IRON STORAGE IN THE NEONATAL PERIOD OF THE HUMAN INFANT.

THIS IS TO CERTIFY

that the Thesis "Iron Storage in the Neonatal Period of the Human Infant" is my own work and has not been submitted as a thesis for a Doctor's degree of another University.

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IRON STORAGE IN THE NEONATAL PERIOD OF THE HUMAN INFANT

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Thesis submitted in partial fulfilment for the degree of Doctor of Medicine (Obstetrics and Gynaecology), University of the Witwatersrand.

[1950]

ACKNOWLEDGMENTS.

I wish to acknowledge my indebtedness to Professor O.S. Heyns for his suggestions which led to the commencement of this work; also for allowing me the facilities of his Department; and further, for his kindness, his help and his constant enthusiasm.

I wish to thank Professor Stammers for allowing me the facilities of the Physiology Department, where the bulk of this work was done; and I am indebted to Dr. Bloomberg for the help and advice he has given me.

My thanks are due to Mr. J.M. Heyns who gave me great assistance in the early and difficult stages of this work, more especially in the field of the chemical investigations.

I must thank Mr. J.G. Truter, of Onderstepoort, for personally demonstrating his method of estimating the iron content of biological materials.

I am very grateful to Dr. Carlisle, of the Bridgman Hospital, for allowing me to obtain

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the bulk of the material on which this thesis is written. For the remainder of the material I thank Dr. Selby, Pediatrician, Coronation Hospital.

Finally, I wish to express my appreciation of the friendly spirit of co-operation with which Miss Krige and her staff of librarians undertook the collecting of references.

CONTENTS.

INTRODUCTION.

INTRODUCTION.

Iron is one of the vital elements of the human body. Without it respiration would be quite impossible. Haemoglobin, the transporter of oxygen throughout the body, has iron as an essential constituent, and it is in this protein complex that the greatest bulk of the metal is found. Iron also plays a very essential part in the respiration of the tissues, as it is a vital element in intracellular respiratory enzymes such as catalase, cytochrome, cytochrome oxidase, etc. Further, it is found in the chromatin material of nuclei in the cells of all the tissues.

As might be expected, the body exercises a most strict economy in the metal. Very little iron is excreted normally, (about 0.1 milligram daily in the urine of an adult male), and the iron from the breakdown of effête red blood cells is recovered most efficiently for repeated use. Normally, the slight daily loss in the urine is made up by the absorption of iron in the diet. If, for any reason, the haemoglobin iron is lost to a

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marked degree, as by a severe acute haemorrhage or by prolonged slight haemorrhages, the stores of iron are drawn on until they become exhausted, and if not replaced by iron in the diet, less and less haemoglobin can be manufactured until a recognizable anaemia develops. The tissue iron is not encroached upon.

Though haemoglobin iron is not lost, a somewhat similar mechanism occurs in young infants, in whom all the iron stores are constantly being drawn on to supply more and more haemoglobin and iron for the growing tissues. If the stores are small and are soon depleted, the haemoglobin iron is gradually used for the tissues, and, relative to the increasing volume of blood in circulation, less and less haemoglobin is produced, until eventually a recognizable anaemia may develop.

The human infant has a certain amount of iron at birth, and with this it must grow and survive until it is about six months old, when it becomes capable of digesting a diet containing iron. Normally, in the first six months it is entirely dependent on its stores of iron, for during this period the main diet is milk, which contains less than one milligram of iron per kilogram of milk, - this being only one part of iron in one million parts of milk ! Thus at

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the end of six months the total iron content of the infant, in spite of its growth, is the same as the total iron content at birth.

The main object of this study was to determine, as accurately as possible, the amount of iron stored in the liver and spleen of the human foetus during the last weeks of pregnancy, and to discover whether or not there was any appreciable increase in the storage of iron in these organs of the infant during the early neonatal period. It had been hoped to include in this work infants up to three or four months, in whom the stores of iron were being drawn upon, to determine, for the average case, when the drain on the iron stores can be said to begin. Unfortunately, up to the date of writing, no suitable material in this age group had been obtained.

As most previous workers in this field had ignored the iron due to the haemoglobin present in the liver, it became a further object to separate the iron due to the blood in the liver from what may be designated the true hepatic iron

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present in the liver tissue itself.

An added aim was to explore what is virtually a virgin field, namely, the Bantu foetus and infant. The only work done on the livers of Bantu infants was that published by Gillman (1945), who investigated a small number of African babies, using mainly histological methods, combined in some cases with a chemical method. The chemical method was used on samples, in the form of very thin slices of the formalin-fixed livers, the organs still retaining their haemoglobin content.

Due to the generally poor diet and relatively high rate of malnutrition existing among these people, it was thought that it would be of interest to see whether or not the foetuses and newborn infants would be affected, and suffer a deficiency in their iron supplies.

Finally, it was hoped that from this work some light might be shed on certain clinical problems, such as whether or not the umbilical cord should be tied early at birth, and also how the iron storage at birth affects the anaemias of early infancy.

HISTORICAL ASPECT.

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Iron has been known to man since prehistoric times. It was the custom of many ancient peoples to attribute the discovery of iron to some god or deity, hence information concerning the earliest use of iron is lost amongst the myths and folklore of the ancients. The Egyptians ascribed the discovery of iron to Osiris, the Greeks to Kadmos or Prometheus, the early Germanic tribes credited Odin with the discovery and the Romans attributed it to Vulcan. (Goldwater, 1935).

The writings of Homer and Hesiod contain allusions to iron, as do the Vedas, the Bible and many other ancient works. In the old annals of China, claim is made that iron was discovered in that country at a time which has been calculated to be about 2900 B.C.

Actual proof that iron was known and used in Egypt at the time of the early Kings of the Fourth Dynasty (about 3000 B.C.) has been adduced from inscriptions and sculptures dating from that period.

The earliest direct reference to iron as a curative agent is found in the writings of Apollodorus, dating from about 2000 B.C. According to this author, Iphycus, one of the Argonauts, was cured of impotence by a certain Melampus of Argas, who prescribed rust which had been scraped from an enchanted sword and dissolved in wine.

It is a familiar fact that the ancient Egyptian pharmacopeias described drugs principally of organic origin. Iron was one of the few inorganic medicines recognized by the early Egyptians. In the Ebers Papyrus which dates from about 1600 B.C. mention is made of the use of iron as a drug.

Hippocrates himself frequently mentions iron in his writings. He states that : "It is impossible for water, which comes from earth where there is iron, to be good. It must of necessity be hard and heating, difficult to pass by the urine, checking defaecation."

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The first test used for iron appears to have been that made by the physician Dioscorides Phakas, a nephew of Ptolemy XIII and a cousin of Cleopatra, in the First Century B.C. (Nierenstein, 1946). Papyrus soaked in aqueous extract of galls would give a definite reaction when mixed in a solution containing iron. Similarly, oak leaves soaked in iron-containing water, when dried, give hieroglyphics which would pass for Greek characters, the reaction being between the tannin in the veins of the oak leaves and the iron present in the solution or water. Dioscorides Phakas used this test for the detection of iron in blood, urine and sputum, after incineration and extracting both the ash and the galls with rainwater. One hundred years later Pliny used this test, and it has become generally known since as Pliny's test for iron, and is still officially in use to this day in Venezuela in the vinegar industry.

During the first century of the Christian Era iron seems to have enjoyed great popularity

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as a therapeutic agent, for we find many writers recommending iron for a great variety of uses. The elder Pliny, for example, states that iron rust is a valuable medicine, and recommends it as a protection against sorcery and ghosts, as well as against sudden pains in the side or chest. The famous Theriacum Andromachi, concocted by Nero's body physician to protect the Emperor from poisons, contained amongst its 64 ingredients some form of iron.

Galen is known to have used iron in his practice, for he specifically mentions employing the sulphate of iron for its astringent effect. Aëtius (about 500 A.D.) in his "Tetrabiblon" speaks of iron as a remedy for splenic engorgement.

Sydenham, the outstanding clinician of the 17th Century, frequently mentions iron in his writings and seems to have held the metal in high esteem for its therapeutic value.

About 1720 Pierre Pomet, chief druggist in the Court of Louis XIV, published his famous "Historie Generale des Drogues". This work was

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translated into English a few years after it appeared, and enjoyed wide circulation. Concerning iron Pomet says the following : "Saffrom of Mars (Crocus Martis) is an excellent remedy for dropsy and to cure the green sickness". He recommended a dose of from 10 - 40 grams, and believed that the most efficacious form of this drug was made by exposing plates of iron to dew, or sprinkling them with rainwater.

In a treatise written in 1715 Ettienne Franscois Geoffroy attributes the efficacy of iron to the fact that it enters into the blood, by which it is "diffused through the whole habit, its astringent virtue being everywhere exerted". Whether or not Geoffroy actually demonstrated the presence of iron in the blood we do not know. Most writers state that this most important discovery was made by Menghinis in 1746. According to Christian, Lemery and Geoffroy demonstrated the presence of iron in the ash of blood as early as 1713, and Menghinis was responsible for

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bringing this discovery to the attention of the medical world.

In 1886, Zaleski demonstrated the presence of iron-bearing bodies in various tissues of the body. This work was later confirmed and extended by Spitzer. The interchangeability of iron in food with iron in human tissues was demonstrated by Dastre and Floresco in 1890. In 1894 McCallum and Hall, working independently, traced the absorption of inorganic iron salts through the mucous membranes of the gastro-intestinal tract in animals. The same was done in man several years later by Hofmann. Quincke and Hochhause in 1895 actually traced the course of iron as it was absorbed from the small intestine into the lymph channels and thence into the blood, and showed that iron was stored in the spleen and liver as a reserve to be drawn on when needed by the blood.

The researches of Bunge and his students from 1885 to about 1905 aroused considerable interest in the problem of iron metabolism,

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chiefly as a result of their analyses of the amount of iron in the bodies of laboratory animals at different ages. It was clearly demonstrated that while the total amount of iron in the body of newborn rabbits, kittens and puppies was stationary or very slightly increased during the suckling period, yet the percentage of iron became markedly decreased. When the mineral composition of the newborn animal was compared with the analysis of the milk of the same species, an essential similarity was observed between the magnitude of the relative proportions of each element in the body and in the milk - except for iron. Milk was found to contain much less iron than many common foods; the newborn animal was found to contain a higher percentage of iron than the adult. From a teleological viewpoint, in order to offset the small amount of iron in the milk the newborn animal was apparently granted a reserve supply of iron that was demobilized for the necessary production of haemoglobin and other iron compounds during the rapid growth of the suckling period.

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In 1899 Hugounenq undertook the determination of iron in the human foetus at different ages. He showed that until the seventh month of gestation the percentage of iron in the foetus was small and was approximately constant per unit of weight. During the last third of the gestation period, however, the deposition of iron in the body of the foetus was very much increased. Hugouneng concluded from his investigations that Bunges' theory of the presence of a reserve supply of iron in the body at birth was also applicable to the human.

In 1932, Gladstone investigated the total iron in the livers of human foetuses and infants, and attempted to distinguish haemoglobin from non-haemoglobin iron by directly estimating the haemoglobin present in the organs. Ramage et al (1933) examined human infant livers spectrographically for iron, their results being the total iron present.

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Most recently of all, work has been done by Pommerenke et al.(1942) on the transmission and storage of iron in the human foetuses, using the radio-active metal. By the use of these radio isotopes it is possible to trace labelled particles of iron in their journeys about the body, and to find them finally in their depôts, the largest depôt always being the liver.

MATERIALS AND METHODS.

MATERIALS AND METHODS.

The material consisted of 49 livers and 22 spleens obtained at post-mortem from the foetuses and infants which were born or had died at the Bridgman Memorial Hospital and at the Coronation Hospital. Only two livers and spleens were obtained from the latter hospital (Numbers 44 and 49), the remainder coming from the Bridgman Hospital.

Without exception, all the livers and spleens were obtained from Bantu foetuses and infants. The age period covered by these specimens was from 28 weeks of gestation to 18 days neonatal; the bulk of the material being full term stillbirths and infants who had died very early in the neonatal period. It was hoped that infants up to four months of age would also be obtained for investigation, but as the main source for these older infants was Coronation Hospital, where less material came to the post-mortem room, combined with the fact that Bantu parents were loath to give permission for post-mortems, no satisfactory specimen was obtained.

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of the dried, powdered and mixed perfused liver were 19.2 milligrams and 19.3 milligrams of iron.

A small section was removed from the right lobe of liver Number 10 before it was perfused. This small piece was analysed for its iron content and from it the total iron of the whole liver was calculated by adjusting the result to the weight of the liver. The total iron in the liver by this means was 51 milligrams. The liver was then perfused free of haemoglobin. A further section was removed from the right lobe and another from the left lobe. These were analysed for iron and on their results the iron content of the perfused liver was calculated. The iron content based on the sample of right lobe was 6.4 milligrams of iron, that based on the sample of left lobe was 7.08 milligrams. The difference in these results again indicates an uneven distribution of the iron, with a slight preponderance in the left lobe of the liver. The results obtained from two samples of the dried, powdered and mixed perfused liver were 7.52 and 7.4 milligrams of iron. The great difference between these

Only premature infants who were stillborn, or those which had lived less than one day were taken. Those that had lived one day or more were discarded, as it was felt that the breakdown of red blood cells with the possible deposition of haemoglobin iron would confuse the picture for this particular investigation. Infants who had died in the neonatal period were only taken if they were full term, as any degree of prematurity would complicate matters. Finally, only full term stillbirths were used to bridge the gap between the premature infants and the neonatal infants.

Except for two cases (Numbers 36 and 49), the post-mortems were done 24 to 48 hours after death, and in a few instances, even after a longer period than this. The chief reasons for this delay were, first of all, the tardy contacting of fathers and family for permission to conduct a post-mortem, and secondly a request by the hospital authorities not to remove the liver and spleen until a burial order had been obtained for the case. As the post-mortem room at the Bridgman Hospital has no refrigerator, infants that had been dead for three days or more were not used.

After opening the abdomen all the peritoneal fluid was drained away, so that when the liver was cut free, the blood that escaped from the hepatic vein could be collected in the hepato-renal pouch without dilution. The gall-bladder and bile duct were always dissected free of the liver and discarded. The liver and spleen were removed whole and placed in a suitable glass jar for transport to the laboratory.

Hitherto, the great majority of workers in this field have estimated the iron content of the human infant liver by removing a small section of the organ and estimating the amount of iron present in it. With this information they have calculated the iron present in the whole liver by adjusting the result obtained relative to the weight of the liver. Not at any stage do they consider the iron that is present in the blood of the organ. That this iron may be a considerable and significant amount is emphasized

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by Barcroft who states : "----- the method which Ramage and his colleagues described for the preparation of liver samples did not exclude the blood that was in the vessels of that somewhat vascular organ. We have, in fact, seen, a few pages back, the iron in the lung used as a measure of the amount of blood in its vessels".

In the field of animal research attempts have been made to separate haemoglobin iron from true hepatic iron. McDougall (quoted by Barcroft) cut the livers of sheep foetuses into slices and then rinsed these in water until they were colourless. More recently, vivi-perfusion has been used, especially in dogs (Kletzien 1933; Bogniard,1932). In this method a modified Ringer-Locke solution was run into a large vein while the blood of the animal was drained away through another large vessel. By this means all the blood can apparently be washed out of the body and the animal kept alive for 20 minutes or more.

Hueck (quoted by Gladstone, 1932), working on adult human liver, washed the organ free of blood apparently by the same method as used by McDougall. Gladstone, in his investigation of

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iron in human infant livers, attempted to estimate the amount of haemoglobin iron present by removing a small portion of the liver, weighing it, mincing it, and making it up to a known quantity of solution with distilled water. He then estimated the amount of haemoglobin present in the filtrate of such a solution by the acid haematin method, and then calculated the amount of iron, using the fact that haemoglobin contains 0.335% iron by weight. He then adjusted the result obtained to the weight of the whole liver. These methods to distinguish non-haemoglobin iron in the human liver, as will be shown later, are probably inaccurate and unsatisfactory.

Efforts at distinguishing haemoglobin iron and non-haemoglobin iron in the liver of the human foetus and infant in this work have been divided into two groups. The first, designated Method 1, was a direct method of approach, in which attempts were made to rid the organ of haemoglobin. The second, designated Method 11, was an indirect method, in which the iron due

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to the blood was calculated, and then subtracted from the total iron in the blood-filled organ.

METHOD 1.

McDougall's method was used in the first attempt at washing out the haemoglobin from the liver. This consisted in cutting the liver into thin slices and washing these in running water. The method was used on liver Number 1. After the organ had been carefully weighed it was cut into thin slices, approximately 2 to 3 millimeters thick, and these were washed in running water for 20 hours. After this period they looked pale and yellow and quite free of haemoglobin. The slices were then placed in an air oven to be dried to constant weight. After four hours the slices were considerably shrunken, and it was seen that a small amount of haemoglobin in solution had oozed out of the contracted tissues, showing that, in spite of the prolonged washing, not all the haemoglobin had been removed. This method was thus abandoned.

The next method of directly removing the haemoglobin from the liver was much more

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successful. It consisted of perfusing normal saline at low pressure through the umbilical vein, and sometimes also through the portal vein, until the organ was blood-free. The remainder of the livers included in Method 1 (Numbers 2 - 14) were dealt with along these lines.

The amount of normal saline required to achieve the desired result varied from ten litres (Number 6) to eighty litres (Number 8). When completely perfused the livers were pale yellow in colour, and when they had been heated and dried to constant weight, they still retained this pale yellow appearance. Had any haemoglobin still been present, the colour would have darkened with the heating and drying, as haemoglobin itself becomes black in colour. In addition, when the filtrate made from a small section of the perfused liver was examined spectroscopically, no evidence of haemoglobin absorption bands were seen. This method of perfusion therefore appeared to be satisfactory in as far as the removal of the haemoglobin was concerned.

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After perfusion the liver was placed in an air oven and dried to constant weight. Two small pieces were then removed from the dried organ for iron analysis. As can be seen from the results obtained in Numbers 2 and 4, the final result calculated from each sample differed considerably. In liver Number 2 the total iron content, in milligrams, of the whole liver was 11.8 from one sample, while from the second sample it was 12.2. In liver Number 4 the difference was even greater, being 3.26 milligrams and 4.8 milligrams of iron respectively. This was taken to indicate that the iron was not evenly distributed throughout the liver, so to overcome this difficulty it was decided to crush the whole of the dried organ and to mix the powder, thus ensuring an even distribution of iron. This was done, the dried liver being crushed in a mortar and pestle to a fine powder, which was then thoroughly mixed by rolling it on a large sheet of paper.

Two specimens taken from this powder yielded results which compared closely with one another,

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usually differing by less than 2%, this difference probably being experimental error. This method of powdering the dried liver and mixing the powder before taking samples for analysis was adopted for all subsequent livers, even those dealt with under Method 11.

This was done with livers Numbers 6 and 7, the results being 8.02 milligrams and 7.88 milligrams of iron for liver Number 6; and 5.29 milligrams and 5.36 milligrams of iron for liver Number 7.

In liver Number 8 a further step was taken. A small section was removed from the fresh unperfused liver and analysed for its iron content. From this result the total iron content of the whole liver was calculated. The result was 70.9 milligrams of iron. This amount included the haemoglobin iron and probably was not a true reflection of the iron content since the metal is irregularly distributed throughout the organ.

The liver was then perfused free of haemoglobin with normal saline. Another small section was removed from the liver at this

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stage and analysed for iron. The result obtained was adjusted to the corrected weight of the whole perfused liver. It was 13.4 milligrams of iron. Next, the perfused liver was dried in the air oven to constant weight, crushed to a fine powder and mixed. Two samples were taken for iron estimation and the iron content of the perfused dried liver calculated from the results obtained. These were 7.1 milligrams of iron from the first sample and 7.05 milligrams of iron from the second sample. The large difference between the results obtained from the section of wet perfused liver and those from the dried, powdered and mixed perfused liver indicates again a probable uneven distribution of iron.

The very large difference in results between the total iron estimated from the section of wet unperfused liver (70.9 milligrams) and those of the dried, powdered and mixed perfused liver 7.1 and 7.05 milligrams) can hardly be accounted for by the haemoglobin iron alone. If it were so, the difference (63.8 milligrams of iron) would represent 95 ccs. of blood containing

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20 grams of haemoglobin per 100 ccs, (taking this latter figure for purposes of argument), and as the original volume of this liver was 145 ccs, the blood in it would make up approximately 65% of the liver's volume - which is absurd. This leads to the conclusion, if the figure for the total iron is nearly correct, that during perfusion not only haemoglobin iron is washed out but also a certain amount of true hepatic non-haemoglobin iron.

Liver No. 9 was perfused free of haemoglobin with normal saline. Then two small sections were taken from the wet liver, one from the right lobe and the other from the left lobe. These were analysed for iron and on their results the total iron for the whole perfused liver was calculated. The result for the whole liver from the section taken from the right lobe was 18.3 milligrams of iron, while that based on the section from the left lobe of the liver was 28.75 milligrams of iron. Such a large difference indicates an uneven distribution of iron in the liver, with a preponderance of the metal in the left lobe. The results obtained from two samples

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of the dried, powdered and mixed perfused liver were 19.2 milligrams and 19.3 milligrams of iron.

A small section was removed from the right lobe of liver Number 10 before it was perfused. This small piece was analysed for its iron content and from it the total iron of the whole liver was calculated by adjusting the result to the weight of the liver. The total iron in the liver by this means was 51 milligrams. The liver was then perfused free of haemoglobin. A further section was removed from the right lobe and another from the left lobe. These were analysed for iron and on their results the iron content of the perfused liver was calculated. The iron content based on the sample of right lobe was 6.4 milligrams of iron, that based on the sample of left lobe was 7.08 milligrams. The difference in these results again indicates an uneven distribution of the iron, with a slight preponderance in the left lobe of the liver. The results obtained from two samples of the dried, powdered and mixed perfused liver were 7.52 and 7.4 milligrams of iron. The great difference between these

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latter results and that of the total iron calculated from the small piece of fresh unperfused liver, again can hardly be accounted for entirely by haemoglobin iron. The difference, 43.5 milligrams of iron, would represent 71 ccs blood containing 20 grams haemoglobin per 100 ccs. This volume of blood is obviously too high, since the original volume of the whole organ was only 120 ccs. The conclusion that must follow is that the large difference in iron content between the fresh and the perfused liver must be due, partly to the haemoglobin iron being removed and in addition, partly to some non-haemoglobin iron of the tissues also being washed out at perfusion. It must be kept in mind that since the distribution of iron appears to be irregular, the result for total iron obtained by means of only a small section of fresh liver may not be accurate.

Two sections of fresh unperfused liver were taken in Number 11 and analysed for iron. The one section was taken from the right lobe and on the result obtained from this piece the total iron of the whole liver was calculated to be 68.9 milligrams. The result based on the portion from the left lobe was 75.6 milligrams of iron. Thus, there is a difference of approximately 9% between these figures, with a slight preponderance in the left lobe of the liver. The difference may again be accounted for by an uneven distribution of iron in the liver tissue and/or a greater congestion of blood in the left lobe of the liver. The organ was then perfused with normal saline till free of haemoglobin. A further section was taken from the right lobe and another from the left lobe and these were investigated for iron. The perfused liver contained 8 milligrams of iron based on the result obtained from the section of right lobe, while the result of the calculation based on the portion of left lobe was 9.16 milligrams of iron. There is a difference of 14.5% between these figures, with the preponderance of iron again in

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the left lobe. The perfused liver was dried, powdered and mixed, and two samples analysed for iron gave the results for the whole perfused liver as 9.01 and 9.12 milligrams, of iron. The difference between the lower of the two results obtained for the total iron in the unperfused liver (68.9 milligrams) and that obtained for the iron content of the perfused and powdered liver is approximately 60 milligrams of iron. This would represent 90 ccs of blood containing 20 grams of haemoglobin per 100 ccs, which, in a liver whose total volume was 140 ccs, is an unreasonably high figure. The conclusion must again be arrived at that the difference of 60 milligrams of iron must be made up not only of the haemoglobin iron that has been perfused out, but also of a certain amount of non-haemoglobin hepatic iron that must have been washed out with it during the perfusion.

Livers Numbers 12 and 13 were dealt with along exactly the same lines as the preceding Liver Number 11, with very similar results. In both, there were startling differences between the total

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iron of the unperfused liver calculated from the right lobe as compared with those calculated from the left lobe. In Number 12, the right lobe sample gave the result of 29 milligrams of iron, whereas the left lobe sample gave the result of 37.9 milligrams of iron. In Number 13 the results varied even more. The total iron in the unperfused liver from the right lobe sample was 51 milligrams of iron, whereas calculated from the left lobe sample it was 75.5 milligrams of iron. In samples from the right and left lobe of the perfused livers marked differences were again found, with higher concentrations of iron in the samples from the left lobe.

The investigation was carried a stage further in Liver Number 14. After the viscus had been weighed and its volume measured, a small section was removed from its anterior edge near the midline, for haemoglobin estimation. From this it was calculated that the whole unperfused liver contained 2.35 grams of haemoglobin; and since haemoglobin contains 0.335% of its weight of iron,

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this represented 7.86 milligrams of iron.

Next, a small piece of right lobe was removed for total iron analysis and likewise a small piece of left lobe. The results obtained for the total iron of the whole unperfused liver were 44.36 milligrams of iron based on the analysis of the piece from the right lobe, and 52 milligrams of iron based on the piece taken from the left lobe. The liver was then perfused with normal saline and the first 2000 ccs perfusate collected for haemoglobin estimation. The greater part of the haemoglobin in the liver is washed out with the first 2000 ccs perfusion fluid. The amount of haemoglobin in this fluid was found to be 2,200 milligrams, representing 7.37 milligrams of iron, which corresponded fairly well with the previous estimation of haemoglobin iron in the liver.

A further small section was taken for iron analysis from the right lobe of the perfused liver and another from the left lobe. The total iron content of the perfused liver based on the analysis of the section from the right lobe was 15.12 milligrams, while that based on the

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analysis of the section from the left lobe was 16.53 milligrams. An analysis of a sample of the dried, powdered and mixed perfused liver gave the total iron content of the perfused liver as 15.3 milligrams.

The difference in results between total iron of the unperfused liver and total iron of the perfused liver should be due to the haemoglobin iron which has been perfused out. Taking the lower of the two figures obtained for total iron in the unperfused liver (namely, 44.36 milligrams) and comparing it with the figure obtained for the iron content of the perfused liver (15.3 milligrams), we see that there is a difference of 29 milligrams of iron. This should represent haemoglobin iron, but we know that only 7.37 to 7.86 milligrams of haemoglobin iron was found by direct experimentation. Hence, the conclusion that non-haemoglobin or tissue iron is being lost on perfusion seems inevitable.

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Since the liver is a main centre of metabolism and is richly supplied with enzymes, it is not inconceivable that autolysis must commence soon after death. In addition, since the post-mortem material is not kept in a refrigerator at the Bridgman Hospital, there is nothing to delay or retard this autolysis. Hence, when these livers are brought to the laboratory they must all have certain autolytic changes present after 24 - 48 hours or more, and certainly one would suspect that the cell membranes do not function as efficient barriers to the intracellular water-soluble contents. Since it is known that a large quantity of the iron in the liver is in the form of ferratin, an iron-containing protein complex, which is soluble in water, then in spite of using normal saline as a perfusing agent, it would not be surprising if a proportion of the non-haemoglobin iron were washed out of the liver tissue during perfusion. Certainly the results obtained from the above experiments strongly indicate that tissue iron is being washed out during perfusion. The same must

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apply if the liver were cut into slices, and the slices washed in running water or saline.

As mentioned before, the total iron of the unperfused liver (Number 14) when calculated from the specimen of right lobe was 44.36 milligrams of iron, while the total iron calculated from the specimen of left lobe was 52 milligrams of iron. This again serves to indicate the uneven distribution of iron in the liver, with the preponderance of iron in the left lobe. Similarly, when specimens of right and left lobe of the perfused liver were analysed, the iron for the perfused liver based on the findings of the right lobe was 15.1 milligrams whereas from the left lobe it was 16.53 milligrams of iron.

The results of the analysis of the liver as described above strongly indicate that iron is irregularly distributed in the liver, inasmuch as that specimens taken from the left lobe always, in this series at least, showed a greater iron content than specimens taken from the right lobe.

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It therefore follows that suspicion must fall on the accuracy of the results obtained by other workers in this field of investigation, for they all based their calculations on small sections taken from the livers they were investigating. Liver Number 13 of this series illustrates very well the degree of inaccuracy that may be attained by using small sections only, since from one section the total iron was calculated as 51 milligrams, whereas from another section the result was 75.5 milligrams - a difference of 48% ! Hence, for accurate results, the whole liver must be used, or some method must be adopted by which a homogeneous distribution of the iron is ensured. The simplest method seems to be by drying the whole liver to constant weight, crushing it to a fine powder and then thoroughly mixing the powder, before a sample is taken for analysis.

No attempt will be made to explain the constant preponderance of iron in the left lobe of the livers of this series, nor any effort made to elucidate its significance.

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The direct approach to the problem of distinguishing haemoglobin iron from non-haemoglobin iron in the liver thus appears unsatisfactory. If the liver is sliced and then washed, not all the haemoglobin is got rid of and a certain amount of non-haemoglobin iron must be washed away; and if the liver is perfused with normal saline the organ can be rendered haemoglobin-free, but non-haemoglobin iron is washed out as well. Thus the direct approach was abandoned.

METHOD 11.

At post-mortem the liver was removed as before, the gall-bladder and bile duct being dissected off and discarded. In cases Numbers 28 - 49 the spleen was removed intact at the same time.

In the laboratory the blood that had oozed out of the liver was collected. Icc was diluted with distilled water to 50 ccs volume. After shaking, it was left overnight in the refrigerator. The next day, from an aliquot,

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usually 2 - 5 ccs, the amount of haemoglobin present was estimated by the alkaline haematin method, the details of which will be described later. From this, the amount of haemoglobin present in 1 cc of the blood was calculated. A further 1 cc of blood was placed in a large pyrex test-tube. This blood was then analysed for its iron content by the method to be described in detail later. The result incorporated the total iron present in 1 cc of blood. In all cases, except Numbers 36 and 49 where the organs were obtained on the day of death, the iron content of the blood was found to be higher than the corresponding amount of haemoglobin estimated to be present in the blood would warrant. At the same time the haemoglobin values were all low, some less than half of what one would have expected. This is not at all surprising considering that the blood must have undergone post-mortem changes with probable breakdown of the complex haemoglobin molecule. Furthermore, these changes should occur especially quickly in the blood of the liver, due to the liver enzymes present and the early autolysis

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that takes place in this organ. In Numbers 36 and 49, where the investigations were done soon after death, the haemoglobin value corresponded well with the iron value of the blood.

Wright (1947) described the iron in the blood as existing in three forms : (i) as haemoglobin iron in the red blood cells; (ii) as so-called "easily split iron" in the plasma, amounting to about 5% of the total blood iron and consisting of various degradation products of haemoglobin, and (iii) serum or plasma iron, amounting to 0.05 - 0.18 % . In the post-mortem blood obtained from the liver it is most probable that the haemoglobin iron is lowered and the iron due to haemoglobin breakdown products is much increased. Thus, it follows that an inaccurate figure for blood iron must be obtained if the quantity of iron is calculated from the total amount of haemoglobin in the organ, as was done by Gladstone (1932), unless investigations were carried out soon after death, before post-mortem changes had appreciably set in.

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Up to this stage the haemoglobin value in 1 cc of the blood which oozed out of the liver, as well as the iron content of 1cc of this blood has been estimated.

After carefully weighing the liver and measuring its volume, the organ was mushed to a fine pulp and made up to 500 ccs volume with distilled water. It was then shaken for ten minutes and allowed to stand for half-an-hour, then shaken again for a further five minutes. The volumetric flask was placed in the refrigerator overnight. The following day all the fine solid particles were found to be deposited at the bottom of the flask, and all the haemoglobin present was in solution. An aliquot of this solution, usually 2 - 5 ccs, was taken and the haemoglobin content estimated by the alkaline haematin method. From this result the haemoglobin present in the 500 ccs solution was calculated,

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this amount representing the total haemoglobin present in the liver. Knowing from a previous estimation how much haemoglobin is present in 1 cc blood, it is a simple calculation to deduce the volume of blood present in the liver. With this knowledge, the iron due to the blood in the liver can be accurately calculated, since it is already known how much iron is present in 1 cc of the blood.

Thus, for example, if 1 cc of blood contains 100 milligrams of haemoglobin and 1cc of the same blood has 0.5 milligram of iron, and the total liver is found to contain 1000 milligrams of haemoglobin, then the volume of blood that must be present in that liver is $\frac{1000}{100}$, which is 10 ccs. And as 1 cc blood contained 0.5 milligram of iron, 10 ccs must contain 5 milligrams of iron. Thus, the iron in the liver due to the blood present is 5 milligrams.

The entire contents of the volumetric flask containing the liver in solution was then poured into a large evaporating dish and dried for 20 - 24 hours in an air oven at 105°C to constant weight. The dried residue, representing

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the complete liver, was carefully weighed. The next step was to crush it to a fine powder, and then mix the powder thoroughly by rolling it on a large sheet of paper.

Samples of this powder were taken and analysed for their iron content and from these results the total iron of the whole liver was calculated. From this result was subtracted the iron due to the blood present in the liver, and this final result then represented the true hepatic iron, or iron in the blood-free organ.

It can be realized, therefore, that the haemoglobin was used merely as a colour index for calculating the volume of blood present in the organ. At no stage was the amount of haemoglobin directly interpreted in terms of blood iron, as this must be an inaccurate procedure since the original amount of haemoglobin present must have undergone partial post-mortem changes.

The spleens were dealt with along exactly the same lines as the livers. The volume of blood in the livers and spleens found here must

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obviously be lower than the volume normally present in the living state, since a good deal of the blood in the large vessels oozes out after removal of the organs at post-mortem.

METHOD OF ESTIMATING HAEMOGLOBIN.

The haemoglobin content of blood and tissue solutions was estimated by the alkaline haematin method of Wu (1922). E.J. King et al. (1944), in an extensive and detailed article on the accuracy of various methods for determining haemoglobin, came to the conclusion that the alkaline haematin method was the most accurate for routine use, being definitely more exact than the popularly used acid haematin method. The most exact method of all, apparently, was the cyanmethaemoglobin method, but as this was much more complicated it was not suited to routine use, and its accuracy was only slightly greater than the alkaline haematin method used here. With the alkaline haematin method, plasma proteins, lipoids and bile pigments have no effect on the intensity of the colour produced by the haemoglobin.

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A standard graph for haemoglobin values was made by using known strengths of a haemoglobin solution, whose haemoglobin content had been accurately estimated with the aid of a standard solution of haemin, the iron content of which was known. (Dr. Bloomberg, Senior Lecturer in Physiology, kindly checked this estimation).

5 ccs of the haemoglobin solution were made up to 10 ccs volume with $^{N}/_{5}$ Sodium hydroxide and the mixture placed in a boiling water bath for five minutes. After cooling to room temperature, it was poured into an Evelyn tube. An Evelyn photo-electric colorimeter was used, with a 540 filter, the transmission being set at 100% with distilled water. Density readings (G) of known strengths of haemoglobin solution were taken and the standard graph constructed from the L values (L being equal to 2 - Log.G. For actual details of the graph see Appendix).

Suitable aliquots of the unknown solutions of haemoglobin were then made up to 10 ccs volume

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with $^{N}/5$ sodium hydroxide. After following the above routine their L values were estimated and were read off against the standard graph to obtain the actual haemoglobin content.

METHOD OF ESTIMATING IRON.

The thiocyanate method as described by Truter (1947) was used throughout this investigation. The reagents used were :

(1) A standard iron solution made up with ferrous ammonium sulphate in such a manner that 1 cc of the solution was equal to 0.1 milligram of iron, this being used as the stock solution. A working standard was made by diluting 100 ccs of the above solution to 1000 ccs, so that 1 cc contained 0.01 milligram of iron. This solution was used to plot the standard graph.

(2) A 50% potassium thiocyanate solution.

(3) Reagent grade concentrated sulphuric acid.

Redistilled concentrated nitric acid.

Redistilled 60% perchloric acid.

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(4) Hydrogen peroxide; 0.1% solution in doubly distilled water, kept in a brown bottle in the refrigerator.

(5) Iso-amyl alcohol.

It is a well known fact that dry ignition of biological materials for iron analysis yields erroneous results if special care if not taken. The wet combustion method has proved not only to be safe but advantageous in many respects. All pyro- or metaphosphates are, for instance, hydrolysed to the ortho form, thus doing away with a special hydrolysis during the sample preparation.

THE COMBUSTION PROCEDURE :

1 cc of blood, or up to 1 Gram of dried liver powder, or up to 3 grams of wet liver tissue, depending on the suspected iron content, is measured into a 8 x 1 inch pyrex tube. A glass bead, 2 ccs of concentrated sulphuric acid, 3 ccs of 60% perchloric acid and 0.5 cc of concentrated nitric acid are added and the material digested over a micro-flame in a fume cupboard. Great care must be taken to avoid loss by spluttering. When the initial, sometimes

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vigorous, oxidation reaction has subsided, nitric acid is added drop-wise until the oxidation is completed and the solution clarified. The final solution should be colourless or, at most, a greenish-yellow colour. If the nitric acid does not clarify the solution the contents are allowed to cool slightly, a further 0.5 to 1 cc perchloric acid added and the heating continued. A few more drops of nitric acid may be necessary to clarify the solution. The heating is continued until the appearance of the characteristic dense white fumes of sulphur trioxide.

At this stage the solution may be expected to be free from perchloric and nitric acids. However, decomposition products of these acids are sometimes tenaciously retained by the concentrated sulphuric acid (e.g. nitrosyl-sulphuric acid), even after prolonged boiling. Hydrolysis with 2 ccs of distilled water and subsequent boiling until white fumes appear,

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effect the removal of these products. The digest is allowed to cool, a further 10 ccs distilled water are added and quantitatively transferred to a 100 ccs volumetric flask. Cool to room temperature and make up to volume with distilled water. The distilled water used throughout was triple glass distilled.

PROCEDURE OF DETERMINATION :

After a test experiment has been done to determine the approximate iron content of the sample, transfer the chosen aliquot to a graduated cylinder fitted with a glass stopper. Adjust the acid concentration if necessary. Add 1 cc of the 0.1% hydrogen peroxide solution and fill up to the 25 ccs mark with distilled water. Add exactly 10 ccs isoamyl alcohol and then add 2 ccs of the 50% potassium thiocyanate solution. Close the cylinder with the stopper and shake for one minute. Allow to stand until the amyl alcohol phase has separated out, then transfer the phase to a centrifuge tube and centrifuge for five minutes at 3,500 r.p.m. to get rid of water particles suspended in the alcohol extract. The water-free amyl alcohol is transferred to an Evelyn tube. A 490 filter is used in the Evelyn photo-electric colorimeter and the light transmission is set at 100% with a reagent blank. The L value is estimated for the unknown solution from its density reading (G). Using L, the iron value is read off a standard curve, made previously by using the standard iron solutions.

R E S U L T S .

RESULTS.

The results of the investigations done on livers Numbers 1 - 14 have already been discussed in the previous section, and no further mention of them will be made here. For the sake of interest, however, these results are set forth in Table No. 1 (Page   ), but the figures will not be used for the general discussion nor for the conclusions, since they are probably inaccurate.

Only the results obtained from livers (Numbers 15 - 49) and spleens (Numbers 28 - 49) dealt with by Method 11, will be considered. In the text and in the tables and graphs, the term "non-haemoglobin iron" is used to denote the actual iron that is present in the hepatic or splenic tissue, and excludes all the iron which is present in the blood of the organ, whether it be in the form of haemoglobin, or of breakdown products of haemoglobin, or as serum iron.

