STUDIES IN ANAEMIA OF INFANCY AND CHILDHOOD

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Part XI.—The effect of iron deficient diets on the size of the red blood cells in rats and in the production of microcytic hypochromic anaemia in their offspring; a contribution to the study of congenital iron-deficiency anaemia in the human infant

The studies of anaemia in infants and young children which have been carried out by our colleagues and ourselves (Parsons et al.^{11, 12, 13, 14}) have shown that the maturation of the red cell is a somewhat more complicated process than that suggested by Witts. For instance, vitamin C and thyroxin are concerned not only with the maturation of the normoblast to the erythrocyte but also act throughout the whole range of erythropoiesis. This statement is based upon the fact that when anaemia occurs as a result of a deficiency of either of these two factors, it is never of the hypochromic and microcytic type, but usually orthochromic and normocytic, although sometimes a macrocytic anaemia may result. Further a true microcytic anaemia is essentially due to a deficient supply of iron to the bone marrow, although it may occur in the absence of certain substances containing the pyrrol ring, possibly also of calcium, and, at any rate experimentally, in the absence of copper and perhaps some other metals which act as mobilizers or catalysts of iron.

In the course of these investigations, Helen Mackay's view⁸ that nutritional anaemia is frequent in infancy has been confirmed; it has also been shown that this form of anaemia may occur as the result of deficiency of dietary substances containing the pyrrol ring, and that it is accompanied by a microcytosis which gives place to a normocytosis when the anaemia is cured by the supply of the missing factors, e.g., iron, copper, pyrrol. Moreover, evidence has been obtained of the existence of a congenital nutritional anaemia which, it is believed, is due to inadequate maternal diet during pregnancy. The experiments described in this present paper were undertaken to test and extend these views on microcytosis and congenital anaemia by means of controlled animal experiments.

Variations in the number and size of red cells of the rat from birth to maturity

Nutritional anaemia in the rat exhibits the same features as regards haemoglobin values, number of red blood cells, and response to iron therapy as the nutritional anaemia of infancy, and indeed also as that of idiopathic hypochromic anaemia of adults. In the study of experimental anaemia, attention has been almost entirely focussed on haemoglobin estimations and to a lesser extent on red cell counts. Moreover the existence of a microcytosis such as occurs in the iron deficiency anaemia of human infants and adults has only been demonstrated by one observer (Foster⁵) who made use of the haematocrit method. It was therefore determined to plan a series of experiments to obtain accurate information, by means of Price Jones' curves, concerning the size of the red cells of rats fed on a diet deficient in iron and of the progeny of such rats; also to follow the changes in size, if any, brought about by the addition of adequate amounts of iron to their diet. In order to assess the importance of any changes found in these rats, it was necessary in the first place to obtain information about any variations that occurred in the number and size of red cells in the normal rat from birth to maturity.

Sure, Kik, and Walker¹⁷ found that there was a continuous increase in the number of red cells from 2·8 millions at birth to 6·0 millions or over at twenty-nine days, whereas according to Wills and Mehta²⁰ the red cells diminish in number until the tenth day of life and then rise rapidly. Smith¹⁶ who made observations on groups of five rats at the following age periods: two days before term, one, two, three, four, five, six, seven, ten, thirteen, nineteen, twenty-three and seventy-three days, and in adult life, was, however, able to confirm and extend the findings of Sure, Kik and Walker, and showed that there was a steady rise in the number of red cells from 2·1 millions at birth to 8 millions in adult life.

The present investigation confirms the statement that a continuous increase in the number of red cells takes place from birth to maturity. For the purpose, normal stock rats (both albino and piebald) were used and counts were made on five rats in each group at ages of one day, one, two, three, four, six, eight, and twelve weeks, and the count was found to increase from 2·8 millions at birth to 7·8 millions at three months of age (see table 1). This striking increase in the number of cells as the animal matures is a fact for which at present no explanation can be offered, and is a phenomenon which is quite unlike anything that occurs in the human infant; in fact, it is the exact opposite of what usually obtains. It is generally accepted that there is a fairly rapid drop in the number of red cells in the first few days of the new-born child's life. Guest⁶ has, however, found that sometimes in the first few days of life there is an actual increase in the number of cells and our colleague, Dr. Margaret Cleland, who is making observations on the blood of new-born children, finds that an increase in the red cell count of something like half a million does sometimes occur in the first two or three days of life. Such an increase is, however, small when compared with the rat and is probably due to dehydration. In this respect the young rat and the infant show a marked difference, but on the other hand the rat shows a continuous fall in the amount of haemoglobin until the fourth week of life which is strictly comparable with the physiological anaemia of infancy.

