fixed films are examined. This possibility had to be considered more closely since Blumenthal and Moravitz, and Price Jones both describe an increase of lymphocytes in the bone marrow following haemorrhage. From careful examination of the cells, however, in wet films fixed in saturated corrosive sublimate and stained with diluted Ehrlich's Triacid stain, the characters of the cell nuclei and the varying amount of haemoglobin in the protoplasm, could be much more easily seen. It became practically certain in this way that the groups of cells were composed solely of erythroblasts. Further support in the same direction was afforded by the application of the Altmann-Schridde method of staining granules in lymphocytes. In none of the cells under discussion could such granules be demonstrated, whereas in a lymphatic gland stained as a control the granules were perfectly depicted.

The presence in the bone marrow of these masses of erythroblasts in all stages of development, can be correlated with the appearances found in the peripheral blood. Erythrocytes are normally derived from the smallest erythroblasts, which in the rabbit have a diameter of 6.5μ, and give as a rule a purely eosinophil reaction in their protoplasm. The erythroblasts of a stage further back are 8μ or more in diameter, and their protoplasm is distinctly basophil. It is quite evidently from these latter cells that the basophil megalocytes, which are so characteristic of the blood picture induced by the haemolytic serum, are derived. In the early stages of the anaemia the first abnormal cells recognisable in the blood films

are normoblasts and basophil megalocytes. These two types of cells represent actually the same stage in development from a megaloblast The former type develops by mititic division,and by its protoplasm becoming gradually more eosinophil; the latter type develops simply by loss of the nucleus of the erythroblasts.

Changes in other organs. These are not of nearly the same importance as the foregoing. It is evident that the action of the haemolytic serum is of the nature of an extra-cellular lysis of the blood corpuscles in the circulating blood,and does not depend on the activity of any cells in the organs. This is in contrast to what is found to be the case with other blood destroying agents,e.g. Toluylendiamin. Phagocytosis of red corpuscles does occur in the spleen sinuses in this anaemia,and pigment is also deposited. These changes are,however,relatively slight.

In all the acute cases,and in the majority of the more chronic ones a well marked haemosiderin reaction was obtained in the liver, kidneys,and spleen. The reaction was most marked in the acute experiments,and it is noteworthy that a very considerable reaction could be induced in an animal injected less than twenty-four hours beforehand. In the kidneys the reaction was always most intense where haemoglobinuria had been present. The reaction in the organs was in every case diffuse,except in one experiment where the rabbit was allowed to live for over seventy days; in this animal fine granules were found in the cells of the liver and kidneys. It is interesting to observe that the haemosiderin reaction in the kidneys was found in an exactly similar situation to where it occurs in pernicious anaemia in man,the cells of the convoluted tubules,and

of Henle's tubules, giving the prussian blue test alone.

CONCLUSIONS.

1. The anaemia produced by the haemolytic serum used is characterised by the early appearance of basophil (polychromatophil) megalocytes. These cells afterwards increase greatly in number, so that the anaemia assumes a very definitely megalocytic type.

2. Coincident with the megalocytic type of blood, the Colour Index becomes markedly raised.

3. Nucleated red cells appear in the peripheral blood, but are never very numerous. Both normoblasts and megaloblasts occur.

4. In association with the changes in the blood, there is a marked erythroblastic reaction in the bone marrow.

5. Phagocytosis of red corpuscles in the spleen and other organs is not a prominent feature of this, as it is of other, experimental anaemias.

6. An intense haemosiderin reaction is given by liver, kidneys, and spleen, especially in the acute experiments.

7. When recovery from the anaemia is taking place, the regeneration of the blood is very rapid, an average daily increase of 500,000 red corpuscles per c.mm. being not uncommon.

8. No myeloid transformation is found in the spleen or other organs, as occurs in some experimental anaemias.

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DESCRIPTION OF FIGURES.

Fig. 1. Film of blood from Experiment III., on the third day after the first injection. Shows "shadows", and imperfectly stained corpuscles. Two basophil megalocytes are also seen.

Fig. 2. Film of blood from Experiment VII., on the second day after the first injection. Shows three large basophil megalocytes.

Fig. 3. Film of blood from the same experiment as the preceding, on the fifth day of the experiment. Shows two normoblasts, and one corpuscle with punctate basophilia.

Fig. 4. Film of blood still from the same experiment, on the eighth day. Shows a megaloblast and a large basophil megalocyte.

Fig. 5. Film of blood from Experiment VIII., on the seventh day. Shows marked variations in the size of the corpuscles and slight poikilocytosis.

Fig. 6. Film of blood from the same experiment as the preceding, six days later. Shows the rapid return to comparative regularity in the appearance of the corpuscles.

Fig. 7. Film of blood from Experiment XI., during the stage of regeneration. Shows marked polychromatophilia, punctate basophilia, and a large proportion of megalocytes. Jenner's Stain.

Fig. 8. Film of bone marrow from Experiment VII., showing the marked erythroblastic reaction which occurs during the process of recovery. The large cells with greenish nuclei and faintly haemoglobin-tinted protoplasm are megaloblasts. Ehrlich's triacid Stain.

(All figures x 1000.)

Fig. 9. Section of kidney from Experiment II., showing great numbers of haemoglobin casts in the convoluted tubules.

(x 100)

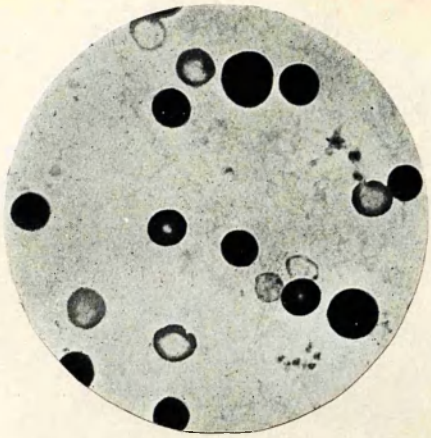

Fig. I.

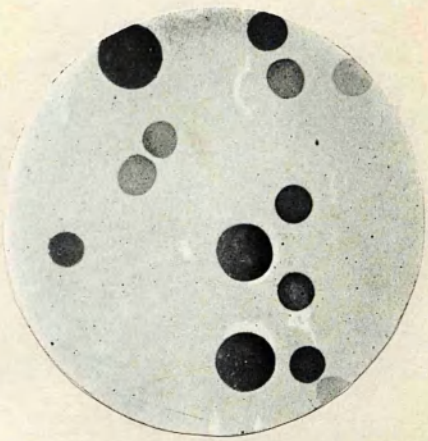

Fig. II.

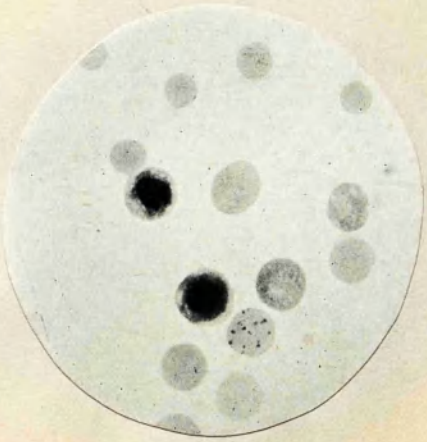

Fig. III.

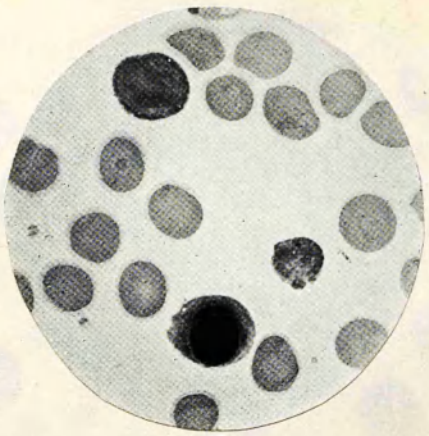

Fig. IV.

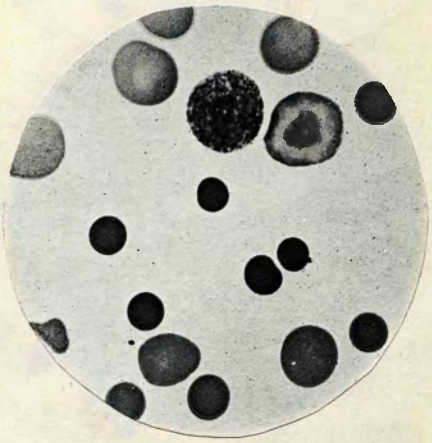

Fig. V.

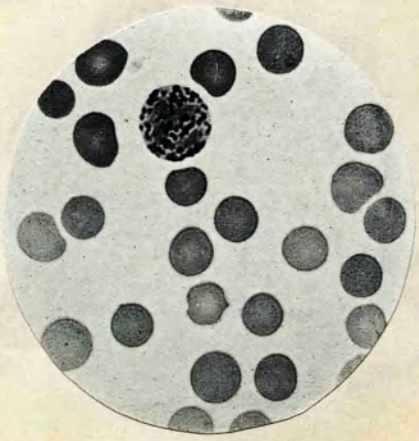

Fig. VI.

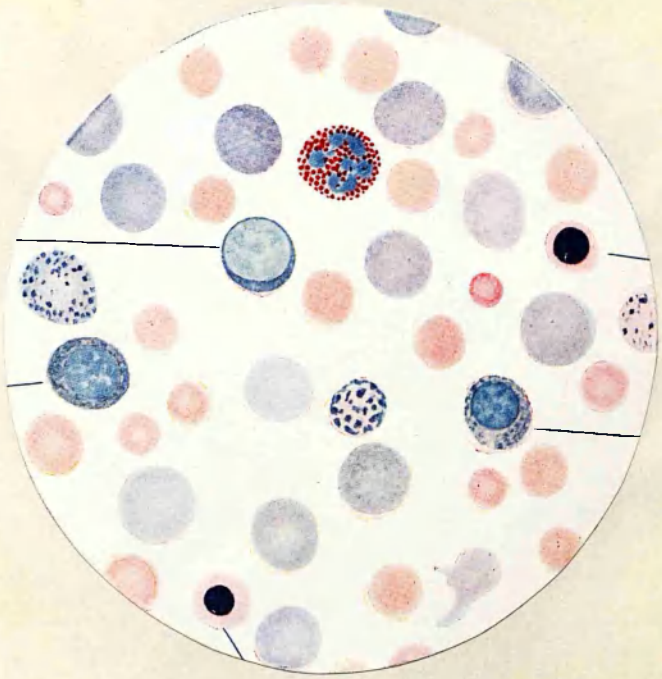

Fig. VII

Fig. VIII.

Fig 9.

B.

THE ANAEMIA PRODUCED BY THE INJECTION OF THE HAEMOLYSIN OBTAINED FROM STREPTOCOCCI.

Although various bacteria are known to be able to produce toxins which are haemolytic, very few of these haemolytic bacterial toxins have been used to bring about experimental anaemias in animals. Staphylococcci, streptococcci, the cholera-like vibrios, B. tetani, and B. megatherium all produce haemolysins, and while all of them have been shown to possess a lytic effect within the animal body as well as in the test-tube, only staphylolysin and vibriolysin have been used experimentally to cause anaemia.

Kraus and St. Ludwig (1902), by injecting cultures of actively haemolytic but relatively avirulent staphylococcci or cholera-like vibrios, were able to bring about an anaemia of secondary type in rabbits. They record a fall of 2 to 3 million red corpuscles, and the appearance of normoblasts and poikilocytes in the circulating blood. They obtained similar results with filtrates of these cultures, but give no details of the experiments.

Streng (1902) also produced anaemia in rabbits by injecting staphylolysin, while Schur (1903) confirmed the results of Kraus and St. Ludwig's experiments.

Cesaris-Demel (1897) and Czeczowiczka (1903) studied the amount and distribution of the haemosiderin in the organs of animals injected with staphylolysin, and found the deposit most abundant in the spleen. The last named author describes localised foci of necrosis in the livers of some of his experimental animals.

The haemolysin obtained from streptococci has never apparently

been used before in experimental work on anaemia. This seems extraordinary when the prominence which has been given to the work of William Hunter in connection with his theory of oral sepsis and streptococci in the etiology of pernicious anaemia is considered. The probable reason for the absence of such experiments up till now is that until very lately only very weakly haemolytic filtrates were obtained from streptococci. Many authors have indeed failed to obtain haemolytic filtrates at all, but active filtrates have been obtained, however, by Besredka (1901), Ruediger (1903), Kerner (1905), Tchitchikine (1906), Jupille (1911), McLeod (1912), and Braun (1912). None of these observers were able to prove the occurrence of haemolysis in the circulating blood of animals, so that they did not suspect that an anaemia, such as occurred in the experiments to be described here, could be brought about.

Now from the point of view of analogy with human pathology experiments with streptolysin should be peculiarly interesting. Streptolysin differs from all other haemolytic bacterial toxins except those obtained from B. tetani and B. megatherium in being actively haemolytic for human blood, as well as for that of certain other mammals; also it is a toxin produced from an organism capable of invading the human tissues, unlike the other two mentioned. Further it varies from all others bacteriolysins yet described in that no formation of anti-substance follows its repeated injection into animals, and no immunity to its haemolytic effects is obtained. All these points, in reference to Hunter's theory of pernicious anaemia, suggested that the study of an experimental anaemia produced by streptolysin would be of considerable interest and value.

Only some strains of streptococci produce a haemolysin, and the one used in the following experiments was the most actively haemolytic of some seventy varieties investigated by McLeod. It was obtained originally from an acute suppuration in the knee-joint of a man, and has retained its active qualities as far as the production of a haemolysin goes for a prolonged period in sub-culture.

For the haemolysin used in these experiments I am indebted to Dr. J. W. McLeod, who has already published the method used in its preparation. While making the haemolysin for these experiments, he was able to improve on his published method, and in fact, he prepared the most actively haemolytic toxins for human blood, derived from bacteria, which have been so far described.

For clearness in following how the doses injected were tested and their haemolytic power estimated, the method used to prepare the haemolysis will be briefly given here.

Method of McLeod. "Bouillon of double of the usual concentration is prepared, 1 lb. of meat being used to make 500 c.c. of broth, and to this 2 per cent of peptone is added. The alkalinity of the bouillon is titrated with phenol-phthalein, and adjusted so that 0.4 c.c. of normal caustic soda solution per 100 c.c. of broth is required to bring the medium to the neutral point. To this bouillon 20 to 25 per cent of fresh horse serum, previously heated at 57°C., is added. A loopful of culture is rubbed down with a platinum needle into about 2 c.c. of salt solution, and 0.01 c.c. to 0.02 c.c. of this dilute emulsion is used for inoculation. After incubation for eighteen hours filtration is carried out through a Maassen filter. The filtrate is tested, before injection, on a

2 per cent suspension of defibrinated unwashed rabbit's blood in 0.85 per cent salt solution. The more active filtrates have a haemolytic dose of 0.05 to 0.01 c.c. for 1 c.c. of this suspension, after incubation at 37°C. for two hours, the ratio of haemolytic effect on human blood compared to that on rabbits' is about 2 : 3."