The iron content of the livers varied considerably, even in livers of similar size and weight. This applied to both total and

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non-haemoglobin iron. Two sets of twins were investigated in this series, both sets being binovular and premature (Numbers 16 and 17; 42 and 43). The iron content of the livers corresponded fairly closely for one set of twins, the total iron content being 6.5 milligrams and 7.4 milligrams for Numbers 42 and 43 respectively. Their non-haemoglobin iron content also compared well, being 3.8 milligrams and 3.6 milligrams respectively. There was, however, a great variation in the iron content of the livers of the other set of twins (16 and 17), being 18.8 milligrams and 36.2 milligrams respectively for total iron. For non-haemoglobin hepatic iron the results were 10.1 milligrams and 23.2 milligrams iron respectively.

/ TABLE No. 1 ....

TABLE No. 1.

DATA AND RESULTS OF LIVERS, NUMBERS 1 - 14, DEALT WITH BY METHOD 1.

No.	AGE	BIRTH WEIGHT	WEIGHT OF LIVER (Grams)	TOTAL IRON IN LIVER (Mgms)	NON-HAEMO-GLOBIN IRON IN LIVER (Mgms)
1.	F.T. S.B.	8lbs.6ozs.	142		
2.	F.T. 10 d.	6lbz.1oz.	70.		12
3.	F.T. S.B.	7lbs.	130		
4.	S.B. 36 w.	4lbs.14 ozs.	99		4
5.	F.T. S.B.	6lbs.8ozs.			
6.	F.T. 6 d.	6lbs.	100		7.95
7.	F.T. S.B.	7lbs.10ozs.	148		5.3
8.	F.T. S.B.	8lbs.	160	70.9	7
9.	F.T. S.B.	7lbs.9ozs.	191.5		19.25
10.	F.T. S.B.	8lbs.	136.5	51	7.5
11.	F.T. S.B.	8lbs.8ozs.	155.5	Av.72.25	9
12.	F.T. 1 d.	6lbs.	109.5	Av.33.5	9
13.	F.T. S.B.	6lbs.2ozs.	106.5	Av.63.25	11
14.	F.T. 6 d.	5lbs.10ozs.	113	Av.48.2	15.3

F.T. = FULL TERM
S.B. = STILLBIRTH
Av. = AVERAGE

d. = DAYS
w. = WEEKS.

The non-haemoglobin, or true hepatic iron, showed a well defined absolute increase from 28 weeks to full term. From the livers investigated in premature infants, values of 3.6 milligrams of iron were obtained at 28 weeks, gradually increasing to 15.7 milligrams at 36 weeks and finally increased to an average of 33.27 milligrams of iron at full term. (Table No. 2).

Calculated in relation to the weight of both the wet and dried livers there is clearly a relative increase as well in the amount of iron deposited in the liver tissue. (Table No. 3.). If 100 grams of the wet liver is taken as a standard, the deposit of iron at 28 weeks was found to be 10.1 milligrams, at 36 weeks 26.8 milligrams, and the average for full term stillbirths was 28.16 milligrams. Using 100 grams of dried liver as a more accurate standard, at 28 weeks there was 55 milligrams of iron, at 36 weeks 108 milligrams of iron and at full term 140.6 milligrams of iron.

/ TABLE No. 2. ...

TABLE NO. 2.

DATA AND RESULTS OF LIVERS AND SPLEENS, NUMBERS 15 - 49, DEALT WITH BY METHOD 2.

No.	Age.	Birth Weight lbs. ozs.	Weight of Liver. (Grams)	Weight of Dried Liver (Grams)	Total Iron in Liver (Mgms)	Non-Hb. Iron in Liver (Mgms)	Weight of Spleen (Grams)	Weight of Dried Spleen (Grams)	Total Iron in Spleen (Mgms)	Non-Hb. Iron in Spleen (Mgm)
43	SB. 28w.	2. 0.	35	6.2	7.4	3.6	1.5	0.25	.24	.005
42	S.B.28w.	2. 4.	37.5	6.9	6.5	3.8	2	0.25	.28	0.02
47	S.B.29w.	2. 8.	39.8	7.3	10.4	6.6	1.8	0.4	.5	0.37
33	S.B.33w.	4. 4.	49.3	10.5	8.9	7	6.3	1.3	1.8	1.5
40	S.B.34w.	4. 0.	53	9.7	6.8	4.3	3.7	0.7	.95	0.6
17	S.B.35w.	4.12.	68	14	36.2	23.2				
16	S.B.35w.	5. 0.	68.5	11	18.8	10.1				
20	S.B.36w.	4.14.	58.5	14.5	20.6	15.7				

F.T. = FULL TERM   d = days

S.B. = STILLBIRTH   w = weeks

NON-Hb. = NON-HAEMOGLOBIN.

TABLE No. 2 - Continued ...

TABLE No. 2 - Continued.

DATA AND RESULTS OF LIVERS AND SPLEENS, NUMBERS 15 - 49, DEALT WITH BY METHOD 2.

No.	Age.	Birth Weight lbs.ozs.	Weight of Liver (Grams)	Weight of Dried Liver (Grams)	Total Iron in Liver (Mgms)	Non-Hb. Iron in Liver (Mgms)	Weight of Spleen (Grams)	Weight of Dried Spleen (Grams)	Total Iron in Spleen (Mgms)	Non-Hb Iron In Spleen (Mgms)
41	F.T. S.B.	6. 0.	113.5	20.5	41.7	32.5	4.4	0.7	2.9	2.5
29	F.T. S.B.	6. 4.	156	32.5	53.1	34.4	5.5	1	2.6	1.7
36	F.T. S.B.	7. 0.	97.5	20.5	22.6	16.7	4.5	1	0.61	0.37
15	F.T. S.B.	7. 2.	116.5	25.7	47.9	35.5				
30	F.T. S.B.	7. 6.	99.5	24	37.4	30.2	6.5	1.25	3.2	1.9
26	F.T. S.B.	7.12.	108	19	44.6	34				
34	F.T. S.B.	7.12.	103.5	18.2	55.3	46	12.1	2.6	4.8	2.1
21	F.T. S.B.	7.13.	104.5	15.5	45.1	35.4				
32	F.T. S.B.	8.0	110	21.8	26.3	21.8	7	1	1.26	0.6
37	F.T. S.B.	8. 0.	175.6	34	94.7	72.2	8.3	2	4	2
24	F.T. S.B.	8.10.	141.5	28	33.1	20.2				
39	F.T. S.B.	9. 4.	126.5	24.5	42	30.3	10	2.8	4.3	2.4

F.T. = FULL TERM   d = days
S.B. = STILLBIRTH   w = weeks
NON- Hb. = NON-HAEMOGLOBIN.

TABLE No. 2 - Continued ...

TABLE No. 2 - Continued.

DATA AND RESULTS OF LIVERS AND SPLEENS, NUMBERS 15 - 49, DEALT WITH BY METHOD 2.

No.	Age.	Birth Weight Lbs.ozs.	Weight of Liver (Grams)	Weight of Dried Liver (Grams)	Total Iron in Liver (Mgms)	Non-Hb. Iron in Liver (Mgms)	Weight of Spleen (Grams)	Weight of Dried Spleen (Grams)	Total Iron in SPLEEN (Mgms)	Non-Hb. Iron in Spleen (Mgms)
38	F.T. 1d.	7. 0.	105.5	20.2	41.2	30.4	5.2	1.2	2.8	2.3
49	F.T. 3d.	7. 0.	109.5	23.7	30.2	26.6	7.8	1.8	3.98	3.5
25	F.T. 4d.	6. 4.	92.3	22.3	42.6	37				
18	F.T. 7d.	5. 8.	82	16	32.8	25.5				
22	F.T. 7d.	5. 6.	76.5	18	25.3	19.2				
35	F.T. 7d.	5.12.	78.8	18.8	28.7	26.3	6	1.5	4.8	4.4
46	F.T. 7d.	7.11.	94.3	24.3	37.4	30	3.5	0.8	3.3	3.1
23	F.T. 8d.	5.14.	75	16	45.8	40.1				
27	F.T. 8d.	5. 6.	101	20.5	104	93.5				
28	F.T. 8d.	6. 3.	113.5	21.5	111	97.5	10.3	1.8	4	3.1
31	F.T. 8d.	6. 8.	82.6	17.6	19.9	15.2	6.5	1.5	2.6	2.1
19	F.T. 9d.	5.10.	83	16	51.7	45.7				
44	F.T.10d.	7. 8.	109.8	22.2	61.9	53.9	14.8	3	8.5	5.5
48	F.T.10d.	8. 4.	139.8	30.3	73	61	7.5	1.8	15.9	14.4
45	F.T.18d.	7. 0.	100.7	22.1	80	69.8	6.5	1.3	4.5	3.6

F.T. = FULL TERM   d = days
S.B. = STILLBIRTH   w = weeks

NON-Hb. = NON-HAEMOGLOBIN.

There were many individual variations, but taken generally, in the early neonatal period there appeared a progressive increase, both absolute and relative, in the amount of iron deposited in the liver. This applied to both total and non-haemoglobin iron. The individual differences were too varied and an insufficient number of cases was done to be able to draw a graph depicting this rise in the iron deposits, but the scatter diagrams, (No. 1 and No. 2, clearly illustrate the general upward trend.

The average total iron content of the liver of the full term stillbirth was found to be 45.6 milligrams, while 18 days after birth it was found to have increased to 80 milligrams. There were two infants (Numbers 27 and 28), both eight days old, in whose livers the iron deposits were actually over 100 milligrams (104 and 111 milligrams respectively, for the total iron). The non-haemoglobin iron content of the liver of the average full term stillbirth

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of this series was 33.27 milligrams, whereas eighteen days after birth the amount had risen to 69.8 milligrams. (Again Numbers 27 and 28 had higher values, being 93.5 and 97.5 milligrams of non-haemoglobin iron respectively).

It is interesting and instructive to note here that the blood iron of the liver forms approximately 50% of the total iron content of this organ at 28 weeks; approximately 27% of the total iron at full term; while 18 days after birth the blood iron was found to be responsible for only 12.5% of the total iron content of the liver.

Investigations of the spleens yielded results somewhat similar to those obtained for the livers. The actual amounts of iron were always very much smaller than those found in the corresponding livers. Though the iron contents varied a good deal in spleens of

/ approximately ...

approximately the same size and weight, the variations were not as marked as in the case of the livers.

Only minute amounts of iron were found in the spleens of 28 weeks foetuses. The total iron for spleen Number 43 (28 week foetus) was 0.24 milligram, while the non-haemoglobin iron was only 0.005 milligram.

The twin foetus (Number 42) had a total of 0.28 milligram of iron in its spleen, with a non-haemoglobin iron content of only 0.02 milligram. The spleen of a 34 weeks foetus (Number 40) contained 0.95 milligram of iron altogether and when the iron due to the blood present was subtracted, the actual tissue iron was found to be only 0.6 milligram.

/ TABLE No. 3 ...

TABLE No. 3.

TABLE SHOWING THE RELATIVE AMOUNTS OF NON-HAEMOGLOBIN IRON IN LIVERS AND SPLEENS.

No.	AGE.	Non-Hb. Iron per 100 Grams Wet Liver. (Mgms)	Non-Hb. Iron per 100 Grams Dried Liver (Mgms)	Non-Hb. Iron per 100 Grams Wet Spleen (Mgms)	Non-Hb. Iron per 100 Grams Dried Spleen. (Mgms).
43	S.B. 28w.	10.3	58	0.33	2
42	S.B. 28w.	10.1	55	1	8
47	S.B. 29w.	16.6	90.5	20.3	92.5
33	S.B. 33w.	14.2	66.5	24.8	115.5
40	S.B. 34w.	12.8	70	16.2	86
17	S.B. 35w.	34	166		
16	S.B. 35w.	14.7	91.5		
20	S.B. 36w.	26.8	108		
41	F.T. S.B.	28.8	78	57	358
29	F.T. S.B.	22	106	31	170
36	F.T. S.B.	17.1	81.5	8.2	37
15	F.T. S.B.	30.4	138		
30	F.T. S.B.	30.4	126	29.2	190
34	F.T. S.B.	44.5	253	17.4	81
26	F.T. S.B.	31.4	179		
21	F.T. S.B.	34	228		
32	F.T. S.B.	19.8	100	8.6	60
37	F.T. S.B.	41	212	24	100
24	F.T. S.B.	14.3	72		
39	F.T. S.B.	24	124	24	81

F.T. = FULL TERM d = days
S.B. = STILLBIRTH w = weeks NON-Hb.= Non-Haemoglobin.

/ TABLE No. 3 Continued ...

TABLE No. 5 - Continued.

TABLE SHOWING THE RELATIVE AMOUNTS OF NON-HAEMOGLOBIN IRON IN LIVERS AND SPLEENS.

No.	AGE.	Non-Hb. Iron per 100 Grams Wet Liver (Mgms)	Non-Hb. Iron per 100 Grams Dried Liver (Mgms)	Non-Hb. Iron per 100 Grams Wet Spleen (Mgms)	Non.-Hb. Iron per 100 Grams Dried Spleen (Mgms)
38	F.T. 1d.	28.8	150	44	192
49	F.T. 3d.	24.2	112	45	194
25	F.T. 4d.	40	116		
18	F.T. 7d.	31	159		
22	F.T. 7d.	25	107		
35	F.T. 7d.	32	140	73.4	294
46	F.T. 7d.	32	123	89	368
23	F.T. 8d.	53.5	250		
27	F.T. 8d.	92.5	456		
28	F.T. 8d.	86.5	454	30	172
31	F.T. 8d.	18.4	86.5	32.4	140
19	F.T. 9d.	55	285		
44	F.T. 10d.	49	242	37	183
48	F.T. 10d.	43.6	204	192	800
45	F.T. 18d.	69	317	55.5	277

F.T. = FULL TERM d = days
S.B. = STILLBIRTH w = weeks

NON-Hb. = NON-HAEMOGLOBIN.

The average total iron content of the spleen at full term was found to be 2.96 milligrams, the average non-haemoglobin iron present in the splenic tissue was 1.7 milligrams. Eighteen days after birth the total iron content of the spleen was 4.5 milligrams and the non-haemoglobin iron content had increased to 3.6 milligrams. This shows a very distinct increase in the actual amount of iron stored by the spleen from 28 weeks to full term and also in the early neonatal period.

Table No. 2 shows the individual results obtained from the spleen analyses and scatter diagram No. 3 illustrates the general trend of increased iron storeage in this organ from the 28 weeks foetus to the 18 day old infant.

There is also a distinct relative increase in the amount of iron stored by the spleen. With 100 grams of wet spleen as a

/ standard ...

standard the 28 weeks foetal organ contained 0.33 to 1 milligram of non-haemoglobin iron, the 34 weeks spleen 16.2 milligrams of iron, while the spleen of the 18 day old infant contained 55.5 milligrams of iron. A more accurate standard is to calculate the amount of non-haemoglobin iron per 100 grams of dried spleen. On this standard the 28 weeks foetal spleen has 2 to 8 milligrams of iron, the 34 weeks spleen 86 milligrams, the full term spleen an average of 134 milligrams and the spleen of the 18 day old infant 277 milligrams of iron. The iron storage of the individual spleens is shown in Table No. 3 and the general trend towards increasing storage is clearly illustrated in the scatter diagram No. 4.

/ TABLE No. 4. ...

TABLE No. 4.

TABLE SHOWING PERCENTAGE MOISTURE IN LIVERS, NUMBERS 15 - 49.

No.	AGE.	BIRTH WEIGHT Lbs.	ozs.	WEIGHT OF WET LIVER (Grams)	WEIGHT OF DRIED LIVER (Grams)	PERCENTAGE MOISTURE IN LIVER.
43	S.B. 28w.	2.	0.	35	6.2	82
42	S.B. 28w.	2.	4.	37.5	6.9	81.5
47	S.B. 29w.	2.	8.	39.8	7.3	81.5
33	S.B. 33w.	4.	4.	49.3	10.5	79
40	S.B. 34w.	4.	0.	53	9.7	81.5
17	S.B. 35w.	4.	12.	68	14	79.5
16	S.B. 35w.	5.	0.	68.5	11	84
20	S.B. 36w.	4.	14.	58.5	14.5	75.5

Average % moisture in livers of premature stillbirths = 80.6% (Standard Deviation 2.3)

/TABLE No. 4 - continued ...

TABLE No. 4 - continued.

TABLE SHOWING PERCENTAGE MOISTURE IN LIVERS NUMBERS 15 - 49.

No.	AGE.	BIRTH Lbs.	WEIGHT. ozs.	WEIGHT OF WET LIVER (Grams)	WEIGHT OF DRIED LIVER (Grams)	PERCENTAGE MOISTURE IN LIVER.
41	F.T. S.B.	6.	0.	113. 5	20.5	82
29	F.T. S.B.	6.	4.	156	32.5	79
36	F.T. S.B.	7.	0.	97.5	20.5	79
15	F.T. S.B.	7.	2.	116.5	25.7	78
30	F.T. S.B.	7.	6.	99.5	24	76
26	F.T. S.B.	7.	12.	108	19	82
34	F.T. S.B.	7.	12.	103.5	18.2	82.5
21	F.T. S.B.	7.	13.	104.5	15.5	85
32	F.T. S.B.	8.	0.	110	21.8	80
37	F.T. S.B.	8.	0.	175.6	34	80.5
24	F.T. S.B.	8.	10.	141.5	28	80
39	F.T. S.B.	9.	4.	126.5	24.5	81

Average % moisture in livers of full term stillbirths = 80.4% (Standard Deviation 2.27)

/ TABLE No. 4 - continued ...

TABLE No. 4 - continued.

TABLE SHOWING PERCENTAGE MOISTURE IN LIVERS NUMBERS 15 - 49.

No.	AGE.		BIRTH WEIGHT. Lbs.	ozs.	WEIGHT OF WET LIVER (Grams)	WEIGHT OF DRIED LIVER (Grams)	PERCENTAGE MOISTURE IN LIVER.
38	F.T.	1d.	7.	0.	105.5	20.2	81
49	F.T.	3d.	7.	0.	109.5	23.7	75
25	F.T.	4d.	6.	4.	92.3	22.3	76
18	F.T.	7d.	5.	8.	82	16	80.5
22	F.T.	7d.	5.	6.	76.5	18	76.5
35	F.T.	7d.	5.	12.	78.8	18.8	76
46	F.T.	7d.	7.	11.	94.3	24.3	74.5
23	F.T.	8d.	5.	14.	75	16	78.5
27	F.T.	8d.	5.	6.	101	20.5	79.5
28	F.T.	8d.	6.	3.	113.5	21.5	81
31	F.T.	8d.	6.	8.	82.6	17.6	78.5
19	F.T.	9d.	5.	10.	83	16	81
44	F.T.	10d.	7.	8.	109.8	22.2	80
48	F.T.	10d.	8.	4.	139.8	30.3	78.5
45	F.T.	18d.	7.	0.	100.7	22.1	78

Average % moisture in the livers of full term neonatal infants = 78.3% (Standard Deviation 2.16)

/ as ...

As would be expected the blood iron for this series of spleens represented more than 90% of the total iron of the spleen in the 28 weeks foetus, approximately 40% of the total iron of the spleen of the full term foetus and only about 20% of the total iron of the spleen of the 18 day old infant. These figures must be only very approximate as the individual variations are so great, but they do tend to emphasize indirectly the marked increase of iron stored in the tissue of the spleen relative to age.

Three cases of interest will be mentioned here. Infants numbers 27 and 48 had their cause of death signed up as Icterus Gravis Neonatorum. Both these infants were found to be very jaundiced and somewhat pale at post-mortem. Both were full term females, the one (Number 27) having lived for eight days, the other (Number 48) for 10 days. In both the actual non-haemoglobin iron content of the liver was high. In

Number 27 it was 93.5 milligrams and in Number 48 it was 61.15 milligrams of iron. This high iron content may be explained by an originally high iron storage at birth, but it seems more reasonable to explain it partly or wholly on the excess blood destruction that takes place in this condition, with the concomitant large and rapid iron storage.

Unfortunately, the spleen in Number 27 was not investigated. In Number 48 the spleen yielded the extraordinarily high figure of 14.4 milligrams for the non-haemoglobin iron content.

The third case of interest was one of Haemorrhagic Disease of the Newborn, (Number 49). This infant, a full term female, died three days after birth with a very low haemoglobin content in the blood: Analysis of the liver revealed a relatively normal value for non-haemoglobin iron, namely 26.6 milligrams. The spleen contained 3.5

/ milligrams ...

milligrams of non-haemoglobin iron definitely more than was found in the spleen of the average full term stillbirth.

The weights of the livers at post-mortem varied a great deal. In the cases of the two sets of premature twins each liver compared closely with the liver of its fellow twin. From 28 weeks to full term the weight of the liver increased markedly. At 28 weeks it was 35 to 37.5 grams in this series, while at full term it was over 100 grams in weight. At full term and in the early days of infancy the weights varied within rather wide limits and strangely enough, were not always in proportion to the weight of the foetus or infant. Thus, a relatively light infant may have a large liver, such as Number 29, whose birth weight was 6 lbs. 4 ozs. and whose liver weighed 156 grams; while in Number 30 the infant weighed 7 lbs. 6 ozs. but its liver only weighed 99.5 grams.

/ The ....

The spleen shows a definite increase in weight from the 28 weeks foetus up to full term, being 1.5 to 2 grams for the former and an average of 7.3 grams for the full term stillbirth. Thereafter it varies greatly and appears to have no relation to the weight of the liver or the foetus. Thus, for example, Number 46, an infant aged 7 days, weighing 7 pounds 11 ounces, whose liver weighed 94 grams, possessed a spleen weighing, at the time of post-mortem, 3.5 grams. By comparison, Number 44, a 10 day old infant weighing 7 pounds 8 ounces, whose liver weighed 109.8 grams, had a spleen weighing 14.8 grams. In this instance extremes were taken to emphasize the point.

The volume of blood in the liver calculated by Method 11 is obviously not the same as that existing in the organ during life. The figures obtained in this work are merely for the post-mortem liver which has been removed from the body. However, when

/ the ...

the volumes obtained were standardised relative to a 100 grams of wet liver, it was found that though they varied considerably they still remained within reasonable limits, the lowest being 8 ccs. in an exsanguinated liver and the highest 26 ccs in a congested liver. The average for the livers of premature foetuses was higher than the average for the livers of full term stillbirths, the figures being 18.6 ccs as compared to 15.3 ccs. The neonatal infant livers showed an average of 13.5 ccs. This indicates that the liver of the premature foetus possesses relatively more blood, or is more congested, than the liver of either the full term foetus or the neonatal infant.

The percentage moisture in all the livers examined was relatively constant, varying between 74.5 per cent and 85 per cent. (Table No. 4). The average percentage moisture in the livers of premature stillbirths was 80.6 per cent (Standard Deviation 2.3);

/ in ..

in the livers of full term stillbirths 80.4 per cent (S.D. 2.27); And in the livers of full term neonatal infants 78.3 per cent (S.D. 2.16).

Certain averages for the full term stillbirths of this series may be repeated here:

The average total iron content of the liver was found to be 45.6 milligrams of iron (S.D.17.6).

The average non-haemoglobin iron content of the liver was 33.27 milligrams of iron (S.D.13.85).

The average total iron content of the spleen was 2.96 milligrams of iron (S.D. 1.36).

The average non-haemoglobin iron content of the spleen was found to be 1.7 milligrams of iron (S.D. 0.735).

The average weight of the liver in 20 full term stillbirths was 131.13 grams (S.D. 26.4).

And finally, the average weight of the spleen in 8 full term stillbirths was 7.3 grams (S.D. 2.54).

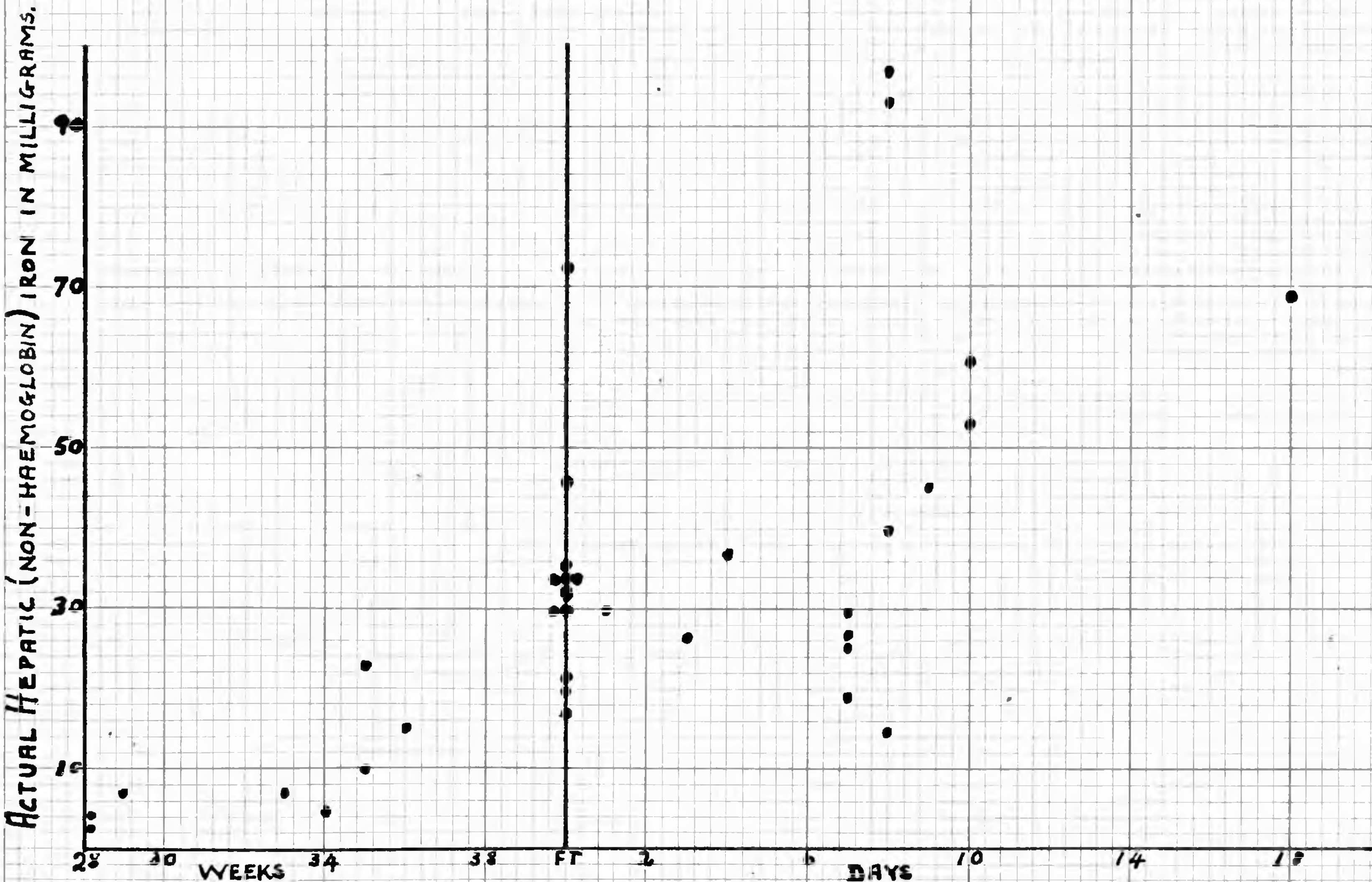
/ Thus ...

Thus, briefly, the main conclusions that may be deduced from the above results are :

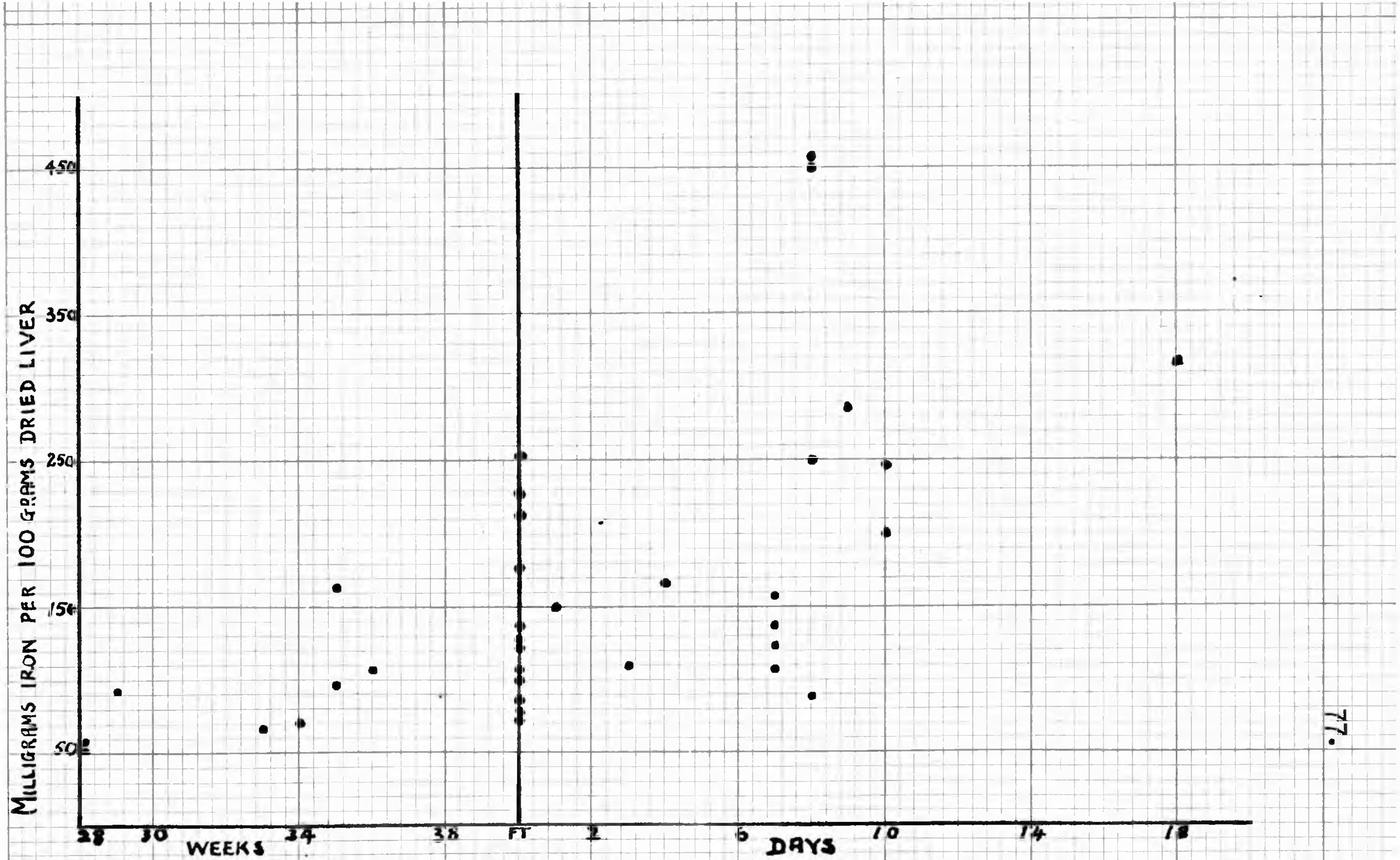
(1) There is a definite increasing storage of iron in the liver, both absolutely and relatively, in the twelve weeks preceding birth and also in the early neonatal period.

(2) There is a definite increasing storage of iron in the spleen, both absolutely and relatively, in the last twelve weeks of pregnancy and also in the early neonatal period. In all cases the amounts of iron were much smaller than those found in the liver, indicating that the spleen is of secondary importance as a storehouse for iron.

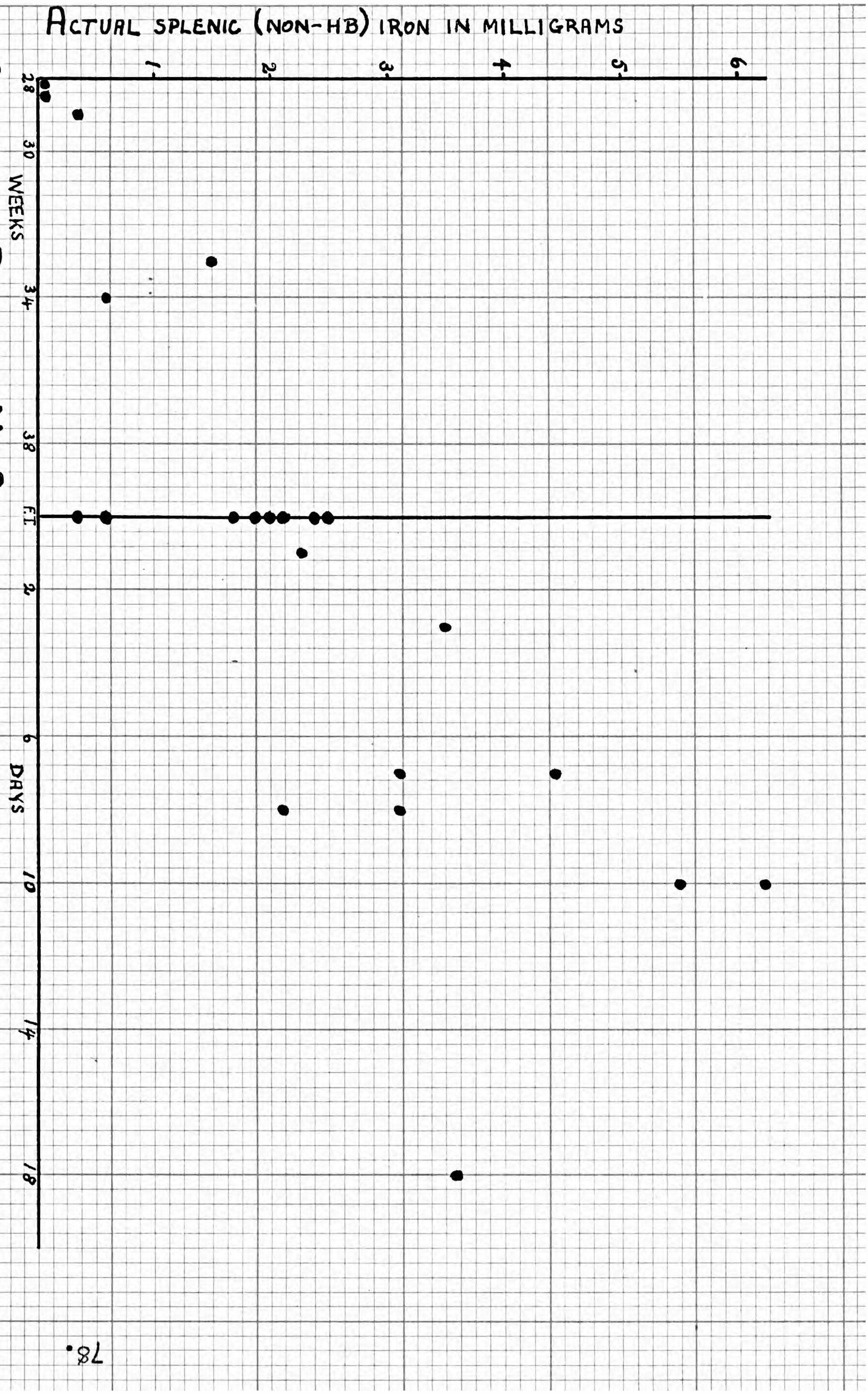
(3) In Icterus Gravis Neonatorum there appears to be a heavy deposit of iron both in the liver and in the spleen.



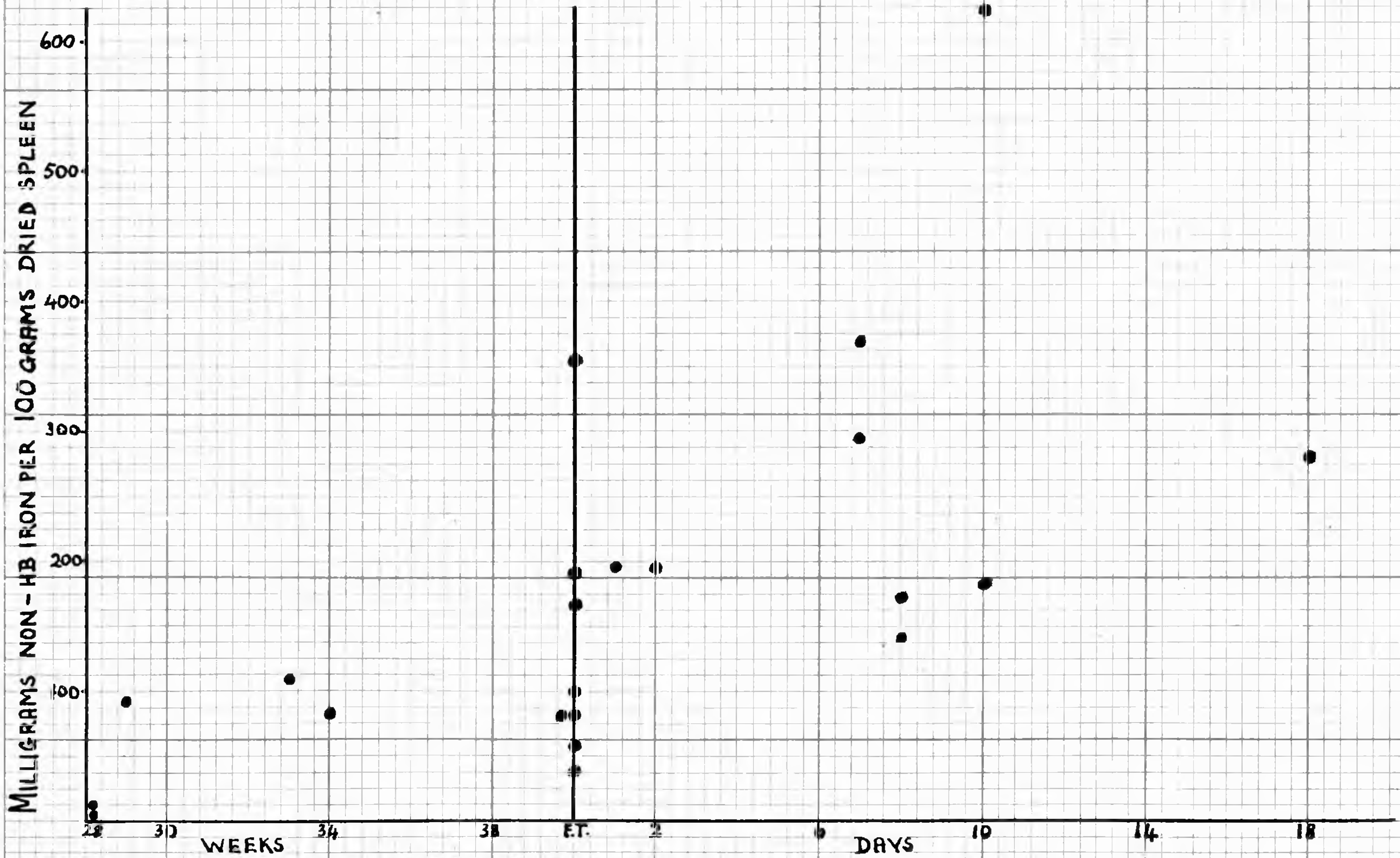
SCATTER DIAGRAM No. I. TO SHOW ACTUAL INCREASE OF IRON IN LIVER



Scatter Diagram No. 2. To show relative increase of iron in liver



SCATTER DIAGRAM. No. 3. TO SHOW ACTUAL INCREASE OF IRON IN SPLEEN



SCATTER DIAGRAM No. 4. TO SHOW RELATIVE INCREASE OF IRON IN SPLEEN

79.

REVIEW OF THE LITERATURE.

REVIEW OF THE LITERATURE.

It is natural to commence this section by mentioning the work of Hugounenq, who was the first to investigate human foetuses for iron. He determined the total iron in the ash of the entire foetus. His results show a gradual and steady increase in the amount of iron present in relation to the age of the foetus. Thus, for a 4½ months foetus weighing 522 grams the total iron content was 42 milligrams, for a 5½ months foetus of 1,115 grams the total was 74 milligrams of iron, and for a 6½ months foetus weighing 1,285 grams the total iron content was 88 milligrams. The results of Brubacker and Camerer and Söldner, who also investigated human foetuses for total iron, are shown together with the results of Hugounenq in Table No. 5.

