Iron Metabolism and Haemoglobin Formation in the Embryonated Hen Egg

3. IRON IN THE LIVER AND BLOOD OF THE HATCHING CHICK

By W. N. M. RAMSAY

Department of Biochemistry, University of Edinburgh

(Received 25 October 1951)

It is generally recognized that in many animals the liver stores an important quantity of iron at all ages. Thus, calculations based largely on figures quoted by Brückmann & Zondek (1939) and by Barcroft (1946) show that in both the adult man and the newborn lamb the liver contains roughly one-third of the non-haem iron in the body. In the course of a study which extended over both sexes of many species, Widdowson & McCance (1948) showed that at 20 weeks of age the livers of pullets contained 14.7 mg. non-haem iron/100 g. wet weight, and similar figures were obtained in pullets of 16 weeks by Chapman, Maw & Common (1950). Ramsay (1951) found that during the last few days of incubation some 200 μ g. haem iron disappears from the chick embryo membranes. As there is an apparently corresponding increase in the rate of accumulation of non-haem iron in the embryo body, it seemed at first sight likely that the membrane haemoglobin was being destroyed (bile pigments also appear at this time), and that the non-haem iron liberated was being accumulated as a store. The present experiments were therefore undertaken with a view to finding out whether a significant proportion of the iron was being concentrated in the liver.

As the total amount of non-haem iron in the hatching chick (excluding the 'spare' yolk) is of the order of 350–400 μ g. (Ramsay, 1951), it may be calculated that if the state of affairs is similar to that in the animals already mentioned, the liver should contain more than 100 μ g. non-haem iron. McFarlane & Milne (1934) determined total iron in the liver of the chick at all stages from 9 days after the eggs were set in the incubator until 3 days after the chicks were hatched. In the hatched chick they found the liver to contain 41 μ g. iron, and in the only two livers tested by the method of Hill (1931), that the non-haem iron was 52 and 66 % of the total, or roughly 25 μ g./liver. This figure is much less than might be expected, so it seemed important to make a re-investigation of the question. The results described in this paper establish that the analyses of McFarlane & Milne (1934) were correct and that the liver of the chick at hatching is much

poorer in iron than any other liver so far studied, including that of the chicken at later stages of development.

This finding suggested the possibility that many chicks might be at least on the verge of iron deficiency, so that reliable blood-haemoglobin figures for late chick embryos and young chicks might prove interesting. As such figures did not seem to be readily available, total blood iron was determined in as many chicks as possible in the present series. It was felt that the results would be likely to afford a better estimate of haemoglobin concentration than the usual colorimetric methods, because chick blood contains nucleated erythrocytes and is often quite exceptionally lipaemic. It seemed probable that any error resulting from the presence of traces of non-haemoglobin iron in the plasma or the cells would be very much less than the pronounced errors which may be caused by turbidity in any of the simple dilution methods. The work of Rostorfer (1949), on ducks, suggests that only gasometric analyses (impracticable in this case) would give better results than total iron determinations.

EXPERIMENTAL

Newly hatched chicks from the stock of Brown Leghorn hens maintained by the Poultry Research Centre, Edinburgh, were anaesthetized with ether and bled by cardiac puncture, using an oxalated syringe and needle. The chicks were then killed by a blow on the back of the neck. The livers were removed, taking great care not to permit contamination from the 'spare' yolk, which was carefully removed from the abdomen at the commencement of the dissection. The livers were weighed and glass-homogenized (Potter & Elvehjem, 1936) with 2.5 ml. water.

The blood specimens were analysed for total Fe and the liver homogenates for total and non-haem Fe by the methods previously described (Ramsay, 1951). A volume of 0.03–0.05 ml. blood was used for each analysis, and the high catalase activity of both blood and liver made it necessary to heat all specimens to 100° before adding H_2O_2 . Reduction and colour development with 2,2'-dipyridyl were as described by Ramsay (1951). In all three methods the coefficient of variation (s.d. expressed as a percentage of the mean) was of the order of 3%.

RESULTS

Blood iron. The average iron content of thirteen blood specimens was 35.5 mg./100 ml. (range 31.6–38.0, s.d. 2.0 mg./100 ml.). An isolated value of 44.5 mg./100 ml. was not included in the series. A few analyses were made on blood specimens obtained 19 days (three chicks), 22 days (two chicks) and 23 days (three chicks) after the onset of incubation, but the results all fell within the apparent normal limits for the 21st day (mean $\pm 2 \times$ s.d.). They did not suggest that any pronounced trend in haemoglobin concentration is associated with the hatching process.

Liver iron. The results of the analyses on twenty-one livers at 21 days are summarized in Table 1. The total iron averaged 45.4 μ g./liver (range 32.8–85.4, s.d. 12.5 μ g./liver). The percentage of non-haem iron in the total liver iron was 37–69 (mean 52 %, s.d. 8.7).