RECORDS OF EXPERIMENTS.

Ten experiments have been carried out on rabbits. The blood of all these animals was in all cases but one tested beforehand by McLeod to estimate its susceptibility to the toxin. A variation in susceptibility was found, and the animals could be divided into two groups as a result of this test. At the outset, the more susceptible rabbits were chosen as it was thought an anaemia would more readily occur in them, but they all died after one or two injections, before any degree of anaemia could be brought about. In the less susceptible animals death did not occur after repeated doses, and they were killed at varying intervals up to twenty-three days.

Four animals belong to the susceptible group, dying as a result of a toxic effect of the filtrate. The tables below give details of two of these experiments.

EXPERIMENT 1.
Rabbit C. weight 1630 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin%.
0[1].	7,330,000	12,000	60
1	6,240,000	7,000	60
2[2].	...	...	...
3[3].	6,150,000	9,600	60

1. 9.5 c.c. of filtrate (M.H.D.- 0.035) injected intravenously.
2. 9.5 c.c. of filtrate (M.H.D.- 0.075) : :
3. 9.5 c.c. of filtrate (M.H.D.- 0.025) : :

[The minimum haemolytic dose (M.H.D.) represents in all the experiments the amount of haemolytic filtrate which produced complete lysis of 1 c.c. of a 5. per cent suspension of defibrinated unwashed rabbit's blood, after two hours' incubation.]

The animal was found dead on the morning after the third injection.

EXPERIMENT 11.

Rabbit E. weight 1740 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0[1]	8,150,000	7,100	66
1[2]	6,750,000	12,200	63
2	7,530,000	9,000	63

1. 10 c.c. of filtrate (M.H.D.- 0.035) injected intravenously.
2. 8 c.c. of filtrate (M.H.D.- 0.025) : :

The animal was found dead on the day after the second injection.

The changes found in the blood of these susceptible animals, and in their organs post-mortem, can be dismissed very briefly. Haemoglobinaemia was marked in all, and in three of the animals haemoglobinuria occurred after the injections. No marked degree of anaemia was produced, and the only changes noted in the blood were the presence of "shadows" and of scanty normoblasts soon after injection of the filtrate. Post-mortem, the organs of all the animals gave a definite haemosiderin reaction. In three of them a definite

lesion was found in the liver, a fairly marked focal necrosis, evidently of toxic origin, being present. This necrotic change was most distinctly seen in Rabbit C. (see Fig. I), where a cirrhosis of old standing was also found to be present.

In all the six animals of the less susceptible group a definite anaemia was produced, although it varied in degree. Below are given details of three of these experiments.

EXPERIMENT. 111.

Rabbit A. weight 1570 gms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0 1.	6,890,000	9,000	60
1	4,880,000	10,000	54
2	6,230,000	12,000	53
3 2.	5,710,000	7,000	52
4	6,610,000	5,300	53
5 3.	5,150,000	6,500	52
6	5,150,000	8,400	48
7 4.	4,770,000	14,200	45
8	4,990,000	8,100	47
9 5.	4,580,000	10,000	47
10	5,130,000	9,000	47
11 6.	...	...	...
12	6,270,000	11,500	50
13	...	...	...
14 7.	...	...	...
15	5,990,000	5,500	52

Experiment 111. (continued).

16	...	...	...
17[8]	6,190,000	6,200	52
18	5,560,000	6,000	50

[1] 10 c.c. of filtrate (M.H.D.- 0.05) injected intravenously.

[2] 10 c.c. : : 0.08 : :

[3] 10 c.c. : : 0.08 : :

[4] 9 c.c. : : 0.1 : :

[5] 9 c.c. : : 0.08 : :

[6] 10 c.c. : : 0.15 : :

[7] 12 c.c. : : 0.04 : :

[8] 15 c.c. : : 0.04 : :

EXPERIMENT 1V.

Rabbit G. weight 1390 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0[1]	5,250,000	7,100	55
1	5,300,000	6,900	55
2[2]	...	...	...
3	4,830,000	10,000	50
4[3]	...	...	...
5	4,330,000	12,400	45
6[4]	...	...	...
7	4,520,000	16,800	42
8[5]	...	...	...
9[6]	3,890,000	14,000	42
10	3,720,000	17,000	40

Experiment IV. (continued).

11[7]	4,030,000	16,200	42
12	5,190,000	7,000	44
13[8]	6,000,000	10,000	47
14	5,560,000	7,200	47
15[9]	...	...	...
16[10]	6,150,000	8,000	50
17	5,760,000	11,900	48

1. 5 c.c. of filtrate (M.H.D.- 0.035) injected intravenously.
2. 6.5 c.c. : : 0.035 : :
3. 8 c.c. : : 0.06 : :
4. 10 c.c. : : 0.04 : :
5. 3 c.c. : : 0.01 : :
6. 6 c.c. : : 0.02 : :
7. 10 c.c. : : 0.04 : :
8. 10 c.c. : : 0.04 : :
9. 10 c.c. : : 0.02 : :
10. 10 c.c. : : 0.05 : :

EXPERIMENT V.

Rabbit M. weight 1280 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0[1]	5,610,000	6,250	68
1[2]	5,820,000	7,900	68
2[3]	6,000,000	11,500	60
3[4]	4,740,000	8,700	50
4[5]	4,800,000	8,000	40

Experiment V. (continued).

5[6.]	3,830,000	8,000	38
6[7.]	3,210,000	6,500	36
7[8.]	3,540,000	14,000	36
8	3,960,000	7,800	39

1. 7 c.c. of filtrate (M.H.D.- 0.035) injected intravenously.
2. 10 c.c. : : 0.03 : :
3. 20 c.c. : : 0.07 : :
4. 20 c.c. : : 0.1 : :
5. 15 c.c. : : 0.05 : :
6. 11 c.c. : : 0.06 : :
7. 10 c.c. : : 0.04 : :
8. 12 c.c. : : 0.08 : :

As is shown in the tables, a definite anaemia resulted in all these experiments. The most marked anaemia was produced in Experiment V. and the changes in the blood of this animal were very striking. The red cell count fell from 5,610,000 to 3,210,000 in seven days, and the haemoglobin percentage decreased from 68 to 36. It will be observed from this that the Colour Index was not raised above the normal. The blood was examined within an hour or two after the first injection, and "shadows" were found to be fairly numerous. By the fourth day a number of basophil macrocytes had appeared, and nucleated red cells were also present. The polychromatophil macrocytes, which were in size quite equal to those found after the injection of the haemolytic serum, gradually increased in number. On the seventh day, when the blood count was lowest, they were very numerous, while erythroblasts

of both types were fairly abundant. Scanty punctate basophilia was observed, but was much more marked in some of the other experiments. Variation in the size of the red cells (anisocytosis) became a striking feature of the films as the experiment advanced, and continued until the animal was killed on the ninth day (see Fig. 2).

In the other less susceptible animals the changes in the blood were similar in character, although not so marked in degree. In Experiment IV., for example, the red cell count fell from 5,250,000 to 3,720,000, and the haemoglobin was reduced from 55 to 40 per cent. On the fourth day of this experiment definite phagocytosis of red cells by polymorphonuclear leucocytes was seen in the blood films, but this was never observed again in this or in any other experiment. Punctate basophilia appeared about the fifth day, and soon became very frequent, so that about the eighteenth day when the animal was killed a very large number of red cells showed the condition very distinctly. In the remaining experiments detailed a fall of from 2 to 2½ million corpuscles per c.mm. was produced, with a corresponding drop in the haemoglobin percentage.

The leucocytes were as a rule somewhat increased in number during the period of anaemia, but not markedly so. In differential counts no alteration in the relative proportion of the different types of white cells was made out.

The changes found post-mortem in these animals will now be described, certain features common to all being referred to first, and then the individual differences dealt with.

A haemosiderin reaction was given by the organs in every experiment, but it varied in degree. A point of importance is that the

liver always gave fully as well marked a reaction as the kidneys and spleen. This contrasts with the result found in some of the other experimental anaemias, and is another resemblance to what is found in human pathology in pernicious anaemia. Microscopically, the liver and kidneys were normal in every case, except for the deposits of haemosiderin. No lesion was ever found in the heart muscle, although it was always examined.

The bone marrow presented changes of very great interest. In five of the six experiments a marked hyperplasia was met with, the fat spaces being greatly encroached upon. The proliferation involved both the erythroblastic and leucoblastic portions of the marrow, and it is notable that in the majority of the experiments the reaction affected the two types of cells in a practically equal degree. Mitotic figures in myelocytes were frequent, and nucleated red cells of all sizes were collected in patches throughout the marrow. In Experiment V. the erythroblastic reaction surpassed the leucoblastic considerably in intensity, and this was the case in which the most marked morphological changes were seen in the blood films. The fat spaces were almost obliterated, and erythroblasts extremely numerous (see Fig.3.). In Experiment VIII. the details of which are not recorded in the tables, a marrow picture of a different nature from that seen in the other animals was discovered. The marrow was in a condition of gelatinous degeneration, and the number of cells in it was below the normal. This experiment was the longest of the series, its duration being twenty-three days.

The spleen was enlarged in all the six experiments. In Experiment IV. the enlargement was enormous, the organ weighing nearly

4 grms., and being very firm in consistence. In Experiment lll. the spleen was three times as large as that of a control animal of similar weight. In the other experiments the enlargement was comparatively slight. Microscopically the changes found were fairly uniform, except in the very large spleen of Experiment lV., which will be described by itself. In the pulp, granules of haemosiderin were always abundant, but were more extra-cellular than within cells. The only other noteworthy change was a widening of the trabeculae of the pulp, and the presence of an undue number of nucleated red corpuscles.

In the spleen of Experiment lV., a very curious myeloid transformation was met with. The Malpigian bodies were much increased in size, and were found surrounded by a distinct ring of myelocytes, many of which showed mitotic figures (see Figs. 4 and 5). The centres of the Malpigian bodies were occupied in many instances by a mass of similar myeloid cells, with frequent mitotic figures in the nuclei. In serial sections it was found that these central islets were in reality continuous with the zone of myeloid tissue round the periphery. It is not easy to explain this curious myeloid change, and it cannot be said with certainty to have commenced only as a result of the injections. No similar appearance has been met with before in experiments on rabbits, so far as can be discovered.

In order to make certain that the anaemia and changes in the organs were brought about by the haemolysin alone, four animals were injected with portions of filtrate in which the haemolytic power had been destroyed by incubation at 37°C. for 12 hours. It is

sufficient to state here that in none of these animals did any anaemia result, nor could any pathological changes be found in the organs post-mortem.

CONCLUSIONS.

1. Rabbits vary in their susceptibility to the haemolysin obtained from streptococci.

2. Susceptible animals die after one or two injections, evidently from some toxic effect. Haemoglobinaemia and haemoglobinuria occur in these animals, so that the toxic and haemolytic properties of the filtrate seem closely allied.

3. In less susceptible animals a definite anaemia follows the injection. Polychromatophil megalocytes appear, of large size, but not in great numbers. The blood picture is somewhat megalocytic in type, but the Colour Index is never greater than 1. Other morphological changes found in the blood are marked anisocytosis, frequent punctate basophilia, and erythroblasts of both normoblastic and megaloblastic types.

4. The bone marrow in the longer experiments shows marked hyperplasia. The reaction affects both leucoblastic and erythroblastic portions of the marrow equally.

5. The organs show a definite haemosiderin reaction, especially well-marked in the liver as compared with other experimental anaemias.

6. In the spleen of one animal a curious myeloid transformation was met with, but the relations of this to the injections are not quite clear.

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DESCRIPTION OF FIGURES.

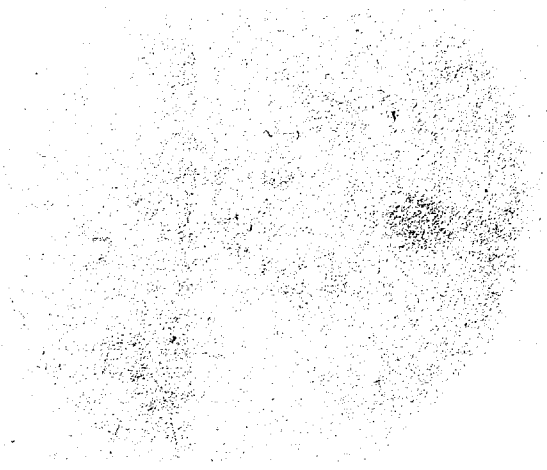
Fig. 1. Section of liver of Experiment 1.,showing focal necrosis. A cirrhosis is also present,but of long standing,and not related to the experiment. (x 50.)

Fig. 2. Blood film in Experiment V.,on the ninth day,showing a megaloblast,variation in size of the red cells,and slight poikilocytosis. (x 1000.)

Fig. 3. Section of bone marrow in Experiment V.,showing a marked hyperplasia. In this animal the erythroblastic surpassed the leucoblastic reaction in intensity. (x 300.)

Fig. 4. Section of spleen in Experiment VIII.,where the organ weighed nearly 4 grms. A Malpighian body is shown surrounded by a zone of myelocytes. In the centre of the lymphoid tissue another zone of myeloid tissue is seen. (x 30)

Fig. 5. The zone of myelocytes seen in Fig. 4. under higher magnification. Several mitotic figures are seen. (x 300)



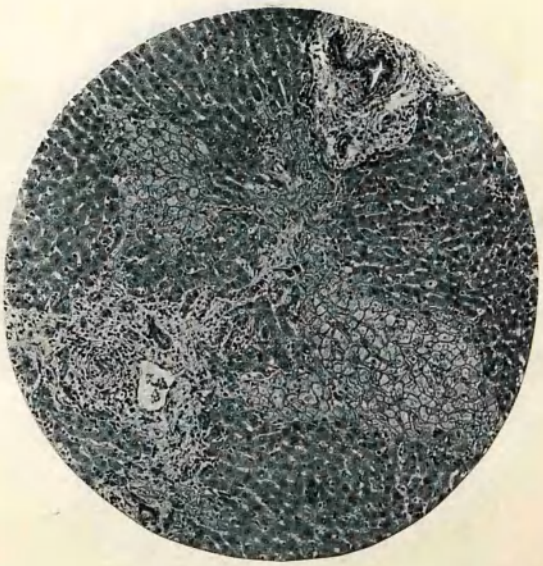


Fig. 1.