Wills and Mehta in the paper already mentioned recorded some measurements of the size of the red cells using a simple halometer method; the only measurements made by the Price Jones' technique are contained in Smith's paper. She proved that during growth there was a progressive reduction in the size of the cell from a mean diameter of 9.91μ in the foetus two days before term to 6.08μ in the adult rat.

The size of the red cells in rats in the present investigation have been measured by means of Price Jones' curves and a similar result obtained, the average mean diameter ranging from 8.79μ at birth to 6.19μ at three months. The full details of the red cell counts, the size of the red cells, the number of reticulocytes and the amount of haemoglobin are given in table 1,

FIG. 1.—Composite curves for five rats showing the alteration in the mean diameters of the red cells from birth to maturity.

and in figure 1 are shown a series of composite curves of the diameters of the cells from birth to three months.

This gradual diminution in the size of the red cell also occurs in the human infant, but to a much less degree; thus van Creveld² has shown that there is a megalocytosis at birth, the mean diameter being 8.0μ , but that the cell diameter diminishes after the neonatal period and is 7.72μ in the eighth week of life. It is clearly important, therefore, that in any study of the possible alteration in the size of the red cells as the result of diet, this physiological change in the size of the cell must be borne in mind, and for this reason the Price Jones' curves of the red cells of anaemic rats have been checked by parallel measurements of the red cells of normal stock animals of the same age. Similar controls have also been used for all haematological observations.

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**Effect of a low iron diet on the number and size of red cells
of rats and of their progeny**

In a previous paper (Parsons and Hickmans¹²) it has been shown that nutritional anaemia in rats could be prevented by the addition of dried yeast to a milk diet after the end of a normal period of lactation, but that the offspring of rats so fed showed a profound anaemia even during the lactation period. Although such a diet does not contain an adequate amount of iron it is not ideal for the purpose of these experiments because the amount of milk makes the diet so bulky that it is impossible for pregnant rats to take enough food to keep up their weight. A solid diet was therefore constructed which, although low in iron, is not, according to present knowledge, deficient in other respects. This diet consists of:—

Rice starch	...	...	...	...	60	per cent.
Casein	...	...	...	...	20	„ „
Palm kernel oil	...	...	...	...	10	„ „
McCullum's salt mixture (omitting the iron compound)	...	...	...	...	5	„ „
Yestamin	...	...	...	...	5	„ „

Fifteen grammes of this mixture made into a thick paste with cows milk and supplemented by two drops of cod-liver oil and two drops of wheat germ oil were given to each adult rat daily. Stock rats fed on this diet from the time of weaning (at about three weeks of age) to three months of age show practically normal growth and do not appear pale or anaemic, their haemoglobin being approximately 100 per cent. (16 gm.); indeed, in only one instance has a percentage as low as 70 been found, this being in a pregnant rat. The red cells, however, show a relatively large increase in number, the count usually ranging from 9 millions to 11 millions as compared with a normal count of 7 to 8 millions per c.mm.; moreover the red cells are smaller than normal, the diameter varying from 5.5μ to 5.8μ as compared with the normal diameter of 6.19μ .