The great increase in total iron content in the last third of pregnancy is well illustrated by these figures. At 7 months the

/ total ...

total iron content was only 82 milligrams whereas at full term the total iron amounted to 343 milligrams. Hugounenq thought this rapid increase in total iron was partly due to the rapid growth of the blood volume in the last third of intra-uterine life and concluded that at birth 57% of the total iron could be accounted for by the blood alone.

More recently, while determining the mineral composition of the ash of human foetuses Givens and Macy (1933) came to the conclusion that there was a greatly augmented demand for minerals during the last two or three months of intra-uterine life. They found that there were marked variations between foetuses of the same length but that there appeared to be a definite relationship between the age and the average composition of the ash.

LIVER IRON :

In 1912 Ashby, while investigating the anaemias of infancy and childhood, determined the amount of iron present in the livers of eight

/ infants ..

infants, varying in age from a full term stillborn infant to a seven months old infant. He found the highest amount of iron in the liver of the stillborn infant, the figure being 58 milligrams of iron per 100 grams fresh liver. This result represents the total iron present, including the iron due to blood. In the liver of a two months old infant he found 50 milligrams of iron per 100 grams fresh liver; in the liver of a three months old infant 30 milligrams, the amount gradually diminishing until in the seven months old infant there was a total of only 13 milligrams of iron. He thus concluded that the store of iron in the liver at birth is used up by the seventh month of life.

/ TABLE No. 5. ...

TABLE No. 5.

THE IRON CONTENT OF THE ENTIRE BODY OF THE HUMAN FETUS AND NEWBORN INFANT. (REPRODUCED FROM THE WHITE HOUSE CONFERENCE ON CHILD HEALTH AND PROTECTION.)

AGE (Months)	BODY WEIGHT (Grams)	TOTAL IRON (milligrams)	AUTHOR.
4.5	522	42	Hugounenq
5.0	570	43	"
5.0	800	51	"
5.5	1,115	74	"
6.0	1,165	83	"
6.5	1,285	88	"
7.0	1,169	82	Brubacker
8.5	1,875	131	"
	THE NEWBORN INFANT.		
	2,476	343	Camerer and Söldner
	2,616	392	" "
	2,683	385	" "
	2,720	268	Hugounenq
	2,755	343	Camerer and Söldner
	3,084	343	" "
	3,300	295	Hugounenq
	3,348	364	Camerer and Söldner

/ Sheldon ...

Sheldon (1932) investigating the influence of copper and manganese on the activity of iron, found in his series that the liver at birth contained an average of 7 milligrams of copper and 50 milligrams of iron.

The White House Conference on Child Health and Protection (1932) gave 15 milligrams as a conservative figure for the total amount of iron present per 100 grams of the newborn infant's liver. It is admitted that this is probably a rather low figure.

Gladstone (1932) investigated the iron content of the livers of 24 human foetuses and infants varying from the fourth month of intra-uterine life to the third month of post-natal life. He calculated both total and non-haemoglobin iron in livers and from his figures came to the conclusion that there was no large or progressive deposition of iron in the liver during intra-uterine life. Nevertheless, he found the total iron

/ content ...

content in the livers of full term still-births to be about 49 milligrams. He states that the iron content of the liver of the human infant reaches its maximum not at birth, but from one to ten weeks after birth and the large amount of iron in this organ results not from accumulation during intra-uterine existence, but from the destruction of blood that occurs in the infant shortly after birth.

Theones and Aschaffenburg (1934) (quoted by Stearns et al. 1937) found the total amount of iron present in the liver of the full term newborn to be 35 milligrams.

Swanson and Iob (1938; 1939) investigated 28 human foetuses of various ages. Fourteen were examined for total body iron and in the second fourteen the liver iron was calculated. They found that the iron content per Kilogram of fat-free dried substance (whole foetus) remained practically

/ unchanged ...

unchanged during the foetal growth period, but the liver iron similarly calculated increased as the foetus approached term. The analysis of the liver iron did not differentiate between the haemoglobin and the non-haemoglobin iron. They concluded that even though the liver may become less vascular towards full term the iron present increases in amount. The value they gave for the total iron present in the liver of a three pounds foetus was 29.6 milligrams, for a six pound infant 72 milligrams, and for an eight and three-quarter pounds infant 118.5 milligrams.

Brückmann and Zondek (1939) examined a large number of livers of infants and adults for their total and non-haemin iron content. Amongst this group were 17 livers obtained from full term stillbirths and infants up to four months of age. Their average for total iron per 100 grams of wet liver for full term stillbirths was 40.6

/ milligrams ...

milligrams and non-haemin iron 31.2 milligrams. For a 13 day old infant liver their figure for total iron was 75 milligrams and non-haemin iron 68 milligrams per 100 grams wet liver. In the four months old infant they found 21 milligrams total iron per 100 grams wet liver and non-haemin iron 20 milligrams. They conclude that in spite of the great variability of the iron content of the livers, typical distribution according to ages could be recognized. No. differences were observed due to the sex of the individual.

Gillman et al. (1945) investigated the total iron content of the livers of nine Bantu infants varying in age from full term stillbirth to four months. Their results are as follows :

/ Liver ...

LIVER No.	AGE.	IRON % Dry Weight
3	S.B.	.02
27	S.B.	.07
5	1 day	.02
22	1 day	.03
14	5 days	.05
25	2½ months	.03
4	3 months	.02
8	3 months	.03
38	4 months	.07

The figures show the total iron present in the liver, including the iron due to the blood in the organ. The above results are all very low in comparison with those obtained in my own series of Bantu livers. My average total iron present in the livers of 12 Bantu full term stillbirths, expressed in the above terms, was 0.191% iron (dry weight), being thus from 3 to 9 times as much as Gillman's figures for stillbirths.

/ The ...

The figure .191% iron (dry weight of liver) compares well with those results obtained by other authors working on European foetuses and infants, though generally the results for European material are slightly higher than this figure. (Brückmann and Zondek 0.186%; Ramage .30%; Swanson and Iob .31%). Thus, Gillman's suggestion that in the first six months of life, the livers of African babies may contain up to twenty times less iron than babies in Europe and England, can apply to individual cases but does not hold for the average.

The iron content of the blood-free liver has been investigated by fewer authors. Gladstone (1932) estimated the amount of haemoglobin present in a sample of liver and from this calculated the total haemoglobin present in the organ and then translated the result into milligrams of iron. This amount was subtracted from the total iron also estimated from a small sample of liver. The average figure obtained for the livers of

/ full ...

full term stillbirths was 32 milligrams of non-haemoglobin iron.

Theones and Aschaffenburg give the figure as 26 milligrams of non-haemoglobin iron per 100 grams wet liver for full term stillbirths. Toverud (quoted by Fullerton, 1937) gives the figure of 60 milligrams of non-haemoglobin iron present in the liver of the average full term stillbirth.

Brückman & Zondek (1939) claim to determine the non-haemin iron in the liver by extracting a sample of the organ in sodium pyrophosphate and 20% trichloroacetic acid and examining an aliquot for iron. They then analysed a second sample of liver for total iron. They state that 30 - 60% of the total iron present in the liver was in the form of blood iron. They express their result for the non-haemin iron in the liver of the average full term stillbirth as 155 milligrams per 100 grams dried liver. This equals approximately 31.2 milligrams of iron per 100

/ grams ...

grams of wet liver.

For the sake of comparison the results obtained by the various authors, for the iron found in the liver of the full term stillbirth, are presented together in Table No. 6.

All the authors quoted used small samples of liver for their investigations and, as has been shown in a previous section, this method is open to a fair margin of error.

SPLENIC IRON.

At the White House Conference on Child Health and Protection (1932), the iron content of the spleen of the newborn infant was given as 2 milligrams. The spleen in my own series of full term stillbirths was found to contain an average of 2.97 milligrams of total iron, and 1.7 milligrams of non-haemoglobin iron.

No further literature giving actual figures for iron in the spleen of human foetuses and newborn infants could be found.

TABLE No. 6.

THE IRON CONTENT OF THE LIVER OF THE FULL TERM STILLBIRTH.

AUTHOR.	No. of Cases.	Total Fe in Liver (Mgms)	Non-Hb. Iron in Liver. (Mgms)	Total Fe 100G. Wet Liver (Mgms).	Total Fe 100G. Dry Liver. (Mgms)	Non-Hb. Fe/100G. Wet Liver (Mgms)	Non-Hb. Fe/100G. Dry Liver (Mgms).
Own Results	12	45.6	33.27	37.5	191.5	28.1	141.5
Ashby	1	-	-	58	-	-	-
Sheldon	-	50	-	-	-	-	-
White House Conference	-	-	-	15	-	-	-
Gladstone	7	49	32	-	-	-	-
Theones & Aschaffenburg	-	35	-	-	-	26	-
Toverud	-	-	60	-	-	-	-
Swanson & Iob	2	72	-	-	310	-	-
Brückman & Zondek	4	-	-	40.6	186	31.2	155
Gillman	2	-	-	-	20 & 70	-	-
Ramage	6	73.3	-	-	250	-	-

/ Lewin ...

Lewin (1929) and Gladstone (1932) investigated the spleens of foetuses and infants histologically for iron. Lewin examined 48 spleens of premature foetuses whose ages varied up to 7 months of intra-uterine life. In these he found very little or no iron at all. In a second series of 37 prematures aged from 7 months to full term he found gradually increasing deposits of iron present in the spleen, the greatest amounts being found in the full term foetuses. His confidence in the constant and regular deposition of splenic iron during the last third of intra-uterine life is so great that he claims to be able to judge the age of the foetus from the amount of iron found in the spleen.

Gladstone's results were similar, the amount of splenic iron becoming greater as full term was approached. He points out, however, that the greatest deposition of iron occurs in the spleen during the early neonatal

/ period ...

period when there is a considerable breakdown of blood.

It is interesting to mention here again the one case of my own (Number 48) who died of Icterus Gravis Neonatorum, who had a remarkably high iron content in the spleen.

Coppoletta (1933) gives the average weight of the liver at birth as 78 grams. This figure is much lower than that given by Scammon (1923) who found the weight to be 135 grams; and lower than the figure in my own series, 131 grams. There is, however, as mentioned before, a very wide range of variation. At birth the liver usually weighs from 4 - 5 % of the body weight, which is twice the corresponding weight in the adult. (Ashby, 1912).

Coppoletta found the weight of the spleen at birth to be 8 grams; the figure in my own series was 7.3 grams.

There is general agreement in the literature that there is an actual increase

/ in ....

in the amount of iron stored in the foetal liver during the last three months of pregnancy. Certain authors (Gladstone, 1932; Swanson and Iob, 1938, 1939) state, however, that there is not a relative increase of iron stored in the liver during this period, claiming that the amount of iron present is proportionate to the weight and that, with more foetal growth and therefore more weight, there is a corresponding increase in iron content. My own findings contradict this claim and instead, support those authors who believe that there is both an absolute and relative increase in the amount of iron stored in the foetal liver during the last third of pregnancy. There is also general agreement that because of the breakdown of excess haemoglobin in the blood there is increased deposition of iron in the liver and spleen during the neonatal period.

D I S C U S S I O N.

DISCUSSION.

Iron is distributed in the human body in the blood, in all the tissues, in the chromatin material of nuclei and in the reticulo-endothelial system, especially in the liver and the spleen. Some 60% of the total body iron is circulating as haemoglobin in the blood and a further 20% is held in the tissues in a form potentially available for haemoglobin synthesis. Of the remaining body iron (20%) about one-third is in myohaemoglobin and two-thirds in such cell enzymes as cytochrome. None of this latter iron is available for haemoglobin synthesis even in extreme iron depletion. (Hynes,1948).

The greatest amount of the metal found circulating in the blood is in the form of haemoglobin (90 - 95%), the remainder consisting of breakdown products of haemoglobin (5 - 10%) and plasma iron (.05 - .18%). Haemoglobin has been studied more completely than any other iron compound of the body.

/ Although ...

Although at one time it was believed that muscle haemoglobin differed from that found in the blood stream, more recent investigations by Whipple and Kennedy have shown the two to be almost certainly identical compounds. Zinoffsky (1886) crystallized haemoglobin and his analysis of the pure crystals showed an iron content of 0.335%. This work has been repeatedly confirmed.

On treatment with dilute acids haemoglobin is split into haematin and a protein fraction. The haemoglobin contains all of the iron. This compound is probably identical in all species, the differences in various haemoglobins being due to the protein portion of the molecule. As a result of their spectroscopic studies, Anson and Mirsky (1925) came to the conclusion that the iron-pyrrole portion of the haemoglobin molecule, which they term haem, is very widely distributed in Nature. They found it in the wing muscles of the fly, bee and other insects,

/ in ...

in plant tissues, in yeast, in animal tissues, - in short, they have been led to regard haemoglobin as a special form of a universally distributed haem .

The iron due to breakdown products of haemoglobin forms only about 5% of the iron in the blood stream of the normal adult. It would naturally be higher in those conditions where excessive haemoglobin is taking place, such as occurs in the early neonatal period. Plasma or serum iron forms only 0.05 - 0.18% of the blood iron. The exact nature of this serum iron is unknown, but it is apparently the form in which iron transport takes place from the bowel via the blood to the tissues. Serum iron is probably in the ferrous form and combined in a complex way with serum proteins. Substantial changes in serum iron take place after ingestion of therapeutic doses of iron salts (Wright 1947), but under ordinary conditions

/ of ...

of iron food intake the fluctuations in serum iron are slight.

Substances giving a reaction for iron have been found consistently in the chromatin material of the nucleus of animal and plant cells, in the cytoplasm of non-nucleated organisms and in the prozymogen granules. It was recognized long ago that iron in tissues exists in several different forms. Molisch, in 1891, introduced the term "masked iron" to denote those compounds such as haemoglobin that respond to iron tests only after special treatment. In addition, distinctions between so-called inorganic and organic iron compounds were introduced to classify compounds in the body, in foods, or the iron salts used in therapy. "Inorganic iron" usually refers to the ionizable form, or to protein compounds that readily yield ionized iron. The term "organic iron" has been used to denote that bound in complex form, such as in ferro- and ferricyanide haematogen and other compounds.

/ The ...

The iron compounds of the cells are primarily concerned with the oxidations and reductions by which the potential energy of the food is rendered available to the organism as kinetic energy. This function completes the work of haemoglobin as a carrier of oxygen from lungs to tissues and intimately associates the iron compounds of the body with the fundamental properties of living matter.

The combination of oxygen and haemoglobin forms oxyhaemoglobin in the lungs; in the capillaries of the tissues, where oxygen tension is low, this is broken down to reduced haemoglobin and oxygen. Analyses of the quantitative relationship between oxygen capacity and the iron content of haemoglobin have shown that for each atom of the metal one molecule of oxygen is combined.

Salts of iron and certain other metals have long been known to act outside the body as efficient catalysts in the oxidation of

/ many ...

many organic compounds. Warburg extended the catalysis - by - iron theory to tissue oxidations and claimed that the utilization of oxygen by living cells at body temperature could only be explained as due to an enzymic activity of the iron compounds of the cell. According to the newer view-point, the respiratory ferment is a haematin. This is assumed to combine with oxygen, thus :

$$X.Fe + O_2 \rightleftharpoons X.FeO_2$$

Organic substrates (A) were then capable of being oxidised, according to the following equation :

$$X.FeO_2 + 2\,A \rightleftharpoons X.Fe + 2\,AO$$

Undoubtedly the other oxidases, such as catalase, glutathione, etc., do play some part in tissue oxidation as well. Concerning the claim that the respiratory ferment is identical with haematin, Keilin's work has thrown considerable doubt upon the haematin theory as the only explanation of tissue oxidations.

/ However ...

However, as Dixon has concluded, this does not mean that the respiratory ferment does not contain iron, nor does it obscure the fact that iron compounds may account in general for about two-thirds of the oxidizing power of the tissues. Scott has shown that apparently abnormal iron metabolism occurs in neoplasms.

Iron also appears in the reticulo-endothelial system especially in the liver and the spleen. In the liver it is held in the Kupffer cells and also in the hepatic cells. In the spleen the iron pigment predomonantly lies in the pulp reticular cells, in smaller quantities in sinus endothelial cells and only in extremely small quantities in the capsular cells, the connective tissue and the trabeculae. (Lewin, 1929). In these deposits in the liver and spleen the pigment generally appears as brownish-yellow granules and has received the name of haemosiderin. There is little doubt that this pigment is

/ the ...

the iron residue of haemoglobin that results from the destruction of the red blood cells. Cook has isolated haemosiderin from the tissues and shown that it contained, in addition to a yellow-brown pigment, an iron fraction that gave on analysis : 55% iron, 12.5% hydrogen and 27% oxygen. This compound may represent a most important endogenous source of iron to the body and its structure is therefore of considerable interest. (White House Conference, 1932). In contrast to our present knowledge of haemoglobin, haematin, and haemosiderin, very little is known about the nature of the other iron compounds of the body.

A water soluble iron-protein compound called ferritin, found in the liver, spleen and bone marrow of mammals, including man, is described by Hahn (1943) and by Granick (1942). Ferritin consists of a protein fraction, apoferritin, linked together with micelles of a special type of colloidal ferric hydroxide, the crystals containing as

/ much ...

much as 23% of iron.

Recent work was done using radio-active iron (Hahn 1943), by which actual particles of iron could be followed and traced about the body. Radio-active iron, Fe^{59}, is prepared by bombarding ordinary iron, Fe^{58}, with deuterons. (Wright 1947). It has a half life of 47 days and behaves in the body exactly like ordinary iron, except that it can be readily traced by physical methods. Hahn et al., using this radio-active isotope in the form of ferric ammonium citrate, demonstrated that it is mostly in the form of ferritin that iron is stored in the body and exists in greatest amount in the liver. It has also been established that the haemoglobin iron of the red blood cells, after breakdown of the haemoglobin, is partly converted into ferritin iron in the liver and the spleen.

In 1944 Libet isolated yet a different iron-protein complex from the liver, which

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they called "ferrin". It has been shown that this substance behaves in the same way as iron salts in catalysis of phospholipid oxidation. Ferrin, however, has the advantage over iron salts of being soluble in neutral solutions. Analysis shows that it contains 15.7 % iron. It seems probable that much of the non-haemin iron in the liver is present in some form of combination with protein, either as ferritin or as ferrin.

In the normal adult human being very little iron is absorbed and very little is excreted. Excretion takes place in the urine and the daily amount lost is on an average only 0.1 milligram of iron, though this figure has a slight individual variation. This loss is made up from the diet, and it therefore follows, that as the average daily ingestion of iron is 12 milligrams, practically all the iron in the diet is unused and passes out again in the faeces.

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The hydrochloric acid of the gastric juice helps to split off the iron from its organic combinations in the food and liberates it in an inorganic, soluble and more absorbable form. Absorption takes place in the duodenum and upper small intestine into the portal vein blood (Wright 1947). It is generally agreed today that the total iron content of the individual is controlled through some function vested in the intestine, whereby the amount of dietary iron absorbed is carefully limited by the needs of the body. (Gillman, 1947). This fact,originally suggested by Widdowson and McCance, has since been conclusively established by the experiments conducted with radio-active iron by Hahn, Whipple and other workers. After ingesting radio-active iron the level of plasma iron (in the inorganic form) increases after one hour, gradually rises to its peak at 12 hours and then falls to normal in 24 hours. Within two hours of ingestion radio-active iron appears in the circulating

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red blood corpuscles (Wright 1947), the amount increasing steadily for about one week. It is quite clear that the iron rapidly reaches the bone marrow and is soon used in the formation of new red blood cells; small amounts of radio-active iron also appear in the liver. The experiments also showed that the amount of iron absorbed depends upon the physiological state of the body.

Hahn et al. on the basis of their findings postulated the existence of some "mucosal acceptor" for iron functioning in the intestine. Physiological saturation of this acceptor resulted in the development of a"mucosal block" to further entry of iron through the intestine into the body. According to Granick's attractive hypothesis ferrous iron enters the epithelial cells of the intestine, is there altered to a ferric state, combined with the protein apoferritin to form ferritin and is held up in the mucosa

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until liberated into the blood stream in the ferrous state. The ferric iron in ferritin acts both as a reservoir for blood iron and as a "mucosal Block".

The mononuclear phagocytic reticulo-endothelial cells and lymphatics play a prominent rôle in transporting iron from the intestine to the associated mesenteric lymph glands. Not only do the phagocytes remove iron, but it appears that iron diffuses directly into the lymphatics and perhaps into the blood vessels themselves. Granick (1944), working with radio-active iron given intravenously, found that very little is "spilled over" in the urine, and none is excreted by the bowel. (Wright 1947; Hynes, 1948). After 13 days, over 80% of the iron was found stored in the liver. Of the crystalline ferritin isolated, three-fourths of the iron in the ferritin had come from the injected iron. One hour after the injection the liver had already taken up

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over 40% of the total injected iron. The storage of iron by the spleen was apparently negligible.

During pregnancy the absorption of iron is greatly increased. Instead of the usual 0.1 - 0.2 milligram of iron being absorbed daily, the amount increases, to an average of 3.2 milligrams daily over the whole duration of the pregnancy. (Macy et al. 1934; Coons 1932). The absorption of iron, however, was not equal at all stages of pregnancy, being highest in the earlier months, with a definite tendency to lower absorption during the very last month. The mother has not only to supply the foetus with iron, but she also requires iron for the hypertrophy of muscles and other organs. Without doubt, the early high iron retentions are for maternal benefits, aside from the immediate foetal needs. The iron content of the foetus at birth is almost 400 milligrams, of the placenta and uterus 150 milligrams, and of the blood lost during the

/ average ...

average delivery 170 milligrams (Wright 1947). Macy and Hunscher (1934) from metabolic studies on 23 gravid women calculated that the mother stores in toto an average of 57, 192, 340, 477, 565, 681, 754 and 799 milligrams of iron from the third to the tenth lunar months respectively. The total maternal retention of 799 milligrams of iron as calculated above, is slightly in excess of the amount contained in the foetus at birth plus that required for the maternal needs of pregnancy, (a total of 720 to 740 milligrams of iron.) The part played by this excess iron may be significant in the preparation for lactation and for the preservation of the maternal reserves during this period. However, no studies are available on the iron metabolism of lactating women.

From analyses of human foetuses it has been shown that the daily average transfer of iron from mother to foetus

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amounts to approximately 0.4 milligram during the first two-thirds of pregnancy. During the last third of pregnancy, however, the accretion was ten times greater, or about 4.7 milligrams of iron daily (Coons, 1932), though this latter figure would appear to be too high. The greatest mineral drain on the maternal tissues then comes during the last three months of gestation. As one would expect, when the mother's diet is rich in iron the foetus will store more iron than when the diet is poor in this metal. The point has been stressed by Marshall (1932).

The precise manner of transfer of iron from the mother to the foetus still awaits demonstration. From early pregnancy to term, vessels in the chorionic villi bring foetal blood into close proximity to a liberal supply of iron, namely, the decidual pools. We know that the barriers presented by the walls of the blood vessels and adjacent tissues will not permit the passage of red blood cells from mother to child, but this close proximity does meet the requirements of an osmotic connection. Tips of the villi may actually project into the maternal blood vessels. Stander (Williams' Obstetrics Textbook) states that the foetus takes up iron from degenerated maternal erythrocytes through the syncitial covering of the chorionic villi. Scholten and Veit observed that the chorionic villi are quite capable of dissolving red blood corpuscles of the circulating blood just as dissolution of red blood cells can be produced by placental extracts.

Recently Kropp (1940)and Dempsey (1944), using micro-incineration methods, noted that the decidual and connective tissue cells contain more iron than either the amniotic or chorionic epithelium. Iron was also detected in the syncitial and cytotrophoblastic elements of the placenta, as well as within the foetal connective

/ tissue ...

tissue of the villi. The syncitium exhibited a small quantity of iron dispersed as punctate aggregates of ash throughout the cytoplasm, and a denser concentration of ferrous material aggregated around all of the nuclei. On the other hand, the cytotrophoblastic cells were characterised by a heavy concentration of iron in the interstitial ground substance between them, whereas the cytoplasm and nuclear membranes contained very little. This iron present at the placental barrier probably represents iron that is passing from the maternal blood to the foetal blood, as well as the iron of the respiratory enzymes that must be present in the syncitium, since this is a region where active oxidative metabolism occurs.

Pommerenke et al. (1942) investigated the transmission of iron to the foetus using radio-active iron. They administered a measured quantity of radio-active iron solution in water or fruit juice to the

/ mothers ...

mothers at selected times prior to the termination of their pregnancy. They noted that minute but measurable amounts of this isotope could be demonstrated in the foetal circulation in transport in the plasma in as little time as 40 minutes after feeding the mother. It seems, therefore, quite improbable that the only route of transmission of iron from the mother to the foetus is through the complicated chain of events expressed in most texts, namely : maternal plasma iron to red cell iron; passing of the latter to the placenta; destruction of the red cell with liberation of the contained haemoglobin iron; absorption and finally elaboration of the iron into the haemoglobin of the foetal blood cells. It seems doubtful that the radio-active iron could follow all these steps before it appears in the foetal plasma in as little time as 40 minutes following the feeding of the mother. It seems more likely that at least part of the iron is conveyed to the

/ placenta ...

placenta in the form of serum or plasma iron and from this form is transferred across the placental barrier to the foetal circulation.

According to the increase in the iron content of the foetus from month to month, the amount of iron passing over daily to the foetus during the fifth and sixth months was 0.5 milligram (Macy and Hunscher 1934), although Coons (1932) places the figure at 0.4 milligram. Macy and Hunscher found that the uptake of iron by the foetus increased to 3.4 milligrams daily during the ninth month and to 6.6 milligrams daily during the tenth month. Swanson and Iob (1939), from their investigations, showed that the iron retention by the foetus during the last two months of pregnancy formed 67% of the total foetal iron found at birth. Thus,foetuses born prematurely must necessarily have low iron stores.

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A total iron content of 375 milligrams in the mature human foetus is accepted as a fair average figure from the various analyses reported by Hugounenq, Brübacker, Camerer and Söldner. Macy and Hunscher (1934), however, found the total iron content to be only 246 milligrams at the tenth month of intra-uterine life and Swanson and Iob (1939) give the average total iron as 280 milligrams. The bulk of this iron is in the blood. The figure given for the blood iron of the full term human foetus at the White House Conference (1932), was 134 milligrams, but it must be much higher than this. The average volume of blood in circulation in the newborn infant is 360 ccs (de Marsh 1942) and as this blood contains on an average 22 grams of haemoglobin per 100 ccs blood (Stearns et al.1937), then the haemoglobin iron alone would account for approximately 264 milligrams of iron. At the White House Conference the amount of iron present in the muscle tissue of the

/ newborn ...

newborn was given as 39 milligrams. The average amount of iron stored in the liver of the newborn, as shown by my own series, was approximately 33 milligrams and in the spleen almost 2 milligrams of iron. These latter figures for liver and spleen are for Bantu infants, and are probably lower than in European infants, since malnutrition is more common amongst the Bantu people. Strauss (1933) has shown that the liver storage is inconstant and may be negligible if the diet of the mother has been low in iron.

In general, it is probable that the average full term infant stores from two to three times as much iron from the breakdown of the excess haemoglobin in the blood as is already stored in the liver at birth. Thus, though there is a storage of iron at birth, the bulk is not accumulated until after the post-natal blood destruction is complete.

Of the blood iron present at birth, 60 milligrams, calculated by Swanson (1939), may be liberated and stored when the breakdown of the excess blood in the circulation has taken place. Stearns and McKinley (1937), put the figure as high as 150 milligrams of iron but the average is probably nearer the former figure (about 80 - 90 milligrams of iron). Evidence that not all the released iron is stored is found in the studies of Beck (1932 - quoted by Stearns and McKinley) and of Langstein and Edelstein (1914). Beck observed that iron excretion in the newborn increased from the second day after birth and reached a maximum between the thirtieth and fortieth day, then gradually decreased. Thus, it appears that during the early weeks of life the excretion of iron by the infant almost invariably exceeds the intake, but that after the second month the iron balance, though variable, tends to approach zero. Stearns and McKinley (1937) state that an average artificially fed full term infant may be expected to lose up to 1.25 milligrams of iron daily for the first

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two months of life, but will approach equilibrium soon after that period. The figures given at the White House Conference for the negative iron balance of normal full term breast fed babies were generally lower than this, varying from 0.04 to 1.28 milligrams daily, and the duration of negative balance was not so prolonged,being only about two weeks. It thus appears that very young infants do not conserve as much of the iron freed by haemoglobin breakdown when given cow's milk,as when they are fed on human milk. (Stearns 1937).

Lichtenstein (quoted in the White House Conference) performed twelve iron metabolism studies on four premature infants. A noteworthy feature is the fact that the experimental periods were of ten days duration. His data showed that in all the cases there was a negative iron balance averaging a daily loss of 0.25 milligram of iron.

It is interesting to note here that some of the earliest studies with radio-active iron showed that the iron liberated by the destruction of effete red blood cells was immediately re-used for haemoglobin synthesis in preference to the reserve iron stores.

The amount of haemoglobin in the blood at birth is higher than at any time subsequently and averages 22 to 23 grams per 100 ccs of blood. (Stearns and McKinley, 1937; Hicks 1942; and Wright 1947). A rapid decrease occurs during the first weeks of life. Two weeks after birth the haemoglobin value has dropped to 17 grams per 100 ccs. At three weeks the haemoglobin value is 15.6 grams. (Stearns,1937). Wide individual differences are observed in the duration of the period of haemoglobin destruction, but by the tenth week of life the amount of haemoglobin has usually decreased to 10.5 grams per 100 ccs of blood. (Stearns, 1937 and Wright, 1947). The shortest period reported for completion of haemoglobin destruction was 21 days, and the longest was 76 days.

It is known that foetal haemoglobin differs in some respects from adult haemoglobin. (Janssen 1943; Kendrew 1949). It is necessary, however, to emphasize that their resemblances are far more striking than their differences. Both have the same molecular weight, about 67,000. In both, the prosthetic group, to which the oxygen becomes attached, is the same iron porphyrin compound (ferroprotohaem) and in both of them, four of these haem groups are contained in each protein molecule. Any differences between these two must, therefore, be due to differences in the protein moiety, which is called globin. Just after birth there is a mixture of about 80% foetal and 20% adult haemoglobin.

One of the properties of a protein is its solubility and the two types of haemoglobin have been found to differ most strikingly in this respect. Foetal haemoglobin

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is over six times more soluble in concentrated phosphate buffers than is the adult haemoglobin. The two kinds of haemoglobin also differ in electric charge; their iso-electric points are not the same. It is possible to separate them by electro-phoresis. It has been found also that human adult and foetal haemoglobin differ in crystal form and symmetry.

The average erythrocyte count immediately after birth is approximately 5,500,000 cells per cubic millimeter though the values are very variable in the first week of life. Haemolysis starts very soon and progresses rapidly, so that by the twelfth postnatal day the count has dropped to 4,500,000 cells per cubic millimeter. (Lucas et al. 1921). The red blood cells of the newborn undergo haemolysis more easily than those of the adult, since there is greater fragility of the cell membranes in the first ten days of life. (Gordon 1933). By the sixth week of life the average erythrocyte count

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has decreased to 3,920,000 cells per cubic millimeter, and by the eleventh week has reached the minimal count of 3,400,000 cells per cubic millimeter. The number of erythrocytes then increases gradually till at six months the count is 4,000,000 cells per cubic millimeter. (Stearn et al. 1937).

Although both blood destruction and icterus are present during the neonatal period, there is no demonstrable relationship between the degree of blood destruction, as indicated by changes in the packed cell volume, and the severity of the icterus, as indicated by the icteric index. (Salmon et al. 1943). Lucas et al. (1921) found that the haemoglobin level of their most jaundiced infants was just as high as that in infants without jaundice, suggesting that the degree of jaundice is determined by liver function. The amount of bilirubin increases after birth and normally reaches its maximum concentration between the first and seventh days of

/ life ...

life. (Gordon, 1933).

Several recent publications have stressed the relationship existing between the iron deficiency during pregnancy and the hypochromic anaemia that appears before the sixth month of life. This anaemia of infancy when not affected by some associated disease is not due to congenital abnormal blood formation since these infants have normal blood at birth. Furthermore, post-natal nutrition of all infants during the first four months is much the same because the food is restricted to milk and vitamin supplements.

In 1931 Mackay published a comprehensive study on nutritional anaemia in infancy, in which a comparative study was made between infants born to moderately anaemic mothers (54 - 70% haemoglobin) and infants born to mothers with normal haemoglobin values. The infants born to the anaemic mothers showed a lower haemoglobin level at every month for the first six months of life than did infants born to non-anaemic mothers.

A later paper by Neale and Hawksley emphasizes the finding of anaemia in twins born to anaemic and non-anaemic mothers. The demand upon a mother for iron in a twin pregnancy is considerably more than in single pregnancies and, even though the mother's haemoglobin may be normal, all her stores may be consumed and one or both of the twins will develop anaemia. Finally, the most conclusive study on this subject is that of Strauss (1933) since it compares a series of 14 mothers with less than 45% haemoglobin with a series of 12 mothers with haemoglobin over 70%. There was no significant difference in the haemoglobin values between the infants of these two groups at birth. However, when these infants were examined one year later, it was found that the infants born to anaemic mothers had an average haemoglobin value of 46% compared with 67% for the infants born to non-anaemic mothers; thus indicating lower

/ iron ...

iron stores in the infants born to anaemic mothers. (Swanson and Iob, 1939).

During normal physiological birth the infant retrieves much of the blood which is in the placental and umbilical vessels. This is effected by uterine contractions on the placenta in the third stage of labour and probably also by vaso-constriction of the umbilical arteries and their branches. When the umbilical cord is clamped immediately after birth over 100 ccs of blood can usually be obtained from the placental end of the cord. (Grodberg et al. 1938). Haselhorst and Allmeling (quoted by De Marsh 1941 and 1942) found that the average amount of blood in the placental vessels of 120 infants was 104 ccs. They recorded accurately the increasing weights of infants during the inflow of placental blood. They also showed that 51% of the placental blood flowed into the infant in the first minute after delivery, 79% reached the child in the first five

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minutes, and 91% in the first ten minutes. Goodall and his colleagues obtained an average of 125 ccs of blood from the placenta for use in a blood bank. Grodberg salvaged as much as 215 ccs of placental blood from one case.

De Marsh et al. (J.A.M.A. 1941) found that the infant receives an additional amount of blood between the time the cord stops pulsating (average 3 minutes after birth) and the time the uterus contracts firmly with separation of the placenta, this amount varying approximately from 40 to 60 ccs. They claim that only a few ccs of the blood can be obtained from the umbilical cord after separation of the placenta. Thus, it appears that practically all the placental blood flows into the infant if the clamping of the cord is delayed until the placenta begins to descend into the vagina.

The actual total volume of blood of the human foetus, complete with its cord and placenta, has never been determined directly. De Marsh et al. (1942), using Evans blue dye,

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calculated the blood volume of 35 newborn infants and found that they varied between 300 and 370 ccs. Thus, if the volume of the placental blood is taken as about 104 ccs, this would represent from one-third to one-quarter of the volume of blood in the infant. The loss of this blood, De Marsh points out, would thus be equivalent to submitting the infant to a rather severe haemorrhage and this may be critical for premature infants and infants who have suffered a long and difficult labour. He quotes Engel who, in the latter part of the nineteenth century, reported twice as great a mortality in premature infants whose cords were tied early as in those whose cords were tied late. Deprivation of the placental blood also results in a relatively large loss of iron to the infant.

Blood volumes were calculated by De Marsh et al. (1942) on two series of newborn infants. In the first series,

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consisting of 18 cases, the umbilical cords were clamped immediately after birth. In the second series, 17 cases, the cord was only clamped after the placenta had descended into the vagina. Blood volumes were done on the first and third day. For the group deprived of their placental blood the average blood volume for the first day was 314 ccs. and on the third day 294 ccs. For the second group, who had received their placental blood, the figures were 364 ccs for the first day and 360 ccs for the third day. The plasma volume in both groups was found to be remarkably constant, averages varying only a few ccs from a mean of 139 ccs. Average haemocrit values of 53% on the first day and 51% on the third day were found in the first group and 61% on the first day and 60% on the third day in the second group, who had obtained their placental blood.

Blood estimations done during the first week of life on infants whose cords

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were clamped immediately, revealed an average erythrocyte count of 5,450,000 cells per cubic millimeter and a haemoglobin value of 19.5 grams per 100 ccs blood. Estimations done on the second group whose cords were clamped late gave an average erythrocyte count of 6,200,000 cells per cubic millimeter and a haemoglobin value of 22.8 grams per 100 ccs of blood. (De Marsh et al. 1941). There is, therefore, a significant decrease in the erythrocyte count and in the haemoglobin content of the blood in those infants whose cords were clamped early.

One may assume that the average amount of blood lost to the infant by early clamping of the cord is 104 ccs. Since the average haemoglobin content of the cord is 16 grams per 100 ccs of blood (Wilson et al. 1941) by immediate clamping of the cord the infant is deprived of 56 milligrams of iron. As the infant is dependent on the iron present at birth for the normal formation

/ of ...

of haemoglobin later, the loss of this amount of iron can result in a lowering of the haemoglobin content of the blood, at six months of age, from 12 to 9 grams per 100 ccs.

Reviewing briefly a few points that have already been mentioned, we know that the infant is largely dependent on the supply of iron present at birth for growth and for the formation of haemoglobin during the first six months of life. The amount of iron obtained from the diet over this period is minimal. The iron reserve of the infant at birth is contained in the circulating haemoglobin and, to a lesser extent in the tissues, especially the liver. The iron liberated during the physiological destruction of blood is stored and becomes available for re-utilization. At the end of six months the total iron content of the infant is about the same as at birth, but the haemoglobin content per 100 ccs of blood is reduced to almost half the amount present at birth. At this age the iron reserves are practically depleted and further growth, with its necessary iron quota for every cell, might occur at the expense of the haemoglobin concentration of the blood,with the development of a hypochromic anaemia. The smaller the stores of iron available the earlier will the anaemia appear.

It is obvious that the metal is at a premium throughout the first six months of life and, should either the iron stores in the liver be low at birth, or a large quantity of iron be lost due to the placental blood not being received, there must be a decided tendency for anaemia to develop in early infancy. Should the infant have a low iron storage in the liver and also have its cord clamped immediately after birth, then the predisposition to anaemia must be very great and this can be witnessed in the anaemia of premature babies where these two factors obtain.

CONCLUSIONS.

CONCLUSIONS.

1. When investigating the infant liver for iron storage the whole organ should be taken for analysis - not samples.

2. For accurate results the iron due to the blood in the liver must be taken into account.

3. The iron storage in the foetal liver and spleen increases both absolutely and relatively during the last third of pregnancy.

4. The liver is a much larger storehouse for iron than the spleen.

5. The iron stores at birth of the average Bantu infant, in this series, are slightly lower than those of the average European infant reported in the literature.

/ 6. The ...

6. The iron stores in the liver and spleen are increased absolutely and relatively in the early neonatal period.

7. From the work of De Marsh, Windle and other authors, early clamping of the umbilical cord at birth robs the infant of a considerable amount of blood and thereby reduces the amount of iron stored in the neonatal period.

8. It is suggested that a low iron store at birth combined with early clamping of the umbilical cord will predispose most strongly to anaemia developing in early infancy.

S U M M A R Y .

S U M M A R Y.

1. A method of investigating non-haemoglobin iron in livers and spleens is described.

2. 49 livers and 22 spleens of Bantu infants and foetuses were analysed for iron and the results obtained presented.

3. A review of the literature is given.

4. A discussion on the incidence of iron in normal adults, in pregnant women and in newborn infants is presented.

APPENDIX.

NUMBER 1.

MARY SIHLABAKA (B.M.H. No. 1296) Full term male. Birth weight 8lbs 6 ozs. Forceps delivery. Stillbirth.

Weight of Crucible + liver	=	317 Grams.
Weight of Crucible	=	175 Grams.
Therefore Weight of Liver	=	142 Grams.