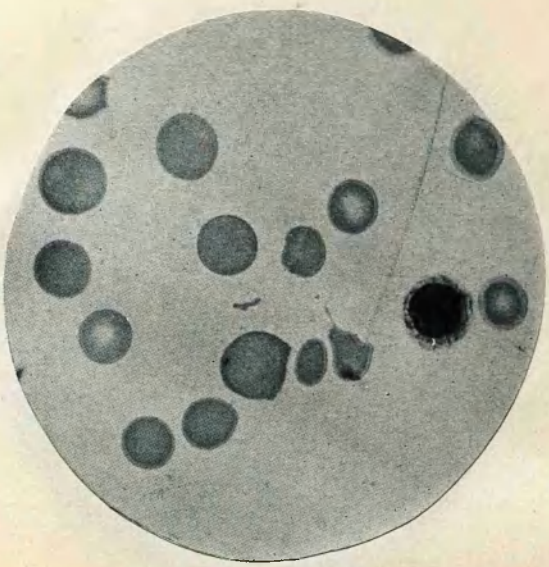


Fig. 2.

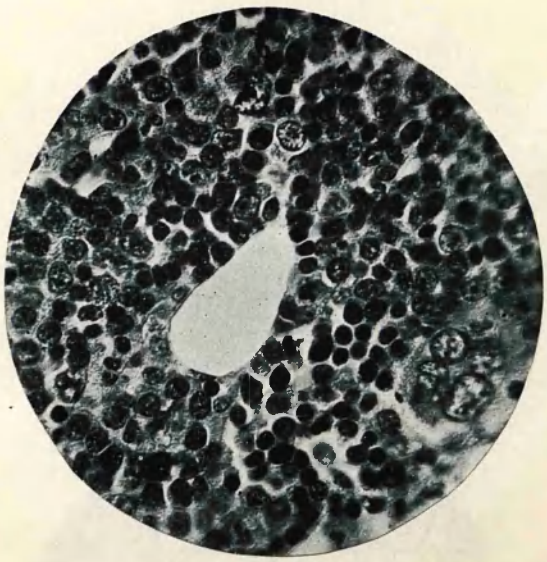


Fig. 3.

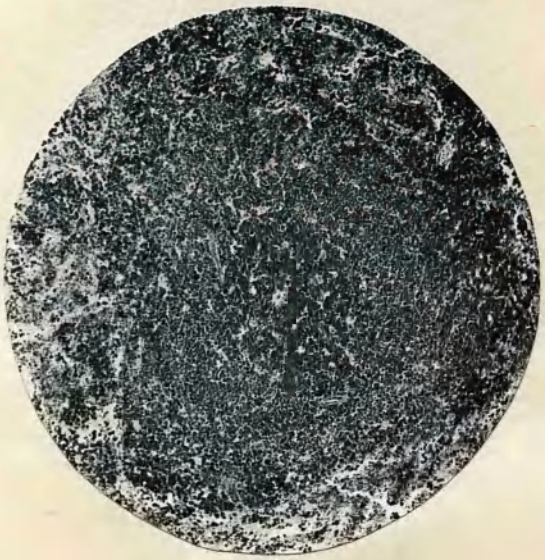


Fig. 4.

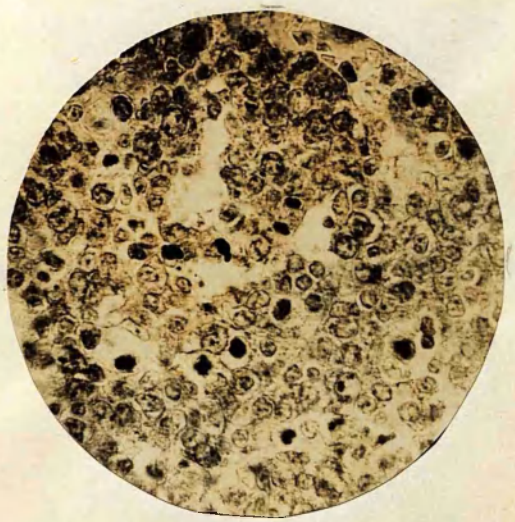


Fig. 5.

C.

THE ANAEMIA PRODUCED BY THE INJECTION OF SAPONIN.

The powerful haemolytic effect exerted by Saponin on red blood corpuscles in test-tube experiments has been long recognised,but the results of injecting the drug directly into the blood stream of the living animal have comparatively seldom been investigated. Among the various observations on the action of the drug in animal experiments,the chief detailed descriptions of the changes which ensue in the organs are to be found in the work of Bunting (1906),in America,and of Isaac and Möckel (1910),in Germany. Their experiments were all carried out on rabbits.

Bunting found that with the specimen of saponin used by him,the lethal dose was about 1.5 to 2 mg. per kilo. of body weight. He used somewhat smaller doses than this to bring about anaemia. Isaac and Möckel used sapotoxin (Merck),derived from Quillaia bark; but they point out,what is quite evident from the work of Kobert, that all the numerous varieties of sapotoxin or saponin are quite identical in action. In their experiments the doses found by them to be non-lethal,and to produce anaemia,varied from 0,3 to 1.0 mg. per kilo. of body weight. They pointed out that large doses of the drug seemed to kill the animals by a direct effect on the nervous system,before any haemolysis had time to occur.

Bunting soon found that the action of the drug in the animal body differs markedly from that "in vitro",the haemolytic effect on the red corpuscles being obviously very much less in the living body than in the test-tube. It is very interesting in connection with

this observation to turn to the work of Ransom and of Windaus, who both showed that the haemolytic effects of saponin in test-tube experiments could be completely prevented by the addition of sufficient cholesterin to the mixture. It seems probable that the modification of the haemolytic effect in the animal body is due to the presence of cholesterin in the blood serum, suprarenals, and tissues generally. Certainly, as I have proved by experiment, the effect of adding fresh blood serum to washed red corpuscles before adding saponin to the test-tube, inhibits the action of the drug to a considerable extent. The results which Bunting obtained by injecting non-fatal doses may be briefly given here, since a criticism of some of them will appear below. In the peripheral blood, he found no evidence of destruction of leucocytes, but haemolysis of red cells was indicated by reduction of the red cell count, and by the presence of many "shadows" of erythrocytes. A very characteristic and constant phenomenon following an injection was the appearance of many normoblasts in the peripheral blood. This "nucleated red" reaction took place within an hour or two after administration of the drug, and was repeated after each subsequent dose. By repeated non-lethal doses, Bunting induced what he describes as "a picture exactly similar to that of a primary anaemia in man." He notes among other changes the occurrence of anisocytosis, some poikilocytosis, polychromatophilia, and a constant presence of nucleated red cells. With regard to the leucocytes, he obtained in differential counts a relatively increased percentage of lymphocytes, a condition which, as he points out, is as a rule also a feature of pernicious anaemia in man.

In the tissues of the animals, he was much struck by finding

lesions confined almost entirely to the haemopoietic system, and especially to the bone marrow and spleen. In animals dying within forty-eight hours, he found the marrow much depleted of mature cells, considerable degeneration in such cells as remained, and a curious diffuse haemmorhage from the capillaries of the marrow, which he thought must result from a direct toxic action of the drug on the delicate walls of the capillaries. In animals surviving repeated doses of saponin, the bone marrow was found to have undergone a regular sclerosis, the marrow tissue proper being replaced by a fibrous tissue. Bunting was at a loss at first to explain how an animal could retain a red cell count of 3,000,000 per c.mm. or more, when its bone marrow was chiefly changed into fibrous tissue. A solution was afforded by the examination of the spleen, in the peripheral sinuses of which he found the most active blood regeneration going on. He found myelocytes and erythroblasts in great numbers, and even the characteristic giant cells (megakaryocytes), normally only found in the bone marrow. He makes no suggestion as to how this extraordinary myeloid transformation may have been brought about. In some animals dying acutely, he found in the liver a toxic necrosis of the central portions of some of the lobules, and in experiments of longer standing a fibrous change had commenced in the necrotic areas. It is worth mentioning here, as the point will not be returned to, that Bunting also tried the effect of introducing saponin subcutaneously. In such experiments he found that the toxin was absorbed too slowly to damage the bone marrow; no normoblasts appeared in the circulation, and as a result of a somewhat inconsiderable haemolysis of red corpuscles a bone marrow picture was induced resembling that found after simple

bleeding.

Isaac and Möckel confirm in their work many of the important findings of the previous author, as regards the changes in the blood and bone marrow. They give, however, certain additional facts of much interest. They point out, for example, that in the anaemia following the injection of saponin, the Colour Index is as a rule less than 1. In a single experiment of theirs, nevertheless, the Index rose to 1.3. They amplify Bunting's statement with regard to the keeping up of the blood count after repeated doses of the drug. They found in several instances that the blood count could return to absolutely normal when the injections were stopped, and this apparently in spite of an extreme fibrosis of the marrow. As regards the leucocytes, in two of their experiments a definite leucopenia resulted, the counts varying from 3,400 to 1,200 per c.mm. They refused to attach any special significance, however, to either qualitative or quantitive changes in the leucocyte count of the rabbit, affirming that even in normal animals the daily variations are extreme. The same view has been previously put forward by Gruber, but Bunting categorically denies it, stating that in successive daily examinations in rabbits he has found only slight differences either in the total or differential counts of the leucocytes. As regards the changes in the bone marrow Isaac and Möckel simply confirm the earlier work. They also observed the myeloid transformation in the spleen in most of their cases, and suggest that the appearances closely resemble those found in leukaemia in man. In the liver they noted necrotic changes, but in addition they observed occasionally the presence of small groups of myelocytes and erythroblasts in some of the dilated vascular capillaries.

RECORDS OF OWN EXPERIMENTS.

The specimen of saponin used was a preparation made by Merck. It is a white powder which readily dissolves in water or in physiological salt solution. In my experiments the drug was in every case injected into the ear vein of rabbits, dissolved in sterile 0.85% salt solution.

The individual experiments will now be given in detail.

EXPERIMENT 1.

Rabbit 28. weight 1690 grms.

The blood count in this animal before injection was as follows. Red Corpuscles 7,510,000, Leucocytes 21,500, Haemoglobin 68%. At 3 p.m. the animal was given 180 mg. of saponin intravenously, a very considerable dose. Next morning, the animal was found dead, but still warm. Urine passed ante-mortem gave a faint but distinct spectrum of oxy-haemoglobin. In urine taken from the bladder post-mortem abundant haemoglobin was found.

Post-mortem. The liver and spleen gave macroscopically a very definite haemosiderin reaction, a point of importance, since Bunting never found this in any of his experiments, and remarks on its absence. The kidneys gave practically no reaction for iron. On examining the bone marrow microscopically, the following points emerged. In films stained with Ehrlich's Triacid stain the only notable change observed was the presence of definite karyorrhexis and pyknosis of cell nuclei. In sections of the marrow the pyknotic changes were even better seen, while scattered over the whole section masses of red corpuscles were seen, which evidently

corresponded to the "capillary haemorrhages" described by Bunting. These latter do not appear in many instances to be actual haemorrhages at all, the capillaries of the marrow being simply dilated and crowded with erythrocytes. The marrow did not in this experiment show great depletion of mature cells, but seemed to be rather unusually cellular. There was a considerable amount of golden-yellow pigment present, contained in large cells of endothelial type, with pale staining nuclei.

In neither spleen nor liver was any marked abnormality made out.

The kidneys showed a condition of severe catarrhal nephritis, there being great destruction and desquamation of cells lining the tubules, and much haemoglobin and unaltered red corpuscles lying free in the tubules themselves.

EXPERIMENT. 11.

Rabbit 29. weight 2000 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1].	6,720,000	10,000	72	1.0
1[2].	7,000,000	21,000	72	1.0
2[3].	6,510,000	11,000	64	0.9
3[4].	5,310,000	10,600	54	0.9

1. 1.5 mg. of saponin injected intravenously.
2. 3 : : :
3. 6 : : :
4. 10 : : :

On the day after the first injection, definite changes were found in the circulating blood. Numerous normoblasts were present, some with very distinct punctate basophilia of their protoplasm, and a number of "shadows" were also seen. On succeeding days, normoblasts continued to be present, but not in such numbers as on the first day noted. No macrocytes ever appeared, but some polychromatophil red cells of quite normal size were observed. On the morning of the fourth day of the experiment, the animal was found dead. Post-mortem. The liver, kidneys, and spleen, all gave a definite haemosiderin reaction.

The bone marrow appeared very bright red in colour, and a film of it stained with diluted Ehrlich's Triacid stain, showed great numbers of non-nucleated red cells, evidently in a state of partial disintegration. Many of these cells stained badly, but a number appeared quite normal. In both nucleated red cells and in myelocytes, pyknotic change was very marked in the nuclei. In a section of marrow the whole capillary system was found to be crowded with non-nucleated red cells, so that the erythroblasts and myelocytes seemed to be greatly diminished in quantity. Giant cells (megakaryocytes) were extremely scanty, only one, on an average, being seen in a complete transverse section of the femur marrow.

The spleen in sections stained with Ehrlich's stain showed a remarkable state of affairs, similar to that drawn attention to by both Bunting, and Isaac and Möckel, in their experiments. The peripheral sinuses were found filled with great numbers of cells belonging normally to the bone marrow. Megakaryocytes were present in abundance, appearing in greater numbers than is seen as a rule in

sections of normal marrow. Myelocytes were found in as great numbers as normally in the marrow, while nucleated red cells were also very numerous. Mitotic figures, indicating proliferation, could be found without difficulty in both types of cells. The question of how this condition in the spleen is brought about will be discussed at the end of the experiment.

The liver exhibited considerable distention of all the vascular capillaries, and these were filled up to a great extent with a mixture of myelocytes and erythroblasts. Giant cells of the marrow were also seen, beside ordinary erythrocytes in great number. These collections of cells were most noticable near the portal areas of the lobules, the capillaries being most distended there.

The kidneys showed also a considerable number of myelocytes, most of them contained within the glomeruli, and staining well with Ehrlich's stain. In the vascular capillaries between the convoluted tubules, scanty myelocytes and nucleated red cells were also found.

EXPERIMENT. 111.

Rabbit 30. weight 1280 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1]	7,620,000	4,400	78	1.0
1[2]	5,350,000	6,200	60	1.1

[1] 1.5 mg. of saponin injected intravenously.

[2] 1.5 : : :

On the day after the first injection scanty normoblasts were found in the blood, and a great number of "shadows". The animal

died within four hours after the second injection.

Post-mortem. The spleen was found small and rather shrivelled. The liver spleen, and kidneys all gave a distinct haemosiderin reaction. This reaction was peculiar in the liver, in that its destribution was patchy, being confined to the zone surrounding the portal tracts.