The result of this diet is, therefore, to produce a microcytosis and a lowered colour index, although the haemoglobin percentage remains unchanged. Moreover, the litters produced by these rats are particularly interesting. At birth their haemoglobin and red cell count are generally only slightly sub-normal, but on comparatively rare occasions haemoglobin readings as low as 10 per cent. have been observed. During the lactation period, however, the haemoglobin is rapidly reduced to a level below that found in the physiological anaemia of early life, often falling as low as 10 per cent.; further, the number of red cells rises less rapidly than in the normal rat. The red cells at birth are smaller than normal and they steadily diminish in size during the first three weeks of life, as do the cells of the normal rat. If, when weaned, these young rats are given the same iron-deficient diet as their parents the haemoglobin rises rapidly and the red cells increase up to 11 millions; nevertheless the mean diameter continues to be small, viz., 5.7μ as compared with 6.12μ for the stock animals. If instead of this special diet they are fed only on milk their haemoglobin percentage continues to fall and the red cells diminish to less than 4 millions,

and their diameter falls to about 5.4μ . A similar, although not so marked, diminution in haemoglobin and in the number and size of the red cells occurs when rats born of stock parents are given milk only for the first three months of life. These results are set out in detail in table 1. In fig. 2 the variations are compared by means of graphs with those of rats on stock diet, and in fig. 3, Price Jones' curves of the size of the red cell at various ages are depicted, together with the corresponding curves for rats of the same age on stock diet.

From these results it is obvious that a diet has been devised which, although low in iron, contains just sufficient iron to enable the adult rat to maintain a normal haemoglobin level, but insufficient to allow the normal complete maturation of the red cell to take place. On such a diet many

FIG. 2.—Showing the Hb., red blood cell count, reticulocytes and mean diameters of red cells of rats:

1. Born of parents fed on stock diet
2. " " " " iron-deficient diet and continued from lactation on similar diets or milk only.

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of the red cells are smaller than normal but the haemoglobin is kept at the normal level by the development of a polycythaemia. We believe that in all iron deficiency anaemias the bone marrow hypertrophies in an attempt to produce large numbers of small envelopes (microcytes) to hold

FIG. 3.—Showing microcytosis at various ages in rats born of parents fed on an iron deficient diet and continued on the same iron deficient diet or on milk only.

the available haemoglobin and thus to offer the largest possible surface area of haemoglobin for gaseous exchange. Although it may appear somewhat paradoxical, we believe that this occurrence of polycythaemia, microcytosis, and a normal haemoglobin value, is the first sign of an iron deficiency anaemia. Such a view receives confirmation from the fact that sometimes in the course of the cure of nutritional anaemia of infancy by the exhibition of iron, what is known as Hagen's phenomenon occurs, i.e., before the haemoglobin reaches a normal figure the red cell count may exceed 6 million per c.cm. but when the haemoglobin does become normal the red cells become reduced to 5 million. The defects of this special diet are, however, even more strikingly shown by the animals in the succeeding generation because the pregnant rat on this diet is unable to transfer sufficient iron to the foetus, with the result that the progeny, during their lactation period, develop a severe degree of microcytic anaemia, a state of affairs which clearly shows that the diet of the mother during pregnancy and lactation has a direct bearing on the blood picture of her young. In previous papers (Parsons¹¹, Parsons and Hickmans¹², Parsons and Hawksley¹³) the occurrence of congenital nutritional anaemia both in the rat and the human infant has been described, and the existence of such a condition has thus been confirmed by the present series of experiments. One important point of difference between the human mother and the rat has, however, been observed. The human mother can sacrifice her own haemoglobin for the sake of the child, and although becoming profoundly anaemic herself, more often than not gives birth to a child whose blood is normal, whereas in none of the rat experiments has any pronounced diminution in haemoglobin in the mother rat occurred. This congenital nutritional anaemia of the rat is microcytic in type and becomes progressively worse if milk feeding is continued beyond the lactation period. If, on the other hand, these anaemic rats are fed on the special diet herein described from the end of the lactation

period, the haemoglobin rises and polycythaemia develops but microcytosis remains—a blood picture identical with that of their parents.

Effect of administration of copper and iron on congenital nutritional anaemia

The anaemia and microcytosis in these rats of the second generation can be rapidly cured by the addition to the daily diet of ferrous sulphate (0.5 mgm. Fe) and a trace of copper sulphate (0.025 mgm. Cu); typical Price Jones' curves before and after such treatment are given in fig. 4 and show that the mean diameter increases from 5.73μ to 6.73μ .

FIG. 4.—Showing the alteration in the size of the cells before and after treatment with FeSO_4 and CuSO_4 .

Before, mean diameter = 5.73μ .

After, mean diameter = 6.73μ .