The liver was cut into slices about 2 mms thick, which were then washed in running water for 18 hours. After this period they appeared to be quite free of blood, being pale greyish in colour. They were then placed in an air oven at 60°C to dry them to constant weight. After 4 hours it was seen that a fair amount of haemoglobin had oozed out of the liver tissue, which had now collapsed, indicating that the washing had not removed all the blood present in the liver.

Nevertheless, the slices were dried to constant weight.

Weight of crucible + dried liver	=	193 Grams
Weight of crucible	=	175 Grams
Therefore weight of dried liver	=	18 Grams.

/ A ....

NUMBER 1 - Continued.

A small chip of dried liver was taken for iron estimation :

Weight of watchglass + sample = 3.2264 Grams.
Weight of watchglass = 3.0720 Grams.
Therefore weight of liver sample = 0.1544 Gram.

This sample was digested and made up to 100 ccs solution.

4 ccs solution taken for colorimetry.

G = 36 Therefore L = .444.

From graph this = 0.0134 mgm iron

= amount in 4 ccs solution.

Therefore :

In 100 ccs solution amount iron = $\left(.0134 \times \frac{100}{4}\right)$ mgm

= 0.335 mgm iron

= amount in 0.1544 Gram dried liver.

Therefore in 18 Grams dried liver amount iron = $\left\{.335 \times \frac{18}{.1544}\right\}$ mgm

= 39 mgms iron

This result, however, must be disregarded, since the dried liver still contained a certain amount of haemoglobin. Therefore this figure does not represent either the total iron in the liver nor the non-haemoglobin iron in the liver, but something between these two figures.

/ NUMBER 2 ...

NUMBER 2.

Maisy Ngwenga (B.M.H. No. 1150) Full term.
Female. Birth Weight 6 lbs.1 oz. Aged 10 days.
Died of bilateral broncho-pneumonia.

Weight of crucible + liver	=	280 Grams
Weight of crucible	=	210 Grams
Therefore weight of Liver	=	70 Grams.

Liver was perfused through umbilical and portal veins with water for 16 hours. Organ was yellowish grey in colour after this period and all blood appeared to be washed out. Liver was then put into an air oven for 24 hours. After this period the liver had been dried to constant weight and was still a yellowish-grey, indicating the absence of haemoglobin.

Weight of crucible + dried liver	=	226 Grams
Weight of crucible	=	210 Grams
Therefore weight of dried liver	=	16 Grams

Two small pieces of dried liver were taken for iron estimation.

/ A. Weight ...

NUMBER 2 - Continued.

A.

Weight of watchglass + sample	= 3.2796 Grams.
Weight of watchglass	= 3.0700 Grams.
Therefore weight of sample	= 0.2096 Gram.

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry.

$G = 76^2$ therefore $L = .1163$

From graph this = .0031 mgm iron
= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron =

$$\left(.0031 \times \frac{100}{2} \right) \text{ mgm}$$

= 0.155 mgm iron

= amount in 0.2096 Gram dried liver

Therefore in 16 Grams dried liver amount iron =

$$\left(.155 \times \frac{16}{.2096} \right) \text{ Mgms.}$$

= 11.83 mgms iron

= actual iron (or non-haemoglobin iron)in liver.

/ B. Weight ...

NUMBER 2 - continued.

B.

Weight of watchglass + sample	=	3.241 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of sample	=	0.171 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

$G = 79^2$ therefore $L = .0996$.

From graph this = 0.0026 mgms iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron =

$$\left(.0026 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.13 mgm iron

= amount in 0.171 Gram dried liver

Therefore in 16 Grams dried liver amount iron =

$$\left(.13 \times \frac{16}{.171}\right) \text{ mgms}$$

= 12.2 mgms iron

= Actual iron in liver (non-haemoglobin iron).

Average between A and B = 12 mgms iron.

(For comment on difference in results between A and B see text).

/ NUMBER 3 ....

NUMBER 3. Martha Masweu. (B.M.H. No. 1355).

Full term. Female. Stillbirth. Birth Weight 7 lbs.

Weight of Crucible + liver	=	331 Grams
Weight of Crucible	=	201 Grams
Therefore weight of liver	=	130 Grams.

Liver was perfused free of haemoglobin. It was placed in a vacuum oven overnight, but the temperature did not rise above 37° C. A very luxuriant fungus growth was found covering the liver the following day, so the specimen was discarded.

/ NUMBER 4 ...

NUMBER 4. Selina (B.M.H. No. 1393) Premature (35 - 36 weeks) Birth weight 4 lbs 14 ozs.

Weight of crucible + liver	=	274.5	Grams
Weight of crucible	=	175.5	Grams
Therefore weight of liver	=	99	Grams

The liver was perfused with normal saline through umbilical vein and portal vein till it was free of haemoglobin. It was then placed in air oven and dried to constant weight.

Weight of crucible + dried liver	=	179.3	Grams
Weight of crucible	=	175.5	Grams
Therefore weight of dried liver	=	3.8	Grams

A small piece was removed from right side of dried liver (A) and another from the left side (B).

A.

Weight of watchglass + sample (right side)	=	13.3544	Grams
Weight of watchglass	=	12.777	Grams
Therefore weight of sample	=	0.5774	Gram

/ This ...

NUMBER 4 - continued.

This was digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$G = 43$ therefore $L' = 0.367$

From graph this = .0097 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0097 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.485 mgm iron

= amount in 0.5774 Gram dried liver.

Therefore in 3.8 Grams dried liver amount iron

$$= \left(.485 \times \frac{3.8}{.5774}\right)$$

= 3.26 mgm iron.

= non-haemoglobin iron in liver.

/ B. ....

NUMBER 4 - continued.

B.

Weight of watchglass + sample (left side)	= 3.344 Grams
Weight of watchglass	= 3.07 Grams
Therefore weight of sample	= 0.274 Gram.

This was digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 55 therefore L = .2596.

From graph this = .0069 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0069 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.345 mgm iron

= amount in 0.274 Gram dried liver

Therefore in 3.8 Grams dried liver amount iron

$$= \left(.345 \times \frac{3.8}{.274}\right) \text{ mgms}$$

= 4.8 mgms iron.

= non-haemoglobin iron in liver.

Therefore average from A and B = 4 mgms iron.

(For comment on differences in results between A and B see text).

/ NUMBER 5 ...

NUMBER 5. Magdeline (B.M.H. No. 1449)
Full term. Male. Birth weight 6 lbs 8 ozs.
Stillbirth.

On opening abdomen and thorax a very large diaphragmatic hernia was found into the right pleural cavity. The hernia consisted of the liver and the entire small gut. The specimen was left intact and was presented to the Anatomy Department.

/ NUMBER 6 ....

<u>NUMBER 6.</u> Sara (B.M.H. No. 1353) Full term. Female. Birth weight 6 lbs. Aged 6 days.

Weight of crucible + liver	=	286 Grams
Weight of crucible	=	186 Grams
Therefore weight of liver	=	100 Grams.

Liver was perfused with normal saline till free of haemoglobin. In this case it required only ten litres to achieve this. The liver was then placed in an air oven and dried to constant weight.

Weight of crucible + dried liver	=	195.2 Grams
Weight of crucible	=	186 Grams
Therefore weight of dried liver	=	9.2 Grams.

Because of the relatively large differences in results obtained when different pieces of liver were taken for iron estimation (such as in Numbers 2 and 4 preceding), it was decided to crush this dried liver to a fine powder and mix it thoroughly to ensure an even distribution of iron.

Two samples were taken for iron estimation :

/ A. ....

NUMBER 6 - Continued.

A. Weight of watchglass + sample = 3.4656 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of sample = 0.3956 Gram.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$G = 54^2$ therefore $L = .2557$

From graph this = .0069 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$= \left(.0069 \times \frac{100}{2} \right)$ mgm

= 0.345 mgm iron

= amount in 0.3956 Gram liver powder.

Therefore in 9.2 Gram dried liver amount iron

$= \left(.345 \times \frac{9.2}{.3956} \right)$ mgm

= 8.02 mgms iron.

/ B. ....

NUMBER 6 - continued.

B.

Weight of watchglass + sample = 14.1780 Grams
Weight of watchglass = 12.777 Grams
Therefore weight of sample = 1.401 Gram.

Sample digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry.

$G = 35^2$ therefore $L = .450$

From graph this = .0120 mgm iron

= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron

= 1.2 mgm iron

= amount in 1.401 Gram dry liver powder.

Therefore in 9.2 Grams dried liver amount iron

$= \left(1.2 \times \frac{9.2}{1.401}\right)$ mgm

= 7.88 mgms iron.

Therefore average from (A) and (B) = 7.95 mgm Fe

= non-haemoglobin iron in liver.

/ NUMBER 7. ...

NUMBER 7. Maria Mathe. (B.M.H. No.1578) Full term. Stillbirth. Male. Birth Weight 7 lbs.10 ozs.

Weight of crucible + liver	= 361 Grams
Weight of crucible	= 213 Grams
Therefore weight of liver	= 148 Grams.

Liver was perfused with normal saline through the umbilical and portal veins till free of haemoglobin. It was then heated in an air oven till it had reached constant weight.

Weight of crucible + dried liver	= 221.5 Grams
Weight of crucible	= 213 Grams
Therefore weight of dry liver	= 8.5 Grams.

The dry liver was crushed to a fine powder and mixed.

Two samples were taken for iron estimation.

/ A. ...

NUMBER 7 - continued.

A.

Weight of watchglass + sample	= 3.7770	Grams
Weight of watchglass	= 3.07	Grams
Therefore weight of sample	= 0.707	Gram.

Sample was digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry.

G = 47 therefore L = .328

from graph this = .0088 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0088 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.44 mgm iron

= amount in 0.707 Gram dried liver.

Therefore in 8.5 Grams dried liver amount iron

$$= \left(.44 \times \frac{8.5}{.707}\right) \text{ mgms}$$

= 5.29 mgms iron.

= non-haemoglobin iron in liver.

/ B. ...

NUMBER 7 - continued.

B.

Weight of watchglass + sample	= 3.5054 Grams
Weight of watchglass	= 3.07 Grams
Therefore weight of sample	= 0.4354 Gram.

Sample was digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry.

G = 62 therefore L = .2076.

From graph this = .0055 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0055 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.275 mgm iron

= amount in 0.4354 Gram dried liver.

Therefore in 8.5 Grams dried liver amount iron

$$= \left(.275 \times \frac{8.5}{.4354}\right) \text{ mgm}$$

= 5.36 mgms iron.

= Non-haemoglobin iron.

Average between A and B = 5.325 mgms iron

= non-haemoglobin iron in liver.

/ NUMBER 8 ....

<u>NUMBER 8.</u> Anna Mabjela (B.M.H. No. 1847). Full term. Male. Birth weight 8 lbs. Stillbirth.

Weight of crucible + liver	= 335 Grams
Weight of crucible	= 175 Grams
Therefore weight of liver	= 160 Grams.

(1) A small section was cut from the right lobe of the liver :

Weight of watchglass + liver specimen	= 13.825 Grams
Weight of watchglass	= 12.777 Grams
Therefore weight of liver specimen	= 1.048 Grams.

This small piece of fresh, unperfused liver was then digested and made up to 100 ccs solution.

2 ccs solution were taken for colorimetry.

G = 443 therefore L = .349.

From graph this = .0093 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0093 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.465 mgm iron

= amount in 1.048 Gram unperfused liver.

Therefore in 160 Grams unperfused liver amount Fe

$$= \left(.465 \times \frac{160}{1.048}\right) \text{ mgm}$$

= <u>70.9 mgms iron.</u>

= <u>Total iron in unperfused liver.</u>

/ (2) ...

NUMBER 8. - continued.

(2) Liver was then perfused with normal saline, it requiring 80 litres of normal saline to render it blood-free.

Perfused liver, due to water absorption, had increased in weight to 165 Grams.

(Corrected weight $= 165 \times \frac{160}{158.9} = 166$ Grams)

A small section was removed from the right lobe for iron estimation.

Weight of watchglass + section of perfused liver	= 13.95 Grams
Weight of watchglass	= 12.777 Grams
Therefore weight of liver section	= 1.173 Grams.

This small section of perfused liver was digested and made up to 100 ccs solution.

10 ccs solution taken for colorimetry :
G = 44' therefore L = .354
From graph this = .0095 mgm iron

= amount in 10 ccs solution.

Therefore in 100 ccs solution amount iron

= .095 mgm

= amount in 1.173 Gram perfused liver.

Therefore in 166 Grams perfused liver amount iron

$= \left(.095 \times \frac{166}{1.173}\right)$ mgm

= <u>13.4 mgm iron</u>
= <u>non-haemoglobin iron in liver.</u>

/ (3) ...

NUMBER 8 - continued.

(3) Perfused liver was then dried to constant weight in an air oven.

Weight of crucible + dried liver	= 187.2	Grams
Weight of crucible	= 175	Grams
Therefore weight of dried liver	= 12.2	Grams

This was crushed to a fine powder and mixed.

Two samples were taken for iron estimation :

A.	Weight of watchglass + sample	= 3.3736	Grams
	Weight of watchglass	= 3.070	Grams
	Therefore weight of sample	= .3036	Gram

Digested and made up to 100 ccs solution

2 ccs solution taken for colorimetry :

G = 72' therefore L = .1412.

From graph this = .0037 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0037 \quad x \quad \frac{100}{2}\right) \text{mgm}$$

= 0.185 mgm iron

= amount in 0.3036 Gram dry liver.

Therefore in 12.2 Grams dried liver amount iron

$$= \left(.185 \quad x \quad \frac{12.2}{.3036}\right) \text{mgms}$$

= 7.104 mgms iron.

/ B. ...

NUMBER 8 - continued.

B. Weight of watchglass + sample = 3.6064 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of sample = 0.5364 Gram.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry.

$G = 58^2$ therefore $L = .2328$.

From graph this = .0062 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0062 \times \frac{100}{2} \right) \text{ mgm}$$

= 0.31 mgm iron

= amount in 0.5364 Gram dry liver.

Therefore in 12.2 Grams dried liver amount iron

$$= \left(.31 \times \frac{12.2}{.5364} \right) \text{ mgms}$$

= 7.05 mgms iron.

Therefore average from (A) and (B) = 7.077 mgms Fe

= non-haemoglobin iron in liver.

(The fact that the results of (2) and (3) do not correspond tends to indicate an uneven distribution of iron in the liver. The very large difference between the results of (1) and (3) can hardly be accounted for by haemoglobin iron alone, so this rather indicates that iron is being lost, probably, with the perfusion.)

/ NUMBER 9 ...

NUMBER 9. Edith Tshabalala. (B.M.H. No.1864)

Stillbirth. Full term. Female. Birth Weight

7 lbs. 9 ozs.

Weight of crucible + liver	= 410.5 Grams
Weight of crucible	= 219 Grams
Therefore weight of liver	= 191.5 Grams.

Liver perfused with normal saline, it requiring 40 litres to render the organ blood free. The perfused liver then weighed 135 Grams.

(1) Two pieces were taken from the perfused liver for iron estimation, one piece from the right lobe, and the other from the left lobe.

A. SPECIMEN TAKEN FROM RIGHT LOBE.

Weight of watchglass + liver specimen	= 15.2234 Grams
Weight of watchglass	= 12.7770 Grams
Therefore weight of liver specimen	= 2.4464 Grams

This was digested and made up to 100 ccs solution.

2.5 ccs solution taken for colorimetry.

G = 49. therefore L = .310

From graph this = .0083 mgm iron

= amount in 2.5 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0083 \times \frac{100}{2.5}\right) \text{ mgm}$$

= 0.332 mgm iron

= amount in 2.4464 Grams perfused liver

/ Therefore ...

NUMBER 9 - continued.

Therefore in 135 Grams perfused liver, amount

$$\text{iron} = \left(.332 \times \frac{135}{2.4464}\right) \text{ mgms iron}$$

= 18.3 mgms iron

= non-haemoglobin iron in liver.

B. SPECIMEN TAKEN FROM LEFT LOBE.

Weight of watchglass + liver specimen = 4.67 Grs.
Weight of watchglass = 3.07 Grs.
Therefore weight of liver specimen = 1.6 Gram.

This was digested and made up to 100 ccs solution.

2.5 ccs solution taken for colorimetry:

G = 48' therefore L = .317

From graph this = .0085 mgm iron

= amount in 2.5 ccs solution

therefore in 100 ccs solution amount iron

$$= \left(.0085 \times \frac{100}{2.5} \right) \text{ mgm}$$

= 0.34 mgm iron

= amount in 1.6 Gram perfused liver.

Therefore in 135 Grams perfused liver amount iron

$$= \left(.34 \times \frac{135}{1.6} \right) \text{ mgms}$$

= 28.75 mgms iron

= non-haemoglobin iron in liver.

NUMBER 9 - continued.

(2) The remainder of the liver mass dried to constant weight in air oven.

Weight of crucible + dried liver	= 230 Grams
Weight of crucible	= 219 Grams
Therefore weight of dried liver	= 11 Grams

Corrected weight of liver $= \left(11 \times \frac{135}{130.9536} \right)$ Grams

$= \underline{11.33 \text{ Grams}}$

This was crushed to a fine powder and mixed.

Two samples were taken for iron estimation :-

(C)

Weight of watchglass + sample	= 13.343 Grams
Weight of watchglass	= 12.777 Grams
Therefore weight of sample	= 0.566 Gram.

This was digested and made up to 100 ccs.

1 cc solution taken for colorimetry :

$G = 43^3$ therefore $L = .359$

From graph this = .0096 mgm iron

= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron

= 0.96 mgm iron

= amount in 0.566 Gram dry liver.

/ Therefore ...

NUMBER 9 - continued.

Therefore in 11.33 Grams dried liver amount Fe

$$= \left(.96 \times \frac{11.33}{.566} \right) \text{ mgms}$$

= 19.2 mgms iron

= non haemoglobin iron in liver.

(D)

Weight of watchglass + sample	=	3.585 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of sample	=	0.515 Gram.

Sample digested and made up to 100 ccs.

1 cc solution taken for colorimetry.

G = 47 therefore L = .328

From graph this = .0088 mgm iron

= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron

= .88 mgm iron

= amount in 0.515 Gram dry liver.

Therefore in 11.33 Grams dried liver amount iron

$$= \left(.88 \times \frac{11.33}{.515} \right) \text{ mgms}$$

= 19.3 mgms iron

= non-haemoglobin iron in liver.

Average between (C) and (D) = 19.25 mgms iron.

= non-haemoglobin iron in liver.

/ (The ...

NUMBER 9 - continued.

(The difference in the results obtained from (A) and (B) in (1) above indicate again an uneven distribution of iron in the liver. As with liver No. 4, there appears to be a greater quantity of iron in the portion taken from the left lobe of the liver as compared to that from the right lobe of the liver.)

/ NUMBER 10 ...

NUMBER 10. Agnes Zwane. (B.M.H. No. 1899)

Full term. Male. Stillbirth. Birth Weight 8 lbs.

Volume of liver	= 120 ccs
Weight of crucible + liver	= 338 Grams
Weight of crucible	= 201.5 Grs.
Therefore weight of liver	= 136.5 Grs.

(1) A small piece was removed from the right lobe of the unperfused liver :

Weight of watchglass + liver section	= 14.106 Grs.
Weight of watchglass	= 12.777 Grs.
Therefore weight of liver section	= 1.329 Grs.

This section was digested and made up to 100 ccs solution. 2 ccs solution taken for colorimetry.

G = 42' therefore L = .374

From graph this = .0099 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0099 \times \frac{100}{2} \right) \text{mgm}$$

= 0.495 mgm iron

= amount in 1.329 Gram unperfused liver.

Therefore in 136.5 Grams unperfused liver amount Fe

$$= \left(.495 \times \frac{136.5}{1.329} \right) \text{Mgms}$$

= <u>51 mgms iron</u>

= <u>Total iron in liver.</u>

/ (2) ...

NUMBER 10 - continued.

(2) Liver was perfused with normal saline till it was free of haemoglobin. It then weighed 87.5 Grams. Corrected weight $= \left(87.5 \times \frac{136.5}{135.171}\right)$

$= 88$ Grams.

A small section (A) was removed from the right lobe of the perfused liver, and another section (B) from the left lobe :

(A).

Weight of watchglass + specimen	=	13.769 Grams
Weight of watchglass	=	12.777 Grams
Therefore weight of specimen	=	.992 Gram

Digested and made up to 100 ccs solution.

5 ccs solution taken for colorimetry.

G = 72^3 therefore L = .1382

From graph this = .0036 mgm iron

= amount in 5 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0036 \times \frac{100}{5}\right)$ mgm

= .072 mgm iron

= amount in .992 Gram perfused liver.

/ Therefore ...

NUMBER 10 - continued.

Therefore in 88 Grams perfused liver amount

$$\text{iron} = \left(.072 \times \frac{88}{.992} \right) \text{ mgms}$$

= 6.4 mgms iron

= non-haemoglobin iron in liver.

(B)

Weight of watchglass + specimen = 3.89 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of specimen = 0.82 Gram

Digested and made up to 100 ccs solution.

5 ccs solution taken for colorimetry.

G = 75 therefore L = .1249

From graph this = .0033 mgm iron

= amount in 5 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0033 \times \frac{100}{.5} \right) \text{ mgm}$$

= .066 mgm iron

= amount in 0.82 Gram of perfused liver.

Therefore in 88 Grams perfused liver amount

$$\text{of iron} = \left(.066 \times \frac{88}{.82} \right) \text{ Mgms}$$

= 7.08 mgms iron

= non-haemoglobin iron in liver.

/ (3) ...

NUMBER 10 - continued.

(3) Liver was then dried to constant weight in air oven.

Weight of crucible + dried liver	=	209.5 Grams
Weight of crucible	=	201.5 Grams
Therefore weight of dried liver	=	8 Grams

Corrected weight $= \left(8 \times \frac{88}{86.188}\right)$ Grams = 8.15 Grs.

The dried liver was crushed to a fine powder and mixed. 2 samples were taken for iron estimation.

(C).

Weight of watchglass + sample	=	13.157 Grams
Weight of watchglass	=	12.770 Grams
Therefore weight of sample	=	0.387 Gram

Sample digested and made up to 100 ccs .

2 ccs solution taken for colorimetry.

G = 54 therefore L = .2676.

From graph this = .00715 mgm iron = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.00715 \times \frac{100}{2}\right)$ mgm

= 0.3575 mgm iron

= amount in 0.387 Gram dry liver.

Therefore in 8.15 Gram dry liver amount iron

$= \left(.3575 \times \frac{8.15}{.387}\right)$ mgms iron

= <u>7.52 mgms iron</u>

= <u>non-haemoglobin iron in liver.</u>

/ (D) ...

NUMBER 10 - continued.

(D)

Weight of watchglass + sample	=	3.334 Grams
Weight of watchglass	=	3.070 Grams
Therefore weight of sample	=	0.264 Gram

Sample digested and made up to 100 ccs

2 ccs solution taken for colorimetry :

G = 65^3 therefore L = .1821

From graph this = .0048 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0048 \times \frac{100}{2} \right) \text{ mgm}$$

= .24 mgm iron

= amount in 0.264 Gram dry liver.

Therefore in 8.15 Grams dried liver amount iron

$$= \left(.24 \times \frac{8.15}{.264} \right) \text{ mgms}$$

= 7.4 mgms iron.

= non-haemoglobin iron in liver.

Therefore average between (C) and (D) = 7.46 mgms iron.

/ (The ...

NUMBER 10 - continued.

(The difference in results between (A) and (B) in (2) above indicate again an uneven distribution of iron in the liver, with again, a slight preponderance in the left lobe. The very great difference between the results of (1) and (3) can hardly be accounted for by haemoglobin iron alone, and it tends to indicate that iron must be lost, probably during perfusion.)

/ NUMBER 11 ...

<u>NUMBER 11.</u> Kate Kubeka (B.M.H. No.3036) Full term. Male. Birth weight $8\frac{1}{2}$ pounds. Stillbirth.

<u>(1).</u> Two small pieces were removed from the unperfused liver, the one (A) from the right lobe, the other (B) from the left lobe.

<u>(A)</u>

Weight of watchglass + specimen	=	13.566 Grams
Weight of watchglass	=	12.777 Grams
Therefore weight of liver specimen from right lobe	=	.789 Gram

This was digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$$G = 54^3 \quad \text{therefore } L = .2616$$

From graph this = .007 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.007 \times \frac{100}{2} \right) \text{ mgm}$$

= 0.35 mgm iron
= amount in .789 Gram liver

Therefore in 155.5 Grams unperfused liver amount

of iron $= \left(.35 \times \frac{155.5}{.789} \right)$ mgms

= <u>68.9 mgm iron</u>

= <u>Total iron in liver.</u>

/ (B) ...

NUMBER 11 - continued.

(B)

Weight of watchglass + specimen	=	4.046 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of specimen from left lobe	=	0.976 Gram

This was digested and made up to 100 ccs solution

2 ccs solution taken for colorimetry :

G = 44' therefore L = .354

From graph this = .0095 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0095 \times \frac{100}{2} \right) \text{mgm}$$

= 0.475 mgm iron

= amount in 0.976 Gram liver

Therefore in 155.5 Grams unperfused liver amount of iron

$$= \left(.475 \times \frac{155.5}{.976} \right) \text{mgm}$$

= 75.6 mgms iron

= Total iron in liver.

/ (2) ...

NUMBER 11 - continued.

(2) The liver was perfused with normal saline till free of haemoglobin. This required 40 litres of normal saline. Weight of perfused liver = 123.5 Grams. Two small sections were removed from the perfused liver, one section (C) from the right lobe, and the other (D) from the left lobe.

Corrected weight of perfused liver = $\left(123.5 \times \frac{155.5}{153.7}\right)$

= 124.9 Grams.

(C)

Weight of watchglass + specimen = 13.509 Grams
Weight of watchglass = 12.777 Grams
Therefore weight of liver specimen from right lobe = 0.732 Gram

Specimen digested and made up to 100 ccs solution

10 ccs solution taken for colorimetry :

G = 66 therefore L = .1805

From graph this = .0047 mgm iron

= amount in 10 ccs solution

Therefore in 100 ccs solution amount iron

= .047 mgm

= amount in 0.732 Gram perfused liver.

Therefore in 124.9 Grams perfused liver amount

of iron = $\left(.047 \times \frac{124.9}{.732}\right)$ mgms

= 8 mgms iron

= non-haemoglobin iron in liver

/ (D) ...

NUMBER 11 - continued.

(D)

Weight of watchglass + specimen	=	3.479 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of liver specimen from left lobe	=	0.409 Grams

Specimen digested and made up to 100 ccs solution.

10 ccs solution taken for colorimetry :

$$G = 77 \text{ therefore } L = .1135$$

From graph this = .003 mgm iron

= amount in 10ccs solution

Therefore in 100 ccs solution amount iron

= .03 mgm

= amount in .409 Gram perfused liver.

Therefore in 124.9 Grams perfused liver the amount

of iron $= \left(.03 \times \frac{124.9}{.409} \right)$ mgm

= 9.16 mgms iron

= non-haemoglobin iron in liver.

---

(3) The perfused liver was now dried to constant weight in an air oven.

Weight of crucible + dried liver	=	201 Grams
Weight of crucible	=	186 Grams
Therefore weight of dried liver	=	15 Grams
Corrected weight = $\left(15 \times \frac{124.9}{123.759}\right)$	=	15.1Grams

This dried liver was crushed to a fine powder and mixed. Two samples were taken for iron estimation :

/ (E) ....

NUMBER 11 - continued.

(E)

Weight of watchglass + sample	=	13.541 Grams
Weight of watchglass	=	12.770 Grams
Therefore weight of sample	=	0.771 Grams

Sample digested and made up to 100 ccs

1 cc solution taken for colorimetry:

G = 67 therefore L = .1739

From graph this = .0046 mgm iron

= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

= .46 mgm

= amount in .771 Gram dry liver

Therefore in 15.1 Grams dried liver amount iron

$$= \left(.46 \times \frac{15.1}{.771} \right) \text{ mgms}$$

= 9.01 mgms iron

= non-haemoglobin iron in liver.

/ (F) ...

NUMBER 11 - continued.

(F)

Weight of watchglass + sample	= 3.4174 Grams
Weight of watchglass	= 3.07 Grams
Therefore weight of sample	= 0.3474 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

G = 69 therefore L = .1612

From graph this = .0042 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0042 \times \frac{100}{2} \right) \text{ mgm}$$

= .21 mgm iron

= amount in 0.3474 Gram dry liver

Therefore in 15.1 Grams dried liver amount iron

$$= \left(.21 \times \frac{15.1}{.3474} \right) \text{ mgms}$$

= 9.12 mgms iron

= non-haemoglobin iron in liver.

(The difference in results between (A) and (B) in (1), and between (C) and (D) in (2), again indicate an uneven distribution of iron in the liver. The left lobe again contains more iron than the right lobe. The large difference in the results of (1) and (3) can hardly be accounted for by haemoglobin iron alone, and thus indicates that some iron must be lost, probably during perfusion.)

/ NUMBER 12 ...

NUMBER 12. Cecilia (B.M.H. No. 3018) Full term. Birth Weight 6 lbs. Age: 1 day. Died of congenital heart disease.

Volume of liver	= 100 ccs
Weight of crucible + liver	= 323 Grams
Weight of crucible	= 213.5 Grams
Therefore weight of liver	= 109.5 Grams

(1) Two small pieces were removed from the unperfused liver, the one piece (A) from the right lobe, the other piece (B) from the left lobe.

(A).

Weight of watchglass + specimen	= 13.401 Grams
Weight of watchglass	= 12.777 Grams
Therefore weight of specimen from right lobe of liver	= 0.624 Gram

Specimen digested and made up to 100 ccs

2 ccs solution taken for colorimetry

G = 75 therefore L = .1249

From graph this = .0033 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0033 \times \frac{100}{2} \right) \text{mgm}$$

= 0.165 mgm iron

= amount in 0.624 gram liver

Therefore in 109.5 Grams liver amount iron

$$= \left(.165 \times \frac{109.5}{.624} \right) \text{mgms}$$

= 29 mgms iron = Total iron in whole liver.

/ (B) ....

NUMBER 12 - continued.

B.

Weight of watchglass + specimen = 3.561 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of specimen from left lobe of liver = 0.491 Gram.

Specimen digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

$G = 74$ therefore $L = .1308$

From graph this = .0034 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0034 \times \frac{100}{2} \right) \text{ mgm}$$

= .17 mgm iron

= amount in 0.491 Gram liver.

Therefore in 109.5 Grams liver amount iron

$$= \left(.17 \times \frac{109.5}{.491} \right) \text{ mgms}$$

= 37.9 mgms iron

= Total iron in whole liver.

---

(2) Liver was perfused with normal saline till it was free of haemoglobin.

Weight of perfused liver = 92.5 Grams

$$\text{Corrected weight} = \left(92.5 \times \frac{109.5}{108.385} \right)$$

= 93.4 Grams

/ Two ....

NUMBER 12 - continued.

Two small pieces were taken from the perfused liver, the one piece (C) from the right lobe, and the other (D) from the left lobe.

(C).

Weight of watchglass + specimen	= 13.443 Grams
Weight of watchglass	= 12.777 Grams
Therefore weight of specimen from right lobe of perfused liver	= 0.666 Gram

Digested and made up to 100 ccs solution.

10 ccs solution taken for colorimetry :

G = 59[3] therefore L = .2236

From graph this = .0059 mgm iron

= amount in 10 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0059 \times \frac{100}{10} \right)$$

= .059 mgm iron

= amount in .666 Gram perfused liver

Therefore in 93.4 Grams perfused liver amount iron

$$= \left(.059 \times \frac{93.4}{.666} \right) \text{ mgm}$$

= 8.27 mgms iron

= non-haemoglobin iron in liver.

/ (D) ....

NUMBER 12 - continued.

(D)

Weight of watchglass + specimen = 3.581 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of specimen from
left lobe of perfused liver = 0.511 Gram

Digested and made up to 100 ccs solution.

10 ccs solution taken for colorimetry :

G = 58 therefore L = .2366

From graph this = .0063 mgm iron

= amount in 10 ccs solution

Therefore in 100 ccs solution amount iron

= .063 mgm

= amount in .511 Gram perfused liver

Therefore in 93.4 Grams perfused liver amount of

iron $= \left(.063 \times \frac{93.4}{.511} \right)$ mgms

= <u>11.5 mgms iron</u>

= <u>non-haemoglobin iron in liver.</u>

<u>(3)</u> Perfused liver was now dried to constant weight in an air oven.

Weight of dried liver = 10 Grams

Corrected weight $= \left(10 \times \frac{93.4}{92.223} \right)$ Grams

= <u>10.1 Grams.</u>

/ The ....

NUMBER 12 - continued.

The dried liver was crushed to a fine powder and mixed. Two samples were taken for iron estimation.

(E).

Weight of watchglass + sample	=	13.551 Grams
Weight of watchglass	=	12.77 Grams
Therefore weight of sample	=	0.781 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 29^3 therefore L = .527

From graph this = .014 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.014 \times \frac{100}{2} \right) \text{ mgm}$$

= 0.7 mgm iron

= amount in 0.781 Gram dry liver

Therefore in 10.1 Grams dried liver amount iron

$$= \left(.7 \times \frac{10.1}{.781} \right) \text{ mgm}$$

= 9.05 mgm iron

= non-haemoglobin iron in liver.

/ (F) ....

NUMBER 12 - continued.

(F)

Weight of watchglass + sample = 3.421 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of sample = 0.351 Gram

Digested and made up to 100 ccs solution

4 ccs solution taken for colorimetry :

G = 34 therefore L = .469

From graph this = .0125 mgm iron

= amount in 4 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0125 \times \frac{100}{4} \right)$$

= 0.3125 mgm iron

= amount in 0.351 Gram dried liver.

Therefore in 10.1 Grams dried liver amount iron

$$= \left(.3125 \times \frac{10.1}{.351} \right) \text{ mgms}$$

= 8.99 mgms iron

= non-haemoglobin iron in liver.

/ (The ....

NUMBER 12 - continued.

(The difference in results between (A) and (B) in (1) again indicate an uneven distribution of iron, with a preponderance of iron in the left lobe of the liver. There is a difference of 31% between the results of (A) and (B) and this shows the degree of error one may have if the total iron of the liver is estimated from just a small section taken from the mass of the liver.
In (2) there is again a large difference between the results of (C) and (D) with the usual preponderance of iron in the left lobe of the liver.)

/ NUMBER 13 ...

NUMBER 13 - Margaret (B.M.H. No. 2054). Full term. Female. Stillbirth. Birth Weight 6 lbs.2 ozs.

Volume of liver	=	96 ccs
Weight of crucible + liver	=	282 Grams
Weight of crucible	=	175.5 Grams
Therefore weight of liver	=	106.5 Grams

(1). Two small pieces were taken from the liver, one piece (A) from the right lobe, and the other (B) from the left lobe.

(A).

Weight of watchglass + specimen	=	14.9 Grams
Weight of watchglass	=	12.777 Grams
Therefore weight of specimen	=	2.123 Grams

Specimen digested and made up to 100ccs.

1 cc solution taken for colorimetry:

G = 41' therefore L = .385

From graph this = 0.0103 mgm iron
= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron
= 1.03 mgm
= amount in 2.123 Gram liver

Therefore in 106.5 Grams liver amount iron

$$= \left(1.03 \times \frac{106.5}{2.123}\right) \text{ mgms}$$

= 51 mgms iron

= Total iron in whole liver.

/ (B) ....

NUMBER 13 - continued.

(B)

Weight of watchglass + specimen	=	4.34	Grams
Weight of watchglass	=	3.07	Grams
Therefore weight of specimen	=	1.27	Gram

Specimen digested and made up to 100 ccs.

1 cc solution taken for colorimetry :

G = 46 therefore L = .337

From graph this = .009 mgm iron

= amount in 1 cc solution

Therefore in 100 ccs solution amount iron = 0.9 mgm

= amount in 1.27 Gram liver.

Therefore in 106.5 Grams liver amount iron

$$= \left(.9 \times \frac{106.5}{1.27} \right) \text{ mgms}$$

= 75.5 mgms iron

= Total iron in whole liver.

---

(2). Liver was perfused with normal saline till it was free of haemoglobin. It then weighed 109 Grams.

Corrected weight $= \left(109 \times \frac{106.5}{103.107} \right)$

= 112.2 Grams.

Two small sections were taken from the perfused liver, one (C) from the right lobe, and the other (D) from the left lobe.

/ (C) ....

NUMBER 13 - continued.

(C)

Weight of watchglass + specimen = 14.108 Grams
Weight of watchglass = 12.777 Grams
Therefore weight of specimen from right lobe = 1.331 Gram

Specimen was digested and made up to 100 ccs solution. 10 ccs solution taken for colorimetry :

G = 32' therefore L = .490

From graph this = 0.013 mgm iron

= amount in 10 ccs solution

Therefore in 100 ccs solution amount iron

= .13 mgm

= amount in 1.331 Gram perfused liver.

Therefore in 112.2 Grams perfused liver the

$$\text{amount iron} = \left(.13 \times \frac{112.2}{1.331} \right) \text{mgms}$$

= 10.95 mgms iron

= non-haemoglobin iron in Liver.

/ (D) ....

NUMBER 13 - continued.

(D)

Weight of watchglass + specimen	=	4.27 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of specimen from left lobe	=	1.2 Gram.

Specimen was digested and made up to 100 ccs solution. 10 ccs solution taken for colorimetry:

G = 25^3 therefore L = .589

From graph this = 0.0157 mgm iron

= amount in 10 ccs solution

Therefore in 100 ccs solution amount iron

= .157 mgm

= amount in 1.2 Gram perfused liver.

Therefore in 112.2 Grams perfused liver the

amount iron = $\left(.157 \times \frac{112.2}{1.2} \right)$ mgms

= 14.7 mgms iron

= non-haemoglobin iron in liver.

---

(3) Perfused liver was now dried to constant weight in air oven.

Dried liver weighed 7.5 Grams.

Corrected weight = $\left(7.5 \times \frac{112.2}{109.669} \right)$ Grams

= 7.68 Grams

/ The ....

NUMBER 13 - continued.

The dried liver was now crushed to a fine powder and mixed. Two samples were taken for iron estimation :

(E).

Weight of watchglass + sample	=	13.375 Grams
Weight of watchglass	=	12.777 Grams
Therefore weight of sample	=	0.598 Gram

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry :

$$G = 47^2 \text{ therefore } L = .323$$

From graph this = .00865 mgm iron

= amount in 1 cc solution

Therefore in 100 ccs amount iron

= .865 mgm iron

= amount in .598 Gram dry liver

Therefore in 7.68 Grams dried liver amount of

$$\text{iron} = \left(.865 \times \frac{7.68}{.598} \right) \text{ mgms}$$

= 11.1 mgms iron

= non-haemoglobin iron in liver.

/ (F) ....

NUMBER 13 - continued.

(F)

Weight of watchglass + sample	=	3.329 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of sample	=	0.259 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$G = 53'$ therefore $L = .2736.$

From graph this = .0073 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

= 0.365 mgm

= amount in 0.259 Gram dry liver

Therefore in 7.68 Grams dried liver the amount

of iron $= \left(.365 \times \frac{7.68}{.259} \right)$ mgms

= 10.82 mgms iron

= non-haemoglobin iron in liver.

/ (The ....

NUMBER 13 - continued.

(The difference in results between (A) and (B) in (1) again indicates the uneven distribution of iron in the liver, the left lobe having a higher concentration of iron than the right lobe. There is a difference of 48% between results of (A) and (B) and this serves to indicate the degree of error that may be obtained if the total iron of the liver is estimated from just a small section of the liver.
In (2) there is again a large difference in the results between (C) and (D) indicating the uneven distribution of iron, with the preponderance again being in the left lobe).

/ TECHNIQUE ...

TECHNIQUE USED FOR HAEMOGLOBIN ESTIMATION.

Alkaline Haematin method :

5 ccs of varying known strengths of Haemoglobin solutions taken and to these 5 ccs of $N/_5$ NaOH. were added. Boiled in a water bath for 5 minutes : cooled and put into Evelyn Tubes. Filter 540 was used, and distilled water used for 100% transmission.