The marrow showed similar distention of the capillary system with red corpuscles to that noted in the previous experiment. Adult cells both myelocytes and erythroblasts, were extremely scanty as compared with the normal, and the fat spaces appeared in contrast much enlarged.

In the spleen all the peripheral sinuses were engorged with erythrocytes; scanty myelocytes were seen, but no giant cells.

In the liver the vascular capillaries were distended with red corpuscles, among which, however, a number of myelocytes could be recognised. The changes in this organ were not nearly so marked as in the previous experiment.

The kidneys showed catarrhal change, but otherwise nothing of interest.

EXPERIMENT IV.

Rabbit 31. weight 1860 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0[1.]	8,760,000	7,000	92	1
1	7,600,000	37,200	84	1.03
2[2.]	6,130,000	30,300	78	1.2
3	6,150,000	30,500	74	1.1
4[3.]	5,930,000	20,000	72	1.1
5	6,940,000	19,000	68	0.9
6	7,250,000	11,400	68	0.9
7[4.]	6,130,000	11,900	68	1.0
8	8,060,000	13,700	68	0.8
9[5.]	7,980,000	27,200	66	0.79
10	6,350,000	10,000	60	0.9
11	6,200,000	11,900	60	0.9
12[6.]	6,170,000	13,100	60	0.98
13	7,100,000	13,100	62	0.8
14	7,320,000	11,500	60	0.77
15	6,730,000	11,000	60	0.89

1. 1.5 mg. of saponin injected intravenously.
2. 1.5 : : :
3. 2.0 : : :
4. 2.0 : : :
5. 2.5 : : :
6. 2.0 : : :

This experiment, the longest of the series, was kept up over a

period of a fortnight, and results of very great interest were brought about.

In the blood on the day after the first injection, normoblasts were fairly numerous, and a few very definite megaloblasts were seen. A marked leucocytosis, chiefly affecting the polymorphs, was present and the occurrence of an unusual number of mast cells with large basophil granules was a feature throughout the whole experiment. The nucleated red cells present were counted in a film against the leucocytes, and estimated to number 4,300 per c.mm. on this day. On the succeeding days nucleated red cells continued to be present, although not in such numbers, and a new feature became prominent, namely, the occurrence of punctate basophilia in the red corpuscles. This became more and more prominent as the experiment progressed, until in the last few days a very large proportion of the red cells showed it very beautifully. Macrocytes were never a feature of the films, although basophil cells of normal, or slightly more than normal, size were fairly abundant. This lack of basophil megalocytes is in great contrast to what was found in the anaemia produced by the haemolytic serum and by streptolysin. The animal was killed on the fifteenth day.

Post-mortem. The liver, spleen, and kidneys all gave a haemosiderin reaction, very marked in the two organs first named.

The bone marrow in films showed widespread degenerative changes in the cells, many of which retained the stain very poorly. In section the marrow was found to be irregularly sclerosed (see Fig. 2) and there was considerable new formation of young connective tissue. Even in the areas of marrow not directly implicated by this connectiv

tissue formation, the essential cellular elements of the marrow were extremely scanty, and the majority of them seemed to be degenerating. Not a single giant cell was found after looking through several different sections.

In the spleen very abundant myelocytes and nucleated red cells were found in the peripheral sinuses. The nucleated red cells were definitely aggregated into clumps, without an indication of marked proliferation and multiplication of these cells in the spleen sinuses. Giant cells of the marrow in fair numbers were seen in the sinuses (see Fig.3). Abundant yellowish pigment, both intra- and extra-cellular in destribution, and giving the iron reaction, was found scattered throughout the organ.

The liver showed similar changes to those noted previously. In the dilated capillaries myelocytes, nucleated red cells, and megakaryocytes were all seen, but not in great numbers.

The lungs were in this experiment alone, carefully examined microscopically, and myelocytes and the nuclei of megakaryocytes were recognised without difficulty in the capillaries.

EXPERIMENT. V.

Rabbit 32. weight 1250 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.	Colour Index.
0 [1.]	6,910,000	12,100	70	1.0
1 [2.]	5,550,000	11,600	59	1.07
2	4,560,000	20,300	50.	1.1

1. 2.5 mg. of saponin injected intravenously.
2. 5.0 : : :

In this experiment an attempt was made to induce a more rapid anaemia by using larger doses of the drug,but the animal died as a result of the second injection. Following the first injection,no important changes were observed in the blood. Following the second injection,however,normoblasts were present in considerable numbers. On this day too the animal passed a quantity of dark red urine,in which casts of haemoglobin from the kidney tubules could be recognised. No unaltered red cells were found in the urine.

Post-mortem. The liver and kidneys gave only a very slight and indefinite haemosiderin reaction; the spleen a more marked reaction.

The bone marrow showed exactly similar changes to those noted in Experiment 1.; namely,much pyknotic change in the nuclei of cells, and diffuse masses of erythrocytes filling out the capillaries. In the spleen no myeloid transformation was apparent.

The liver showed some definite focal areas of necrosis.

The kidneys exhibited only catarrhal changes.

RESULTS OF EXPERIMENTS.

The results of these experiments bring out very strongly the mode in which saponin acts as an agent which can produce anaemia. Its action is selective and peculiar,being confined in a destructive sense chiefly to the bone marrow as an organ. It is,of course,a fact that saponin must destroy red blood corpuscles in the peripheral circulation,and this is proved by the occurrence of "shadows" in the blood films following its injection. This action of laking cells in the peripheral blood of an animal must,however,be very much inferior to the powers of haemolysis possessed by the drug in test-

tube experiments.

Changes in the bone marrow. The destructive effect on this organ is, as already indicated, one of the chief effects of injection of the drug. In animals dying acutely there is a marked destructive action on all the cells, which show very varied conditions of degeneration, and frequent pyknotic change in the cell nuclei. In the very acute cases the marrow cells seem simply to necrose and disappear, so that the whole organ is almost emptied of its normal cell elements. The other change observed in the acute experiments is what Bunting describes as "haemorrhage from the vascular capillaries", but careful examination shows that this is in many cases not a true haemorrhage at all, the capillaries being simply crowded and distended with erythrocytes, but the capillary wall remaining intact.

In longer continued experiments, these early degenerative changes are succeeded by a diffuse fibrous change, which may invade almost the whole marrow. In the new connective tissue hardly a mature marrow cell can be recognised, while the fat spaces are also much encroached upon and may almost disappear (see Fig.2). This disappearance of the fat as a result of fibrosis corresponds closely to what Flemming described as occurring in the subcutaneous fat after various inflammatory processes.

These marrow changes have a very decided interest when compared with the condition of the bone marrow found in cases of Aplastic anaemia in man.

Changes in the blood. The two early changes noted in the blood, within a very short period after each injection, are, the occurrence of fairly numerous "shadows", and the presence in the peripheral

circulation of many nucleated red corpuscles, almost all normoblastic in type. The "shadows" indicate the direct lytic effect on the blood corpuscles, while the presence of many normoblasts must be taken as the sign of commencing damage to the bone marrow. It is probable that all so-called "blood crises", in whatever type of anaemia they are met with, depend directly on some acute process of destruction or damage going on at that time in the marrow. In the longer experiments other morphological changes are met with. Polychromatophilia is a constant feature, but the cells exhibiting it are of almost normal size and are not to be compared with the large basophil megalocytes seen in the experiments with the haemolytic immune-serum, or to a lesser extent following injections of streptolysin. In short, the anaemia induced by saponin is never of a truly megalocytic type, and bears no real resemblance to the blood picture found in pernicious anaemia in man. This is quite in opposition to the view held by Bunting. Poikilocytosis was never seen in the experiments and from the tables given it will be readily seen that the Colour Index never sank far below, nor rose much above, the normal value. The highest Colour Index found was 1.2 on the second day of Experiment IV., and the lowest recorded was 0.77 on the fourteenth day of the same experiment.

As regards the leucocytes, a leucopenia such as is described by Isaac and Möckel, was never found. On the contrary, in most of the experiments shown in the tables a definite leucocytosis was almost the rule. Differential counts yielded such varying results that their value must be discounted.

Changes in the spleen and liver. The changes in these organs are of great interest, and raise certain very important questions. The myeloid changes in the spleen have been so well described by previous workers, that nothing new falls to be added. In my experiments, normoblasts, megaloblasts, myelocytes, and megakaryocytes were frequently seen in great numbers in the peripheral sinuses, and evidences of their active proliferation in this situation were not wanting. In the liver a similar aggregation of elements of the bone marrow to that described by Isaac and Möckel was found in the blood capillaries in several of my experiments.

In connection with these appearances in the liver and spleen, the question at once arises whether this is a true extra-medullary formation of bone marrow, or whether the cells have simply been dislodged from their normal position in the marrow, and have anchored themselves in these new situations. The question of "myeloid metaplasia", the term by which the presence of bone marrow elements in extra-medullary situations is more commonly known, has attracted widespread attention. Very varied views as to how it occurred are held, but from my observations I am satisfied that in regard to saponin at least, the bone marrow cells have simply been transferred to the liver and spleen as a result of the direct toxic and destructive action of the drug on the marrow. A convincing proof of this view is afforded by the result of examination of the lungs in Experiment IV., in the capillaries of which the nuclei of many giant cells of the bone marrow were discovered, thus making it certain that these cells had been dislodged from their normal position and had circulated in the blood stream. In experiments with Pyrogallol and Phenyl-hydrazin,

v. Dareus found similar myeloid changes in the spleen to those obtained with saponin, but indicates his belief that this is not a formation of bone marrow "de novo", but probably dependant on a simple transference of cells.

It must not be concluded from these remarks, however, that no true extra-medullary bone marrow formation can occur under a different set of conditions. Fuller reference to this point will be found in the part of this thesis dealing with the anaemia induced by simple bleeding. It will be seen that after haemorrhage a new blood formation can be induced in the liver, thymus, etc., simply as a result of the stimulus of continued bleedings. Further, cases have been described especially in young children of definite extra-medullary marrow formation in the hilum of the kidney in severe anaemias of v. Jaksch's type. In several cases too, of pernicious anaemia in man observed by myself, there was found considerable collections of myelocytes and erythroblasts undergoing active proliferation within the capillaries of the liver. Herz has drawn attention to certain cases in the human subject which are of great interest when compared with the experiments with saponin. He observed in certain severe septic conditions going on to a fatal issue, that post-mortem there was an aplastic and depleted bone marrow, and that in the liver and spleen definite bone marrow formation had occurred, which he thought was compensatory in nature.

It is interesting to note, as indicative of the efficacy of the myeloid transformation in the liver and spleen as regards blood regeneration, that in Experiment IV. the red cell count was maintained at a figure of between 6 and 7 millions in spite of the fact that

the bone marrow itself was found post-mortem to be almost completely fibrosed.

Other changes in the organs. In all of my experiments a definite haemosiderin reaction was given by the liver, kidneys, and spleen, when tested in the usual way. It was more marked in the acute experiments than in those of longer duration. Attention must be directed to this point, since both Bunting, and Isaac and Mőckel, state that no such reaction is given. In the kidneys of the animals dying acutely very marked catarrhal changes were found, showing that the drug, or some product of it, exercise a toxic effect on these cells during excretion.

CONCLUSIONS.

1. The haemolytic action of saponin is very much less in the animal body than in test-tube experiments.

2. Following injection of the drug, evidence of a direct haemolysis in the peripheral blood is given by the presence of many "shadows".

3. The chief action of saponin is a destructive one on the bone marrow as an organ. At an early stage, marked degenerative changes occur in the marrow, resulting in the tissue being greatly depleted of mature cells. These changes are succeeded by a more or less complete fibrosis of the marrow.

4. The morphological changes in the blood can in great part be explained on the basis of the findings in the marrow. The presence of normoblasts in the circulating blood soon after each injection is to be regarded as the outward sign of damage to the marrow. The blood

picture never becomes megalocytic, the Colour Index is as a rule not raised above the normal, and the films do not ever really resemble those from a case of human pernicious anaemia.

5. Myeloid metaplasia is found almost as a rule in the spleen, and frequently in the liver. So far as saponin is concerned, this appearance depends on dislodgement of cells from the marrow, which come to rest again in these new situations, to proliferate with vigour there.

6. These myeloid changes in the liver and spleen are sufficient to compensate for an almost complete fibrosis of the marrow, so that an animal with the latter condition may retain a normal blood count.

7. Contrary to the negative findings of Bunting, the organs give a distinct haemosiderin reaction.

8. In the kidneys, the drug induces fairly marked catarrhal change of the tubules.

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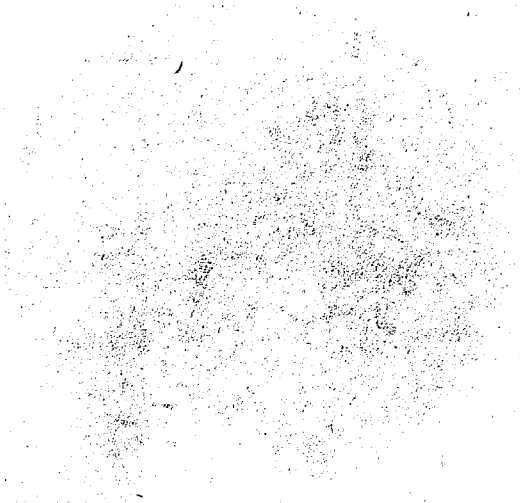
DESCRIPTION OF FIGURES.

Fig. 1. Section of bone marrow from Experiment 11. The animal was found dead on the fourth day. Marked degenerative changes, and depletion of adult marrow cells, are seen. (x 300)

Fig. 2. Section of bone marrow from Experiment 1V. Duration of experiment fifteen days. The marrow is in a state of sclerosis, and very few cells are present. (x 300)

Fig. 3. Section of spleen from Experiment 1V. A megakaryocyte, and fairly numerous myelocytes are seen. (x 300)

Fig. 4. Shows the same changes as Figure 3.



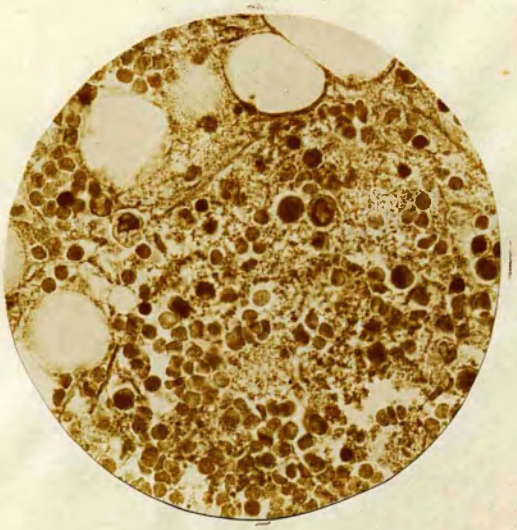


Fig. 1.