In some of their earlier papers Hart, Steenbock et al.⁷ stated that the administration of pure iron to rats suffering from nutritional anaemia did not produce a cure, but this invariably occurred if a small amount of copper was given in addition to the iron. In their opinion this was due to the exhaustion of both iron and copper stores in the bodies of the anaemic rats as the result of the deficient dietary. Later, Elvehjem and Sherman³ showed that the administration of pure iron led to the accumulation of this element in the liver and that it was only mobilized for action by the subsequent administration of copper.

In the present experiments the rats were already anaemic during the lactation period and only a short time beyond the lactation period was

necessary to produce a severe anaemia. It was therefore thought that they might have retained sufficient copper stores to render the simultaneous administration of copper and iron unnecessary and attempts were made to cure the anaemia by the administration of ferrous sulphate only. Most of these were unsuccessful, but in three rats an erratic response with slow improvement occurred and the diameter of the cells eventually became normal. If, however, copper even in minute amounts (0.025 mgm.) was also given daily the return to normal was rapid and consistent. The differences between the response by these congenitally anaemic rats to pure iron and that to iron and copper in respect of haemoglobin, red cells and reticulocytes are shown by a series of graphs in fig. 5.

FIG. 5.—Showing the effect on Hb., red blood cells and reticulocytes of anaemic rats of:

(1) Pure FeSO_4 .

(2) „ „ and CuSO_4 .

Effect of special low-iron diet on the breeding and growth of second generation rats

The first generation of rats fed on the special diet breeds fairly freely and may have three or four litters in the first eighteen months of life. (Some of the stock rats in the same colony may have eight or nine litters in the same time.) Most of these litters survive and grow up on this deficient diet to produce adult rats apparently identical with their parents. These, the second generation, however, breed with difficulty, their litters are few and

during the period covered by these experiments (two to three years) few have survived the lactation period; at about the eighteenth day of age they suddenly assumed the appearance of having had a bath or of having been immersed in their food and often were killed off by the doe. A few have, however, survived and the weights and haemoglobin values of some of the third generation rats are shown in the following table:—

AGE OF RATS OF THIRD GENERATION ON IRON-LOW DIET.	HAEMOGLOBIN.	AVERAGE WEIGHT OF ONE.	AVERAGE WEIGHT OF ONE FROM STOCK LITTER.
Birth	45		
2 days		5 gm.	5 gm.
11 days	35	10 gm.	
16 days		12 gm.	19 gm. (14 days)
18 days	10	22-35 gm.	30-35 gm. (21 days)
8 weeks		36 gm.	96 gm.

Discussion and summary

In a perusal of the literature on experimental nutritional anaemia of the rat it is found that the occurrence of polycythaemia on a iron-deficient diet has been previously described by Eveleth, Bing and Myers⁴.

These writers fed rats on a diet of raw cow's milk until they were anaemic; they were then given intraperitoneal injections containing sub-optimal amounts of pure iron, and as a result the rats not only developed a polycythaemia, but their haemoglobin remained subnormal throughout the experimental period. This would appear to be a stage nearer the typical nutritional anaemia than that shown by the animals in the present experiments, or in other words, the animals showed an earlier stage in the development of this iron deficiency anaemia. Eveleth et al. did not estimate the size of the red cells, and the only reference to microcytosis in milk anaemia in rats found is in the paper by Foster⁵ already mentioned, who demonstrated a diminution in size of the red cells by the haematocrit method. Polycythaemia, marked microcytosis and a reduction of haemoglobin have, however, been demonstrated under apparently quite different conditions, namely as the result of an extreme restriction of all the inorganic constituents of the diet (Smith and Schultz¹⁵, Swanson and Smith¹⁸, Orten and Smith⁹, Orten, Smith and Mendel¹⁹). The diet used in these experiments was extremely deficient in calcium and to a lesser degree in potassium, sodium, phosphorus, manganese, and chloride, and contained less iron than the albino rat is supposed to require. From a study of these contributions the cause of these blood changes was probably a deficiency in iron, and this view appeared to be borne out by the fact that when the Osborne and Mendel salt mixture, which contains adequate quantities of iron, was added to the diet the blood became normal. Orten, Smith and Mendel say that they also suspected that an iron deficiency was the explanation even though an 'iron deficiency usually produces a decrease in the erythrocyte content of the blood'—a statement shown in this paper to be incorrect provided that the iron deficiency is minimal—but that they were also struck by the deficiency of calcium in the diet and therefore planned a series of experiments to test the effect of administering iron and calcium to rats consuming such a mineral deficient ration. They found that the administration of adequate amounts of calcium (purified calcium carbonate in the amounts present in the Osborne and Mendel salt mixture) prevented the occurrence of these blood changes, or cured them if administered after the anaemia had been developed. Purified ferric chloride on the other hand, whilst preventing the development of the changes, showed variable results when used as a curative agent. These investigators also