A specimen of blood was taken from a normal healthy adult Non-European male subject. The haemoglobin of this specimen was accurately estimated using a standard solution of Haemin, the iron content of which was known. (Dr. Bloomberg, Senior Lecturer in Physiology, kindly checked this estimation). The specimen was found to contain 13.62 Grams Haemoglobin per 100 ccs blood.

/ (Standard ...

Standard Haemin was $\equiv \frac{1}{200}$ dilution Hb.

Hb. solution was diluted to $\frac{1}{200}$. 5 ccs of latter was treated as above. On the Evelyn Colorimeter, using a filter 540 and Distilled water as 100% :

Standard Reads G = 39^2

Therefore L = 0.403 (= 10 Grs. Hb.)

Unknown reads G = 28'

L = 0.549 ($\equiv$ 13.62 Grs.Hb.)

5 ccs of this specimen of blood was diluted to 100 ccs with distilled water (Flask 1).

10 ccs of Flask 1 diluted to 100 ccs (Flask 2)

20 ccs of Flask 1 diluted to 100 ccs (Flask 3)

5 ccs of blood contained $\frac{13.62}{20}$ = 0.681 Gr.Hb.

=(Amt.Hb. in Flask 1.)

Therefore in Flask 2 there is $\frac{681}{10}$ = 68.1 mgms Hb./100 ccs.

And in Flask 3 there is 68.1 x 2 = 136.2 mgms Hb./100 ccs.

/ In ....

<u>In Test-Tube 1</u> =

1 cc of Flask 2 + 4cc H_2O + 5 ccs $\frac{N}{5}$ NaOH
(≡ 0.681 mgm Hb)

<u>In Test-Tube 2</u> =

2 ccs of Flask 2 + 3 cc H_2O + 5 ccs $\frac{N}{5}$ NaOH
(≡ 1.362 mgm Hb.)

<u>In Test-Tube 3 =</u>

3 ccs of Flask 2 + 2 cc H_2O + 5 ccs $\frac{N}{5}$ NaOH
(≡ 2.043 mgm Hb)

<u>In Test-Tube 4</u> =

4 ccs of Flask 2 + 1 cc H_2O + 5 ccs $\frac{N}{5}$ NaOH
(≡ 2.724 mgm Hb)

<u>In Test-Tube 5</u> =

5 ccs of Flask 2 + 5 ccs $\frac{N}{5}$ NaOH (≡ 3.405 mgm Hb)

<u>In Test-Tube 6</u> =

3 ccs of Flask 3 + 2 ccs H_2O + 5 cc $\frac{N}{5}$ NaOH
(≡ 4.086 mgm Hb)

<u>In Test-Tube 8</u> =

4 ccs of Flask 3 + 1 cc H_2O + 5 ccs $\frac{N}{5}$ NaOH
(≡ 5.448 mgm Hb)

<u>In Test-Tube 10</u> =

5 ccs of Flask 3 + 5 ccs $\frac{N}{5}$ NaOH (≡ 6.81 mgm Hb)

/ On ....

<u>ON EVELYN COLORIMETER :</u>

Test Tube 1 reads $G = 88^{1}$ therefore $L = 0.0543$
($\equiv$ 0.681 mgm Hb)

Test Tube 2 reads $G = 77^{3}$ therefore $L = .1093$
($\equiv$ 1.362 mgm Hb)

Test Tube 3 reads $G = 68^{3}$ therefore $L = .1627$
($\equiv$ 2.043 mgm Hb)

Test Tube 4 reads $G = 61$ therefore $L = .2147$
($\equiv$ 2.724 mgm Hb)

Test Tube 5 reads $G = 53^{1}$ therefore $L = .2736$
($\equiv$ 3.405 mgm Hb)

Test Tube 6 reads $G = 47^{2}$ therefore $L = .3230$
($\equiv$ 4.086 mgm Hb)

Test Tube 8 reads $G = 36^{2}$ therefore $L = .438$
($\equiv$ 5.448 mgm Hb)

Test Tube 10 reads $G = 28^{1}$ therefore $L = .549$
($\equiv$ 6.81 mgm Hb)

The above L values ($L = 2 - \text{Log } G$) were then used to plot a graph, for subsequent use in estimating unknown solutions of Haemoglobin.

/ LIVER No. 14 ...

<u>LIVER No. 14.</u> Moses Seagwe (No. 2193) (6 days old) B.M.H. Full term male infant. Mother was an eclamptic. Crown-Heel length 20". Birth Weight 5 lbs 10 ozs.

Volume of liver	= 100 ccs
Weight of crucible + liver	= 288.5 Grams
Weight of crucible	= 175.5 Grams
Therefore weight of liver	= 113 Grams

(i) A small section of liver (2.118 Grams) was removed from the anterior edge of the organ in the midline, for haemoglobin estimation.

(ii) First 2,000 ccs of perfusate being collected for haemoglobin estimation.

(iii) A small section was cut from the Right lobe of the liver (1.573 Grams)

(iv) A small section was cut from the Left lobe of the liver (0.973 Gram).

The weight of the perfused liver = 84.5 Grs.

<u>(i)</u>

The 2.118 Grams liver was cut up into very fine pieces and then diluted with distilled water to 100 ccs. This was allowed to stand overnight, after thorough shaking, in the refrigerator.

5 ccs of this solution was taken and treated as / described ...

described previously; ("Technique for Haemoglobin Estimation") the haemoglobin being converted into alkaline haematin.

Put into Evelyn Colorimeter :

$G = 67$ therefore $L = .1739$

From graph this = 2.2 mgm Hb

= amount Hb in 5 ccs soln.

Therefore in 100 ccs solution amount Hb

$= 2.2 \times \frac{100}{5} = 44$ mgms Hb.

= amount in 2.118 Grams liver.

Therefore in 113 Grams liver amount Hb

$= 44 \times \frac{113}{2.118}$

= 2,347.5 mgms Hb.

= <u>2.348 Grams Haemoglobin.</u>

Now since Haemoglobin contains 0.335% iron, the amount of iron present in 2,347.5 mgm Hb.

$= 2,347.5 \times \frac{.335}{100}$

= <u>7.86 mgm iron.</u>

/ (ii) ....

LIVER No.14 - continued.

(ii)

5 ccs of the first 2000 ccs perfusate taken for Haemoglobin estimation. Treated to convert Hb to alkaline haematin.

Placed in Evelyn Photo-electric Colorimeter :

$G = 36^2$ therefore $L = .438$

From graph this = 5.5 mgm haemoglobin

= amount in 5 ccs solution

Therefore in 2000 ccs perfusate amount

haemoglobin $= 5.5 \times \frac{2000}{5}$

= 2,200 mgm Hb

As haemoglobin contains 0.335% iron, the amount of iron in 2,200 mgm Hb $= 2{,}200 \times \frac{.335}{100}$

= 7.37 mgm Iron.

/ (iii) ....

LIVER No. 14 - continued.

(iii)

The portion of right lobe (1.573 Grams) of fresh unperfused liver was digested and made up to 100 ccs Solution.

2 cc of this solution taken for colorimetry.

$G = 34^2$ therefore $L = .462$

From graph this = 0.01235 mgm iron

= amou t in 2 ccs solution

Therefore amount iron in 100 ccs solution

$= .01235 \times \frac{100}{2}$

= 0.6175 mgm iron

= amount iron in 1.573 Grams liver.

Therefore amount iron in 113 Grams liver

$= 0.6175 \times \frac{113}{1.573}$

= 44.36 mgm iron

= Total iron in liver.

/ (iv) ...

LIVER No. 14 - continued.

(iv)

The portion of left lobe (0.973 Gram) of fresh unperfused liver was digested and made up to 100 ccs solution.

2 ccs taken for colorimetry.

$G = 46^2$ therefore L = .332

From graph this = 0.009 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$= .009 \times \frac{100}{2}$

= .45 mgm iron

= amount iron in 0.973 Gram liver.

Therefore in 113 Grams liver amount iron

$= .45 \times \frac{113}{.973}$

= 52.05 mgm iron

= Total iron in liver.

/ (v) ....

LIVER No. 14 - continued.

(v)

A small piece of right lobe removed from the Perfused Liver. (0.8026 Gram). This was digested and made up to 100 ccs solution. 4 ccs of this solution were taken for colorimetry.

G = 62' therefore L = .2059.

From graph this = .0055 mgm iron

= amount iron in 4 ccs soln.

Therefore in 100 ccs solution amount iron

$= .0055 \times \frac{100}{4}$

= 0.1375 mgm

= amount iron in 0.8026 Gram perfused liver.

Corrected weight for perfused liver

$= 84.5 \times \frac{113}{108}$

= 88.4 Grams

Therefore amount iron in 88.4 Grams perfused liver

$= 88.4 \times \frac{.1375}{.8026}$

= 15.116 mgms.

/ (vi) .....

LIVER No. 14 - continued.

(vi)

A small piece of the left lobe was removed from the perfused liver (0.9024). This was digested and made up to 100 ccs solution. 4 ccs of this solution were taken for Colorimetry :

$$C = 56 \quad \text{therefore} \quad L = .2518.$$

From graph this = .00675 mgm iron

= amount in 4 ccs solution

Therefore in 100 ccs solution amount iron

$$= .00675 \times \frac{100}{4}$$

$$= .16875 \text{ mgm}$$

= amount iron in 0.9024 Gram liver.

Therefore in 88.4 Grams perfused liver amount

$$\text{iron} = .16875 \times \frac{88.4}{.9024}$$

$$= \underline{16.53 \text{ mgms.}}$$

/ ((vii) ...

LIVER No. 14 - continued.

(vii)

The perfused liver was now dried to constant weight (12 Grams). The dried liver was then crushed to a fine powder and this was thoroughly mixed by rolling on paper.

Weight of watchglass + liver sample	= 3.4792 Grs.
Weight of watchglass	= 3.0734 Grs.
Therefore weight of sample	= 0.4058 Gr.

This was digested and made up to 100 ccs solution. 2 ccs were taken for colorimetry :

G = 41 therefore L = .387

From graph this = 0.0105 mgm iron

= amount iron in 2 ccs soln.

Therefore in 100 ccs solution amount iron

$$= .0105 \times \frac{100}{2}$$

= 0.525 mgm

= amount iron in 0.4058 Gram dried liver.

Therefore in 12 Grams dried liver amount iron

$$= 0.525 \times \frac{12}{.4058}$$

= 15.3 mgms iron.

/ LIVER No. 15 ....

LIVER No. 15. Mary Mathibe (B.M.H. No.2699)

F.T. S.B. Male Infant. 7 lbs 2 ozs. I.C.H.

---

(1) 1 cc of blood, taken for Haemoglobin Estimation. Diluted with distilled water to 50 ccs. Thoroughly shaken. Allowed to stand overnight. 1 cc taken then for Colorimetry :

$G = 56$ Therefore $L = .2518$

From graph this = 3.175 mgm Haemoglobin.

Therefore 50 ccs Solution (1cc blood) contains $3.175 \times 50 = 158.75$ mgms haemoglobin.

---

(2) 1 cc blood taken for iron estimation.

This was digested and made up to 100 ccs Solution.

2 ccs of this solution taken for Colorimetry.

$G = 33^2$ Therefore $L = .475$

From graph this = 0.0127 mgm Fe

= Amount in 2ccs Solution.

Therefore in 100 ccs Solution amount iron $= .0127 \times \frac{100}{2}$

= 0.635 mgm Fe

= Amount in 1cc blood.

/ (3).

LIVER No. 15 - Continued.

(3) Volume of Liver = 100 ccs
Weight of Crucible + Liver = 330 Grams
Weight of Crucible = 213.5 Grams
Therefore Weight of Liver = 116.5 Grams.

The whole liver was now mushed and pulped and the vascular tree cut into fine pieces. This was all transferred to a flask and made up to 500 ccs Solution with distilled water. It was allowed to stand overnight in the refrigerator. 4 ccs of this solution then diluted to 50 ccs with distilled H_2O.

5 ccs of this resultant solution taken for Colorimetry :

$G = 63^3$ Therefore $L = .1956$.

From Graph this = 2.5 mgms haemoglobin.

Equals the amount of Hb in 5ccs solution.

Therefore in 50 ccs solution amount
Hb = 2.5 x 10 = 25 mgms Hb
= Amount in original 4ccs solution.

Therefore amount haemoglobin present in 500 ccs
Solution = $25 \times \frac{500}{4}$

= 3,125 mgms Hb
= Amount haemoglobin in whole liver.

From (1) above 1 cc blood contains 158.75 mgm. Hb.

Therefore volume of blood present
in Liver = $\frac{3125}{158.75}$ = <u>19.6 ccs.</u>

/ (4) ....

LIVER No. 15 - continued.

(4) 500 ccs solution containing liver now evaporated down to constant weight in a large crucible using an oven temperature of 105°C .

Weight of Crucible + dried liver	=	239.2 Grams
Weight of Crucible	=	213.5 Grams
Therefore weight of dried liver	=	25.7 Grams

This was then crushed to a fine powder and thoroughly mixed. Two samples were taken :

A.	Watchglass + liver	=	13.62 Grams
	Watchglass	=	12.77 Grams
	Therefore weight of sample	=	0.85 Grams.

Digested and made up to 100 ccs Solution.

1 cc Solution taken for Colorimetry :

$G = 24^3$ Therefore $L = 0.606$.

From Graph this = .0161 mgm Fe

= Amount in 1 cc solution.

Therefore 100 ccs solution contains 1.61 mgm Fe.

= Amount in 0.85 Grams Liver

Therefore in 25.7 Grams liver amount of iron

$$= 1.61 \times \frac{25.7}{.85}$$

= 48.6 mgm Fe.

/ B. ....

LIVER No. 15 - continued.

B.

Watchglass + sample	= 3.4134 Grams
Watchglass	= 3.0700 Grams
Therefore weight of sample	= 0.3434 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$$G = 33^{2 + 1} \quad \text{Therefore} \quad L = 0.472$$

From graph this = .01255 mgm iron

= amount in 2 ccs solution

Therefore 100 ccs solution contains :

$$.01255 \times \frac{100}{2} = .6275 \text{ mgm iron}$$

= amount iron in 0.3434 Gr. dried liver

Therefore in 25.7 Grams dried liver amount of

$$\text{iron} = 0.6275 \times \frac{25.7}{.3434} = \underline{47.2 \text{ mgms iron}}$$

Therefore average = <u>47.9 mgm iron for whole liver.</u>

From (3) above this liver contained 19.6 ccs blood.
From (2) above each cc blood contains 0.635 mgm Fe

Therefore 19.6 ccs blood represents :

$$(0.635 \times 19.6) \text{ mgm iron} = \underline{12.45 \text{ mgm iron}}$$

Therefore amount iron actually present in Hepatic

$$\text{Tissue} = 47.9 \times 12.45 = \underline{35.45 \text{ mgms}}$$

/ LIVER No. 16 ...

LIVER No. 16 - Maria Mende. (B.M.H. No.2744)

S.B. First Twin. Premature (± 35 weeks) Weight 5 lbs. Mother was an eclamptic. Binovular Twins. Male.

---

(1)

1 cc of blood taken for haemoglobin estimation: Diluted to 100 ccs with distilled water. 1cc of this solution used for Colorimetry:

G = 80 therefore L = .0969.

From graph this = 1.225 mgms haemoglobin.

Therefore 100 ccs solution contains :

1.225 x 100 = 122.5 mgms Haemoglobin

= Amount haemoglobin in 1 cc blood.

---

(2)

1 cc of blood taken for iron estimation:

Digested and made up to 100 ccs solution.

2 ccs taken for Colorimetry :

G = 37 therefore L = .432.

From graph this = .0116 mgm iron

= amount iron in 2 ccs soln.

Therefore in 100 ccs solution amount iron

$= .0116 \times \frac{100}{2} = .58$ mgm Fe

= amount iron in 1 cc blood.

/ (3) ....

LIVER No. 16 - continued.

(3) Volume of liver	= 60 ccs
Weight of crucible + liver	= 270 Grams
Weight of crucible	= 201.5 Grs.
Therefore weight of liver	= 68.5 Grams

Liver was mushed and pulped and made up to 500 ccs solution. Allowed to stand overnight in refrigerator. 5 ccs of this solution then diluted further to 50 ccs.

5 ccs of this diluted solution taken for

Colorimetry : G = 71 therefore L = 0.1487

From graph this = 1.875 mgm haemoglobin.

Therefore in 50 ccs solution amount haemoglobin

$$= 1.875 \times \frac{50}{5} = 18.75 \text{ mgms}$$

= amount in 5 ccs of original solution.

Therefore in 500 ccs original solution amount Hb

$$= 18.75 \times \frac{500}{5} = \underline{1,875 \text{ mgms}}$$

From (1) above 1 cc blood contains 122.5 mgm Hb.

Therefore volume of blood in liver

$$= \frac{1875}{122.5} = \underline{15.3 \text{ ccs blood.}}$$

/ (4) ...

LIVER No. 16 - continued.

(4) The 500 ccs solution containing the whole unperfused liver heated in air oven at 105°C to a constant weight.

Crucible + liver (dried)	=	212.5 Grams
Crucible weighs	=	201.5 Grams
Therefore weight of dry liver	=	11 Grams

This dried liver was then crushed to a fine powder and thoroughly mixed. Two samples were taken:

(A)	Crucible + liver sample	=	3.686 Grams
	Crucible weighs	=	3.070 Grams
	Therefore sample	=	0.616 Gram.

Digested and made up to 100 ccs solution.

1 cc of this solution taken for colorimetry.

G = 41 therefore L = 0.387

From graph this = 0.01045 mgm iron, i.e. 1 cc solution contains 0.01045 mgm iron.

Therefore 100 ccs solution contains 1.045 mgm Fe.

= amount iron in 0.616 Gram dry liver.

Therefore in 11 Grams dried liver amount iron

$$= 1.045 \times \frac{11}{.616} = \underline{18.66 \text{ mgms Iron.}}$$

/ (B) ....

LIVER No. 16 - continued.

(B) Crucible + liver sample = 13.222 Grams
Crucible weighs = 12.770 Grams
Therefore liver sample = 0.452 Gram

Digested and made up to 100 ccs solution.

1 cc taken for Colorimetry.

G = 51 therefore L = .2924

From graph this = .0078 mgm iron,

i.e. 1 cc solution contains 0.0078 mgm iron.

Therefore 100 ccs solution contains 0.78 mgm Fe.

= amount iron in 0.452 Gram Liver.

Therefore amount iron in 11 Grams dried liver

$$= .78 \times \frac{11}{.452}$$

= 19 mgms iron.

Therefore Average between A and B = 18.83 mgm iron in whole liver.

From (3) above we know this liver contained 15.3 ccs blood. And from (2) above that 1 cc blood contained .58 mgm iron. Therefore amount iron in 15.3 ccs blood = .58 x 15 = 8.7 mgm

Therefore amount iron actually in Hepatic Tissue

= (18.83 - 8.7) mgms = 10.13 mgms Iron.

LIVER No. 17 ....

<u>LIVER No. 17</u> - Maria Mende (B.M.H. No.2744)

S.B. Premature. Second twin. (± 35 weeks).

Binovular twin. Weight 4 lbs 12 ozs. Mother

was an eclamptic. Liver very congested.

<u>(1)</u>

1 cc blood taken for haemoglobin estimation.

Diluted to 50 ccs with distilled water.

1 cc of this solution taken for colorimetry.

G = 51 therefore L = .2924.

From graph this = 3.7 mgms Hb = amount in 1cc solution.

Therefore in 50 ccs solution amount haemoglobin

= (3.7 x 50) = 185 mgms Hb.

= amount in 1 cc blood.

<u>(2)</u>

1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs taken for colorimetry :

G = 29 therefore L = .538

From graph this = 0.0143 mgm Fe = amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$= .0143 \times \frac{100}{2} =$ <u>.715 mgm Fe.</u>

= <u>amount iron in 1 cc blood.</u>

/ (3) ....

LIVER No. 17 - continued.

(3) Volume of Liver	= 60 ccs
Weight of crucible + liver	= 281.5 Grams
Weight of crucible	= 213.5 Grams
Therefore weight of liver	= 68 Grams

Liver thoroughly mushed to a fine pulp and then made up to 500 ccs with distilled water :

Allowed to stand overnight in the refrigerator.

5 ccs solution then diluted to 50 ccs c̄ H_2O.

4 ccs of this solution used for colorimetry :

G = 61 therefore L = .2147

From graph this = 2.7 mgms haemoglobin.

= amount Hb in 4 ccs diluted solution.

Therefore in 50 ccs solution amount haemoglobin

$$= 2.7 \times \frac{50}{4} = 33.75 \text{ mgms Hb.}$$

= amount in 5 ccs original solution.

Therefore in 500 ccs original solution amount Hb

$$= 33.75 \times \frac{500}{5} = \underline{3,375 \text{ mgm Hb.}}$$

From (1) above we know that 1 cc blood had 185 mgms Hb, therefore volume of blood in

$$\text{liver} = \frac{3375}{185} = \underline{18.2 \text{ ccs.}}$$

/ (4) ...

LIVER No. 17 - continued.

(4) 500 ccs containing liver placed in a large evaporating dish and dried to constant weight at 105° C.

Crucible + dried liver	= 227.5 Grams
Crucible weighs	= 213.5 Grams
Therefore dried liver	= 14 Grams

Dried liver crushed to a fine powder and thoroughly mixed. Two samples taken for iron estimation :

A.	Watchglass + sample	= 13.223 Grams
	Watchglass	= 12.770 Grams
	Therefore liver sample	= 0.453 Gram

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry.

G = 36 therefore L = .444

From graph this = 0.0118 mgm Fe = amount Fe in 1cc

Therefore in 100 ccs solution amount iron

= 1.18 mgm = amount in 0.453 Grs. liver.

Therefore in 14 Grams liver amount of iron

$$= 1.18 \times \frac{14}{.453}$$

= 36.4 mgm iron.

/ (B) ...

LIVER No. 17 - continued.

B.	Watchglass + sample	=	3.276 Grams
	Watchglass	=	3.070 Grams
	Therefore liver sample	=	0.206 Gram

Digested and made up to 100 ccs solution.

1 cc taken for colorimetry :

G = 63 therefore L = 0.2007

From graph this = .0053 mgm Fe = amount Fe in 1cc

Therefore in 100 ccs solution amount Fe = .53 mgm

= amount in .206 Gram liver

Therefore amount iron in 14 grams of dried liver

$$= .53 \times \frac{14}{.206}$$

= 36 mgms Iron.

Average of A and B = 36.2 mgms iron.

= Total iron in liver.

From (3) above we know this liver contained 18.2 ccs blood. From (2) above 1 cc blood contained .715 mgm iron.

Therefore 18.2 ccs blood contains (.715 x 18.2) mgm iron

= 13 mgms

Therefore amount of iron actually in Hepatic Tissue

= (36.2 - 13) = 23.2 mgms iron.

/ LIVER No. 18 ...

LIVER No. 18. - Agnes Nxu (B.M.H. No.2973).

Female child. Died aged 7 days. Birth Weight 5 lbs 8 ozs. Full Term. Precipitate labour. Died of Intracranial haemorrhage.

---

(1)

1 cc of blood taken for haemoglobin estimation:

Made up to 50 ccs solution with distilled water.

2 ccs of this solution taken for colorimetry

$$G = 30^{+1} \quad \text{therefore } L = .520.$$

From graph this = 6.55 mgm Hb = amount in 2 ccs solution.

Therefore in 50 ccs solution amount haemoglobin

$$= 6.55 \times \frac{50}{2} = \underline{163.75 \text{ mgm Hb.}}$$

$$= \underline{\text{amount in 1 cc blood.}}$$

---

(2)

1 cc of blood taken for iron estimation :

Digested and made up to 100 ccs solution.

2 ccs of this solution used for colorimetry :

$$G = 30^{2} \quad \text{therefore} \quad L = .516.$$

From graph this = .0138 mgm Fe = amount in 2 ccs solution.

Therefore amount iron in 100 ccs solution

$$= .0138 \times \frac{100}{2} = \underline{0.69 \text{ mgm Iron}}$$

$$= \underline{\text{amount iron in 1 cc blood.}}$$

/ (3) ...

LIVER No. 18 - continued.

(3) Volume of liver	=	76 ccs
Weight of Crucible + liver	=	268 Grams
Weight of crucible	=	186 Grams
Therefore weight of liver	=	82 Grams

Liver mushed and pulped and made up to 500 ccs solution. 10 ccs of this solution taken (after 24 hours in the refrigerator) and made up to 50 ccs with distilled water.

5 ccs of this diluted mixture taken for colorimetry.

$G = 53$ therefore $L = .2756$.

From graph this = 3.475 mgms haemoglobin

= amount Hb in 5 ccs of diluted solution.

Therefore in 50 ccs diluted solution amount Hb

$= 3.475 \times \frac{50}{5} = 34.75$ Mgm Hb.

= amount in 10 ccs of original solution.

Therefore amount Haemoglobin in 500 ccs original

solution $= 34.75 \times \frac{500}{10} = \underline{1,737.5 \text{ mgms Hb.}}$

From (1) above we know 1 cc blood contained 163.75 mgm Hb. Therefore volume of blood in liver

$= \frac{1737.5}{163.75}$ ccs $= \underline{10.6 \text{ ccs.}}$

/ (4) ....

LIVER No. 18 - continued.

(4) 500 ccs solution containing liver placed in evaporating dish and dried to a constant weight at 105°C in an air oven.

Weight of Dish + liver (dried)	=	202 Grams
Weight of evaporating dish	=	186 Grams
Therefore weight of dry liver	=	16 Grams

This was crushed to a fine powder and thoroughly mixed. Two samples taken for iron estimation.

A.	Watchglass + liver sample	=	13.349 Grams
	Watchglass weighs	=	12.770 Grams
	Therefore liver sample	=	0.579 Grams.

Digested and made up to 100 ccs solution.

1 cc taken for colorimetry.

$$G = 36^{+1} \quad \text{therefore} \quad L = .444$$

From graph this = 0.0118 mgm Fe = amount in 1 cc solution.

Therefore in 100 ccs solution Iron = 1.18 mgm Fe.

= amount in 0.579 Gram dry liver.

Therefore in 16 Grams dry liver amount of iron

$$= 1.18 \times \frac{16}{.579} = \underline{32.6 \text{ mgm iron.}}$$

/ (B) ....

LIVER No. 18 - continued.

B. Watchglass + liver sample = 3.3694 Grams
Watchglass weighs = 3.0700 Grams
Therefore liver sample = 0.2994 Gram

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry.

G = 58 therefore L = .2376

From graph this = 0.00617 mgm iron

= amount in 1 cc solution.

Therefore in 100 ccs solution iron = 0.617 mgm Fe

= amount in 0.2994 Gram dry liver.

Therefore in 16 Grams dry liver amount of iron

$$= 16 \times \frac{.617}{.2994}$$

= 33 mgm iron.

Therefore average between A and B = 32.8 mgm iron.

= Total iron in whole liver.

From (3) above this liver contained 10.6 ccs blood

From (2) above 1 cc blood contained 0.69 mgm iron

Therefore 10.6 ccs blood contains (0.69 x 10.6) mgm iron

= 7.3 mgm iron

Therefore actual iron in hepatic tissue

= (32.8 - 7.3) = 25.5 mgms iron.

/ LIVER No. 19 ...

LIVER No. 19. Leah. (No. 3117 - B.M.H.)

Female child. Birth weight 5 lbs 10 ozs.(F.T.)

Died on 9th day. Anaemic looking liver.

---

(1)

1 cc blood taken for haemoglobin estimation.

Made up to 50 ccs solution. 2 ccs of this solution taken for colorimetry.

G = 62 therefore L = .2076.

From graph this = 2.625 mgms haemoglobin

= amount in 2 ccs solution

Therefore amount Hb in 50 ccs solution

$= 2.625 \times \frac{50}{2} = \underline{65.625 \text{ mgms Hb.}}$

= amount in 1 cc blood.

---

(2)

1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs of this solution taken for colorimetry.

$G = 33^{1} + 1$ therefore L = 0.475

From graph this = 0.0126 mgm Fe = amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$= .0126 \times \frac{100}{2} = \underline{0.63 \text{ mgms iron}}$

= amount iron in 1 cc blood.

/ (3) ....

LIVER No. 19 - continued.

(3)	Volume of liver	=	76 ccs
	Crucible + liver	=	296.5 Grams
	Crucible weighs	=	213.5 Grams
	Therefore liver weighs	=	83 Grams

Liver mushed to a fine pulp and made up to 500 ccs solution with distilled water. Shaken and allowed to stand overnight, in the refrigerator. 5 ccs of this solution taken for colorimetry.

$G = 31^{+1}$ therefore $L = .505$

From graph this = 6.35 mgms Hb = amount in 5 ccs soln.

Therefore in 500 ccs solution amount of Hb

$$= 6.35 \times \frac{500}{5} = \underline{635 \text{ mgms Hb}}$$

From (1) above 1 cc blood contains 65.625 mgms Hb

Therefore volume of blood in liver $= \dfrac{635}{65.625}$

$= \underline{9.7 \text{ ccs Blood.}}$

/ (4) ...

LIVER No. 19 - continued.

(4) 500 ccs solution containing liver placed in an evaporating dish and dried to constant weight at 105°C in an air oven.

Dish + liver	=	229.5 Grs.
Dish weighed	=	213.5 Grs.
Therefore weight of dry liver	=	16 Grs.

Liver crushed to a fine powder and thoroughly mixed. Two samples taken for iron estimation.

A.

Watchglass + liver sample	=	13.309 Grs.
Weight of watchglass	=	12.770 Grs.
Therefore weight of liver sample	=	0.539 Grs.

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry.

G = 22 therefore L = 0.658

From graph this = 0.0176 mgm iron
= amount iron in 1 cc solution.

Therefore in 100 ccs solution amount iron

= 1.76 mgm = amount in 0.539 Gram liver

Therefore amount iron in 16 grams of dry liver

$$= 1.76 \times \frac{16}{.539} = \underline{52.06 \text{ mgm iron}}$$

/ (B) ....

LIVER No. 19 - continued.

B.	Watchglass + liver sample	= 3.42 Grams
	Weight of watchglass	= 3.07 Grams
	Therefore weight of liver sample	= 0.35 Gram.

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry :

G = 41 therefore L = .386

From graph this = .01125 mgm iron
= amount iron in 1 cc solution.

Therefore in 100 ccs solution amount iton

= 1.125 mgm = amount in 0.35 Gram liver

Therefore amount iron in 16 Grams dry liver

$= 1.125 \times \frac{16}{.35} =$ 51.45 mgms iron.

Therefore average of A and B = 51.75 mgm iron

= Total iron in whole liver.

From (3) above we know that liver contained 9.7 ccs blood. From (2) above 1 cc blood contained 0.63 mgm iron. Therefore 9.7 cc blood contains (.63 x 9.7) mgm iron = 6.1 mgm Fe.

Therefore iron present acutally in Hepatic Tissue = 51.75 - 6.1 mgm = 45.65 mgms iron.

/ LIVER No. 20 ...

<u>LIVER No. 20.</u> Emily. (B.M.H. No. 3186) Male child. S.B. (36 weeks) Premature. Birth Weight 4 lbs 14 ozs. Liver was pale and yellow.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs solution.

5 ccs of this solution taken for colorimetry:

$$G = 37^{1 + 1} \quad \text{therefore } L = .426.$$

From graph this = 5.4 mgms Hb = amount in 5 ccs solution.

Therefore in 50 ccs solution amount haemoglobin

$$= 5.4 \times \frac{50}{5} = \underline{54 \text{ mgms Hb.}}$$

$$= \underline{\text{amount in 1 cc blood.}}$$

---

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs of this solution taken for colorimetry :

$$G = 33^{+1} \quad \text{therefore } L = .478$$

From graph this = 0.0127 mgm Fe = amount in 2 ccs solution.

Therefore 100 ccs solution contains $.0127 \times \frac{100}{2}$

$= \underline{0.635 \text{ mgms iron}}$ = amount iron present in 1 cc blood.

/ (3) ...

LIVER No. 20 - continued.

(3)	Volume of liver	=	55 ccs
	Crucible + liver weighs	=	277.5 Grams
	Crucible weighs	=	219 Grams
	Therefore liver weighs	=	58.5 Grams.

Liver mushed to a fine pulp and made up to 500 ccs solution. 5 ccs of this solution taken for colorimetry.

G = 46 therefore L = .337

From graph this = 4.225 mgms haemoglobin

= amount in 5 ccs solution

Therefore amount haemoglobin in 500 ccs soln.

$$= 4.225 \times \frac{500}{5}$$

= 422.5 mgms

Therefore from (1) above we know 1 cc blood contained 54 mgms haemoglobin.

Therefore volume of blood in this liver

$$= \frac{422.5}{54} = \underline{7.8 \text{ ccs blood.}}$$

/ (4) ....

LIVER No. 20 - continued.

(4) 500 ccs solution containing liver dried to constant weight.

Weight of dish + liver (dried)	=	233.5 Grams
Weight of evaporating dish	=	219 Grams
Therefore weight of dried liver	=	14.5 Grams.

This was then crushed to a fine powder and thoroughly mixed. Two samples were taken for iron estimation.

A. Weight of watchglass + liver sample	=	13.45 Grams
Weight of watchglass	=	12.77 Grams
Therefore weight of liver sample	=	0.68 Gram.

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry.

$G = 43^{1+1}$ therefore $L = 0.362$

From graph this = 0.0097 mgm Fe = amount Fe in 1 cc solution.

Therefore in 100 ccs solution iron = .97 mgm

= amount in 0.68 Gram dry liver.

Therefore amount iron in 14.5 gram of dried

$$\text{liver} = .97 \times \frac{14.5}{.68} = \underline{20.7 \text{ mgm iron.}}$$

/ (B) ...

LIVER No. 20 - continued.

B.

Watchglass + liver sample = 3.485 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of liver sample = 0.415 Gram.

Digested and made up to 100 ccs solution.

1 cc taken for colorimetry.

G = 60 therefore L = .2218

From graph this = .0059 mgms iron
= amount Fe in 1 cc solution

Therefore in 100 ccs solution iron = .59 mgm

= amount iron in .415 gram dry liver

Therefore amount iron in 14.5 Grams of dried

liver = $.59 \times \frac{14.5}{.415}$ = 20.6 mgms iron

Therefore average between A and B = 20.65 mgms iron.

= Total iron in whole liver.

From (3) above this liver contained 7.8 ccs blood. From (2) above 1 cc blood contained .635 mgm iron. Therefore 7.8 ccs blood contains .635 x 7.8 = 4.95 mgms iron

Therefore Iron actually in Hepatic Tissue

= 20.65 - 4.95 = 15.7 mgms iron.

/ LIVER No. 21 ...

<u>LIVER No. 21</u> - Betty Zondo. (B.M.H. No.

Female. F.T. S.B. Birth weight 7 lbs 13 ozs.

Brown heel measurement 21".

---

(1)

½ cc blood taken for haemoglobin estimation.

Diluted to 50 ccs solution with distilled water. 3 ccs of this solution taken for colorimetry :

G = 60 therefore L = .2218

From graph this = 2.8 mgms haemoglobin

= amount in 3 ccs solution

Therefore amount in 50 ccs solution

$= 2.8 \times \frac{50}{3} = 46.6$ mgm Hb

= amount in ½ cc blood

Therefore in 1 cc blood amount Hb = <u>93.2 mgm</u>

---

(2)

1 cc of blood taken for iron estimation.

Digested and made up to 100 ccs solution.

1 cc of this solution taken for colorimetry :

G = 57 therefore L = .2441

From graph this = .0065 mgm Fe = amount in 1 cc solution.

Therefore in 100 ccs solution amount Fe = <u>.65 mgms</u>

= amount in 1 cc blood.

/ (3) ....

LIVER No. 21 - continued.

(3)

Crucible + liver weighs	= 306 Grams
Crucible weighs	= 201.5 Grams
Therefore liver weighs	= 104.5 Grams
Volume of liver	= 95 ccs

Liver was mushed to a fine pulp and made up to 500 ccs solution. Shaken well. Allowed to stand in refrigerator overnight.

2 ccs of this solution taken for colorimetry.

$G = 36$ therefore $L = .444$

From graph this = 5.6 mgm haemoglobin

= amount in 2 ccs solution

Therefore amount in 500 ccs solution

$$= 5.6 \times \frac{500}{2} = 1{,}400 \text{ mgm Hb}$$

From (1) volume of blood in liver

$$= \frac{1400}{93.2}$$

= 15 ccs blood.

/ (4) ....

LIVER No. 21 - continued.

(4) 500 ccs solution containing liver dried to constant weight at 105°C in air oven.

Weight of crucible + liver	=	217 Grams
Weight of crucible	=	201.5 Grams
Therefore weight of liver	=	15.5 Grams

This was then crushed to a fine powder and thoroughly mixed. Two samples were taken for iron estimation.

A. Watchglass + liver sample	=	3.602 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of liver sample	=	0.532 Gram

Digested and made to 100 ccs. 1 cc solution taken for Colorimetry.

$G = 26^{+1}$ therefore $L = .581$

From graph this = .0155 mgm iron
= amount in 1 cc solution.

Therefore amount iron in 100 ccs solution

= 1.55 mgm = amount in 0.532 Gram liver.

Therefore amount iron in 15.5 Grams dried liver

$$= 1.55 \times \frac{15.5}{.532} = \underline{45.15 \text{ mgms Iron.}}$$

/ (B) ....

LIVER No. 21 - continued.

B.	Watchglass + liver sample	=	13.4778 Grs.
	Weight of watchglass	=	12.77 Grs.
	Therefore weight of liver sample	=	0.7078 Gr.

Digested and made up to 100 ccs solution.

1 cc of this solution taken for colorimetry.

G = 17 therefore L = .770

From graph this = .0206 mgms Iron

= amount in 1 cc solution

Therefore amount iron in 100 ccs solution

= 2.06 mgm

= amount in .7078 Gram liver.

Therefore amount iron in 15.5 Grams dried liver

$$= 2.06 \times \frac{15.5}{.7078} = \underline{45.11 \text{ mgm Fe.}}$$

Average from A and B = 45.13 mgm Fe.

= Total iron in whole liver.

From (3) above this liver contained 15 ccs.blood.

From (2) above each cc blood contained .65 mgm Fe.

Therefore 15 ccs blood contains (.65 x 15) mgm Fe.

= 9.75 mgm Fe.

Therefore Iron actually in Hepatic Tissue

= (45.13 - 9.75) = 35.38 mgm iron.

/ LIVER No. 22 ...

LIVER No. 22. Doreen Majola. (B.M.H.No.3649)
Full term. Female. Aged 7 days. Birth Weight
5 lbs 6 ozs. Died of Bilateral Broncho-
pneumonia.

---

(1)
1 cc of blood taken for Haemoglobin estimation.
Diluted to 50 ccs with distilled water. Allowed
to stand in refrigerator overnight.
2 ccs of this solution taken for Colorimetry.

$G = 39^{2} \ (+\frac{1}{4})$ therefore $L = 0.401$

From graph this = 5.05 mgms haemoglobin
= amount in 2 ccs solution.

Therefore in 50 ccs solution haemoglobin

$= 5.05 \times \frac{50}{2} = \underline{126.5 \text{ mgms}}$

= amount in 1 cc blood.

---

(2)
1 cc of blood taken for Iron estimation.
Digested and made up to 100 ccs solution.
2 ccs of this solution taken for Colorimetry:

$G = 38^{2} \ (+\frac{1}{4})$ therefore $L = 0.412$.

From graph this = 0.011 mgm Fe = Amount in 2 ccs solution.

Therefore 100 ccs solution contain $.011 \times \frac{100}{2}$

= <u>0.55 mgm Iron</u> = amount Fe in 1 cc Blood.

/ (3) ....

LIVER No. 22 - continued.

(3) Volume of Liver	= 70 ccs
Weight of crucible + liver	= 278 Grams
Weight of crucible	= 201.5 Grams
Therefore weight of liver	= 76.5 Grams

Liver mushed and pulped and made up to 500 ccs solution with distilled water. Shaken and allowed to stand overnight in refrigerator.