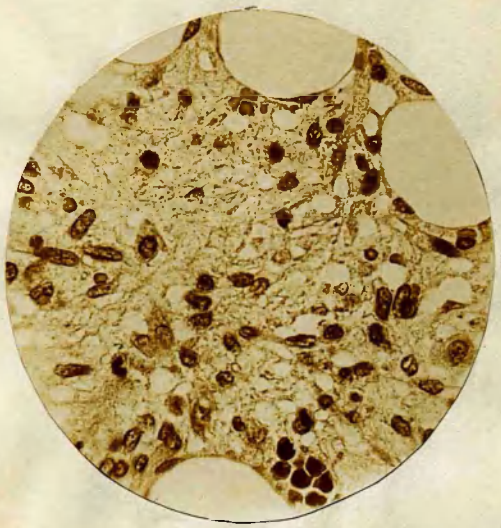


Fig 2.

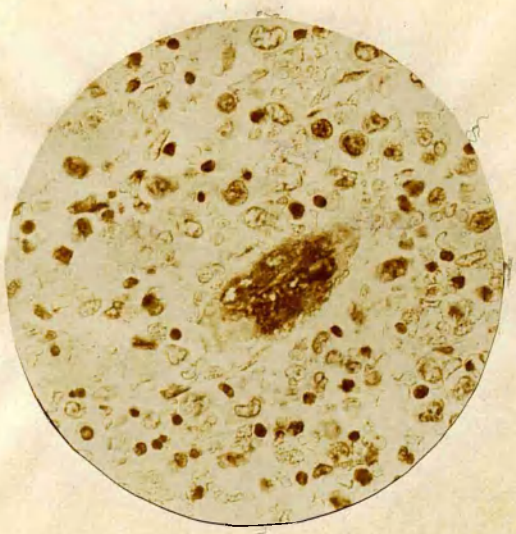


Fig. 3.

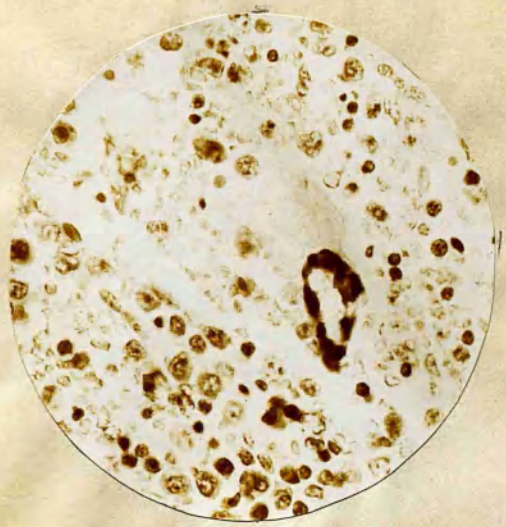


Fig. 4.

D.

THE ANAEMIA PRODUCED BY THE INJECTION OF TOLUYLENDIAMIN.

Toluylendiamin,which in the test-tube has absolutely no haemolytic action on red blood corpuscles,was first discovered to be a blood poison in the animal body by Schmiedeberg nearly thirty years ago. Its action in the living body was first investigated thoroughly by Stadelmann,who found that in dogs the drug was an active blood destroyer,and that severe jaundice followed in the animals to which it was administered. His observations on the jaundice and blood destruction induced were collected by him in his book "Der Icterus und seine verschiedenen Formen",published in 1891. Further reference to his work will be found in the section of this thesis devoted to "Haemolytic Icterus".

Here at present we are concerned more with the action of the drug on the blood corpuscles within the body,and the extent and variety of the anaemia which ensues. For a long time there was no clue to the reason why toluylendiamin,with no action whatever on red corpuscles in the test-tube,should be so definitely haemolytic in the body. It is only recently,since the work of Joannovics and Pick (1909), that an idea has been gained of what may occur. These authors were tempted to investigate the subject by the extremely interesting publication of Faust and Tallquist (1907) in relation to the question of Borthriocephalus anaemia. Faust and Tallquist were able to show that there could be extracted from the proglottis of the worm certain fatty acids (ölsaure),which existed in the body of the parasite combined with cholesterin in the form of esters. They found that these

fatty acids could be split off from the combination,were then absorbed into the blood of the host,and there exercised a haemolytic action. Joannovics and Pick sought to find whether in poisoning by toluylendiamin,the drug might act by producing indirectly some haemolytic substance or substances in the organs. Their experiments were carried out on dogs,and they arrived at the following interesting results. A definite distinction had to be made in their experiments between the acute cases of poisoning,fatal in a few hours,and the chronic cases which were allowed to go on for weeks. In the former cases they found in the extract of the animals' livers a powerfully acting haemolysin,whose development was not influenced in any way by removal of the spleen,and which was almost certainly formed as a direct result of the action of the drug injected. This haemolysin in more chronic cases was found by them in much more sparing amount, being evidently,so they concluded,already fixed to the blood corpuscles. In the chronic cases,however,another haemolytic body was discovered,which made its appearance coincident with the fatty change which ensued in the liver. This latter haemolysin was evidently closely allied to the fatty acids in composition,and in contrast to the other haemolysin the removal of the spleen had a definite effect on its development in the liver. Such,then,is the extent of our knowledge of how toluylendiamin acts in the animal body.

As regards the changes produced apart from the jaundice,the chief published results of the effects on the blood corpuscles,so far as is known to me,are those of Wm. Hunter in his book on Pernicious Anaemia (1901). In his experiments he used toluylendiamin to determine the function of the spleen in regard to blood destruction. He

pointed out the differences of the action of the drug in animals of different species, although this had also been referred to by Stadelmann. In the cat, even minute doses (0.15 grm.) are sufficient to cause haemoglobinuria and death in a very short time. In the dog the action is less toxic, and still less so in the rabbit, where the blood destruction is very moderate in amount. Hunter used rabbits in his experiments because he wished to produce a blood destruction only a little more marked than what occurs in health, and so to simulate more or less completely the slow haemolysis which must occur in pernicious anaemia. The results obtained by Hunter, in his experiments on rabbits, are briefly these.

When small doses of the drug were used, he found simply an exaggeration of the changes which occur in the blood in health, with in addition, however, increased production of bile pigments, and increased deposit of iron in the liver, spleen, and bone marrow. Larger doses increased the above phenomena, but there resulted as well, 1. a marked oligaemia, and 2. morphological changes never seen in health, such as the occurrence of stromata and "schatten" in the blood, and of remains of haemoglobin in the urine. He never produced haemoglobinuria or biliuria. vide Hunter, p. 384.

From a series of experiments in which small, medium, and large doses of the drug were employed, Hunter drew very important conclusions with regard to the site and extent of the blood destruction. He concluded that with small doses the haemolysis is confined entirely to the spleen. With medium doses the haemolytic effects, while still most marked in the spleen and portal blood, are still absent from the general circulation. With large doses the whole circulation is

involved in the haemolysis, but here again the spleen and portal blood bear the brunt of the destructive process. Hunter gives no details of the morphological changes found in the blood in the more chronic cases.

RECORDS OF EXPERIMENTS.

The sample of the drug employed was a product of Kahlbaum, with the formula

$C_6H_3 : CH_3 : NH_2 : NH_2$ 1 : 2 : 4 (ie. the Meta-Toluylendiamin.)

It was in every case injected intravenously into the ear vein of rabbits. Solution in normal saline is only accomplished by boiling, and whenever the solution cools, crystals of the drug readily separate out. This constituted at first a difficulty in administering the drug, until it was found that the addition of a few drops of acetic acid to the saline keeps the drug in perfect solution, even when the saline is allowed to become quite cold before injection. In the later experiments this procedure was always adopted.

The experiments themselves will now be given in detail.

EXPERIMENT 1.

Rabbit, weight 1360 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0[1]	6,820,000	9,000	80
1[2]	6,810,000	8,400	72
2	5,150,000	11,900	70

1. 0.3 grm. of toluylendiamin injected intravenously, in 5 c.c. NaCl.
2. 1.0 : : : 20 :

In this animal no changes in the blood or urine were observed on the day following the injection of the first small dose. After the administration of the second dose, the animal was very ill for some time, but recovered. The same evening, six hours after the injection, a specimen of urine was found to contain abundant biliverdin. Films of blood made at this time showed no evidence of "shadows", nor were any abnormal cells present. Next day the urine again contained abundant bile, which tended to separate out as a layer on the surface. The blood count on this day (see Table) was distinctly lowered, but blood films showed no apparent abnormality whatsoever. On the following day the animal was killed, and the organs examined.

The spleen was found enlarged, dark purple in colour, and gave a very marked haemosiderin reaction. The liver gave a slight reaction for iron, the kidneys none at all. The bone marrow appeared very fatty.

The bone marrow microscopically simply showed large fat spaces, with no degenerative or other changes visible in the cells. Golden-yellow pigment was seen in comparatively large amount, contained within large palely staining cells. This pigment is found normally in the marrow, but here it was obviously much in excess.

The spleen in section was found to have the peripheral sinuses absolutely crowded with erythrocytes, but they all stained normally, and showed no obvious signs of commencing disintegration. No phagocytosis of red cells was appareht, but large splenic phagocytic cells were seen filled with brownish pigment which gave the iron reaction. Masses of pigment were also present extra-cellularly.

The liver, kidneys, and heart showed no changes of interest.

EXPERIMENT 11.

Rabbit, weight 1470 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0[1]	7,780,000	5,600	74
1[2]	6,290,000	6,500	73

[1] 1.0 grm. of toluylendiamin injected intravenously in 20 c.c. NaCl.

[2] 1.0 : : : 20 :

Following the first injection, marked illness ensued. Next morning the urine contained abundant biliverdin, and this was also present soon after the second injection. The animal was found dead on the following morning, before a blood count could be made.

The liver and spleen gave a definite haemosiderin reaction, most marked in the latter. The kidneys gave no reaction.

Microscopically, the changes in the organs were quite similar to those found in the previous experiment, the most striking fact being the great aggregation of normally-staining erythrocytes in the peripheral sinuses of the spleen.

EXPERIMENT 111.

Rabbit, weight 2030 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0	5,170,000	5,300	48
1	4,170,000	5,000	46
2	...	...	...
3	5,030,000	6,000	48
4	...	...	...

Experiment 111. (continued)

8	5,340,000	3,500	52
9	5,650,000	5,600	50
14	...	...	...

0.75 grm. of toluylendiamin injected intravenously.

1.0	:	:	:
1.0	:	:	:
1.0	:	:	:
0.5	:	:	:

In this animal the haemoglobin percentage was very low from the outset. The experiment was carried on for a fortnight, the animal receiving in all 4.25 grms. of the drug. The blood was carefully observed in this case, films being stained daily. On the day after the first injection, a few basophil red cells of normal size were seen but no "shadows". By the third day of the experiment the basophil cells had increased in number, and some of them were slightly but distinctly larger than the usual erythrocytes. Two days later the red cells began to exhibit distinct poikilocytosis, and this persisted until the animal was killed. Nucleated red corpuscles were never present, nor were "shadows" seen at any time during the experiment. The urine contained bile practically throughout the experiment, in greater amount on the days following injections of the drug. The animal was killed on the fourteenth day.

The spleen was found distinctly enlarged, almost black in colour, and gave a marked haemosiderin reaction. Both liver and kidneys gave a less intense but unequivocal iron reaction. The bone

marrow was red, firm, and not at all fatty.

The spleen in section presented an interesting picture. Very marked phagocytosis of erythrocytes by cells of the splenic pulp was found, and a relatively enormous amount of golden-yellow pigment was to be seen. This pigment, which gave a beautiful iron reaction, was here and there aggregated into large masses, but was also found in finely granular form within cells.

The bone marrow showed a distinct, but not very marked, erythroblastic reaction, and the fat spaces were somewhat encroached upon. Pigment was found in many large pale cells of endothelial type, in about the same amount noted in Experiment 1.

RESULTS OF EXPERIMENTS.

In considering the results of the injection of toluylendiamin into rabbits, the first point that will strike the reader is that the anaemia which results is a trifling one, and the morphological changes in the blood slight as compared with what have been described in the other series of experiments.

Previous authors, especially Hunter, have laid great stress upon the occurrence of "shadows" in the peripheral blood of the animal injected, but I was never able to convince myself of the presence of these even at a short interval after an injection. Thus I found no evidence in any of the experiments of a direct lytic effect of the drug in the peripheral circulation. This is really what might be expected, when it is considered how absolutely inactive the drug is, when added to red corpuscles in test-tube experiments.

The most marked changes in my experiments were found in the spleen,

In the more acute cases the peripheral sinuses of this organ were found simply packed with erythrocytes,which,however,stained well and showed no obvious signs of degeneration. In the more chronic case (Experiment 111.) marked phagocytosis of erythrocytes was met with in the spleen,and in passing it may be mentioned that this was a feature also in the experiments on dogs to bring about a haemolytic jaundice. It seemed as if,in the more acute cases,the red corpuscles had been damaged in some way,and were then caught out of the circulation and taken to the spleen. There they must undergo degeneration,but it is not quite clear how the destructive process progresses. It must remain an open question whether the red cells, as Joannovics and Pick have supposed,have a small amount of the haemolysin,formed in the liver,attached to each of them. A possible solution of this question might be arrived at by removing some of the splenic pulp into physiological salt solution,and watching to see whether the washed red corpuscles underwent slow haemolysis in the isotonic solution.

In the chronic case morphological changes were indeed met with in the circulating blood,which depended evidently on a loss of red corpuscles. No nucleated reds ever appeared,and on examination of the marrow no evidence of damage there could be detected. Basophil red cells were seen in the films on the later days,and these must be regarded just as somewhat immature cells coming out into the circulation to make up for those destroyed in the spleen. More definite poikilocytosis was met with in the chronic experiment,than in any of the other experimental anaemias. In the same experiment,too, the bone marrow showed a slight hyperplasia.

The occurrence of bile in the urine, which was a feature of all the experiments, is an important point to draw attention to. In cats, as pointed out by Stadelmann and Hunter, the drug induces copious haemoglobinuria and death; in dogs a severe jaundice with biliuria is also brought about; but no author seems hotherto to have drawn attention to biliuria in rabbits following injection of toluylendiamin. It is interesting that after careful histological examination no marked changes were found in the liver suggestive of jaundice. The increased formation of bile must follow a breaking down of the erythrocytes in the spleen, but no thickened or inspissated bile was ever seen in the liver capillaries. In the bone marrow an interesting change was observed, namely, the presence of a considerable amount of yellowish pigment within endothelial cells. No actual phagocytosis of erythrocytes was, however, seen in this situation in the sections.