found that sodium, potassium, manganese and chloride were not concerned in the production of the blood changes and came to the conclusion that the 'haematological abnormalities which occur in rats as a result of the feeding of the mineral deficient ration are due chiefly, if not entirely, to a lack of calcium and/or iron.'

Nevertheless, in spite of these findings, we are still of the opinion that these blood changes are essentially due to a deficient supply of iron to the bone marrow which, as stated at the commencement of this paper, is believed to be the essential cause of a microcytosis, and that the cause of this deficiency must be looked for in the inter-dependence of calcium and iron metabolism; and that the results obtained by these workers can be adequately explained on this ground. Both calcium and iron are absorbed from the same portion of the alimentary canal; both require an acid medium for adequate absorption and are therefore less readily absorbed if achlorhydria is present. Further, Brock and Diamond¹ showed that the administration of large amounts of iron will produce rickets in rats who are on a non-rachitogenic diet. It is therefore clear that the calcium-iron-phosphorus ratio in the bowel is an important factor in both calcium and iron absorption. In the 'poor salt diet' the extreme deficiency is in calcium, whereas that of phosphorus and iron is relatively much less. In other words, there is a relative excess of phosphorus available to combine with the iron to form insoluble ferrous phosphate and thus to restrict a large portion of already somewhat deficient iron and phosphorus to the intestine and produce a real iron deficiency. Those who have worked with this dietary have themselves produced definite evidence in favour of this explanation in the observations made by Swanson, Timson and Frazier¹⁹ that the blood changes are not so marked if edestin, a protein which contains little, if any phosphorus, is substituted for the phospho-protein casein in the 'poor salt diet.' It would have been interesting to see what would have been the result on the blood if the diet had been modified so as to be extremely deficient in phosphorus and less so in calcium.

An attempt has been made to apply these findings to clinical medicine. In coeliac disease, the absorption of calcium is greatly diminished. We therefore administered to children suffering from the hypochromic microcytic anaemia of that disease large doses of calcium chloride for a period of three to four weeks, and then for a further period calcium chloride and a minimal dose of iron—one-tenth of the usual dose—but without any significant effect on the anaemia.

Summary

The results of the investigations contained in this paper may therefore be summarized as follows:—

(1) A special iron-deficient solid diet has been devised which when fed to rats results in the development of polycythaemia and microcytosis although the haemoglobin remains normal—findings which it is suggested are the first step in the production of an iron-deficiency anaemia.

(2) The offspring of such rats develop in the lactation period an anaemia greater than the physiological anaemia of infancy and one which is microcytic in type.

(3) If these young rats, after weaning, are fed on the special diet, microcytosis persists, but polycythaemia develops and the haemoglobin becomes normal.

(4) If the diet of these young rats after weaning is restricted to milk the anaemia becomes progressively worse and the cells smaller and less numerous than those in the group receiving the special diet.

(5) The cells can be restored to normal size by the administration of adequate amounts of iron and a trace of copper.

(6) Rats fed on the diet for one generation can produce and rear their litters fairly well, but the reproductive powers of the succeeding generation fed on the same diet are greatly diminished, the young are reared with difficulty, are subnormal in weight and size and show an even more pronounced anaemia than the preceding generation.

(7) The analogies between these findings and certain clinical conditions in the human mother and infant are pointed out.

(8) The findings support the suggestion that the infants of women whose diets during pregnancy are low in iron or who suffer from an iron deficiency anaemia may show a congenital nutritional anaemia.

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