2 ccs taken for Colorimetry :

$$G = 35^{2\ (+\frac{1}{4})} \qquad \text{therefore } L = 0.447$$

From graph this = 5.625 mgms Hb = amount in 2 ccs solution.

Therefore in 500 ccs solution amount haemoglobin

$$= 5.625 \times \frac{500}{2}$$

$$= 1406.25 \text{ mgms Hb.}$$

= amount Hb in liver.

From (1) above 1 cc blood contained 126.5 mgms Hb

Therefore volume of blood in liver

$$= \frac{1406.25}{126.5} \text{ ccs}$$

$$= \underline{\underline{11.1 \text{ ccs.}}}$$

/ (4) ....

LIVER No. 22 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight at 105°C in an air oven.

Weight of dish + dried liver	=	219.5 Grams
Weight of evaporating dish	=	201.5 Grams
Therefore weight of dried liver	=	18 Grams

This was crushed to a fine powder and mixed.

Two samples were taken for iron estimation :

A.	Crucible + liver sample	=	3.665 Grams
	Crucible weighs	=	3.075 Grams
	Therefore liver sample	=	0.59 Gram.

Digested and made up to 100 ccs solution.

1 cc solution taken for Colorimetry.

G = 49 therefore L = 0.310.

From graph this = 0.0083 mgm Fe = amount in 1 cc solution.

Therefore in 100 ccs solution Fe = 0.83 mgms

= amount in 0.59 Gram dried liver.

Therefore in 18 Grams of dried liver amount

Iron $= .83 \times \frac{18}{.59}$

= 25.32 mgms Iron.

/ (B) ....

LIVER No. 22 - continued.

B.

Crucible + liver sample	= 3.815 Grams
Crucible weighs	= 3.075 Grams
Therefore liver sample weighs	= 0.74 Grams

Digested and made up to 100 ccs solution

1 cc solution taken for Colorimetry.

G = 41^2 therefore L = 0.382

From graph this = 0.0104 mgm iron
= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron

= 1.04 mgm = amount in 0.74 Gr. liver.

Therefore in 18 Grams dried liver amount iron

$= 1.04 \times \frac{18}{.74} =$ 25.29 mgms iron.

Therefore average from A and B = 25.305 mgms Fe.
= Total iron in whole liver.

From (3) above liver contained 11.1 ccs blood

From (2) above 1 cc blood contained 0.55 mgm Fe.

Therefore 11.1 ccs blood contain 11.1 x .55 mgm Fe.

= 6.1 mgms iron

Therefore amount iron actually in Hepatic Tissue

= 25.305 - 6.1 = 19.205 mgms iron.

/ LIVER No. 23 ...

LIVER No. 23. Lena Kumalo (B.M.H. No.3941)

Full Term. Female. Aged 8 days. Birth

Weight 5 lbs 14 ozs. Died of Bronchopneumonia.

---

(1)

1 cc blood taken for haemoglobin estimation.

Diluted to 50 ccs with distilled water.

Allowed to stand in refrigerator overnight:

2 ccs of this solution taken for Colorimetry.

G = 44' therefore L = 0.354

From graph this = 4.45 mgms Hb = amount in 2 ccs solution

Therefore amount Haemoglobin in 50 ccs solution

$= 4.45 \times \frac{50}{2} =$ 111.25 mgms = amount Hb in 1 cc blood.

---

(2)

1 cc blood taken for iron estimation :

Digested and made up to 100 ccs solution.

2 ccs of this solution taken for Colorimetry.

G = 41 (+ ¼) therefore L = 0.385

From graph this = 0.0102 mgm Fe = amount Fe in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= .0102 \times \frac{100}{2}$ = 0.51 mgm = amount in 1 cc blood.

/ (3) ....

LIVER No. 23 - continued.

(3) Volume of liver	= 70 ccs
Weight of crucible + liver	= 250 Grams
Weight of crucible	= 175 Grams
Therefore weight of liver	= 75 Grams

Liver mushed and pulped and made up to 500 ccs solution with distilled water. Allowed to stand overnight in refrigerator.

2 ccs of this solution taken for Colorimetry :

$$G = 40 \quad \text{therefore } L = 0.398$$

From graph this = 5 mgms Hb = amount in 2 ccs solution.

Therefore in 500 ccs solution amount Hb

$$= \left(5 \times \frac{500}{2}\right) = \underline{1,250 \text{ mgms}}$$

From (1) above 1 cc blood contained 111.25 mgms Hb

Therefore volume of blood in liver $= \frac{1250}{111.25}$ ccs

$= \underline{11.2 \text{ ccs.}}$

(4) Solution containing liver placed in evaporating dish and dried to constant weight in an air oven at 105°C.

Weight of dish + dried liver	= 191 Grams
Weight of dish	= 175 Grams
Therefore weight of dried liver	= 16 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Watchglass + liver sample	= 3.473 Grams
Weight of watchglass	= 3.075 Grams
Therefore weight of liver sample	= 0.398 Gram

/ This ...

LIVER No. 23 - continued.

This was digested and made up to 100 ccs solution.

1 cc of this solution taken for colorimetry :

G = 37' therefore L = .429

From graph this = 0.0114 mgm iron

= amount in 1 cc solution

Therefore amount iron in 100 ccs solution

= 1.14 mgms

= amount in 0.398 Gram liver

Therefore amount iron in 16 grams dried liver

$$= \frac{1.14 \times 16}{.398} = \underline{45.83 \text{ mgms iron}}$$

= Total iron in whole liver.

From (3) above liver contained 11.2 ccs blood

From (2) above 1 cc blood contained .51 mgm Fe

Therefore 11.2 ccs blood contain (11.2 x .51)mgm Fe

= 5.7 mgms iron

Therefore actual amount of iron in Hepatic Tissue

= (45.83 - 5.7) mgms

= 40.13 mgms iron

/ LIVER No. 24 ...

LIVER No. 24 - Beatrice Matlatgi (B.M.H.No.4058)

Full Term. Male. Stillbirth. Birth Weight 8lbs 10ozs.

---

(1) $\frac{1}{2}$ cc blood taken for haemoglobin estimation.

Diluted to 50 ccs with distilled water. Allowed to stand in refrigerator overnight.

1 cc solution taken for colorimetry:

$$G = 75^2 \quad \text{therefore} \quad L = .1221$$

From graph this = 1.55 mgms haemoglobin
= amount in 1 cc solution

Therefore in 50 ccs solution, haemoglobin

= 1.55 x 50 = 77.5 mgms = amount in $\frac{1}{2}$ cc blood

Therefore haemoglobin in 1 cc blood = 155 mgms.

---

(2) $\frac{1}{2}$ cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs of this solution taken for colorimetry:

$$G = 53 \quad \text{therefore} \quad L = .2756$$

From graph this = 0.0074 mgm Fe = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= .0074 \times \frac{100}{2} = .37 \text{ mgm iron}$$

This amount iron is present in $\frac{1}{2}$ cc blood, thus in 1 cc blood amount of iron present is 0.74 mgms

/ (3) ...

LIVER No. 24 - continued.

(3)	Volume of Liver	= 130 ccs
	Weight of crucible + liver	= 343 Grams
	Weight of crucible	= 201.5 Grams
	Therefore weight of liver	= 141.5 Grams

Liver mushed to a fine pulp and made up to 500 ccs with distilled water. Thoroughly shaken and then allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

$G = 37'$ therefore $L = .429$

From graph this = 5.4 mgms haemoglobin

Therefore amount haemoglobin in 500 ccs solution

= 5.4 x 500 = 2700 mgms haemoglobin

= amount haemoglobin in liver

From (1) above 1 cc blood contained 155 mgms Hb

Therefore volume of blood in liver = $\frac{2700}{155}$ ccs

= 17.4 ccs.

/ (4) ....

LIVER No. 24 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	= 229.5 Grams
Weight of dish	= 201.5 Grams
Therefore weight of liver	= 28 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	= 13.339 Grams
Weight of watchglass	= 12.773 Grams
Therefore weight of liver sample	= 0.566 Grams

Digested and made up to 100 ccs solution.

2 ccs of this solution taken for colorimetry:

G = 31' therefore L = .505

From graph this = .0134 mgm iron

= amount in 2 ccs solution.

Therefore amount iron in 100 ccs solution

$$= .0134 \times \frac{100}{2} = 0.67 \text{ mgm}$$

= amount in 0.566 Gram dry liver.

Therefore amount iron in 28 Grams dried liver

$$= \left(.67 \times \frac{28}{.566}\right) \text{ mgms} = \underline{33.1 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

LIVER No. 24 - continued.

From (3) above this liver contained

17.4 ccs blood.

From (2) above 1 cc blood contained

0.74 mgm iron.

Therefore 17.4 ccs blood contain

$(17.4 \times .74)$ mgms iron

= 12.9 mgms iron.

Therefore iron actually present in

Hepatic Tissue = $(33.1 - 12.9)$

= 20.2 mgms.

/ LIVER No. 25 ...

LIVER No. 25. Freda Kubeka. (B.M.H. No.6337)
Female. Full Term. Aged 4 days. Died of bronchopneumonia. Birth weight 6 lbs 4 ozs.

---

(1)

1 cc blood taken for haemoglobin estimation: Diluted to 50 ccs with distilled water. Shaken and allowed to stand overnight in the refrigerator :

2 ccs solution taken for colorimetry :

$G = 59^2$ therefore $L = .2255$

From graph this = 2.85 mgms haemoglobin
= amount in 2 ccs solution.

Therefore in 50 ccs solution haemoglobin

$$= 2.85 \times \frac{50}{2} = \underline{71.25 \text{ mgms}}$$

= amount Hb in 1 cc blood.

(2)

1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry :

$G = 63$ therefore $L = .2007$

From graph this = .0054 mgm iron
= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron
= .54 mgm = amount in 1 cc blood.

/ (3) ....

LIVER No. 25 - continued.

(3) Volume of liver	=	80 + ccs
Weight of crucible + liver	=	267.5 Grams
Weight of crucible	=	175.2 Grams
Therefore weight of liver	=	92.3 Grams

Liver mushed to a fine pulp and made up to 500 ccs with distilled water: Left overnight in refrigerator, after shaking.

2 ccs of this solution taken for colorimetry:

$G = 58^2$ therefore $L = 0.2328$.

From graph this = 2.925 mgm haemoglobin

= amount in 2 ccs solution

Therefore amount Hb in 500 ccs solution

$= 2.925 \times \frac{500}{2}$

= 731.25 mgms haemoglobin.

From (1) above 1 cc blood contained 71.25 mgms Hb

Therefore volume of blood in liver

$= \frac{731.25}{71.25}$ ccs

= 10.3 ccs.

/ (4) ....

LIVER No. 25 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	197.5 Grams
Weight of dish	=	175.2 Grams
Therefore weight of dried liver	=	22.3 Grams

This was crushed to a fine powder and mixed.

Sample was taken for iron estimation.

Weight of watchglass + liver sample	=	3.836 Grams
Weight of watchglass	=	3.071 Grams
Therefore sample of dried liver	=	0.765 Gram

This was digested and made up to 100 ccs solution. 1 cc solution taken for colorimetry :

$G = 28^{(+1)}$ therefore $L = .549$

From graph this = .0146 mgm iron

= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron

= 1.46 mgms = amount in 0.765 Gram dried liver.

Therefore amount iron in 22.3 Grams dried liver

$= \left(1.46 \times \frac{22.3}{.765}\right)$ mgms = 42.6 mgms iron

= Total iron in whole liver.

/ From ...

LIVER No. 25 - continued.

From (3) above this liver contained 10.3 ccs blood.

From (2) above 1 cc blood contained 0.54 mgm iron.

Therefore 10.3 ccs blood contain :

(10.3 x .54) mgms iron = 5.56 mgms

Therefore iron actually present in Hepatic Tissue = (42.6 - 5.56) = 37.04 mgms.

LIVER No. 26 ...

LIVER No. 26. Sare Sikutikwa. (B.M.H.No.6451)
Full Term. Female. Stillbirth. Birth Weight
7 lbs 12 ozs.

---

(1)
1 cc blood taken for haemoglobin estimation:

Diluted to 50 ccs and allowed to stand over-night in refrigerator.

1 cc of this solution taken for colorimetry :

$G = 70$ therefore $L = .1549$

From graph this = 1.975 mgms haemoglobin

= amount in 1 cc solution.

Therefore in 50 ccs solution amount Hb

$= 1.975 \times 50 = \underline{98.75 \text{ mgms}}$ = amount in 1 cc Blood.

---

(2)
1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs taken for colorimetry :

$G = 40'$ therefore $L = .395$

From graph this = .0105 mgm iron

= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$= .0105 \times \frac{100}{2} = \underline{0.525 \text{ mgm iron}}$

= amount in 1 cc blood.

/ (3) ....

LIVER No. 26 - continued.

(3)

Volume of liver	= 95 ccs
Weight of crucible + liver	= 309.5 Grams
Weight of crucible	= 201.5 Grams
Therefore weight of liver	= 108 Grams

Liver mushed and made up to 500 ccs solution with distilled water. Shaken and allowed to stand overnight in refrigerator.

1 cc of this solution taken for colorimetry :

G = 48' therefore L = .317

From graph this = 4 mgms haemoglobin

= amount in 1 cc solution.

Therefore in 500 ccs solution amount Hb

= 4 x 500 = 2,000 mgms haemoglobin.

From (1) above 1 cc blood contained 98.75 mgms Hb

Therefore volume of blood in liver

$$= \frac{2000}{98.75} \text{ ccs}$$

= 20.1 ccs.

/ (4) ...

LIVER No. 26 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of evaporating dish + dried liver	= 220.5 Grams
Weight of evaporating dish	= 201.5 Grams
Therefore weight of dried liver	= 19 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + liver sample	= 13.258 Grams
Weight of watchglass	= 12.770 Grams
Therefore weight of liver sample	= 0.488 Gram.

Digested and made up to 100 ccs solution.

1 cc of this solution taken for colorimetry:

G = 37' therefore L = .429.

From graph this = .0114 mgms iron

= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

= 1.14 mgms = amount in 0.488 Gram dried liver.

Therefore amount iron in 19 Grams dried liver

$= 1.14 \times \frac{19}{.488} =$ 44.6 mgms iron

= Total iron in whole liver.

/ From ...

LIVER No. 26 - continued.

From (3) above this liver contained
20.1 ccs blood.

From (2) above 1 cc blood contained
.525 mgms iron.

Therefore 20.1 ccs blood contain

(20.1 x .525) = 10.6 mgms iron.

Therefore iron actually in Hepatic Tissue

= (44.6 - 10.6) mgms = 34 mgms iron.

/ LIVER No. 27 ...

LIVER No. 27 - Sarah Nmgomezuki - (B.M.H. No. 6595). Full Term. Female. Age : 8 days. Birth Weight 5 lbs 6 ozs. Died of Icterus Gravis Neonatorum.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted with distilled water to 50 ccs. Allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry.

G = 61 therefore L = .2147.

From graph this = 2.7 mgms haemoglobin

= amount in 1 cc solution.

Therefore amount Hb in 50 ccs solution

= (2.7 x 50) mgms = 135 mgms haemoglobin.

= amount in 1 cc blood.

---

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

G = 38' therefore L = .417.

From graph this = .0111 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0111 \times \frac{100}{2}\right)$ mgms

= <u>0.55 mgm iron</u>

= amount in 1 cc blood.

/ (3) ....

LIVER No. 27 - continued.

(3) Volume of Liver	=	90 ccs
Weight of crucible + liver	=	302.5 Grams
Weight of crucible	=	201.5 Grams
Therefore weight of liver	=	101 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

$G = 38^2$ therefore $L = .414$.

From graph this = 5.225 mgms haemoglobin
= amount in 1 cc solution

Therefore amount Hb in 500 ccs solution

= 5.225 x 500 = 2,612.5 mgms Hb

From (1) above 1 cc blood contained :

135 mgms haemoglobin

Therefore volume of blood in liver

$= \frac{2612.5}{135}$ ccs = 19.3 ccs.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	222 Grams
Weight of dish	=	201.5 Grams
Therefore weight of dried liver	=	20.5 Grams

/ This ...

LIVER No.27 - continued.

This crushed to a fine powder and mixed. A

Sample was taken for iron estimation.

Weight of watchglass + sample	=	3.630 Grams
Weight of watchglass	=	3.071 Grams
Therefore weight of sample	=	0.559 Gram

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry :

G = 10 therefore L = 1.000.

This reading is too high for accuracy, so a

repeat was done using ½ cc solution :

G = 29' therefore L = .534

From graph this = 0.0142 mgms iron
= amount in ½ cc solution

Therefore in 100 ccs solution amount iron

= .0142 x 200 = 2.84 mgm
= amount iron in 0.559 Gr. dry liver.

Therefore amount iron in 20.5 Grams dried liver

$$= \left(2.84 \times \frac{20.5}{.559} \right) \text{mgms} = \underline{104.1 \text{ mgms Fe.}}$$

= Total iron in whole liver.

From (3) above this liver contained 19.3 ccs blood.
From (2) above 1 cc blood contained 0.55 mgm iron
Therefore 19.3 ccs blood contain (.55 x 19.3)mgm Fe
= 10.6 mgms iron

Therefore amount iron actually in Hepatic Tissue
= (104.1 - 10.6) mgms
= 93.5 mgms iron.

/ LIVER No. 28 ...

LIVER No. 28. Minah. (B.M.H. No. 686 - 1949).

Full term. Male Child. Died of intracranial haemorrhage. Birth weight 6 lbs 3 ozs. Aged 8 days. Congenital absence of right hand.

---

(1) 1 cc blood taken for haemoglobin estimation.

Diluted to 50 ccs with distilled water and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

G = 49 Therefore L = .310.

From Graph this = 3.9 mgms haemoglobin.
= amount in 2 ccs solution.

Therefore amount Hb in 50 ccs solution = $3.9 \times \frac{50}{2}$

= 97.5 mgms haemoglobin.

= amount in 1 cc blood.

---

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

1 cc of this solution taken for colorimetry:

G = 48 Therefore L = .319.

From Graph this = 0.0085 mgm iron = amount in 1cc solution.

Therefore in 100 ccs solution amount iron
= .85 mgm = Amount in 1 cc blood.

/ (3) ....

LIVER No. 28 - continued.

(3)	Volume of Liver	=	100 ccs
	Crucible + Liver weighs	=	315 Grams
	Crucible weighs	=	201.5 Grams
	Therefore Liver weight	=	113.5 Grams.

LIVER mushed and made up to 500 ccs, solution.

Shaken and allowed to stand overnight in refrigerator.

$1\frac{1}{2}$ ccs solution taken for colorimetry :

$G = 42^1$ Therefore $L = .374$.

From graph this = 4.7 mgms haemoglobin

= amount in $1\frac{1}{2}$ ccs solution.

Therefore in 500 ccs solution haemoglobin

$$= 4.7 \times \frac{500}{1.5}$$

= 1566.6 mgms Hb.

From (1) above 1 cc blood contains 97.5 mgms Hb.

Therefore volume of blood in liver $= \frac{1566.6}{97.5}$

= 16 ccs.

/ (4) ...

LIVER No. 28 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at $105^{\circ}C$.

Weight of dish + dried liver	=	223 Grams
Weight of dish	=	201.5 Grams
Therefore weight of dried liver	=	21.5 Grams.

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.310 Grams
Weight of watchglass	=	3.071 Grams
Therefore weight of sample	=	0.239 Grams.

Digested and made up to 100 ccs solution.

1cc of solution taken for colorimetry :

$G = 34^3$ therefore $L = .459$.

From Graph this = .0123 mgm iron

= amount in 1 cc solution.

Therefore amount iron in 100 ccs solution

= 1.23 mgms Fe

= amount in .239 Gram Liver.

Therefore amount iron in 21.5 Grams dried liver

$$= \left(1.23 \times \frac{21.5}{.239}\right) \text{ mgms}$$

= 110.9 mgm iron.

/ A second ....

LIVER No. 28 - continued.

A second sample was taken :

Weight of watchglass + sample	=	3.422 Grams
Weight of watchglass	=	3.074 Grams
Therefore weight of sample	=	0.348 Grams.

Digested and made up to 100 ccs solution.

1 cc of solution taken for colorimetry :

G = 21 Therefore L = 0.678.

From graph this = .018 mgm iron

= amount in 1 cc solution.

Therefore amount iron in 100 ccs = 1.8 mgms

= amount iron in .348 gram dry liver.

Therefore amount iron in 21.5 Grams dried liver

$$= 21.5 \times \frac{1.8}{.348}$$

= 111.2 mgms iron.

Average from two samples = 111.05 mgms Fe

= Total iron in whole liver.

From (3) above liver contained 16ccs blood.

From (2) above 1 cc blood contained .85 mgm iron

Therefore 16 ccs blood contain(16 x .85) mgm = 13.6 mgm Fe.

Therefore amount iron actually in Hepatic Tissue = (111.05 - 13.6 = 97.45 mgms Fe.

/ (3) SPLEEN ....

LIVER No. 28 - continued.

(3) Volume of Spleen = 9 ccs
Weight of crucible + spleen = 185.5 Grams
Weight of crucible = 175.2 Grams
Therefore weight of spleen = 10.3 Grams.

SPLEEN mushed and made up to 100 ccs with distilled water. Shaken and stood in refrigerator overnight.

3 ccs solution taken for calorimetry :

G = 58 therefore L = .2366

From graph this = 2.957 mgms haemoglobin

= amount in 3 ccs solution

Therefore in 100 ccs solution amount haemoglobin

$$= \left(2.975 \times \frac{100}{3}\right) \text{ mgm}$$

= 99.1 mgms Hb.

From (1) above 1 cc blood contained 97.5 mgms Hb.

Therefore volume of blood in spleen $= \dfrac{99.1}{97.5}$

= 1 cc.

/ (4) ....

LIVER No. 28 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	= 177 Grams
Weight of dish	= 175.2 Grams
Therefore weight of dried spleen	= 1.8 Grams.

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	= 3.251 Grams
Weight of watchglass	= 3.071 Grams
Therefore weight of sample	= 0.18 Gram.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry:

G = 50 therefore L = .301.

From Graph this = .008 mgm iron
= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.008 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.4 mgm

= amount in 0.18 Gram dry Spleen.

Therefore amount iron in 1.8 Grams dried Spleen

= 4 mgms

= Total iron in whole spleen.

/ From ....

LIVER No. 28 - continued.

From (3) above spleen contained 1 cc blood.

From (2) above 1cc blood contains .85 mgm Fe.

Therefore actual amount iron in

splenic tissue = (4 - .85) mgms

= 3.15 mgms iron.

/ Liver No. 29 ...

<u>NUMBER 29</u> - Mary (B.M.H. No. 815) Full term male child. Stillbirth. Birth Weight 6 lbs 4ozs. Intracranial haemorrhage.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator. 1 cc of this solution taken for colorimetry :

G = 63 therefore L = .2007

From graph this = 2.55 mgms haemoglobin

= amount in 1 cc solution

Therefore in 50 ccs solution amount haemoglobin

= 2.55 x 50 = <u>127.5 mgms</u> haemoglobin

= amount haemoglobin in 1 cc blood.

---

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution.

1 cc of this solution taken for colorimetry:

G = 59 therefore L = .2291

From graph this = .0061 mgm iron

= amount in 1 cc solution.

Therefore in 100 ccs solution amount iron

= <u>.61 mgm</u> = amount iron in 1 cc blood.

/ (3) ....

NUMBER 29 - continued.

(3) Volume of liver	= 138 ccs
Weight of crucible + liver	= 370 Grams
Weight of crucible	= 214 Grams
Therefore weight of liver	= 156 Grams.

LIVER mushed and made up to 500 ccs solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

$\frac{1}{2}$ cc of this solution taken for colorimetry.

G = 49 therefore L = .310

From graph this = 3.9 mgms haemoglobin

= amount in $\frac{1}{2}$ cc solution

Therefore in 500 ccs amount haemoglobin

= 3.9 x 500 x 2

= 3,900 mgms haemoglobin

= Total Hb in the whole liver

From (1) above 1 cc blood contained :

127.5 mgms haemoglobin

Therefore volume of blood in liver

$$= \frac{3900}{127.5} = \underline{30.6 \text{ ccs.}}$$

/ (4) ...

NUMBER 29 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	246.6 Grams
Weight of dish	=	214 Grams
Therefore weight of dried liver	=	32.5 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.377 Grams
Weight of watchglass	=	3.071 Grams
Therefore weight of liver sample	=	0.306 Gram

Digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

G = 42' therefore L = .374

From graph this = .01 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= .01 \times \frac{100}{2} = 0.5$ mgm iron

= amount in .306 Gram dried liver

Therefore amount iron in 32.5 Grams dried liver

$= \left(0.5 \times \frac{32.5}{.306}\right)$ mgms = 53.1 mgms iron

= Total iron in whole liver.

/ From ...

NUMBER 29 - continued.

From (3) above liver contained :

30.6 ccs blood.

From (2) above 1 cc blood contained :

0.61 mgm iron

Therefore 30.6 ccs blood contains :

(30.6 x .61) mgm iron

= 18.6 mgm iron

Therefore amount actually in Hepatic Tissue

= (53.1 - 18.6) mgms

= 34.4 mgms iron.

/ (3) SPLEEN ...

NUMBER 29 - continued.

(3) Volume of spleen	=	5 ccs
Weight of crucible + spleen	=	72.5 Grams
Weight of crucible	=	67 Grams
Therefore weight of spleen	=	5.5 Grams

SPLEEN mushed and made up to 100 ccs with distilled water. Shaken and allowed to stand overnight in refrigerator.

2 ccs of this solution taken for colorimetry :

G = 50 therefore L = .301

From graph this = 3.8 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= 3.8 \times \frac{100}{2}$$

= 190 mgms haemoglobin

= Total haemoglobin in spleen

From (1) above 1 cc blood contained :

127.5 mgms haemoglobin

Therefore volume of blood in spleen

$$= \frac{190}{127.5} \text{ ccs}$$

= 1.5 ccs.

/ (4) ...

NUMBER 29 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	68 Grams
Weight of dish	=	67 Grams
Therefore weight of dried spleen	=	1 Gram

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.255 Grams
Weight of watchglass	=	3.071 Grams
Therefore weight of spleen sample	=	0.184 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 44' therefore L = .354

From graph this = .0095 mgm iron

= amount in 2 ccs

Therefore in 100 ccs solution amount iron

$= .0095 \times \frac{100}{2} = 0.475$ mgm

= amount in .184 Gram dried spleen

Therefore amount iron in 1 Gram dried spleen

$= \left(.475 \times \frac{1}{.184}\right)$ mgms = 2.58 mgms iron

= Total iron in spleen.

/ From ...

NUMBER 29 - continued.

From (3) above spleen contained :

1.5 ccs blood.

From (2) above 1 cc blood contained :

.61 mgm iron

Therefore 1.5 ccs blood contain :

$$(1.5 \times .61) \text{ mgm iron}$$

$$= \underline{0.915 \text{ mgm}}$$

Therefore amount iron actually in Splenic

Tissue = (2.58 - .915) mgms

$$= \underline{1.665 \text{ mgms iron.}}$$

/ NUMBER 30 ...

NUMBER 30 - Sanna Mazibuko.(B.M.H.No.779)

Full term male child. Stillbirth. Birth Weight 7 lbs 6 ozs. Intracranial haemorrhage.

---

(1) $\frac{1}{2}$ cc blood taken for haemoglobin estimation: Made up to 50 ccs solution with distilled water. Shaken and allowed to stand overnight in refrigerator.

3 ccs of this solution taken for colorimetry :

$$G = 69 \quad \text{therefore} \quad L = .1612$$

From graph this = 2.05 mgms haemoglobin

= amount in 3 ccs solution

Therefore in 50 ccs solution amount haemoglobin

$$= 2.05 \times \frac{50}{3} = 34.2 \text{ mgms haemoglobin}$$

= amount in $\frac{1}{2}$ cc blood

Therefore amount haemoglobin in 1cc blood

= <u>68.4 mgms.</u>

---

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry:

$$G = 62 \quad \text{therefore} \quad L = .2076.$$

From graph this = .0055 mgm iron = amount in 1cc solution.

Therefore in 100 ccs solution amount iron

= <u>.55 mgm</u> = amount in 1 cc blood.

/ LIVER ....

NUMBER 30 - continued.

(3) LIVER.

Volume of liver	= 87 ccs
Weight of crucible + liver	= 318.5 Grams
Weight of crucible	= 219 Grams
Therefore weight of liver	= 99.5 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry.

G = 52 therefore L = .2840

From graph this = 3.575 mgms haemoglobin

= amount in 2 ccs solution

Therefore amount haemoglobin in 500 ccs solution

$= 3.575 \times \frac{500}{2} = \underline{893.75 \text{ mgms haemoglobin}}$

= Total haemoglobin in whole liver.

From (1) above 1 cc blood contained :

68.4 mgms haemoglobin

Therefore volume of blood in liver

$= \frac{893.75}{68.4} = \underline{13 \text{ ccs.}}$

/ (4) ...

NUMBER 30 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	243 Grams
Weight of dish	=	219 Grams
Therefore weight of dried liver	=	24 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.449 Grams
Weight of watchglass	=	3.071 Grams
Therefore weight of liver sample	=	0.378 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry.

$$G = 36' \quad \text{therefore} \quad L = .441$$

From graph this = .0118 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= .0118 \times \frac{100}{2}$$

$$= \underline{0.59 \text{ mgm iron}}$$

= amount in 0.378 Gram dry liver.

Therefore amount iron in 24 Grams dried liver

$$= \left(.59 \times \frac{24}{.378} \right) \text{mgms} = \underline{37.4 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ....

NUMBER 30 - continued.

From (3) above liver contained :

13 ccs blood.

From (2) above 1 cc blood contained :

0.55 mgm iron

Therefore 13 ccs blood contains :

(13 x .55) mgms iron

= 7.15 mgms iron

Therefore amount iron actually in Hepatic

Tissue = (37.4 - 7.15) mgm

= 30.25 mgms iron.

/ (3) SPLEEN ...

NUMBER 30 - continued.

(3) SPLEEN.

Volume of Spleen	=	- 6 ccs
Weight of crucible + spleen	=	192.5 Grams
Weight of crucible	=	186 Grams
Therefore weight of spleen	=	6.5 Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry.

G = 56 therefore L = .2518

From graph this = 3.175 mgms haemoglobin

= amount in 2 ccs solution

Therefore amount Hb in 100 ccs solution

$$= 3.175 \times \frac{100}{2}$$

= 158.75 mgms haemoglobin

= Total Hb in spleen

From (1) above 1 cc blood contained 68.4 mgms Hb

Therefore volume of blood in spleen $= \frac{158.75}{68.4}$

= 2.3 ccs.

/ (4) ....

NUMBER 30 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	187.25 Grams
Weight of dish	=	186 Grams
Therefore weight of dried spleen	=	1.25 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.249 Grams
Weight of watchglass	=	3.071 Grams
Therefore weight of spleen sample	=	0.178 Gram

Sample digested and made up to 100 ccs

2 ccs solution taken for colorimetry :

G = 46 therefore L = .337

From graph this = .009 mgms iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= .009 \times \frac{100}{2} = .45 \text{ mgm}$$

= amount in .178 Gram dry spleen

Therefore amount iron in 1.25 Grams dried spleen

$$= \left(0.45 \times \frac{1.25}{.178} \right) \text{ mgms iron}$$

= 3.16 mgms iron

= Total iron in spleen.

/ From ...

NUMBER 30 - continued.

From (3) above this spleen contained :

2.3 ccs blood

From (2) above 1 cc blood contained :

0.55 mgm iron

Therefore 2.3 ccs blood contains :

(2.3 x .55) mgms iron

= 1.27 mgms iron

Therefore amount iron actually in Splenic Tissue = (3.16 - 1.27) mgms

= 1.89 mgms iron.

/ NUMBER 31 ...

<u>NUMBER 31</u> - Mary Pilane. (B.M.H. No.1234)
Full term male child. Aged 8 days. Birth Weight 6 lbs 8 ozs. Intracranial haemorrhage.

---

(1)

1 cc blood taken for haemoglobin estimation.

Made up to 50 ccs with distilled water.

Shaken, and allowed to stand in refrigerator overnight.

$2\frac{1}{2}$ ccs solution taken for colorimetry:

$$G = 32' \quad \text{therefore} \quad L = .491$$

From graph this = 6.175 mgms haemoglobin

= amount in $2\frac{1}{2}$ ccs solution

Therefore amount haemoglobin in 50 ccs solution

$$= \left(6.175 \times \frac{50}{2.5}\right) \text{mgms} = \underline{124 \text{ mgms Hb}}$$

= amount in 1 cc blood.

---

(2)

1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$$G = 39' \quad \text{therefore } L = .406$$

From graph this = .0108 mgms iron = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0108 \times \frac{100}{2}\right) = \underline{0.54 \text{ mgms iron}}$$

= amount in 1 cc blood.

/ (3) ....

NUMBER 31 - continued.

(3) LIVER.

Volume of liver	=	75 ccs
Weight of crucible + liver	=	268.8 Grams
Weight of crucible	=	186.2 Grams
Therefore weight of liver	=	82.6 Grams

Liver mushed and made up to 500 ccs solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

$2\frac{1}{2}$ ccs solution taken for colorimetry.

G = 37' therefore L = .429

From graph this = 5.4 mgms haemoglobin

= amount in $2\frac{1}{2}$ ccs solution

Therefore in 500 ccs solution amount Hb

$$= \left(5.4 \times \frac{500}{2.5}\right)$$

= 1080 mgms Hb

From (1) above 1 cc blood contained 124 mgms Hb

Therefore volume of blood in liver

$$= \frac{1080}{124} \text{ ccs}$$

= <u>8.7 ccs.</u>

/ (4) ...

NUMBER 31 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	203.8 Grams
Weight of dish	=	186.2 Grams
Therefore weight of dried liver	=	17.6 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.414 Grams
Weight of watchglass	=	3.070 Grams
Therefore weight of liver sample	=	0.344 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 51 therefore L = .2924

From graph this = .0078 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(\frac{.0078 \times 100}{2} \right) \text{ mgm} = 0.39 \text{ mgm iron}$$

= amount in 0.344 Gram liver

Therefore amount iron in 17.6 Grams dried liver

$$= \left(.39 \times \frac{17.6}{.344} \right) \text{ mgms} = 19.9 \text{ mgms iron}$$

= Total iron in whole liver.

/ From ....

NUMBER 31 - continued.

From (3) above liver contained :

8.7 ccs blood

From (2) above 1 cc blood contained

0.54 mgm iron

Therefore 8.7 ccs blood contains :

(8.7 x .54) mgms iron

= 4.7 mgms.

Therefore amount iron actually present in

Hepatic Tissue = (19.9 - 4.7) mgms

= 15.2 mgms iron.

/ (3) SPLEEN ...

NUMBER 31 - continued.

(3) SPLEEN.

Volume of spleen	= 6 ccs
Weight of crucible + spleen	= 73.2 Grams
Weight of crucible	= 66.7 Grams
Therefore weight of spleen	= 6.5 Grams

Spleen mushed and made up to 100 ccs.

Shaken, and allowed to stand overnight in refrigerator.

$2\frac{1}{2}$ ccs taken for colorimetry.

G = 61 therefore L = .2147

From graph this = 2.7 mgms haemoglobin

= amount in $2\frac{1}{2}$ ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(2.7 \times \frac{100}{2.5}\right)$$

= 108 mgms Haemoglobin

From (1) above 1 cc blood contained 124 mgms Hb

Therefore volume of blood in spleen

$$= \frac{108}{124} \text{ cc}$$

$$= .87 \text{ cc}$$

/ (4) ....

NUMBER 31 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	68.2 Grams
Weight of dish	=	66.7 Grams
Therefore weight of dried spleen	=	1.5 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.340 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of spleen sample	=	0.27 Gram

Digested and made up to 100 ccs solution.

2 ccs taken for colorimetry :

$$G = 44^2 \quad \text{therefore} \quad L = .354$$

From graph this = .0094 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0094 \times \frac{100}{2} \right) \text{ mgm}$$

= 0.47 mgm iron

= amount in 0.27 Gram spleen

Therefore amount iron in 1.5 Gram dried spleen

$$= \left(.47 \times \frac{1.5}{.27} \right) \text{ mgms} = \underline{2.61 \text{ mgms iron}}$$

= Total iron in spleen.

/ From ...

NUMBER 31 - continued.

From (3) above spleen contained :

.87 cc blood

From (2) above 1 cc blood contained

.54 mgm iron

Therefore 0.87 cc blood contains :

(.87 x .54) mgm iron

= 0.47 mgm

Therefore amount iron actually present in

Splenic Tissue = (2.61 - .47)

= 2.14 mgms iron.

/ NUMBER 32 ...

<u>NUMBER 32.</u> - Anna Mabasa (B.M.H. No. 1402)
Full term male. Birth Weight 8 lbs. Still-birth. Craniotomy.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Shaken and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

G = 58 therefore L = .2366

From graph this = 3 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount Hb

$= 3 \times \frac{50}{2} = \underline{75 \text{ mgms Hb}}$

= amount in 1 cc blood.

---

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 43.3 therefore L = .359

From graph this = .0096 mgms iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0096 \times \frac{100}{2}\right)$ mgm

= <u>0.48 mgm iron</u> = amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 32 - continued.

(3) LIVER.

Volume of liver	=	100 ccs
Weight of crucible + liver	=	329.2 Grams
Weight of crucible	=	219.2 Grams
Therefore weight of liver	=	110 Grams

Liver mushed and made up to 500 ccs solution, with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

G = 60 therefore L = .2218

From graph this = 2.8 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 500 ccs solution amount Hb

$= \left(2.8 \times \frac{500}{2}\right)$ mgms

= 700 mgms haemoglobin

= Total Hb in liver.

From (1) above 1 cc blood contained 75 mgms Hb

Therefore volume of blood in liver

$= \frac{700}{75}$ ccs

= <u>9.3 ccs.</u>

/ (4) ....

NUMBER 32 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	= 241 Grams
Weight of dish	= 219.2 Grams
Therefore weight of dried liver	= 21.8 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.4014 Grams
Weight of watchglass	= 3.07 Grams
Therefore weight of liver sample	= 0.3314 Gram

Digested and made up to 100 ccs.

2 ccs solution taken for colorimetry.

G = 50 therefore L = .301

From graph this = .008 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.008 \times \frac{100}{2} \right) \text{ mgm}$$

= 0.4 mgm

= amount in 0.3314 Gram liver

Therefore amount iron in 21.8 Grams dried liver

$$= \left(\frac{.4 \times 21.8}{.3314} \right) \text{ mgms} = \underline{26.3 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

NUMBER 32 - continued.

From (3) above liver contained :

9.3 ccs blood

From (2) above 1 cc blood contained :

.48 mgm iron

Therefore 9.3 ccs blood contains :

(9.3 x .48)

= 4.46 mgms iron

Therefore amount iron actually present in

Hepatic Tissue = (26.3 - 4.46) mgm

= 21.84 mgms iron.

/ (3) SPLEEN ...

NUMBER 32 - continued.

(3) SPLEEN.

Volume of spleen	= 6ccs
Weight of crucible + spleen	= 182.5 Grams
Weight of crucible	= 175.5 Grams
Therefore weight of spleen	= 7.0 Grams.

Spleen mushed and made up to 100 ccs. solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

G = 68 therefore L = .1675

From graph this = 2.125 mgm haemoglobin

= amount in 2 ccs solution

Therefore in 100 ccs solution amount haemoglobin

$$= \left(2.125 \times \frac{100}{2}\right) \text{ mgms}$$

= 106.25 mgms haemoglobin

= Total haemoglobin in spleen

From (1) above 1 cc blood contained 75 mgms Hb

Therefore volume of blood in spleen

$$= \frac{106.25}{75} \text{ ccs}$$

= 1.4 ccs.

/ (4) ...

NUMBER 32 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	176.5 Grams
Weight of dish	=	175.5 Grams
Therefore weight of spleen	=	1 Gram

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.311 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of spleen sample	=	0.241 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 59 therefore L = .2291

From graph this = .0061 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0061 \times \frac{100}{2}\right)$ mgm

= 0.305 mgm

= amount in 0.241 Gram dry spleen

Therefore amount iron in 1 gram dried spleen

$= \left(.305 \times \frac{1}{.241}\right)$ mgms = 1.26 mgm iron

= Total iron in spleen.