CONCLUSIONS.

1. Toluylendiamin, in the living body of the rabbit, gives rise to comparatively slight anaemia.

2. The anaemia produced is not brought about by a direct lytic action in the general circulation, since no "shadows" are ever seen in blood films examined.

3. The blood destruction which occurs takes place chiefly in the spleen, the peripheral sinuses of which become crowded with erythrocytes, most of which must be destroyed there.

4. It must remain an open question how the disintegration of these cells in the splenic sinuses is brought about. Some haemolytic body produced indirectly in the liver by the drug, as Joannovics

and Pick have suggested, may perhaps bring about extra-cellular lysis in the spleen. On the other hand, in chronic cases intra-cellular destruction of erythrocytes by phagocytosis is found.

5. The only morphological changes seen in the peripheral blood are the appearance of a number of basophil red cells of normal size, and the occurrence of poikilocytosis. This latter change is a curious and interesting result of the experiments.

6. Biliuria was a feature of all the experiments, a point which seems to have escaped the notice of other experimenters on rabbits. The bile was always present as biliverdin.

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Wm. Hunter	"Pernicious Anaemia", London, 1901.

E.

THE ANAEMIA PRODUCED BY THE INJECTION OF COBRA-LECITHID.

Renewed interest on the question of the relationships of lipoidal substances to the production of haemolysis was aroused by the work of Faust and Tallquist on Borthriocephalus anaemia, which has been referred to already when the probable mode of action of Toluylendiamin in the animal body was being considered. Our knowledge, however, that certain of the lipoids are able to influence the phenomena of haemolysis, is of much longer date. It goes back to the time when Ransom (1901) discovered that the action of saponin could be inhibited by the presence of the lipoid cholesterin, and more especially to the time when Kyes (1902-3) began his work on the venom of the cobra. Kyes found that cobra venom by itself was physiologically inactive, unless combined with the lipoidal substance lecithin, when the compound was found to possess intensely haemolytic properties. To the substance resulting from the combination of these two bodies, Kyes applied the name "Cobra-Lecithid". Subsequently the same author was able to unite in a similar way the poison of the scorpion with lecithin, and later still Morgenroth and Carpi carried out the same work with the poison of the bee.

Cobra-Lecithid has been the subject of much research in test-tube work on haemolysis, but the attempt has only been made once before to bring about an anaemia in animals by means of it. Morgenroth and Reicher, by injecting the substance into rabbits, were able to show that a degree of anaemia resulted, but they never attempted to follow any morphological changes in the blood or alterations in the organs.

post-mortem. In their experiments they were only concerned with the questions of whether any anaemia did result, and further whether substances such as cholesterin which are anti-haemolytic in the test-tube inhibit the action within the body as well. Both of these questions they were able to answer in the affirmative. In their best experiment the blood count was reduced from 5,600,000 to 3,350,000, and the haemoglobin from 70% to 41%, within a period of three days, by the injection of 10.5 c.c. of an approximately 1 per cent solution of the Cobra-Lecithid. In their other two experiments, the fall in the blood count was about 1,500,000. In rabbits which at the same time were fed with cholesterin dissolved in olive oil, no fall in the blood count whatever followed the injection of the Cobra-Lecithid. In consequence of this latter work Reicher (1908) was tempted to try the effects of administering cholesterin, dissolved in olive oil, to patients suffering from pernicious anaemia, but this treatment must be reckoned a complete failure.

Through the courtesy of Professor Sachs of Frankfurt, a small amount of Cobra-Lecithid came into my possession, only enough unfortunately for a single experiment. The observation, solitary as it is, seems worth recording because in it the condition of the blood and organs was carefully gone into. Further, the results are of considerable interest for purposes of comparison with the experiments made by injecting toluylendiamin.

An account of the experiment will now be given in detail.

Rabbit, weight 1540 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0	6,420,000	5,000	60
1	5,420,000	6,100	58
2	5,800,000	5,600	56
3	...	...	...
4	5,790,000	9,300	46

3 c.c. of approximately 1% solution of Cobra-Lecithid intravenously

10 c.c. : : : :

10 c.c. : 2½% : : :

Following the first two injections, "shadows" were seen in the circulating blood, but they were not very numerous. No haemoglobinuria occurred after either of these injections. Following the third and most powerful dose, haemoglobinuria resulted, and various morphological changes were present in the blood. "Shadows" were extremely numerous, and nucleated red cells, all of normoblastic type, were found fairly abundantly. Basophil erythrocytes also made their appearance on this day, but they did not exceed the normal red cells in size. The animal was found dead two days after the third injection.

Post-mortem. The liver was of light-yellowish colour and very fatty. The spleen was somewhat enlarged and quite black in colour, while the kidneys also appeared darker than normal. The heart was much engorged, and the pericardial sac contained some blood-stained fluid. The liver gave an extremely marked haemosiderin reaction, as marked as in any one of the other experiments. The kidney gave a reaction in the cortex, and as usual the test was also strongly positive in the

spleen.

Microscopically, the bone marrow was found to contain extremely large fat spaces, but no changes of a degenerative or hyperplastic nature could be made out in the cells. The liver and kidneys, except for the iron deposits, showed nothing of interest. In the spleen the most notable finding of the experiment was discovered. The peripheral sinuses were filled with erythrocytes, but unlike what was found after injection of toluylendiamin, the red cells did not stain normally. In fact, what was present was really only the stromata or envelopes of the corpuscles, the haemoglobin apparently having been already removed. (see Fig 1) No phagocytosis was seen, but masses of pigment were fairly numerous extra-cellularly.

RESULTS OF THE EXPERIMENT.

These require only brief discussion of certain points. It is evident that the Cobra-Lecithid causes blood destruction chiefly, if not entirely, by a process of lysis in the peripheral blood. The presence of such numerous "shadows" after the third injection is a sufficient proof of this. It seems, from the appearances in the spleen, as if the haemoglobin was simply extracted from the corpuscles in the circulating blood, without damaging the stromata. These latter appear to be carried thereafter to the spleen, where they are found in great numbers in the peripheral sinuses. The other morphological changes in the blood resemble closely those induced by other blood poisons. A further point of interest in the experiment, on which some slight emphasis may be laid, is the intensity of the haemosiderin reaction found in the liver.

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DESCRIPTION OF FIGURE.

Fig. 1. Section of spleen showing the large number of "shadows" of erythrocytes collected in the splenic sinuses. The section has been stained very intensely with eosin in order to bring out the envelopes of the decolorised red corpuscles. (x 100)

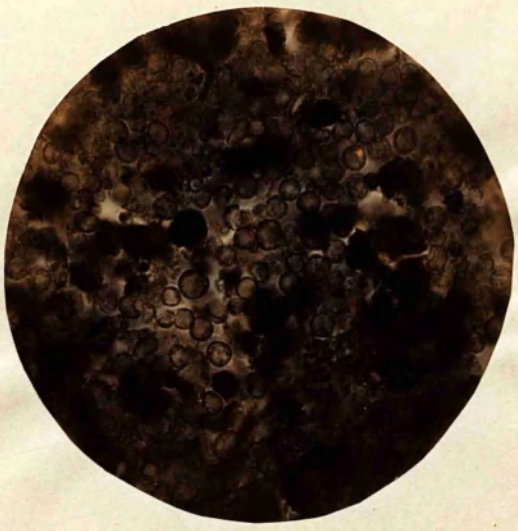

Fig 1.

POST-HAEMORRHAGIC ANAEMIA.

To compare with the anaemia induced by the various blood destroying agents injected into the circulation, I have carried out experiments whereby anaemia was brought about by simple bleeding. This is the type known in the literature as Post-haemorrhagic anaemia, and the rabbit is an animal which by reason of its large ear veins lends itself very readily to such experiments. It is really quite extraordinary what a large quantity of blood can be removed daily from the ear vein, over a long period of time. In one of my experiments it will be seen that over 400 c.c., at least four times the animal's total blood volume in health, was removed within a period of about a month. This, however, is by no means a record, since Boggs and Morris (1912) in their experiments on lipaemia, which will be referred to again, actually obtained 1,468 c.c. of blood from the ear veins of a single rabbit in some 70 bleedings!

The study of the anaemia produced by simple bleeding, in addition to its interest for comparison with the other types of anaemia already dealt with, has yielded much information in connection with the condition in the human subject known as "Aplastic Anaemia". This type of human anaemia, first drawn attention to by Ehrlich (1880 and 1888), was recognised by him as a severe and fatal anaemia, in which regenerative changes in the bone marrow of the long bones remained absent. Senator, at a later date, drew attention to the marked overgrowth in the bone marrow in these cases of a lymphoid type of tissue. This finding is of much interest in connection with what research on

experimental post-haemmorhagic anaemias has yielded.

Post-haemorrhagic anaemias have been chiefly studied in detail by Blumenthal and Morawitz (1908), Morawitz and Rehn (1908), Itami (1909) Ritz (1909), and Lindsay Milne (1912).

Blumenthal and Morawitz by experiments on both dogs and rabbits, found that after long continued bleedings the marrow of the animals came to resemble closely that found in human aplastic anaemia, there being apparently produced a condition of exhaustion of the marrow. There was disappearance of the cells of both the erythroblastic and granular series, and instead of these there was found a considerable development of cells resembling lymphocytes morphologically. The interest of Senator's observation in human aplastic anaemia is now apparent.

Morawitz and Rehn, in a subsequent research, attempted to clear up the morphology of these lymphocyte-like cells, and finally came to the conclusion that they were most probably myeloblasts. In their reasoning they state that the erythroblastic tissues and the granular cells have very close relationship, and that damage to the first group will adversely affect the second group as well. They considered that what occurred in their animals was that following exhaustion of the erythroblastic portion of the marrow by the constant drain of the bleeding, the cells of the granular series were in consequence also affected and held back in development. These latter cells, they would suggest, stop short at the myeloblastic stage, and never develop granules. The Oxydase reaction was not discovered at the time of their work, and the Altmann-Schridde method of staining granules in lymphocytes was apparently not applied. In the anaemia resulting

from injection of the haemolytic immune serum, some difficulty was experienced in reading the marrow picture brought about to define exactly the morphology of certain large cells with rounded nuclei very like large lymphocytes. By the use of the Oxydase reaction and the Altmann-Schridde method, both of which were quite negative, the cells were shown to be neither lymphocytes or myeloblasts, and it was concluded for this and for other reasons that they were early erythroblasts.

The experiments of Itami and of Ritz bring out especially two important characteristics of post-haemorrhagic anaemia. Both observers found that the red corpuscles returned to a normal number much sooner than the haemoglobin percentage, when the bleedings were stopped, and also that in anaemia induced by blood destroying agents of whatever variety, blood regeneration took place far more rapidly than in the anaemia from bleeding. Itami points out the almost certain cause for this latter phenomenon, namely, that in the toxic anaemias the products of the blood destruction are still available within the animal's body for purposes of regeneration. This view is strongly supported by experiments in which Itami, in addition to bleeding animals, injected washed blood corpuscles into their peritoneal cavities, and found that such animals regained a normal red cell count sooner than animals in which no such injections were made.

Neither Blumenthal and Moravitz, nor Itami found more than traces of myeloid metaplasia in any of their experimental animals, although the latter author in some of his toxic anaemias found extensive myeloid change in the organs.

Lindsay Milne's experiments are of especial interest from his

description of the morphological changes in the blood, and from the extensive myeloid metaplasia he was able to bring about by bleeding alone. In the blood at the very early stage of the experiments, erythroblasts were sometimes present, but never later on. Anisocytosis became marked, but poikilocytosis was never seen. A point on which he lays stress was the frequent occurrence of punctate basophilia, which led him to question the older views of the degenerative nature of this change. With regard to the occurrence of myeloid metaplasia, he found this present in both liver and spleen of certain rabbits bled two or three times a week over periods varying from two to three months. In two such animals he found large clumps of myeloid cells in the liver capillaries, these foci quite resembling pieces of bone marrow. In these cases there was also considerable blood regeneration in the spleen, although the organ was not enlarged. Myeloid tissue in small patches was even found in the pyramids of the kidneys ! He makes no mention of having examined the thymus gland for blood regeneration, an omission of importance as will be seen from my own experiments. In all his animals Milne found that lipaemia resulted whenever the anaemia became pronounced, a point previously pointed out and discussed by Boggs and Morris.

My own experiments will now be described.

RECORDS OF EXPERIMENTS.

EXPERIMENT 1.

Rabbit, weight 1530 grms.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0[1]	4,800,000	...	72
1	3,570,000	7,000	54
3[2]	...	...	...
5	3,450,000	4,000	56
6	3,530,000	5,000	56
7[3]	4,160,000	5,300	61
9	2,800,000	5,000	36
10	2,610,000	5,000	40
11	3,900,000	5,100	48
12	4,020,000	4,300	51
13	3,880,000	5,000	50
14	4,080,000	6,200	50
15	5,010,000	6,000	52
16	4,620,000	3,800	52
17	4,610,000	5,300	50
18[4]	...	...	...
20	3,690,000	5,300	49

1. 25 c.c. of blood removed from the ear vein by venesection.
2. 20 c.c. : : : :
3. 25 c.c. : : : :
4. 20 c.c. : : : :

In this experiment the animal was killed on the twenty-fourth day.

The morphological changes in the blood, and the alterations in the number of corpuscles and haemoglobin percentage, were carefully entered into in this animal, to study the process of blood regeneration after simple bleeding. As regards the morphological changes, after the first bleeding only a single erythroblast was seen in the films, and none were ever found subsequently. No other alterations fall to be recorded until after the third bleeding, on the seventh day of the experiment. There was on this occasion destinct lipaemia, the serum after separation from the clot being quite milky and opalescent. Polychromatophil cells made their appearance in the circulation at this time in considerable numbers, and in size most of them were a little larger than the ordinary erythrocytes. Cells less in diameter than the usual erythrocytes were also present, so that there existed a very definite anisocytosis. Many cells were also seen showing beautiful punctate basophilia, and I was able to observe quite readily all stages of gradation between the blue and quite polychromatophil cells and the cells with eosin-tinted protoplasm in which the dark blue granules are seen. It was quite evident that in this anaemia these two phenomena, polychromatophilia and punctate basophilia, were just different stages in the life history of the same type of cells. This observation is another point in favour of putting punctate basophilia among the regenerative phenomena, and not among the degenerative About the ninth and tenth days of the experiment a distinct tendency to poikilocytosis became apparent, a fact not recorded by previous workers. I am satisfied that this alteration in shape did not depend upon technical faults in the making of films, for it could be made out in drops of the blood mounted fresh. Punctate basophilia

continued,however,to be the chief morphological phenomenon in the blood right up to the end of the experiment (see Fig.).

It will be seen that,in this animal,the withdrawel of blood was never succeeded by a leucocytosis.

As the experiment progressed,blood platelets became present in extraordinary numbers. They were counted on the twentieth day of the experiment,by Aynaud's method,and found to number about 1,400,000 per c.mm.,as compared with a normal number for the rabbit of about 300,000 per c.mm.

Following the third bleeding on the seventh day,the animal was left alone for a period of eleven days to observe the progress and rapidity of blood regeneration. Various interesting points are brought out by the blood counts given in the Table. First of all it will be noted that the number of red corpuscles did not rise very rapidly. From 2,800,000 on the ninth day,the count reached 5,010,000 on the fifteenth day,an average daily increase of roughly 350,000. This is considerably less than the average daily increase found in the experiments with the haemolytic immune-serum.

It will also be seen that the percentage of haemoglobin,which was 36 on the ninth day,rose very slowly indeed. On the fifteenth day, the blood count (5,010,000) was higher than that at the commencement of the experiment (4,800,000),whereas the percentage of haemoglobin was only 52 as compared with 72 at the outset. The low colour index, and the fact that the haemoglobin does not return to normal nearly so quickly as the red cell count,have been drawn attention to by all the previous workers on this subject.

The animal being killed with the object of making a blood-volume

estimation, the organs could not be examined histologically.

EXPERIMENT 11.

Rabbit, weight 2220 grms.

In this animal 411 c.c. of blood were removed from the ear veins within a period of 28 days, an average daily amount of 14.7 c.c. The following table gives a record of the bleedings:-

Day.	Amount.	Day.	Amount.
0	25 c.c.	16	20 c.c.
2	25	17	15
3	10	18	18
4	15	19	24
6	20	21	19
8	20	22	20
11	22	24	20
12	20	25	15
13	18	26	22
14	15	27	20
15	18	28	10

Blood counts were made during thr course of the experiment as follows.

Day.	Red Corpuscles.	Leucocytes.	Haemoglobin %.
0	4,710,000	9,000	65
14	3,100,000	10,300	32
17	3,280,000	6,000	40
25	3,620,000	5,000	60
28	2,770,000	4,200	55

Seeing that the morphology of the blood was so fully entered into in connection with the previous experiment, it seems unnecessary to go into details again. Suffice it to say that the changes found were quite similar to those already mentioned. Punctate basophilia was again in this animal a very prominent feature of the films.

The animal lost about 300 grms in weight during the 28 days of the experiment.

Post-mortem. The organs appeared normal macroscopically. The spleen was very small, weighing only 0.49 grms. It gave a slight haemosiderin reaction, but neither liver nor kidneys gave any. The bone marrow of the femur was extremely red.

For microscopic examination, films of bone marrow were fixed wet in a solution of corrosive sublimate in salt and stained with diluted Ehrlich's Triacid stain, and dry films were also stained with Jenner's stain. In this way it was shown that a marked erythroblastic reaction was present, very many nucleated reds being found. Comparatively few of the large cells which are difficult to place in the category of being erythroblasts or myeloblasts or even lymphoidal, were met with in the films. Sections of bone marrow showed the fat spaces well preserved, and the preponderance of nucleated red cells was clearly made out.

The liver in section showed practically nothing abnormal. In a section stained by Triacid a few myelocytes were found lying in the capillaries, but there was really no attempt at myeloid metaplasia.

The spleen showed one curious change. Masses of golden-brown pigment were collected into heaps scattered irregularly through the section. This pigment gave a very marked haemosiderin reaction,

and it seemed as if the animal had been unable to use up this coarse extra-cellular haemosiderin in the process of manufacturing new blood cells.

The thymus was about the size of a large pea, and dark reddish in colour. In section much vicarious blood formation was found to be going on, the lymphoid tissue normally present being replaced considerably by myeloid tissue (see Fig.). Both the erythroblastic and granular series of cells were found proliferating in this unusual situation.

Neither in the kidneys, nor in the lymph glands of the mesentery were any abnormal changes made out.

EXPERIMENT 111.

Belgian Hare, weight 2370 grms.

In this experiment of thirty days duration, the corpuscles were not counted, but a careful account of the haemoglobin percentage was kept. In all, 404 c.c. of blood was removed from the animal in 16 bleedings. The record of the experiment is given below.

Day.	Haemoglobin %.	Bled.
0	74	30 c.c.
1	64	31
2	52	28
3	38	..
4	45	23
5	38	..
6	42	20
7	45	..

Experiment 111 (continued).

8	50	18
9	52	..
10	52	25
11	55	27
12	45	..
13	45	19
14	52	..
15	55	..
16	..	28
17	45	..
19	65	25
21	65	21
22	60	23
24	50	28
25	35	..
26	42	28
27	50	..
28	50	30
30	60	..

A remarkable point in this experiment was the way in which the haemoglobin percentage kept up, in spite of the repeated and severe bleedings. To enable this to be done, it was evident even before the organs were examined histologically that very active blood regeneration must have been going on somewhere. In the blood films, punctate basophilia was again a feature, and polychromatophil cells were also very numerous in the later stages. No poikilocytosis occurred.

Post-mortem. The bone marrow of the femur appeared very red. The other organs seemed normal macroscopically.

Microscopically the bone marrow both in films and in section showed a marked hyperplasia, the fat spaces being considerably diminished in size (see Fig.). The cells of the granular series seemed less in amount than normal, and many cells with round nuclei which were regarded by me as certainly erythroblasts, were present throughout the marrow. The megakaryocytes seemed to be present in increased numbers, which is of interest in connection with Homer Wright's researches on the origin of the blood platelets.

The liver was found to contain scanty myelocytes in the capillaries but no nucleated reds were seen.

In the thymus proliferation of cells of both the bone marrow types was found, just as in the previous experiment.

The spleen was just a replica of that seen in Experiment 11., and in the lymph glands and kidneys nothing unusual was found.

RESULTS OF EXPERIMENTS.

As my thesis deals chiefly with haemolytic anaemias, and these experiments which have just been described above were carried out primarily for purposes of comparison, they will be dealt with less fully than previous sets of experiments. The main points of distinction between haemolytic and post-haemorrhagic anaemias will be discussed immediately.

The outstanding points in the anaemia produced by haemorrhage were found to be as follows. In the films of blood all evidences of destruction are of course wanting, and the chief changes depend on the

regenerative processes to make up for the blood taken away. Thus polychromatophil cells become numerous, but not until several days at least after the first bleeding. They are to be regarded simply as somewhat immature cells, and are only slightly larger than the normal erythrocytes. The most prominent feature in the films is undoubtedly the occurrence of punctate basophilia, which in these experiments is also an evidence in the cells of youth, and not of commencing degeneration. After some time slight poikilocytosis may develop. The Colour Index always becomes lowered and may fall below one half. Blood regeneration is slow, and whereas the number of corpuscles may return to the normal figure in a week or ten days, the haemoglobin percentage always lags far behind. In the bone marrow there was an erythroblastic reaction of considerable intensity in both my experiments. The marrow picture could not be said to resemble closely that of aplastic anaemia, as other observers have described. No overgrowth of lymphoidal cells was apparent, the increase in the cells being almost entirely due to the multiplication of erythroblasts. In the spleen no myeloid metaplasia was met with, nor any evidence whatever of blood regeneration. Phagocytosis was absent, but large extra-cellular masses of pigment giving the iron reaction were present in both animals. It seems curious that this iron pigment was not used up in the process of blood regeneration, and it seems probable that such coarse masses of iron pigment cannot be used by the animal economy for such a purpose. In the liver I met with no such myeloid metaplasia such as Lindsay Milne has described. The bleedings were, however, more frequent in my experiments, and the duration of them was only half of those in which Milne found his curious results.

In the thymus gland in both the animals examined, extensive myeloid changes were found. Both erythroblasts and myelocytes were present in great numbers and actively proliferating, so that the normal thymus tissue was considerably curtailed. These changes in the thymus in post-haemorrhagic anaemia seem to have been missed by all previous observers.

CONCLUSIONS.

1. In post-haemorrhagic anaemia the characteristic changes in the peripheral blood are the occurrence of polychromatophilia in many erythrocytes, and the presence of marked punctate basophilia in many others. These two changes are to be regarded in this form of anaemia simply as different stages of the same process of ripening of the red cells.

2. As all previous authors have pointed out, the Colour Index is low, blood regeneration is somewhat tardy when compared with other experimental anaemias, and the haemoglobin always lags behind the red cells in returning to normal amount.

3. The bone marrow shows an erythroblastic reaction, and when blood regeneration has gone on for some time this reaction may be considerable. No overgrowth of lymphoidal tissue occurred in my experiments at least, and the marrow picture did not coincide even closely with the published descriptions of the condition in the human subject known as aplastic anaemia.

4. No myeloid metaplasia occurs in the liver or spleen, at least within a period of a month from the commencement of the experiment. Marked myeloid change, however, is found in the thymus, where cells of

both the erythroblastic and granular series are found in active proliferation.

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DESCRIPTION OF FIGURES.

Fig. 1. Film of blood from Experiment 1., near the end of the experiment, showing punctate basophilia. (x 1000)

Fig. 2. Section of bone marrow from Experiment 11., showing a hyperplastic reaction. This contrasts with the picture in human aplastic anaemia. (x 80)

Fig. 3. Section of Thymus gland from Experiment 11., showing extensive myeloid metaplasia. The darkly stained tissue represents the lymphoid tissue of the Thymus. The lighter stained tissue is myeloid in nature. (x 80)

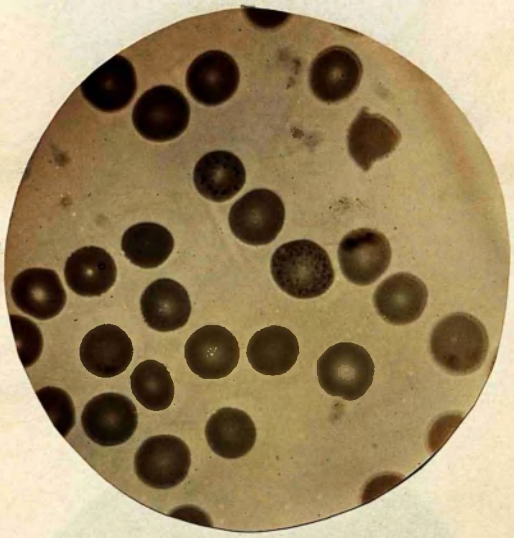

Fig 1.

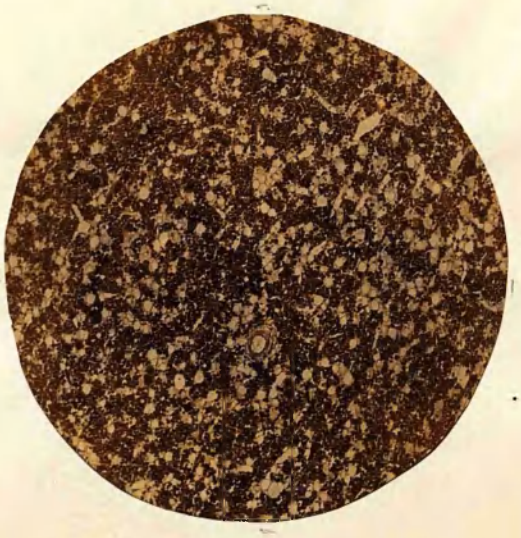

Fig 2.

Fig 3.

CHANGES IN THE HEART MUSCLE AND IN THE LIVER FOLLOWING THE INJECTION OF A HAEMOLYTIC SERUM.

Brief reference has already been made to the fact that,following injection of the haemolytic serum,changes were found in some cases in the heart muscle and in the liver. These will now be entered into more fully,especially the myocardial lesions,since the experimental production of myocarditis is of considerable interest in relation to human cardiac Pathology.

The work of Dudgeon, Panton, and Ross, and of Pearce,with haemolytic sera has made it clear that the action of all of these is not the same,since these authors found marked degenerative changes in various organs following their injection.

With regard to myocardial changes these have been brought about experimentally in rabbits in a considerable variety of ways.

Ribbert (1886) seems to have been the first to induce degenerative changes in the cardiac muscle of rabbits. He injected cultures of staphylococcci introvenously,and produced both endocardial and myocardial lesions. The latter consisted of a central necrotic area, surrounded by a zone of polymorph infiltration.

Litten (1887), Naunyn (1884), and Welch (1888), by keeping rabbits at a terperature of 40°C. for several days brought about marked fatty degeneration and other changes in the fibres of the heart muscle.

Numerous observers have studied the lesions caused in the heart by the diphtheria bacillus and its toxins. Babes (1896) showed in his experiments that the lesions were identical,whether the organism itself,or only its toxins were injected. The degenerative changes

themselves have been carefully worked out by Charrin (1890), Welch and Flexner (1891), Flexner (1897), and Mollard and Regaud (1892 and 1897). The changes found were fatty degeneration of fibres, atrophy of others, and interstitial oedema, with infiltration of leucocytes. The last mentioned authors found that several months after the appearance of these early changes, fibrous tissue had replaced the degenerated muscle fibres.

Baumgarten (1899) in experiments on dogs whereby branches of the coronary arteries were ligatured to produce a sort of cardiac infarct, found areas of degeneration in the cardiac muscle, which were later replaced by connective tissue.

A whole series of interesting researches have been carried out in recent years with Adrenalin, which was found to be an agent capable of bringing about severe arterial changes in rabbits. Lesions also occur in the heart, as the observations of the following authors show.

K. Ziegler (1905), seems to have been the first to observe these myocardial changes after injection with adrenalin. He found distinct fibrous change in animals after about ten days, and was of opinion that the new connective tissue was derived from that normally found surrounding the blood vessels and below the endocardium.

B. Fischer (1905) described similar changes about the same time, noting the occurrence of fibrous and necrotic changes in the heart wall, and in rare instances calcification.

Pearce (1906) repeated the work of the last two authors, and gives a very detailed description of what he found in the heart after repeated doses of adrenalin. The first change noted was the presence of great oedema of the myocardium, so that the individual

fibres became widely separated from one another. In longer experiments he found, in addition to the oedema, hyaline changes in the muscle fibres and sometimes actual necroses. Round the necrotic fibres lymphocytic infiltrations occurred. In still longer experiments definite proliferative changes were present, new connective tissues being laid down. This consisted in some cases simply of a focal accumulation of fine fibrils round about single degenerated fibres or small groups of these. In other cases a diffuse fibrotic change was found invading considerable areas of muscle. He pointed out that the new connective tissue was always found in very close relationship to the myocardial blood vessels, and also that no collections of lymphocytes are seen in the more diffuse fibrous lesions. Pearce examined the cardiac muscle in a large series of animals used for other work, to control his experiments, but never found a myocardial lesion in any of them.