/ From ...

NUMBER 32 - continued.

From (3) above spleen contained :

1.4 cc blood

From (2) above 1 cc blood contained :

.48 mgm Fe

Therefore 1.4 ccs blood contain :

(1.4 x .48) mgm iron

= 0.67 mgm iron

Therefore amount iron actually present in Splenic Tissue = (1.26 - 0.67) mgm

= 0.59 mgm iron

/ NUMBER 33 ...

NUMBER 33- Irene Ngashee (B.M.H. No. 1383)

Premature male child. 33 - 34 weeks. Birth Weight 4 lbs 4 ozs. Stillbirth. Was very pale, and had multiple congenital abnormalities, e.g. complete cleft palate, absence of eyes and supernumerary digits.

(1) 1 cc blood taken for haemoglobin estimation: Made up to 50ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator:

2 ccs solution taken for colorimetry :

G = 66 therefore L = .1805

From graph this = 2.3 mgms haemoglobin
= amount in 2 ccs solution.

Therefore amount Hb in 50 ccs solution

$$= \left(2.3 \times \frac{50}{2}\right) \text{mgms}$$

= 57.5 mgms Hb = amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation: Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry.

G = 66 therefore L = .1805

From graph this = .0048 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0048 \times \frac{100}{2}\right) \text{mgms} = 0.24 \text{ mgms iron}$$

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 33 - continued.

(3) LIVER.

Volume of liver	= 42 ccs
Weight of crucible + liver	= 262.5 Grams
Weight of crucible	= 213.2 Grams
Therefore weight of liver	= 49.3 Grams

Liver mushed and made up to 500 ccs. with distilled water. Shaken, and allowed to stand overnight in refrigerator.

3 ccs solution taken for colorimetry :

G = 62 therefore L = .2076.

From graph this = 2.625 mgms haemoglobin

= amount in 3 ccs solution

Therefore in 500 ccs solution amount Hb

$$= \left(2.625 \times \frac{500}{3}\right)$$

= 437.5 mgms haemoglobin

From (1) above 1 cc blood contains 57.5 mgms Hb

Therefore volume of blood in liver

$$= \frac{437.5}{57.5} \text{ ccs}$$

$$= \underline{7.6 \text{ ccs.}}$$

/ (4) ...

NUMBER 33 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	223.7 Grams
Weight of dish	=	213.2 Grams
Therefore weight of dried liver	=	10.5 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.4064 Grams
Weight of watchglass	=	3.07 Grams
Therefore weight of liver sample	=	0.3364 Grams

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 61 therefore L = .2147

From graph this = .0057 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0057 \times \frac{100}{2}\right) = 0.285$ mgm iron

= amount in 0.3364 Gram dried liver.

Therefore amount iron in 10.5 Grams dried liver

$= \left(.285 \times \frac{10.5}{.3364}\right)$ mgms = 8.9 mgms iron

= Total iron in whole liver.

/ From ...

NUMBER 33 - continued.

From (3) above this liver contained :

7.6 ccs blood

From (2) above 1 cc blood contained

.24 mgm iron

Therefore 7.6 ccs blood contains :

(7.6 x .24) mgms iron

= 1.825 mgms iron

Therefore amount iron actually present in

Hepatic tissue = (8.9 - 1.825) mgms

= 7.075 mgms iron.

/ (3) SPLEEN ...

NUMBER 33 - continued.

(3) SPLEEN

Volume of spleen	= - 6 ccs
Weight of crucible + spleen	= 207.7 Grams
Weight of crucible	= 201.4 Grams
Therefore weight of spleen	= 6.3 Grams

Spleen mushed and made up to 100 ccs solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

G = 78 therefore L = .1079

From graph this = 1.35 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(1.35 \times \frac{100}{2}\right) \text{ mgms} = \underline{67.5 \text{ mgms Hb}}$$

From (1) above 1 cc blood contains 57.5 mgms Hb

Therefore volume of blood in spleen

$$= \frac{67.5}{57.5} \text{ ccs}$$

$$= \underline{1.17 \text{ ccs}}$$

/ (4) ...

NUMBER 33 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen = 202.7 Grams
Weight of dish =201.4 Grams
Therefore weight of dried spleen= 1.3 Gram

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample = 3.290 Grams
Weight of watchglass = 3.07 Grams
Therefore weight of spleen sample = 0.22 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 59 therefore L = .2291

From graph this = .0061 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0061 \times \frac{100}{2}\right) = 0.305$ mgm iron

= amount in 0.22 Gram dried spleen

Therefore amount iron in 1.3 Gram dried spleen

$= \left(.305 \times \frac{1.3}{.22}\right)$ mgms = 1.8 mgms iron

= Total iron in spleen.

From ...

NUMBER 33 - continued.

From (3) above spleen contained :

1.17 ccs blood

From (2) above 1 cc blood contained :

0.24 mgm iron

Therefore 1.17 cc blood contains :

(1.17 x .24) mgm iron

= 0.28 mgm iron

Therefore amount iron actually present in Splenic Tissue = (1.8 - 0.28) mgm

= 1.52 mgms iron.

/ NUMBER 34. ..

NUMBER 34 - Dora Ramatso (B.M.H. No.1543)

Full term male. Stillbirth. Birth weight 7 lbs. 12 ozs. Placenta praevia. No.F.H.H. on admission.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

$G = 42'$ therefore $L = .374$.

From graph this = 4.725 mgms haemoglobin

= amount in 2 ccs solution

Therefore amount Hb in 50 ccs solution

$$= \left(4.725 \times \frac{50}{2}\right) \text{ mgms}$$

= 118 mgms haemoglobin = amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$G = 37'$ therefore $L = .429$

From graph this = .0114 mgm Iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0114 \times \frac{100}{2}\right) \text{ mgms} = 0.57 \text{ mgms iron}$$

= amount in 1 cc blood.

/ (3) LIVER ..

NUMBER 34 - continued.

(3) LIVER.

Volume of liver	=	95 ccs
Weight of crucible + liver	=	279 Grams
Weight of crucible	=	175.5 Grams
Therefore weight of liver	=	103.5 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

G = 50 therefore L = .301

From graph this = 3.8 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

$$= \left(3.8 \times \frac{500}{1} \right) \text{ mgms}$$

= 1900 mgms haemoglobin.

From (1) above 1 cc blood contained 118 mgms Hb

Therefore volume of blood in liver $= \frac{1900}{118}$ ccs

= 16.1 ccs.

/ (4) ...

NUMBER 34 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	193.7 Grams
Weight of dish	=	175.5 Grams
Therefore weight of dried liver	=	18.2 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.455 Grams
Weight of watchglass	=	3.0735 Grams
Therefore weight of liver sample	=	0.3815 Gram

Sample digested and made up to 100 ccs solution.

1 cc taken for colorimetry :

$G = 36^2$ therefore $L = .438$

From graph this = .0116 mgm iron

= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

= 1.16 mgm = amount in 0.3815 Gram dried liver

Therefore in 18.2 Grams of dried liver amount

of iron = $\left(1.16 \times \frac{18.2}{.3815}\right)$ mgms = 55.3 mgms Fe.

= Total iron in whole liver.

/ From ...

NUMBER 34 - continued.

From (3) above liver contained :

16.1 ccs blood

From (2) above 1 cc blood contained

.57 mgm iron

Therefore 16.1 ccs blood contain :

(16.1 x .57) mgms iron

= 9.2 mgms

Therefore amount iron actually in

Hepatic Tissue = (55.3 - 9.2) mgms

= 46.1 mgms iron.

/ (3) SPLEEN ...

NUMBER 34 - continued.

(3) SPLEEN.

Volume of spleen	=	10.5	ccs
Weight of crucible + spleen	=	213.5	Grams
Weight of crucible	=	201.4	Grams
Therefore weight of spleen	=	12.1	Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

$G = 36'$ therefore $L = .441$

From graph this = 5.55 mgms haemoglobin

= amount in 1 cc solution

Therefore in 100 ccs solution amount Hb

= (5.55 x 100) mgms = 555 mgms Hb.

From (1) above 1 cc blood contained 118 mgms Hb

Therefore volume of blood in spleen

$$= \frac{555}{118} \text{ ccs}$$

$$= 4.7 \text{ ccs.}$$

/ (4) ...

NUMBER 34 - continued.

(4) Solution containing spleen placed in evaporating sidh and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	204 Grams
Weight of dish	=	201.4 Grams
Therefore weight of dried spleen	=	2.6 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.288 Grams
Weight of watchglass	=	3.0735 Grams
Therefore weight of spleen sample	=	0.2145 Gram

Sample digested and made up to 100 ccs solution.

2 ccs taken for colorimetry :

G = 50' therefore L = .299

From graph this = .008 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.008 \times \frac{100}{2}\right) \text{ mgm} = 0.4 \text{ mgm}$$

= amount in 0.2145 Gram dried spleen

Therefore amount iron in 2.6 Grams dried

$$\text{spleen} = \left(.4 \times \frac{2.6}{.2145}\right) \text{ mgm}$$

= 4.8 mgms iron = Total iron in spleen.

/ From ...

NUMBER 34 - continued.

From (3) above spleen contained 4.7 ccs blood

From (2) above 1 cc blood contained 0.57 mgm Fe

Therefore 4.7 ccs blood contains :

(4.7 x .57) mgm iron

= 2.68 mgms

Therefore amount iron actually in

Splenic Tissue = (4.8 - 2.68) mgms

= 2.12 mgms iron.

/ NUMBER 35. ...

NUMBER 35 - Irene Kumalo (B.M.H. No.1412)

Full term female child. Very pale. Aged 7 days.

Birthweight 5 lbs 12 ozs.

---

(1) 1 cc blood taken for haemoglobin estimation:

Diluted to 50 ccs solution with distilled water.

Allowed to stand overnight in refrigerator.

2 ccs taken for colorimetry :

G = 46 therefore L = .337

From graph this = 4.25 mgms haemoglobin
= amount in 2 ccs solution.

Therefore in 50 ccs solution amount haemoglobin

$$= \left(4.25 \times \frac{50}{2}\right) \text{ mgms} = \underline{106.25 \text{ mgms}} \text{ haemoglobin}$$

= amount in 1 cc blood.

---

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry:

G = 51 therefore L = .2924

From graph this = .0078 mgms iron = amount in 2ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0078 \times \frac{100}{2}\right) \text{ mgms} = \underline{0.39 \text{ mgms}}$$

= amount iron in 1 cc blood.

/ (3) LIVER ...

NUMBER 35 - continued.

(3) LIVER.

Volume of liver	=	70 ccs
Weight of crucible + liver	=	265 Grams
Weight of crucible	=	186.2 Grams
Therefore weight of liver	=	78.8 Grams

Liver mushed and made up to 500 ccs solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs taken for colorimetry :

$G = 62$ therefore $L = .2076$

From graph this = 2.625 mgms Hb

= amount in 2 ccs solution

Therefore in 500 ccs amount haemoglobin

$$= \left(2.625 \times \frac{500}{2}\right) = 656.25 \text{ mgms Hb.}$$

From (1) above 1 cc blood contained 106.25 mgms Hb

Therefore volume of blood in liver

$$= \frac{656.25}{106.25} \text{ ccs} = \underline{6.2 \text{ ccs.}}$$

/ (4) ...

NUMBER 35 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	205 Grams
Weight of dish	=	186.2 Grams
Therefore weight of dried liver	=	18.8 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.436 Grams
Weight of watchglass	=	3.0735 Grams
Therefore weight of liver sample	=	0.3625 Grams

Sample was digested and made up to 100 ccs solution. 2 ccs taken for colorimetry :

$C = 38$ therefore $L = .417$

From graph this = .0111 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0111 \times \frac{100}{2} \right)$ mgms $= 0.555$ mgm

= amount in 0.3625 Gram dry liver

Therefore amount iron in 18.8 Grams dried liver

$= \left(.555 \times \frac{18.8}{.3625} \right)$ mgms = 28.7 mgms iron

= Total iron in whole liver.

/ From ...

NUMBER 35 - continued.

From (3) above liver contained :

6.2 ccs blood

From (2) above 1 cc blood contained :

0.39 mgms iron

Therefore 6.2 ccs blood contains :

(6.2 x .39) mgms iron

= 2.42 mgms iron

Therefore amount iron actually present in Hepatic Tissue = (28.7 - 2.42) mgm

= 26.28 mgms iron.

/ (3) SPLEEN ....

NUMBER 35 - continued.

(3) SPLEEN.

Volume of spleen	=	5 ccs
Weight of crucible + spleen	=	72.7 Grams
Weight of crucible	=	66.7 Grams
Therefore weight of spleen	=	6 Grams

Spleen mushed and made up to 100 ccs solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs taken for colorimetry :

$G = 66$ therefore $L = .1805$

From graph this = 2.3 mgms Hb

= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(2.3 \times \frac{100}{2}\right) = \underline{115 \text{ mgms haemoglobin}}$$

From (1) above 1 cc blood contained :

106.25 mgms haemoglobin

Therefore volume of blood in spleen

$$= \frac{115}{106.25} \text{ ccs}$$

$$= \underline{\underline{1.08 \text{ ccs.}}}$$

/ (4) ...

NUMBER 35 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	= 68.2 Grams
Weight of dish	= 66.7 Grams
Therefore weight of dried spleen	= 1.5 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	= 3.2055 Grams
Weight of watchglass	= 3.0735 Grams
Therefore weight of spleen sample	= 0.132 Gram

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$$G = 48^3 \quad \text{therefore} \quad L = .312$$

From graph this = .0084 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0084 \times \frac{100}{2}\right) \text{ mgm}$$

= 0.42 mgm iron

= amount in .132 Gram dry spleen

Therefore amount iron in 1.5 Gram dried spleen

$$= \left(.42 \times \frac{1.5}{.132}\right) \text{ mgms} = 4.77 \text{ mgms iron}$$

= Total iron in spleen.

/ From ...

NUMBER 35 - continued.

From (3) above spleen contained :

1.08 ccs blood

From (2) above 1 cc blood contained :

0.39 mgm iron

Therefore 1.08 cc blood contains :

(1.08 x .39) mgm iron

= 0.42 mgm iron

Therefore amount iron actually in

Splenic Tissue = (4.77 - 0.42) mgm

= 4.35 mgm iron.

/ NUMBER 36 ...

NUMBER 36 - Cynthia Thali. (B.M.H. No.1611)

Full term female. Stillbirth. Birth Weight 7 lbs. Died of intracranial haemorrhage. Specimen obtained within 12 hours.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator. 1 cc solution taken for colorimetry :

G = 60 therefore L = .2218

From graph this = 2.8 mgms haemoglobin
= amount in 1 cc solution

Therefore in 50 ccs solution amount haemoglobin

$= \left(2.8 \times \frac{50}{1}\right)$ mgms = <u>140 mgms</u> haemoglobin
= amount in 1 cc blood.

---

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution. 2 ccs taken for colorimetry :

G = 43' therefore L = .364

From graph this = .0098 mgm iron = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0098 \times \frac{100}{2}\right)$ mgms = 0.49 mgms iron
= <u>amount in 1 cc blood.</u>

/ (3) LIVER ...

NUMBER 36 - continued.

(3) LIVER.

Volume of liver	= 85 ccs
Weight of crucible + liver	= 299 Grams
Weight of crucible	= 201.5 Grams
Therefore weight of liver	= 97.5 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

G = 54 therefore L = .2676

From graph this = 3.375 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

$$= \left(3.375 \times \frac{500}{1}\right) \text{ mgms}$$

= 1687.5 mgms haemoglobin

From (1) above 1 cc blood contains 140 mgms Hb

Therefore volume of blood in liver

$$= \frac{1687.5}{140} \text{ ccs} = \underline{12 \text{ ccs.}}$$

/ (4) ...

NUMBER 36 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	222 Grams
Weight of dish	=	201.5 Grams
Therefore weight of dried liver	=	20.5 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.427 Grams
Weight of watchglass	=	3.0735 Grams
Therefore weight of liver sample	=	0.3535 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry:

G = 51 therefore L = .2924

From graph this = .0078 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0078 \times \frac{100}{2}\right) \text{ mgm} = 0.39 \text{ mgms iron}$$

= amount in 0.3535 Gram dry liver

Therefore in 20.5 Gram dried liver amount iron

$$= \left(.39 \times \frac{20.5}{.3535}\right) \text{ mgms iron} = \underline{22.6 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

<u>NUMBER 36 - continued.</u>

From (3) above this liver contained

12 ccs blood

From (2) above 1 cc blood contained

0.49 mgm iron

Therefore 12 ccs blood contain

(12 x .49) mgm iron

= <u>5.88 mgms iron</u>

Therefore amount iron actually present in

Hepatic Tissue = (22.6 - 5.88) mgm

= <u>16.72 mgms iron.</u>

/ (3) SPLEEN ...

NUMBER 36 - continued.

(3) SPLEEN.

Volume of spleen	=	- 4 ccs
Weight of crucible + spleen	=	190.5 Grams
Weight of crucible	=	186 Grams
Therefore weight of spleen	=	4.5 Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

G = 78 therefore L = .1079

From graph this = 1.375 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(1.375 \times \frac{100}{2}\right) \text{ mgms} = \underline{68.75 \text{ mgms}} \text{ Hb}$$

From (1) above 1 cc blood contains140 mgm Hb

Therefore volume of blood in spleen = $\frac{68.75}{140}$ cc

$= \underline{0.5 \text{ cc}}$

/ (4) ...

NUMBER 36 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	187 Grams
Weight of dish	=	186 Grams
Therefore weight of dried spleen	=	1 Gram

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.24 Grams
Weight of watchglass	=	3.0735 Grams
Therefore weight of spleen sample	=	0.1665 Grams

Sample digested and made up to 100 ccs solution.

4 ccs solution taken for colorimetry :

$$G = 69^2 \quad \text{therefore} \quad L = .1580$$

From graph this = .0041 mgm iron
= amount in 4 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0041 \times \frac{100}{4}\right) \text{mgm} = .1025 \text{ mgm iron}$$

= amount in .1665 Gram dry spleen

Therefore amount iron in 1 Gram dried spleen

$$= \left(.1025 \times \frac{1}{.1665}\right) \text{mgm} = \underline{0.615 \text{ mgm}} \text{ iron}$$

= Total amount iron in whole spleen

/ From ...

NUMBER 36 - continued.

From (3) above spleen contained :

0.5 cc blood

From (2) above 1 cc blood contains :

0.49 mgm iron

Therefore 0.5 cc blood contains :

(.5 x .49) mgm iron

= 0.245 mgm iron.

Therefore amount iron actually present in Splenic Tissue = (.615 - .245) mgm

= 0.37 mgm iron.

/ NUMBER 37 ...

<u>NUMBER 37</u> - Jane Nthable (B.M.H.1853) Full term. Male. Stillbirth. Birth Weight 8 lbs. Intracranial haemorrhage.

---

(1) 1 cc blood taken for haemoglobin estimation. Made up to 50 ccs solution with distilled water. Allowed to stand in refrigerator overnight. 2 ccs solution taken for colorimetry :

$$G = 37 \quad \text{therefore} \quad L = .432$$

From graph this = 5.45 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount Hb

$$= \left(5.45 \times \frac{50}{2}\right) \text{ mgms} = \underline{136.25 \text{ mgms Hb}}$$

<u>= amount in 1 cc blood.</u>

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution. 2 ccs solution taken for colorimetry:

$$G = 30' \quad \text{therefore} \quad L = .520$$

From graph this = .0138 mgm iron = amount in 2ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0138 \times \frac{100}{2}\right) \text{ mgm} = \underline{0.69 \text{ mgm iron}}$$

<u>= amount in 1 cc blood.</u>

/ (3) LIVER ...

NUMBER 37 - continued.

(3) LIVER.

Volume of liver	=	160 ccs
Weight of crucible + liver	=	389 Grams
Weight of crucible	=	213.4 Grams
Therefore weight of liver	=	175.6 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

$\frac{1}{2}$ cc solution taken for colorimetry:

G = 44 therefore L = .354

From graph this = 4.45 mgms haemoglobin

= amount in $\frac{1}{2}$ cc solution

Therefore in 500 ccs solution amount Hb

$$= \left(4.45 \times \frac{500}{.5}\right) \text{mgm} = \underline{4{,}450 \text{ mgms Hb.}}$$

From (1) above 1 cc blood contains 136.25mgm Hb

$$\text{Therefore volume of blood in liver} = \frac{4450}{136.25} \text{ ccs}$$

$$= \underline{32.6 \text{ ccs.}}$$

/ (4) ...

NUMBER 37 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver = 247.5 Grams
Weight of dish = 213.4 Grams
Therefore weight of dried liver = 34.1 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample = 3.4842 Grams
Weight of watchglass = 3.074 Grams
Therefore weight of liver sample= 0.4102 Gram

Sample digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry:

G = 37' therefore L = .429

From graph this = .0114 mgm iron
= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

$$= \left(.0114 \times \frac{100}{1} \right) \text{ mgms} = \underline{1.14 \text{ mgms iron}}$$

= amount in 0.4102 Gram dry liver

Therefore amount iron in 34.1 Grams dried liver

$$= \left(1.14 \times \frac{34.1}{.4102} \right) \text{ mgms} = \underline{94.7 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

NUMBER 37 - continued.

From (3) above liver contained

32.6 ccs blood

From (2) above 1 cc blood contained

0.69 mgm iron

Therefore 32.6 ccs blood contain

(32.6 x .69) mgm iron

= 22.5 mgms iron.

Therefore amount iron actually in

Hepatic Tissue = (94.7 - 22.5) mgm

= 72.2 mgms iron.

/ (3) SPLEEN ...

NUMBER 37 - continued.

(3) SPLEEN.

Volume of spleen	= 7 ccs
Weight of crucible + spleen	= 75 Grams
Weight of crucible	= 66.7 Grams
Therefore weight of spleen	= 8.3 Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

G = 48 therefore L = .310

From graph this = 3.9 mgms haemoglobin

= amount in 1 cc solution

Therefore in 100 ccs solution amount haemoglobin

= (3.9 x 100) mgm = 390 mgms haemoglobin.

From (1) above 1 cc blood contains 136.25 mgms Hb

Therefore volume of blood in spleen

$$= \frac{390}{136.25} \text{ ccs} = \underline{2.88 \text{ ccs.}}$$

/ (4) ...

NUMBER 37 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	68.7 Grams
Weight of dish	=	66.7 Grams
Therefore weight of dried spleen	=	2 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.313 Grams
Weight of watchglass	=	3.074 Grams
Therefore weight of spleen sample	=	0.239 Grams

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

$$G = 43^{3} \quad \text{therefore} \quad L = .359$$

From graph this = .0096 mgm iron
= amount in 2 ccs solution

Therefore amount iron in 100 ccs solution

$$= \left(.0096 \times \frac{100}{2} \right) \text{ mgms} = 0.48 \text{ mgms iron}$$

= amount in 0.239 Gram dry spleen

Therefore amount iron in 2 grams dried spleen

$$= \left(.48 \times \frac{2}{.239} \right) \text{ mgms} = \underline{4 \text{ mgms iron}}$$

= <u>Total iron in whole spleen</u>

/ From ...

<u>NUMBER 37 - continued.</u>

From (3) above spleen contained

2.88 ccs blood

From (2) above 1 cc blood contained

0.69 mgm iron

Therefore 2.88 ccs blood contain

(2.88 x .69) mgm iron

= <u>1.98 mgm iron</u>

Therefore amount iron actually in

Splenic Tissue = (4 - 1.98) mgms

= <u>2.02 mgms iron.</u>

/ NUMBER 38 ...

NUMBER 38 - Alina Nkoko (B.M.H. No.1757)

Full term female. Aged 1 day. Birth weight 7 lbs.

Intracranial haemorrhage.

---

(1) 1 cc blood taken for haemoglobin estimation: diluted to 50 ccs with distilled water. Allowed to stand in refrigerator overnight.

2 ccs solution taken for colorimetry:

G = 40 therefore L = .398

From graph this = 5 mgms Hb = amount in 2 ccs solution

Therefore in 50 ccs solution amount haemoglobin

$$= \left(5 \times \frac{50}{2}\right) \text{ mgms} = \underline{125 \text{ mgms Hb}}$$

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 32' therefore L = .491

From graph this = .013 mgm iron = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.013 \times \frac{100}{2}\right) \text{ mgms} = 0.65 \text{ mgm iron}$$

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 38 - continued.

(3) LIVER.

Volume of liver	= 94 ccs
Weight of crucible + liver	= 324.5 Grams
Weight of crucible	= 219 Grams
Therefore weight of liver	= 105.5 Grams

Liver mushed and made up to 500 ccs. with distilled water. Shaken, and allowed to stand in refrigerator overnight.

1 cc taken for colorimetry :

G = 47 therefore L = .328

From graph this = 4.15 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

= (4.15 x 500) mgms = 2,075 mgms Hb

= amount of haemoglobin in whole liver.

From (1) above 1 cc blood contains 125 mgms Hb

Therefore volume of blood in liver = $\frac{2075}{125}$ ccs

= 16.6 ccs.

/ (4) ...

NUMBER 38 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	239.2 Grams
Weight of dish	=	219 Grams
Therefore weight of dried liver	=	20.2 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.496 Grams
Weight of watchglass	=	3.074 Grams
Therefore weight of sample	=	0.422 Grams

Sample digested and made up to 100 ccs.

1 cc solution taken for colorimetry:

$$G = 47^{3} \quad \text{therefore} \quad L = .321$$

From graph this = .0086 mgm iron
= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

$$= (.0086 \times 100) \text{ mgms} = 0.86 \text{ mgm}$$

= amount in 0.422 Gram dried liver

Therefore amount iron in 20.2 Grams dried liver

$$= \left(.86 \times \frac{20.2}{.422} \right) \text{ mgms} = \underline{41.19 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

<u>NUMBER 38 - continued.</u>

From (3) above liver contained

16.6 ccs blood

From (2) above 1 cc blood contained

0.65 mgm iron

Therefore 16.6 ccs blood contain

(16.6 x .65) mgm iron

= <u>10.8 mgms iron</u>

Therefore amount iron actually present in

Hepatic Tissue = (41.19 - 10.8) mgms

= <u>30.39 mgms iron.</u>

/ (3) SPLEEN ...

NUMBER 38 - continued.

(3) SPLEEN.

Volume of spleen	= 4 ccs
Weight of crucible + spleen	= 180.2 Grams
Weight of crucible	= 175 Grams
Therefore weight of spleen	= 5.2 Grams

Spleen mushed and made up to 100 ccs solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

G = 70 therefore L = .1549

From graph this = 1.975 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(1.975 \times \frac{100}{2} \right) \text{mgms} = \underline{98.75 \text{ mgms Hb}}$$

= amount of haemoglobin in whole spleen.

From (1) above 1 cc blood contains 125 mgm Hb

Therefore volume of blood in spleen $= \frac{98.75}{125}$ cc

$= \underline{0.79 \text{ cc}}$

/ (4) ...

NUMBER 38 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	176.2 Grams
Weight of dish	=	175 Grams
Therefore weight of dried spleen	=	1.2 Gram

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	=	3.312 Grams
Weight of watchglass	=	3.074 Grams
Therefore weight of spleen sample	=	0.238 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry.

$$G = 37^2 \quad \text{therefore} \quad L = .426$$

From graph this = .0113 mgm iron

= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0113 \times \frac{100}{2} \right) \text{mgm} = 0.565 \text{ mgm Fe}$$

= amount in 0.238 Gram dried spleen

Therefore in 1.2 Gram dried spleen amount iron

$$= \left(.565 \times \frac{1.2}{.238} \right) \text{mgms} = \underline{2.84 \text{ mgms iron}}$$

= Total iron in whole spleen.

/ From ...

NUMBER 38 - continued.

From (3) above spleen contained

0.79 cc blood

From (2) above 1 cc blood contained

0.65 mgm iron

Therefore 0.79 cc blood contains

(.79 x .65) mgm iron

= 0.514 mgm iron

Therefore amount iron actually present in

Splenic Tissue = (2.84 - 0.514) mgm

= 2.326 mgms iron.

/ NUMBER 39 ...

NUMBER 39 - Salome Mofekeng (B.M.H. No.2002)

Full term male. Birth Weight 9 lbs 4 ozs.

Age 12 hours. Died of intracranial haemorrhage.

Mother's W.R. +.+.

(1) 1 cc blood taken for haemoglobin estimation.

Made up to 50 ccs with distilled water. Allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

$$G = 66 \quad \text{therefore} \quad L = .1805$$

From graph this = 2.3 mgms haemoglobin
= amount in 1 cc solution

Therefore in 50 ccs solution amount haemoglobin

= (2.3 x 50) mgm = 115 mgms

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution

2 ccs of this solution taken for colorimetry:

$$G = 36' \quad \text{therefore} \quad L = .441$$

From graph this = .0118 mgm iron = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0118 \times \frac{100}{2} \right) \text{mgms} = 0.59 \text{ mgm iron}$$

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 39 - continued.

(3) LIVER.

Volume of liver	=	114 ccs
Weight of crucible + liver	=	340 Grams
Weight of crucible	=	213.5 Grams
Therefore weight of liver	=	126.5 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

$G = 43'$ therefore $L = .364$

From graph this = 4.6 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

= (4.6 x 500) mgms = 2,300 mgms haemoglobin

= amount of haemoglobin in liver.

From (1) above 1 cc blood contains 115 mgms Hb

Therefore volume of blood in liver = $\frac{2300}{115}$ ccs

= 20 ccs.

/ (4) ...

NUMBER 39 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	= 238	Grams
Weight of dish	= 213.5	Grams
Therefore weight of dried liver	= 24.5	Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.499	Grams
Weight of watchglass	= 3.0715	Grams
Therefore weight of sample	= 0.4275	Grams

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

G = 28 therefore L = .553

From graph this = .0147 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0147 \times \frac{100}{2}\right) \text{ mgms} = 0.735 \text{ mgms iron}$$

= amount in 0.4275 Gram dried liver

Therefore in 24.5 Grams dried liver amount iron

$$= \left(.735 \times \frac{24.5}{.4275}\right) \text{ mgms} = \underline{42.12 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

NUMBER 39 - continued.

From (3) above liver contained

20 ccs blood

From (2) above 1 cc blood contained

0.59 mgm iron

Therefore 20 ccs blood contain

(20 x .59) mgms iron

= 11.8 mgms iron

Therefore amount iron actually present in

Hepatic Tissue = (42.12 - 11.8) mgm

= 30.32 mgms iron.

/ (3) SPLEEN ...

NUMBER 39 - continued.

(3) SPLEEN.

Volume of spleen	=	8 ccs
Weight of crucible + spleen	=	196.2 Grams
Weight of crucible	=	186.2 Grams
Therefore weight of spleen	=	10 Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

G = 51 therefore L = .2924

From graph this = 3.675 mgms haemoglobin

= amount in 1 cc solution

Therefore in 100 ccs solution amount Hb

= 367.5 mgms haemoglobin.

= amount haemoglobin in spleen.

From (1) above 1 cc blood contains 115 mgms Hb

Therefore volume of blood in spleen = $\frac{367.5}{115}$ ccs

= 3.2 ccs.

/ (4) ...

NUMBER 39 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at $105^{o}C$.

Weight of dish + dried spleen	=	189 Grams
Weight of dish	=	186.2 Grams
Therefore weight of dried spleen	=	2.8 Grams

This was crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.3 Grams
Weight of watchglass	=	3.0715 Grams
Therefore weight of sample	=	0.2285 Gram

Sample digested and made up to 100 ccs.

4 ccs solution taken for colorimetry:

G = 29 therefore L = .538

From graph this = 0.0143 mgm iron
= amount in 4 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0143 \times \frac{100}{4} \right) \text{ mgm} = 0.3575 \text{ mgm iron}$$

= amount in 0.2285 Gram dry spleen

Therefore in 2.8 Grams dried spleen amount iron

$$= \left(.3575 \times \frac{2.8}{.2285} \right) \text{ mgms} = \underline{4.3 \text{ mgms iron}}$$

= Total iron in spleen.

/ From ...

NUMBER 39 - continued.

From (3) above spleen contained

3.2 ccs blood

From (2) above 1 cc blood contained

0.59 mgm iron

Therefore 3.2 ccs blood contain

(3.2 x .59) mgm iron

= 1.88 mgm iron

Therefore amount iron actually present in

Splenic Tissue = (4.3 - 1.88) mgm

= 2.42 mgm iron.

/ NUMBER 40 ...

NUMBER 40. Eliza Lithuli. (B.M.H.No.3390)

Female. Stillbirth. Premature (33 - 34 weeks)

Birth weight 4 lbs.

(1) 1 cc blood taken for haemoglobin estimation.

Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

$G = 68'$ therefore $L = .1659$

From graph this = 2.1 mgm haemoglobin
= amount in 1 cc solution

Therefore in 50 ccs solution amount haemoglobin

= (2.1 x 50) mgms = 105 mgms haemoglobin

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$G = 45'$ therefore $L = .344$

From graph this = .0092 mgm iron = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0092 \times \frac{100}{2}\right)$ mgm = 0.46 mgm iron

= amount in 1 cc blood.

/ (3) LIVER ..

NUMBER 40 - continued.

(3) LIVER.

Volume of liver	= 48 ccs
Weight of crucible + liver	= 254.5 Grams
Weight of crucible	= 201.5 Grams
Therefore weight of liver	= 53 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

G = 67 therefore L = .1739

From graph this = 2.2 mgms Hb = amount in 2 ccs solution

Therefore in 500 ccs solution amount haemoglobin

$= \left(2.2 \times \frac{500}{2}\right)$ mgms = 550 mgms haemoglobin

= amount haemoglobin in liver.

From (1) above 1 cc blood contains 105 mgms Hb

Therefore volume of blood in liver = $\frac{550}{105}$ ccs

= 5.25 ccs.

/ (4) ....

NUMBER 40 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	211.2 Grams
Weight of dish	=	201.5 Grams
Therefore weight of dried liver	=	9.7 Grams

This dried liver was crushed to a fine powder and mixed. A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.56 Grams
Weight of watchglass	=	3.0715 Grams
Therefore weight of liver sample	=	0.4885 Grams

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$G = 55^{3}$ therefore $L = .2537$

From graph this = .0068 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0068 \times \frac{100}{2} \right)$ mgm = 0.34 mgm iron

= amount in 0.4885 gram dried liver

Therefore in 9.7 Grams dried liver amount of iron

$= \left(.34 \times \frac{9.7}{.4885} \right)$ mgms = 6.75 mgms iron

= Total iron in whole liver.

/ From ...

NUMBER 40 - continued.

From (3) above liver contained

5.25 ccs blood

From (2) above 1 cc blood contained

0.46 mgm iron

Therefore 5.25 ccs blood contain

(5.25 x .46) mgm iron

= 2.42 mgms iron

Therefore amount actually present in

Hepatic Tissue = (6.75 - 2.42) mgms

= 4.33 mgms iron.

/ (3) SPLEEN ...

NUMBER 40 - continued.

(3) SPLEEN.

Volume of spleen	= 3 ccs
Weight of crucible + spleen	= 70.4 Grams
Weight of crucible	= 66.7 Grams
Therefore weight of spleen	= 3.7 Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry.

G = 75 therefore L = .1249

From graph this = 1.6 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$= \left(1.6 \times \frac{100}{2}\right)$ mgms = 80 mgms Hb

= amount of haemoglobin in spleen.

From (1) above 1 cc blood contains 105 mgms Hb

Therefore volume of blood in spleen = $\frac{80}{105}$ ccs

= 0.76 ccs

/ (4) ....

NUMBER 40 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	= 67.4 Grams
Weight of dish	= 66.7 Grams
Therefore weight of dried spleen	= 0.7 Gram

This dried spleen was crushed to a fine powder and mixed. A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.2694 Grams
Weight of watchglass	= 3.0715 Grams
Therefore weight of spleen sample	= 0.1979 Gram

Sample was digested and made up to 100 ccs.

4 ccs solution taken for colorimetry :

$$G = 43^{3} \quad \text{therefore} \quad L = .359$$

From graph this = .0096 mgm iron
= amount in 4 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0096 \times \frac{100}{4}\right) \text{mgm} = 0.24 \text{ mgm iron}$$

= amount in .1979 Gram dried spleen

Therefore amount of iron in 0.7 Gram dried spleen $= \left(\frac{.24 \times .7}{.1979}\right)$ mgm = 0.95 mgm iron

= Total iron in whole spleen.

/ From ...

NUMBER 40 - continued.

From (3) above spleen contained :

0.76 cc blood

From (2) above 1 cc blood contained

0.46 mgm iron

Therefore 0.76 cc blood contains

(.76 x .46) mgm iron

= 0.35 mgm iron

Therefore amount iron actually present in

Splenic Tissue = (0.95 - 0.35) mgm

= 0.6 mgm iron.

/ NUMBER 41 ...

<u>NUMBER 41</u> - Maria Nxumalo (B.M.H. No.3458)

Full term male. Birth weight 6 lbs. Lived 5 hours. Died of intracranial haemorrhage.

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(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs solution with distilled water. Allowed to stand overnight, in refrigerator.

2 ccs solution taken for colorimetry.

G = 56 therefore L = .2518

From graph this = 3.175 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount Hb

$$= \left(3.175 \times \frac{50}{2}\right) \text{ mgms} = \underline{79.4 \text{ mgms Hb}}$$

= <u>amount in 1 cc blood.</u>

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

G = 47 therefore L = .328

From graph this = 0.0088 mgm iron
= amount in 2 cc solution

Therefore in 100 ccs solution amount iron

$$= \left(.0088 \times \frac{100}{2}\right) \text{ mgm} = \underline{0.44 \text{ mgm iron}}$$

= <u>amount in 1 cc blood.</u>

/ (3) LIVER ..

NUMBER 41 - continued.

(3) LIVER.

Volume of liver	=	100 ccs
Weight of crucible + liver	=	327 Grams
Weight of crucible	=	213.5 Grams
Therefore weight of liver	=	113.5 Grams

Liver mushed and made up to 500 ccs solution with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

G = 53 therefore L = .2756

From graph this = 3.475 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

= (3.475 x 500) mgms = 1737.5 Mgms Hb

= amount haemoglobin in liver.

From (1) above 1 cc blood contains 79.4 mgms Hb

Therefore volume of blood in liver = $\frac{1737.5}{79.4}$ ccs

= 20.8 ccs.

/ (4) ...

NUMBER 41 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105° C.

Weight of dish + dried liver	=	234 Grams
Weight of dish	=	213.5 Grams
Therefore weight of dried liver	=	20.5 Grams

Dried liver was crushed to a fine powder and mixed. A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.3866 Grams
Weight of watchglass	=	3.0716 Grams
Therefore weight of liver sample	=	0.315 Gram

Sample was digested and made up to 100 ccs solution. 2 ccs solution taken for colorimetry:

G = 33 therefore L = .482

From graph this = .0128 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0128 \times \frac{100}{2}\right) \text{mgm} = 0.64 \text{ mgm iron}$$

= amount in 0.315 Gram dried liver

Therefore in 20.5 Grams dried liver amount

$$\text{iron} = \left(.64 \times \frac{20.5}{.315}\right) \text{mgms} = \underline{41.7 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

NUMBER 41 - continued.

From (3) above liver contained 20.8 ccs blood

From (2) above 1 cc blood contained 0.44 mgm Fe

Therefore 20.8 ccs blood contain :

(20.8 x .44) mgms iron

= 9.17 mgms iron

Therefore amount iron actually in

Hepatic Tissue = (41.7 - 9.17) mgm

= 32.53 mgms iron.

/ (3) SPLEEN ...

NUMBER 41 - continued.

(3) SPLEEN.

Volume of spleen	=	4 ccs
Weight of crucible + spleen	=	205.9 Grams
Weight of crucible	=	201.5 Grams
Therefore weight of spleen	=	4.4 Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

4 ccs solution taken for colorimetry :

G = 49[3] therefore L = .303.

From graph this = 3.675 mgms haemoglobin

= amount in 4 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(3.675 \times \frac{100}{4}\right) \text{mgms} = \underline{91.875 \text{ mgms Hb}}$$

= amount of haemoglobin in spleen.

From (1) above 1 cc blood contains 79.4 mgms Hb

Therefore volume of blood in spleen $= \frac{91.875}{79.4}$ ccs

= <u>1.16 ccs.</u>

/ (4) ...

NUMBER 41 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105° C.

Weight of dish + dried spleen	=	202.2 Grams
Weight of dish	=	201.5 Grams
Weight of dried spleen	=	0.7 Gram

Dried spleen was crushed to a fine powder and mixed. A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.289 Grams
Weight of watchglass	=	3.0716 Grams
Therefore weight of spleen sample	=	0.2174 Gram

Sample was digested and made up to 100ccs solution. 1.25 ccs solution taken for colorimetry:

$G = 37$ therefore $L = .432$

From graph this = 0.0115 mgm iron
= amount in 1.25 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(0.0115 \times \frac{100}{1.25}\right)$ mgms = 0.92 mgms iron

= amount in 0.2174 Gram dried spleen.

Therefore in 0.7 Gram dried spleen amount iron

$= \left(.92 \times \frac{.7}{.2174}\right)$ mgms = 2.96 mgms iron

= Total iron in spleen.

/ From ...

NUMBER 41 - continued.

From (3) above spleen contained :

1.16 ccs blood

From (2) above 1 cc blood contained

0.44 mgm iron

Therefore 1.16 ccs blood contain

(1.16 x 0.44) mgm iron

= 0.51 mgm iron.

Therefore amount iron actually in

Splenic Tissue = (2.96 - 0.51) mgms

= 2.45 mgms iron.

/ NUMBER 42 ...

NUMBER 42 - Hermina Matabane (B.M.H.No.3468)

Male. Stillbirth. Premature (27 - 28 weeks)

First twin (binovular) Birth weight 2 lbs 4 ozs.

---

(1) 1 cc blood taken for haemoglobin estimation.

Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

$G = 53^3$ therefore $L = .2696$

From graph this = 3.4 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount Hb

$= \left(3.4 \times \frac{50}{2}\right)$ mgms = 85 mgms haemoglobin

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry:

$G = 54^2$ therefore $L = .2636$

From graph this = .007 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.007 \times \frac{100}{2}\right)$ mgms = 0.35 mgm iron

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 42 - continued.

(3) LIVER.

Volume of liver	= 33 ccs
Weight of crucible + liver	= 256.5 Grams
Weight of crucible	= 219 Grams
Therefore weight of liver	= 37.5 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

$G = 62^2$ therefore $L = .2041$

From graph this = 2.575 mgms Hb

= amount in 2 ccs solution

Therefore in 500 ccs solution amount haemoglobin

$= \left(2.575 \times \frac{500}{2}\right)$ mgms = 643.75 mgms Hb

= amount haemoglobin in liver.

From (1) above 1 cc blood contained 85 mgms Hb

Therefore volume of blood in liver = $\frac{643.75}{85}$ ccs

= <u>7.6 ccs.</u>

/ (4) ...

NUMBER 42 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	225.9 Grams
Weight of dish	=	219 Grams
Therefore weight of dried liver	=	6.9 Grams

Dried liver crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.3956 Grams
Weight of watchglass	=	3.0716 Grams
Therefore weight of liver sample	=	0.324 Gram

Sample digested and made up to 100 ccs.

2 ccs taken for colorimetry:

G = 58 therefore L = .2366

From graph this = .0063 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0063 \times \frac{100}{2} \right) \text{ mgm} = 0.315 \text{ mgm iron}$$

= amount in 0.324 Gram dried liver

Therefore in 6.9 Grams dried liver amount

$$\text{iron} = \left(.315 \times \frac{6.9}{.324} \right) \text{ mgms} = \underline{6.5 \text{ mgms Fe.}}$$

= Total iron in whole liver.

/ From ..

NUMBER 42 - continued.

From (3) above liver contained :

7.6 ccs blood

From (2) above 1 cc blood contained :

0.35 mgm iron

Therefore 7.6 ccs blood contains :

(7.6 x .35) mgm iron

= 2.66 mgm iron.

Therefore actual amount iron in

Hepatic Tissue = (6.5 - 2.66) mgms

= 3.84 mgms iron.

/ (3) SPLEEN .

NUMBER 42 - continued.

(3) SPLEEN.

Volume of spleen	= - 2 ccs	
Weight of crucible + spleen	= 177.2	Grams
Weight of crucible	= 175.2	Grams
Therefore weight of spleen	= 2	Grams

Spleen mushed, and made up to 100 ccs solution with distilled water. Allowed to stand overnight in refrigerator, after shaking.

4 ccs solution taken for colorimetry :

$G = 63$ therefore $L = .2007$

From graph this = 2.525 mgms haemoglobin

= amount in 4 ccs solution

Therefore in 100 ccs solution amount Hb

$= \left(2.525 \times \frac{100}{4}\right)$ mgms = $\underline{63.1 \text{ mgms Hb}}$

= amount of haemoglobin in spleen

From (1) above 1 cc blood contained 85 mgms Hb

Therefore volume of blood in spleen = $\frac{63.1}{85}$ ccs

= $\underline{0.74 \text{ ccs.}}$

/ (4) ...

NUMBER 42 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	175.45 Grams
Weight of dish	=	175.2 Grams
Therefore weight of dried spleen	=	0.25 Gram

Dried spleen crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.168 Grams
Weight of watchglass	=	3.0716 Grams
Therefore weight of spleen sample	=	0.0964 Gram

Sample digested and made up to 100 ccs.

5 ccs solution taken for colorimetry :

G = 63 therefore L = .2007

From graph this = .0053 mgm iron
= amount in 5 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0053 \times \frac{100}{5} \right) \text{ mgm} = 0.106 \text{ mgm iron}$$

= amount in 0.0964 Gram dried spleen

Therefore in 0.25 Gram dried spleen amount

$$\text{iron} = \left(.106 \times \frac{.25}{.0964} \right) \text{ mgm} = \underline{0.275 \text{ mgm Fe}}$$

= Total iron in spleen.

/ From ...

NUMBER 42 - continued.

From (3) above spleen contained

0. 74 cc blood

From (2) above 1 cc blood contained

0. 35 mgm iron

Therefore 0.74 cc blood contained :

(.74 x .35) mgm iron

= 0.259 mgm iron

Therefore actual amount of iron in

Splenic Tissue = (0.275 - 0.259) mgm

= 0.016 mgm iron

/ NUMBER 43 ...

<u>NUMBER 43</u> - Hermina Matabane (B.M.H.No.3468)

Male. Stillbirth. Premature (27 - 28 weeks)

Second twin. (binovular) Birth weight : 2 lbs.

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator : 2 ccs solution taken for colorimetry :

$$G = 43' \quad \text{therefore} \quad L = .364$$

From graph this = 4.6 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount haemoglobin

$$= \left(4.6 \times \frac{50}{2}\right) \text{ mgms} = \underline{115 \text{ mgms haemoglobin}}$$

= <u>amount in 1 cc blood.</u>

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution. 2 ccs solution taken for colorimetry :

$$G = 47 \quad \text{therefore} \quad L = .328$$

From graph this = .0088 mgm iron
= amount in 2 ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0088 \times \frac{100}{2}\right) \text{ mgm} = \underline{0.44 \text{ mgm iron}}$$

= <u>amount in 1 cc blood.</u>

/ (3) LIVER ...

NUMBER 43 - continued.

(3) LIVER.

Volume of liver	= 32 ccs
Weight of crucible + liver	= 221.2 Grams
Weight of crucible	= 186.2 Grams
Therefore weight of liver	= 35 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry :

$G = 48^2$ therefore $L = .314$

From graph this = 3.95 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 500 ccs solution amount Hb

$= \left(3.95 \times \frac{500}{2}\right)$ mgms = 987.5 mgms Hb

= amount haemoglobin in liver.

From (1) above 1 cc blood contained 115 mgms Hb

Therefore volume of blood in liver = $\frac{987.5}{115}$ ccs

= 8.6 ccs.

/ (4) ...

NUMBER 43 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	= 192.4 Grams
Weight of dish	= 186.2 Grams
Therefore weight of dried liver	= 6.2 Grams

Dried liver crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	= 3.4024 Grams
Weight of watchglass	= 3.0716 Grams
Therefore weight of liver sample	= 0.3308 Gram

Sample digested and made up to 100 ccs.

2 ccs taken for colorimetry :

$$G = 50^2 \quad \text{therefore} \quad L = .297$$

From graph this = .0079 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0079 \times \frac{100}{2} \right) \text{mgm} = 0.395 \text{ mgm iron}$$

= amount iron in 0.3308 Gram dry liver.

Therefore in 6.2 Grams dried liver amount

$$\text{iron} = \left(.395 \times \frac{6.2}{.3308} \right) \text{mgms} = \underline{7.39 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

<u>NUMBER 43 - continued.</u>

From (3) above liver contained

8.6 ccs blood

From (2) above 1 cc blood contained

0.44 mgm iron

Therefore 8.6 ccs blood contains

(8.6 x .44) mgm iron

= <u>3.78 mgms iron</u>

Therefore amount iron actually present

in Hepatic Tissue = (7.39 - 3.78) mgms

= <u>3.61 mgms iron.</u>

/ (3) SPLEEN ...

NUMBER 43 - continued.

(3) SPLEEN.

Volume of spleen	= - 1.5 ccs
Weight of crucible + spleen	= 68.2 Grams
Weight of crucible	= 66.7 Grams
Therefore weight of spleen	= 1.5 Grams.

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

4 ccs solution taken for colorimetry :

$G = 63^3$ therefore $L = .1956$

From graph this = 2.475 mgms haemoglobin

= amount in 4 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(2.475 \times \frac{100}{4} \right) \text{mgm} = \underline{61.875 \text{ mgms Hb}}$$

= amount of haemoglobin in spleen.

From (1) above 1 cc blood contained 115 mgms Hb

$$\text{Therefore volume of blood in spleen} = \frac{61.875}{115} \text{ cc}$$

$$= \underline{0.54 \text{ cc.}}$$

/ (4) ...

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105^0 C.

Weight of dish + dried spleen	= 66.95 Grams
Weight of dish	= 66.7 Grams
Therefore weight of dried spleen	= 0.25 Gram

Dried spleen crushed to a fine powder and mixed. A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.1724 Grams
Weight of watchglass	= 3.0716 Grams
Therefore weight of spleen sample	= 0.1008 Gram

Sample digested and made up to 100 ccs solution.

5 ccs solution taken for colorimetry :

$$G = 65 \quad \text{therefore} \quad L = .1871$$

From graph this = .0049 mgm iron
= amount in 5 ccs solution

Therefore in 100 ccs solution amount of iron

$$= \left(.0049 \times \frac{100}{5} \right) \text{mgm} = .098 \text{ mgm iron}$$

= amount in 0.1008 dried spleen

Therefore in 0.25 Gram dried spleen amount

$$\text{iron} = \left(.098 \times \frac{.25}{.1008} \right) \text{mgm} = \underline{0.243 \text{ mgm Fe}}$$

= Total iron in spleen

/ From ...

NUMBER 43 - continued.

From (3) above spleen contained :

0.54 cc blood

From (2) above 1 cc blood contained :

0.44 mgm iron

Therefore 0.54 cc blood contains

(.54 x .44) mgm iron

= 0.2376 mgm iron

Therefore amount iron actually present in

Splenic Tissue = (0.243 - 0.2376) mgm

= 0.0054 mgm iron.

/ NUMBER 44 ...

NUMBER 44 - Matobi Modisekeng (C.H.No.3830[8])

Full term. Male. Birth weight 7 lbs 8 ozs.

Age:10 days. Was a forceps delivery.

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator. 2 ccs solution taken for colorimetry :

G = 42 therefore L = .369

From graph this = 4.65 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount Hb

$= \left(4.65 \times \frac{50}{2}\right)$ mgms = 116.25 mgms Hb

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution. 2 ccs solution taken for colorimetry :

G = 34 therefore L = .469

From graph this = .0124 mgm iron
= amount iron in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0124 \times \frac{100}{2}\right)$ mgm = 0.62 mgm iron

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 44 - continued.

(3) LIVER.

Volume of liver	= 100 ccs
Weight of crucible + liver	= 285 Grams
Weight of crucible	= 175.2 Grams
Therefore weight of liver	= 109.8 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

G = 33 therefore L = .482

From graph this = 6.025 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 500 ccs solution amount of Hb

$$= \left(6.025 \times \frac{500}{2}\right) \text{ mgms} = \underline{1506.25 \text{ mgms Hb}}$$

= amount haemoglobin in liver.

From (1) above 1 cc blood contained 116.25 mgm Hb

Therefore volume of blood in liver $= \dfrac{1506.25}{116.25}$ ccs

$= \underline{12.9 \text{ ccs.}}$

/ (4) ...

NUMBER 44 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	= 197.4 Grams
Weight of dish	= 175.2 Grams
Therefore weight of dried liver	= 22.2 Grams

Dried liver crushed to a fine powder and mixed. A sample was taken for iron estimation :

Weight of watchglass + sample	= 3.5084 Grams
Weight of watchglass	= 3.075 Grams
Therefore weight of liver sample	= 0.4334 Grams

Sample digested and made up to 100 ccs solution

1 cc solution taken for colorimetry :

G = 35 therefore L = .456

From graph this = 0.0121 mgm iron
= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

= (.0121 x 100) mgm = 1.21 mgm iron

= amount in .4334 Gram dried liver

Therefore in 22.2 Grams dried liver amount of

iron = $\left(1.21 \times \frac{22.2}{.4334}\right)$ mgms = 61.9 mgms iron

= Total iron in whole liver.

/ From ...

NUMBER 44 - continued.

From (3) above liver contained

12.9 ccs blood

From (2) above 1 cc blood contained

0.62 mgm iron

Therefore 12.9 ccs blood contain

(12.9 x .62) mgm iron

= 8 mgms iron

Therefore amount iron actually in

Hepatic Tissue = (61.9 - 8) mgms

= 53.9 mgms iron.

/ (3) SPLEEN ...

NUMBER 44 - continued.

(3) SPLEEN.

Volume of spleen	=	12 ccs
Weight of crucible + spleen	=	81.5 Grams
Weight of crucible	=	66.7 Grams
Therefore weight of spleen	=	14.8 Grams

Spleen mushed, and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

G = 36 therefore L = .444

From graph this = 5.6 mgms haemoglobin

= amount in 1 cc solution

Therefore in 100 ccs solution amount Hb

= 560 mgms

= amount haemoglobin in spleen

From (1) above 1 cc blood contained 116.25 mgm Hb

Therefore volume of blood in spleen = $\frac{560}{116.25}$ ccs

= 4.8 ccs.

/ (4) ...

NUMBER 44 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	= 69.7 Grams
Weight of dish	= 66.7 Grams
Therefore weight of dried spleen	= 3 Grams

Dried spleen was crushed to a fine powder and mixed. A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.216 Grams
Weight of watchglass	= 3.075 Grams
Therefore weight of sample	= 0.141 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

G = 50 therefore L = .301

From graph this = .008 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.008 \times \frac{100}{2} \right) \text{mgm} = 0.4 \text{ mgm}$$

= amount iron in 0.141 Gram dried spleen

Therefore in 3 grams dried spleen amount iron

$$= \left(.4 \times \frac{3}{.141} \right) \text{mgms} = \underline{8.51 \text{ mgms iron}}$$

= Total iron in spleen.

/ From ...

NUMBER 44 - continued.

From (3) above spleen contained

4.8 ccs blood

From (2) above 1 cc blood contained

0.62 mgm iron

Therefore 4.8 ccs blood contain

(4.8 x .62) mgm iron

= 2.976 mgm iron

Therefore amount of iron actually present

in Splenic Tissue = (8.51 - 2.976) mgm

= 5.534 mgms iron.

/ NUMBER 45 ...

NUMBER 45. - Kitty. (B.M.H. No.3244) Full term. Female. Birth weight 7 lbs. Age 18 days. Died of broncho-pneumonia.

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator. 2 ccs of this solution taken for colorimetry:

$G = 39$ therefore $L = .409$

From graph this = 5.15 mgms haemoglobin
= amount in 2 ccs solution.

Therefore in 50 ccs solution amount haemoglobin

$= \left(5.15 \times \frac{50}{2}\right)$ mgms = 128.75 mgms haemoglobin

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution. 2 ccs solution taken for colorimetry:

$G = 30^2$ therefore $L = .516$

From graph this = .0138 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0138 \times \frac{100}{2}\right)$ mgms = 0.69 mgms iron

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 45 - continued.

(3) LIVER.

Volume of liver	= 90 ccs
Weight of crucible + liver	= 302.4 Grams
Weight of crucible	= 201.7 Grams
Therefore weight of liver	= 100.7 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry:

G = 50 therefore L = .301

From graph this = 3.8 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

= (3.8 x 500) mgms

= 1,900 mgms haemoglobin.

= amount of Hb in liver.

From (1) above 1 cc blood contained 128.75 mgms haemoglobin

Therefore volume of blood in liver = $\frac{1900}{128.75}$ ccs

= 14.75 ccs.

/ (4) ....

NUMBER 45 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	223.8 Grams
Weight of dish	=	201.7 Grams
Therefore weight of dried liver	=	22.1 Grams.

Dried liver crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.3736 Grams
Weight of watchglass	=	3.075 Grams
Therefore weight of sample	=	0.2986 Gram

Sample digested and made up to 100 ccs solution.

1 cc solution taken for colorimetry:

$G = 39'$ therefore $L = .406$

From graph this = 0.0108 mgm iron
= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

= (.0108 x 100) mgm = 1.08 mgm iron

= amount in 0.2986 Gram dry liver.

Therefore in 22.1 Grams dried liver amount of

iron = $\left(1.08 \times \frac{22.1}{.2986}\right)$ mgms = 80 mgms iron

= Total iron in whole liver.

/ From ...

NUMBER 45 - continued.

From (3) above liver contained

14.75 ccs blood

From (2) above 1 cc blood contained

0.69 mgm iron

Therefore 14.75 ccs blood contain

(14.75 x .69) mgms iron

= 10.2 mgms iron.

Therefore amount iron actually present

in Hepatic Tissue = (80 - 10.2) mgm

= 69.8 mgms iron.

/ (3) SPLEEN ...

NUMBER 45 - continued.

(3) SPLEEN.

Volume of spleen	= 5.5 ccs
Weight of crucible + spleen	= 192.7 Grams
Weight of crucible	= 186.2 Grams
Therefore weight of spleen	= 6.5 Grams

Spleen mushed, and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs of this solution taken for colorimetry:

$G = 56$ therefore $L = .2518$

From graph this = 3.175 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(3.175 \times \frac{100}{2} \right) \text{mgms} = \underline{158.75 \text{ mgms Hb}}$$

= amount haemoglobin in spleen.

From (1) above 1 cc blood contained 128.75 mgm Hb

$$\text{Therefore volume of blood in spleen} = \frac{158.75}{128.75} \text{ ccs}$$

$$= \underline{1.23 \text{ ccs.}}$$

/ (4) ...

NUMBER 45 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105° C.

Weight of dish + dried spleen	= 187.5 Grams
Weight of dish	= 186.2 Grams
Weight of dried spleen	= 1.3 Gram

Dried spleen crushed to a fine powder and mixed. A sample was taken for iron estimation.

Weight of watchglass + sample	= 3.201 Grams
Weight of watchglass	= 3.075 Grams
Therefore weight of sample	= 0.126 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry:

G = 47' therefore L = .325

From graph this = .0087 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0087 \times \frac{100}{2} \right) \text{mgm} = 0.435 \text{ mgm iron}$$

= amount iron in 0.126 Gram dried spleen

Therefore amount iron in 1.3 Grams dried spleen

$$= \left(.435 \times \frac{1.3}{.126} \right) \text{mgm} = \underline{4.48 \text{ mgms iron}}$$

= Total iron in spleen.

/ From ...

NUMBER 45 - continued.

From (3) above spleen contained :

1.23 ccs blood

From (2) above 1 cc blood contained

0.69 mgm iron

Therefore 1.23 ccs blood contain

(1.23 x .69) mgm iron

= 0.85 mgm iron

Therefore amount iron actually present in

Splenic Tissue = (4.48 - 0.85) mgm

= 3.63 mgms iron.

/ NUMBER 46 ...

NUMBER 46. Agnes (B.M.H. No. 3562) Full term. Male. Birth weight 7 lbs 11 ozs. Aged 7 days.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator. 2 ccs solution taken for colorimetry :

$$G = 34^2 \quad \text{therefore} \quad L = .462$$

From graph this = 5.8 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount haemoglobin

$$= \left(5.8 \times \frac{50}{2}\right) \text{mgms} = 145 \text{ mgm haemoglobin}$$

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution 2 ccs solution taken for colorimetry:

$$G = 30^2 \quad \text{therefore} \quad L = .516$$

From graph this = .0138 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0138 \times \frac{100}{2}\right) \text{mgm} = 0.69 \text{ mgm iron}$$

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 46 - continued.

(3) LIVER.

Volume of liver	= 85 ccs
Weight of crucible + liver	= 313.5 Grams
Weight of crucible	= 219.2 Grams
Therefore weight of liver	= 94.3 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry:

G = 57 therefore L = .2441

From graph this = 3.075 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

= (3.075 x 500) mgms = 1537.5 mgms Hb

= amount haemoglobin in liver.

From (1) above 1 cc blood contained 145 mgms Hb

Therefore volume of blood in liver $= \frac{1537.5}{145}$ ccs

= 10.6 ccs.

/ (4) ...

NUMBER 46 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	= 243.5 Grams
Weight of dish	= 219.2 Grams
Therefore weight of dried liver	= 24.3 Grams

Dried liver crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.436 Grams
Weight of watchglass	= 3.075 Grams
Therefore weight of liver sample	= 0.361 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry :

G = 38' therefore L = .417

From graph this = 0.0111 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0111 \times \frac{100}{2} \right) \text{mgm} = \underline{0.555 \text{ mgm iron}}$$

= amount in 0.361 Gram dried liver

Therefore in 24.3 Grams dried liver amount

$$\text{iron} = \left(.555 \times \frac{24.3}{.361} \right) \text{mgms} = \underline{37.36 \text{ mgms Fe.}}$$

= Total iron in whole liver.

/ From ...

NUMBER 46 - continued.

From (3) above liver contained :

10.6 ccs blood

From (2) above 1 cc blood contained

0.69 mgm iron

Therefore 10.6 ccs blood contain :

(10.6 x .69) mgm iron

= 7.314 mgms iron

Therefore amount iron actually present in

Hepatic Tissue = (37.36 - 7.314) mgms

= 30.046 mgms iron.

/ (3) SPLEEN ...

NUMBER 46 - continued.

(3) SPLEEN.

Volume of spleen	= - 3 ccs
Weight of crucible + spleen	= 189.7 Grams
Weight of crucible	= 186.2 Grams
Therefore weight of spleen	= 3.5 Grams

Spleen mushed, and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

4 ccs solution taken for colorimetry:

$G = 67'$ therefore $L = .1724$

From graph this = 2.175 mgms haemoglobin

= amount in 4 ccs solution

Therefore in 100 ccs solution amount Hb

$= \left(2.175 \times \frac{100}{4}\right)$ mgms = $\underline{54.4 \text{ mgms Hb}}$

= amount haemoglobin in spleen

From (1) above 1 cc blood contained 145 mgms Hb

Therefore volume of blood in spleen = $\frac{54.4}{145}$ cc

= $\underline{0.375 \text{ cc}}$

/ (4) ....

NUMBER 46 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	= 187 Grams
Weight of dish	= 186.2 Grams
Therefore weight of dried spleen=	0.8 Gram

Dried spleen crushed to a fine powder and mixed. A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.176 Grams
Weight of watchglass	= 3.075 Grams
Therefore weight of sample	= 0.101 Gram

Sample digested and made up to 100 ccs

2 ccs solution taken for colorimetry :

$$G = 48^2 \quad \text{therefore} \quad L = .314$$

From graph this = .0084 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount of iron

$$= \left(.0084 \times \frac{100}{2} \right) \text{mgm} = 0.42 \text{ mgm iron}$$

= amount in 0.101 Gram dried spleen.

Therefore in 0.8 Gram dried spleen amount

$$\text{iron} = \left(.42 \times \frac{.8}{.101} \right) \text{mgms} = 3.32 \text{ mgm iron}$$

= Total iron in spleen.

/ From ...

<u>NUMBER 46 - continued.</u>

From (3) above spleen contained :

0.375 cc blood

From (2) above 1 cc blood contained

0.69 mgm iron

Therefore 0.375 cc blood contains :

(.375 x .69) mgm iron

= <u>0.258 mgm iron</u>

Therefore amount iron actually present

in Splenic Tissue = (3.32 - 0.258) mgms

= <u>3.062 mgms iron</u>

/ NUMBER 47 ...

NUMBER 47. Sinah Mokozo. (B.M.H. No. 3740)

Female. Stillbirth. Premature (28 - 29 weeks)

Birth weight 2lbs 8 ozs.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator. 2 ccs solution taken for colorimetry:

$$G = 52^{3} \quad \text{therefore} \quad L = .2777$$

From graph this = 3.5 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount Hb

$$= \left(3.5 \times \frac{50}{2}\right) \text{mgms} = \underline{87.5 \text{ mgms Hb}}$$

= <u>amount in 1 cc blood.</u>

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution. 2 ccs solution taken for colorimetry :

$$G = 41^{2} \quad \text{therefore} \quad L = .382$$

From graph this = .0101 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0101 \times \frac{100}{2}\right) \text{mgm} = 0.505 \text{ mgm iron}$$

= <u>amount in 1 cc blood</u>.

/ (3) LIVER ...

NUMBER 47 - continued.

(3) LIVER.

Volume of liver	= 35 ccs
Weight of crucible + liver	= 253.5 Grams
Weight of crucible	= 213.7 Grams
Therefore weight of liver	= 39.8 Grams.

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

$$G = 62 \quad \text{therefore} \quad L = .2076$$

From graph this = 2.625 mgms haemoglobin

= amount in 2 ccs solution

Therefore in 500 ccs solution amount Hb

$$= \left(2.625 \times \frac{500}{2}\right) \text{mgms} = \underline{656.25 \text{ mgms Hb.}}$$

= amount haemoglobin in liver.

From (1) above 1 cc blood contained 87.5 mgms Hb

$$\text{Therefore volume of blood in liver} = \frac{656.25}{87.5} \text{ ccs}$$

$$= \underline{7.5 \text{ ccs.}}$$

/ (4) ....

NUMBER 47 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at $105^{\circ}C$.

Weight of dish + dried liver	=	221 Grams
Weight of dish	=	213.7 Grams
Therefore weight of dried liver	=	7.3 Grams

Dried liver crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.4078 Grams
Weight of watchglass	=	3.075 Grams
Therefore weight of liver sample	=	0.3328 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry:

$G = 44$ therefore $L = .357$

From graph this = 0.0095 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0095 \times \frac{100}{2} \right) \text{mgm} = 0.475 \text{ mgm iron}$$

= amount in 0.3328 Gram dried liver

Therefore in 7.3 Gram dried liver amount iron

$$= \left(.475 \times \frac{7.3}{.3328} \right) \text{mgms} = \underline{10.4 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

NUMBER 47 - continued.

From (3) above liver contained 7.5 ccs blood

From (2) above 1 cc blood contained .505 mg iron

Therefore 7.5 ccs blood contain :

(7.5 x .505) mgms iron

= 3.78 mgm iron

Therefore amount iron actually in

Hepatic Tissue = (10.4 - 3.78) mgms

= 6.62 mgms iron.

/ (3) SPLEEN ...

NUMBER 47 - continued.

(3) SPLEEN.

Volume of spleen	=	1 cc +
Weight of crucible + spleen	=	68.5 Grams
Weight of crucible	=	66.7 Grams
Therefore weight of spleen	=	1.8 Grams

Spleen mushed and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

5 ccs solution taken for colorimetry :

G = 78 therefore L = .1079

From graph this = 1.375 mgms haemoglobin

= amount in 5 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(1.375 \times \frac{100}{5}\right) \text{ mgms} = \underline{27.50 \text{ mgms Hb}}$$

= amount haemoglobin in spleen

From (1) above 1 cc blood contained 87.5 mgms Hb

Therefore volume of blood in spleen $= \frac{27.5}{87.5}$ ccs

$= \underline{0.31 \text{ cc}}$

/ (4) ....

NUMBER 47 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	= 67.1 Grams
Weight of dish	= 66.7 Grams
Therefore weight of dried spleen	= 0.4 Gram

Dried spleen crushed to a fine powder and mixed.

A sample was taken for iron estimation :

Weight of watchglass + sample	= 3.182 Grams
Weight of watchglass	= 3.075 Grams
Therefore weight of spleen sample	= 0.107 Gram

Sample digested and made up to 100 ccs.

4 ccs solution taken for colorimetry :

$d = 61$ therefore $L = .2147$

From graph this = .0057 mgm iron
= amount in 4 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0057 \times \frac{100}{4} \right)$ mgms = 0.1425 mgm iron

= amount in 0.107 gram dried spleen

Therefore in 0.4 Gram dried spleen amount

iron = $\left(.1425 \times \frac{.4}{.107} \right)$ mgm = 0.533 mgm iron

= Total iron in whole spleen

/ From ...

NUMBER 47 - continued.

From (3) above spleen contained

0.31 cc blood

From (2) above 1 cc blood contained

0.505 mgm iron

Therefore 0.31 cc blood contains

(.31 x .505) mgm iron

= .1566 mgm iron

Therefore amount iron actually in

Splenic Tissue = (.533 - .1566) mgm

= 0.376 mgm iron.

/ NUMBER 48 ...

NUMBER 48 - Violet Honi (B.M.H. No.3557)

Full term. Female. Age : 10 days. Birth Weight 8 lbs 4 ozs. Haemolytic disease of the Newborn. Child pale and jaundiced.

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

$G = 72$ therefore $L = .1427$

From graph this = 1.8 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount haemoglobin

$= \left(1.8 \times \frac{50}{2}\right)$ mgms = 45 mgms haemoglobin

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation.

Digested and made up to 100 ccs solution.

2 ccs solution taken for colorimetry :

$G = 42'$ therefore $L = .374$

From graph this = .01 mgm iron = amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.01 \times \frac{100}{2}\right)$ mgm = 0.5 mgm iron

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 48 - continued.

(3) LIVER.

Volume of liver	=	120 ccs
Weight of crucible + liver	=	341.5 Grams
Weight of crucible	=	201.7 Grams
Therefore weight of liver	=	139.8 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

1 cc solution taken for colorimetry :

$G = 68$ therefore $L = .1675$

From graph this = 2.125 mgms haemoglobin

= amount in 1 cc solution

Therefore in 500 ccs solution amount Hb

= (2.125 x 500) mgms = 1062.5 mgms Hb

= amount haemoglobin in liver.

From (1) above 1 cc blood contained 45 mgms Hb

Therefore volume of blood in liver = $\frac{1062.5}{45}$ ccs

= 23.7 ccs.

/ (4) ....

NUMBER 48 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried liver	=	232 Grams
Weight of dish	=	201.7 Grams
Therefore weight of dried liver	=	30.3 Grams

Dried liver crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.3426 Grams
Weight of watchglass	=	3.075 Grams
Therefore weight of sample	=	0.2676 Grams

Sample digested and made up to 100 ccs

2 ccs solution taken for colorimetry:

$G = 33$ therefore $L = .482$

From graph this = .0129 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$= \left(.0129 \times \frac{100}{2} \right)$ mgm $= 0.645$ mgm iron

= amount in 0.2676 Gram dry liver

Therefore in 30.3 Grams dried liver amount

iron $= \left(.645 \times \frac{30.3}{.2676} \right)$ mgms = <u>73 mgms iron</u>

= <u>Total iron in whole liver.</u>

/ From ...

NUMBER 48 - continued.

From (3) above liver contained :

23.7 ccs blood

From (2) above 1 cc blood contained

0.5 mgm iron

Therefore 23.7 ccs blood contains :

(23.7 x .5) mgm iron

= 11.85 mgms iron

Therefore amount iron actually present

in Hepatic Tissue = (73 - 11.85) mgm

= 61.15 mgms iron.

/ (3) SPLEEN

NUMBER 48 - continued

(3) SPLEEN.

Volume of spleen	=	6.5 ccs
Weight of crucible + spleen	=	182.7 Grams
Weight of crucible	=	175.2 Grams
Therefore weight of spleen	=	7.5 Grams

Spleen mushed, and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

G = 61 therefore L = .2147

From graph this = 2.7 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(2.7 \times \frac{100}{2} \right) \text{ mgms} = \underline{135 \text{ mgms Hb}}$$

= amount haemoglobin in spleen.

From (1) above 1 cc blood contained 45 mgms Hb

Therefore volume of blood in spleen = $\frac{135}{45}$ ccs

= $\underline{3 \text{ ccs.}}$

/ (4) ...

NUMBER 48 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of dish + dried spleen	=	177 Grams
Weight of dish	=	175.2 Grams
Therefore weight of dried spleen	=	1.8 Gram

Dried spleen crushed to a fine powder and mixed.

A sample was taken for iron estimation.

Weight of watchglass + sample	=	3.197 Grams
Weight of watchglass	=	3.075 Grams
Therefore weight of sample	=	0.122 Gram

Sample digested and made up to 100 ccs

1 cc solution taken for colorimetry :

G = 39 therefore L = .409

From graph this = .0108 mgm iron
= amount in 1 cc solution

Therefore in 100 ccs solution amount iron

= 1.08 mgm iron = amount in 0.122 Gram dried spleen

Therefore in 1.8 Gram dried spleen amount

iron $= \left(1.08 \times \frac{1.8}{.122} \right)$ mgms = 15.9 mgms Fe

= Total iron in spleen.

/ From ...

NUMBER 48 - continued.

From (3) above spleen contained :

3 ccs blood

From (2) above 1 cc blood contained

0.5 mgm iron

Therefore 3 ccs blood contain (3 x .5) mgm iron

= 1.5 mgm iron

Therefore amount iron actually present

in Splenic Tissue = (15.9 - 1.5) mgm

= 14.4 mgms iron.

/ NUMBER 49 ...

NUMBER 49. Franscina. (C.H. No. ---)

14/6/49. Wards 5 & 6. Full term. Female child. Aged 3 days. Birth weight 7 lbs. Died of Haemorrhagic disease of the newborn. Haemoglobin value just before death = 6.5 Grams per 100 ccs blood.

---

(1) 1 cc blood taken for haemoglobin estimation. Diluted to 50 ccs with distilled water. Allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

$$G = 65^2 \quad \text{therefore} \quad L = .1838$$

From graph this = 2.325 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 50 ccs solution amount haemoglobin

$$= \left(2.325 \times \frac{50}{2}\right) \text{ mgms} = \underline{58.25 \text{ mgms}} \text{ Hb}$$

= amount in 1 cc blood.

(2) 1 cc blood taken for iron estimation. Digested and made up to 100 ccs solution.

4 ccs solution taken for colorimetry:

$$G = 46^3 \quad \text{therefore} \quad L = .330$$

From graph this = .0088 mgm iron = amount in 4ccs solution.

Therefore in 100 ccs solution amount iron

$$= \left(.0088 \times \frac{100}{4} \right) \text{ mgms} = \underline{0.22 \text{ mgm iron}}$$

= amount in 1 cc blood.

/ (3) LIVER ...

NUMBER 49 - continued.

(3) LIVER.

Volume of liver	= 98 ccs
Weight of crucible + liver	= 311.2 Grams
Weight of crucible	= 201.7 Grams
Therefore weight of liver	= 109.5 Grams

Liver mushed and made up to 500 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

G = 50 therefore L = .301

From graph this = 3.8 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 500 ccs amount haemoglobin

$$= \left(3.8 \times \frac{500}{2} \right) \text{ mgms} = \underline{950 \text{ mgms Hb}}$$

= amount haemoglobin in liver.

From (1) above 1 cc blood contained 58.25 mgm Hb

Therefore volume of blood in liver $= \frac{950}{58.25}$ ccs

$= \underline{16.3 \text{ ccs.}}$

/ (4) ...

NUMBER 49 - continued.

(4) Solution containing liver placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of crucible + dried liver	=	225.4 Grams
Weight of crucible	=	201.7 Grams
Therefore weight of dried liver	=	23.7 Grams

Dried liver crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	=	3.3000 Grams
Weight of watchglass	=	3.0746 Grams
Therefore weight of sample	=	0.2254 Gram

Sample digested and made up to 100 ccs.

2 ccs solution taken for colorimetry:

G = 61 therefore L = .2164

From graph this = .00575 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.00575 \times \frac{100}{2} \right) \text{ mgm} = 0.2875 \text{ mgm iron}$$

= amount in 0.2254 Gram dried liver

Therefore in 23.7 Gram dried liver amount iron

$$= \left(.2875 \times \frac{23.7}{.2254} \right) \text{ mgms} = \underline{30.2 \text{ mgms iron}}$$

= Total iron in whole liver.

/ From ...

NUMBER 49 - continued.

From (3) above liver contained :

16.3 ccs blood

From (2) above 1 cc blood contains :

0.22 mgm iron

Therefore 16.3 ccs blood contain

(16.3 x .22) mgm iron

= 3.59 mgm iron.

Therefore amount iron actually present

in Hepatic Tissue = (30.2 - 3.59) mgms

= 26.61 mgms iron.

/ (3) SPLEEN ...

NUMBER 49 - continued.

(3) SPLEEN.

Volume of spleen	= 7 ccs
Weight of crucible + spleen	= 74.5 Grams
Weight of crucible	= 66.7 Grams
Therefore weight of spleen	= 7.8 Grams

Spleen mushed, and made up to 100 ccs with distilled water. Shaken, and allowed to stand overnight in refrigerator.

2 ccs solution taken for colorimetry:

$$G = 63 \quad \text{therefore} \quad L = .2007$$

From graph this = 2.55 mgms haemoglobin
= amount in 2 ccs solution

Therefore in 100 ccs solution amount Hb

$$= \left(2.55 \times \frac{100}{2} \right) \text{ mgms} = \underline{127.5 \text{ mgms Hb}}$$

= amount haemoglobin in spleen.

From (1) above 1 cc blood contained 58.25 mgms Hb

$$\text{Therefore volume of blood in spleen} = \frac{127.5}{58.25} \text{ ccs}$$

$$= \underline{2.2 \text{ ccs.}}$$

/ (4) ...

NUMBER 49 - continued.

(4) Solution containing spleen placed in evaporating dish and dried to constant weight in air oven at 105°C.

Weight of crucible + dried spleen	= 68.5 Grams
Weight of crucible	= 66.7 Grams
Therefore weight of dried spleen	= 1.8 Gram

Dried spleen crushed to a fine powder and mixed.

A sample was taken for iron estimation:

Weight of watchglass + sample	= 3.2124 Grams
Weight of watchglass	= 3.0746 Grams
Therefore weight of sample	= 0.1378 Gram

Sample digested and made up to 100 ccs

2 ccs taken for colorimetry :

G = 59 therefore L = .2291

From graph this = .0061 mgm iron
= amount in 2 ccs solution

Therefore in 100 ccs solution amount iron

$$= \left(.0061 \times \frac{100}{2} \right) \text{mgm} = 0.305 \text{ mgm iron}$$

= amount in 0.1378 Gram dried spleen

Therefore in 1.8 gram dried spleen amount

$$\text{iron} = \left(.305 \times \frac{1.8}{.1378} \right) \text{mgms} = \underline{3.98 \text{ mgms iron}}$$

= Total iron in whole spleen.

/ From ...

NUMBER 49 - continued.

From (3) above spleen contained

2.2 ccs blood

From (2) above 1 cc blood contained

0.22 mgms iron

Therefore 2.2 ccs blood contains

(2.2 x .22) mgm iron

= 0.485 mgm iron

Therefore amount iron actually present

in Splenic Tissue = (3.98 - 0.485) mgs

= 3.495 mgms iron.

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