Grober (1907) has described an actual cardiac hypertrophy, with definite thickening of the muscle, and without degenerative changes, as a sequel to repeated adrenalin injections.

Fleischer and Loeb (1909) added a further chapter to the work with adrenalin by their published results showing that in 60 per cent of rabbits so treated, an extensive myocardial lesion can be brought about by a single injection of 0.2 c.c. of 1 in 1000 adrenalin solution, to which had been added 0.012 grm. of Spartein Sulphate, or 0.025 grm. of Caffein-Sodium-Benzoate. How these two latter substances amplify the action of adrenalin is uncertain, and by themselves they have no activity, but it is easily proved that the same single dose of adrenalin without their presence is not nearly so potent.

The lesions they described were in the main identical with those indicated by Pearce,but they were able,in a very large series of animals,to follow the development of the changes in sequence from periods of minutes up to about six weeks after the solitary injection. In this way they found,what is of considerable importance in regard to my own observations,that proliferative changes in the heart muscle may begin as early as two days after the injection,and by the end of four days may be quite marked. The short period of time necessary to allow of the occurrence of fibrous changes in heart muscle is really quite extraordinary. They pointed out,in addition,that the very early changes are first noticeable round about the blood vessels and near the pericardium and endocardium. This is interesting in connection with the phenomena now to be described.

RECORDS OF ANIMALS.

The cardiac muscle was examined in the majority of the animals injected with the haemolytic serum,but in only four out of the number was a myocardial lesion discovered. In all four instances the changes were of a proliferative nature,and as will be seen below they varied considerably in intensity. A brief resume of the experiments in which myocarditis was found,with a description of the lesion in order of severity,will now be given.

Rabbit 8, weight 1590 grms.

This was an acute experiment,the red count being reduced from 6,060,000 to 2,160,000,and the haemoglobin from 74% to 40%,within fifteen hours. Marked haemoglobinuria ensued,although only 1 c.c.

of the haemolytic serum had been injected. The animal was killed nineteen hours after the injection, as it appeared to be dying.

Heart. A very early myocardial lesion was found, one small circular patch of muscle fibres presenting the following appearances. The individual fibres were separated from one another, and surrounded by a somewhat scanty infiltration of round cells resembling lymphocytes The patch was sharply marked off from the healthy muscle round about. No fibroblasts, or other sign of proliferative change, was made out. At one edge of the lesion a small capillary was seen cut in longitudinal section, and this was found quite filled up with similar cells to those surrounding the fibres. The portion of cardiac muscle examined which was taken from the wall of the left ventricle, was cut in serial sections, and a few other smaller areas of round cell infiltration, all in close proximity to small blood vessels, were found. The appearances of this early lesion are shown in Fig. 1.

Rabbit 3, weight 1360 grms.

This was another acute case, with copious haemoglobinuria. The blood count was reduced from 7,200,000 to about 460,000 within forty hours. The total amount of serum injected was 1.5 c.c., and the animal was killed about fifty hours after the first injection.

Heart. The muscle from the left ventricle, cut in longtudinal section, showed a well marked lesion in one small area contiguous with the pericardium. The fibres in this area were in places widely separated by inflammatory oedema, while in other parts well marked proliferative changes were evident. There was no round cell infiltration, but the fibres were separated by a very early and loosely

formed connective tissue. The nuclei of the muscle fibres stained much more faintly here than elsewhere, but no actual necrosis or atrophy of muscle fibres were seen. The lesion is represented in Fig. 2.

Rabbit 22, weight 1260 grms.

This case was rather more chronic than the previous ones, and was unaccompanied by haemoglobinuria, although 2.75 c.c. of the haemolytic serum was injected in three doses. The blood count was reduced from 4,600,000 to 1,880,000, and the haemoglobin from 58% to 28%. The animal was killed ninety and a half hours after the first injection. Heart. In the muscle of the left ventricle a very widespread but early fibrous change was met with. The extent of this change is very well brought out in Fig. 3. In this lesion certain very interesting points could be made out, especially with relation to the site of origin of the connective tissue. It was very evident that this tissue was always to be found most abundantly in the near neighbourhood of blood vessels. Further, atrophy of the muscle fibres surrounded by the new formed fibrous tissue was better seen here than in any of the other lesions discovered. This latter point is very clearly shown in the photograph.

Rabbit 15, weight 2150 grms.

In this animal the anaemia was very acute, the red count being reduced from 7,200,000 to 2,080,000, and the haemoglobin from 85% to 34%, within forty-six hours. The total amount of serum injected was 1 c.c., given in two doses, and the animal was found dead on the third

day of the experiment, probably a little less than seventy hours after the first injection.

Heart. In this animal the most marked myocardial change of the whole series was discovered. The changes, indeed, were so marked that it is difficult to imagine that they were brought about in their entirety within the short period of the experiments duration. This point will be referred to again. In the portion of left ventricle taken for microscopic examination, very numerous areas of well formed connective tissue, some of quite considerable size, were found. In many of the patches of fibrous tissue no trace of muscle fibres could be seen, while in others a few atrophic fibres could be picked out. No necrosis, or other active degenerative change, was detected in those parts of cardiac muscle not involved in the fibrosis. In this lesion also, a definite relationship of the areas of connective tissue to the blood vessels was very evident, and this is well seen in Fig. 4.

These were all the definitely proliferative changes met with, but in several of the other experimental animals separation of the cardiac muscle fibres was observed, suggestive of a condition of oedema. These cases, however, require no description.

DISCUSSION OF RESULTS.

The above observations really call for little discussion. They are chiefly recorded as being evidence of an interesting and hitherto unknown method of bringing about experimental myocarditis in animals. In the first three rabbits to which reference was made, the lesions produced are very similar indeed to those described

by Pearce, and by Fleischer and Loeb, in their published works. A striking similarity to the experiment with adrenalin is, that the fibrous change which follows the injection of a haemolytic serum, begins also in close proximity to the blood vessels. This seems a clear proof that a toxic material is carried in the blood stream, and affects most the parts first supplied by the plasma. In the fourth animal, the fibrous lesion is so marked and the connective tissue so well formed, that it is practically inconceivable that no myocardial lesion was present before the experiment began. It must be pointed out, however, that just as was done by Pearce I have examined the cardiac muscle in a considerable number of laboratory rabbits, used for other experimental work, and have never found myocarditis in any of them.

Changes in the liver. A lesion was found in the liver in only one animal injected with the haemolytic serum, although this organ was carefully examined microscopically in every case. This compares very strangely with some work of Pearce on "Experimental cirrhosis of the liver", where he actually used a haemolytic serum obtained by injecting rabbits with dogs' corpuscles, to bring about degenerative liver changes. With the serum he produced almost as a rule lesions of the following nature. He found within forty-eight hours after an injection marked necrotic areas in the liver, which had a very definite relation to vascular thrombi composed of masses of semi-fused red blood corpuscles. In animals which survived these initial necrotic changes the degenerated areas were replaced by connective tissue, so that in a few weeks (he examined animals up to thirty-six days after the first

injection) a very definite cirrhosis of the liver had resulted. My only observation on degenerative changes in the liver is given below.

Rabbit 8.

This was the animal in which a very early myocardial lesion has been described. In the liver a very marked condition of necrosis was present, affecting chiefly the central parts of the lobules, in close relationship to the central veins. Although the lesion seemed always to commence in the centre, in many places the whole of the lobules right out to their periphery was necrotic. In the necrotic areas, marked cellular infiltration was present, and the cells were on examination found to be chiefly polymorpho-nuclear leucocytes, and not lymphocytes. The lesion being an early one, no attempt at connective formation was seen.

CONCLUSIONS.

1. In animals injected with the haemolytic immune-serum, definite myocardial changes occurred in four out of about twenty animals. These changes consisted, briefly, in the development of a fibrous myocarditis. It is evident from the experiments of others that haemolytic sera differ in the amount of damage they produce in organs other than those belonging to the haemopoietic system.

2. In one animal a marked necrosis of liver tissue followed the injection. By means of a haemolytic serum prepared in a different way, Pearce has been able to produce almost invariably necrotic changes in the livers of rabbits.

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DESCRIPTION OF FIGURES.

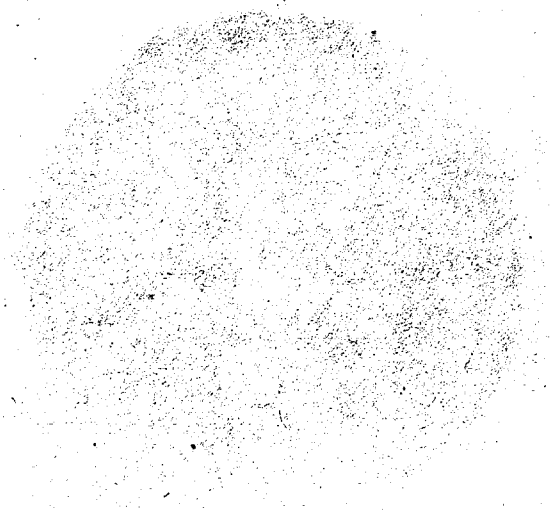
Fig. 1. Heart muscle of Rabbit 8, showing a very early lesion. (x 80)

Fig. 2. Heart muscle of Rabbit 3, showing an early proliferative change. (x 80)

Fig. 3. Heart muscle from Rabbit 22, showing a diffuse fibrous change in the neighbourhood of blood vessels. Atrophy of muscle fibres is well seen. (x 80)

Fig. 4. Heart muscle of Rabbit 15, showing patches of advanced fibrosis. (x 80)

Fig. 5. Section of liver of Rabbit 8, showing well marked necrosis of a lobule. (x 80)



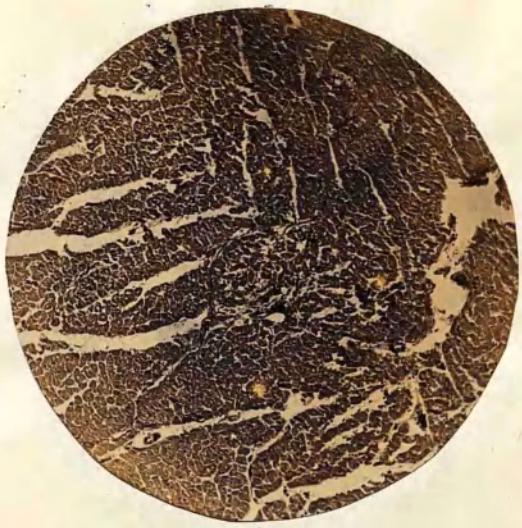


Fig. 1.

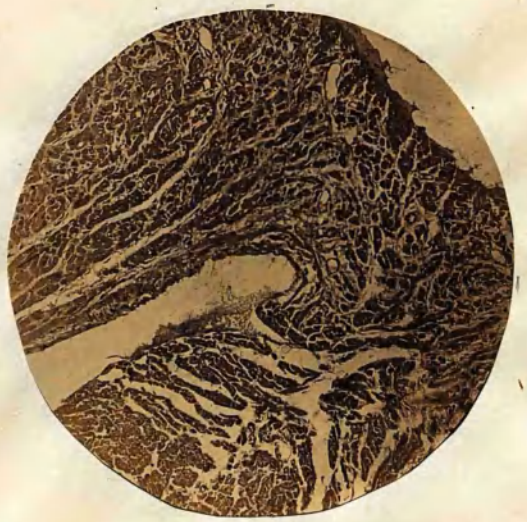


Fig. 2.

Fig. 3.

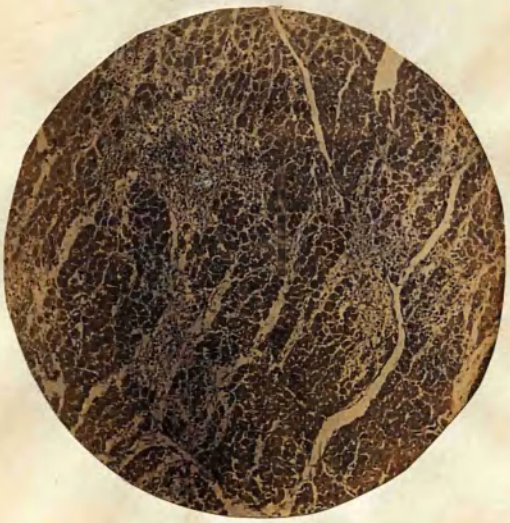


Fig. 4.

Fig. 5.

GENERAL SUMMARY AND COMPARISON OF RESULTS.

In concluding this thesis,I have considered that any summary of the results of my work would be incomplete without comparing,in a general way,the changes brought about in experimental anaemia from blood destruction within the body (haemolytic anaemia),with those produced by simple removal of blood (post-haemorrhagic anaemia). In this way an idea is given of how the two types of experimental anaemia contrast,just as a comparison is made between what are called in man primary and secondary anaemia.

It must be stated at once that such a comparison cannot be made with all the experimental haemolytic anaemias investigated. For example,those produced by saponin and toluylendiamin have no real similarity as regards the blood picture brought about to primary anaemia in man. The anaemias induced by the injection of the haemolytic serum,and in a lesser degree that brought about by streptolysin, have on the other hand exceedingly striking similarities,both as regards the blood picture and the changes in the organs,with primary or pernicious anaemia in the human subject.

When the haemolytic anaemia produced by the two above mentioned agents is contrasted with that produced in animals by simple bleeding, it is seen that one by one the points of distinction compare just as in the text-book descriptions of the differentiation of human anaemias.

In the haemolytic anaemia there develops,just as in human primary anaemia,a megalocytic type of blood with a high colour index.

Nucleated red cells of both types appear in the circulation, marked anisocytosis is present, large polychromatophil megalocytes occur in numbers, and other changes such as punctate basophilia etc., are also found. In the organs post-mortem, the deposits of haemosiderin are quite similar in extent and distribution to those of the human disease.

In post-haemorrhagic anaemia in rabbits, the changes found are quite similar to those in severe secondary anaemia in man. In the films anisocytosis and to some extent poikilocytosis occur, nucleated red cells are seen but seldom, and the blood platelets become extremely numerous. The colour index is uniformly low. A slight point of difference perhaps in the animal experiments is the extent to which punctate basophilia is met with.

The close correspondence of the results found in the two types of anaemia in animals with those of the two varieties in man, seem to me to further strongly the view that Primary or Pernicious Anaemia in man is a true haemolytic anaemia, dependant on some process of blood destruction in the body, all the other changes being secondary to this haemolysis. What the nature of the haemolytic substance in Pernicious Anaemia is, only further work can show. To my mind, the recent researches of Faust and Tallquist on the cause of Borthriocephalus Anaemia are very suggestive for future investigation of the subject.

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