Table 1. *Iron in the liver of twenty-one newly hatched chicks*

	Liver wt. (g.)	Total iron		Non-haem iron		
		(μ g./liver)	(mg./100 g.)	(μ g./liver)	(mg./100 g.)	(% of total)
Mean	0.80	45.4	5.7	22.7	2.9	52
Range	0.56–0.94	32.8–85.4	3.8–10.5	14.4–38.4	1.7–4.7	37–69
s.d.	0.108	12.5	1.51	5.9	0.75	8.7

DISCUSSION

Blood iron. The blood-iron figures on these chicks were similar to, but generally slightly higher than, some made on adult birds from the same flock. The danger of direct comparison between birds of such very different ages is real, but it does seem likely that the chicks were not, in fact, seriously deficient in iron.

If it is assumed that the relation between total iron and haemoglobin is the same in the blood of the newly hatched chick as in that of mammals (cf. Rostorfer, 1949), it may be calculated that the observed iron concentrations correspond to haemoglobin concentrations of about 9.5–11.5 g./100 ml. Zorn & Dalton (1937) made haemoglobin determinations on similar material, but their paper unfortunately lacks the technical information which would have made a useful comparison possible. They record striking fluctuations in haemoglobin concentration during and after incubation, but do not state either the method used or the number of analyses on which their conclusions were based.

Liver iron. The total iron, at 45.4 μ g./liver, agrees well with the figure of 41 μ g./liver reported by McFarlane & Milne (1934). Moreover, the two isolated values which they gave for the proportion of non-haem iron in the liver fall within the limits

determined in the present work. The two earlier figures were 52 and 66 %, and the mean for the present work was 52 % (s.d. 8.7 %). It is unfortunate that the present author, in a previous paper (Ramsay, 1950) expressed the view that the analyses of McFarlane & Milne (1934) were erroneously quoted. This view was based on analogy with other species, on a series of calculations in the paper of McFarlane & Milne (1934), which purported to show that during the greater part of incubation and after hatching a substantial proportion of the total iron in the body was concentrated in the liver, and on the fact that these authors failed to notice that the chick liver is actually very poor in iron. The present work emphasizes the last point, shows the analogy to be quite false, and demonstrates that the original analyses of McFarlane & Milne (1934) were correctly reported, while the calculations under discussion were erroneous.

It is interesting to note that the proportion of non-haem iron in the liver, at 52 %, is much lower than the 75 % or over which is commonly found in mammalian livers (Brückmann & Zondek, 1939; McCance & Widdowson, 1940; Scott & McCoy, 1944). It might be thought that this difference was an analytical one, as the chick livers were not washed or perfused to remove blood; they were merely dabbed with filter paper. These livers, however, are rather fatty (20–22 % ether-soluble material; Entenman, Lorenz & Chaikoff, 1940; A. K. Lough, private communication), and contain little blood. Moreover, the proportion of non-haem iron was not less than usual in livers which contained much haem iron (and might therefore have been contaminated with larger volumes of blood). The difference is therefore probably a real one, and would be expected from the knowledge that at this stage the chick liver is not carrying a large store of non-haem iron.

Other chicks analysed at the same time gave figures for the quantity and distribution of iron similar to those reported in the previous paper (Ramsay, 1951). Calculations based on the figures in the present work and on values taken from the graphs given by Ramsay (1951) show that the non-haem iron in the liver amounts to no more than 2.0–2.5 % of the total iron in the body or 6–8 % of the non-haem iron. Recalculation from the analyses of McFarlane & Milne (1934) shows their figures to

be in good agreement with the present ones in this respect also, except that they did not make determinations of non-haem iron in the whole chick. This state of affairs may be in part a reflexion of the small size of the liver, which at hatching amounts to only 2-3 % of the body weight, while according to Hamilton, Boyd & Mossman (1945) the human liver at birth may be 5 % of the body weight. The difference in proportion of functional tissue weight may be very much greater than these figures suggest because, as has already been noted, fully one-fifth of the wet weight of the chick liver consists of ether-soluble material. This particular problem in comparative biochemistry would seem to be an ideal one for attack by the method advocated by Vendrely & Vendrely (1949) and Davidson & Leslie (1950), in which deoxyribonucleic acid determinations are made and the number of cells calculated from the results with the aid of certain fundamental assumptions.

The question arises whether iron normally stored in the liver is displaced in the hatching chick to other sites, or whether a very large proportion of the non-haem iron in the body is to be regarded as belonging to the 'parenchymatous iron' fraction (Hahn & Whipple, 1936) which is not believed to be readily available for haemoglobin formation. Neither the kidney analyses of McFarlane & Milne

(1934) nor a few spleen analyses made in this laboratory suggest that these organs usurp the function of the liver in respect to iron storage. The idea is tempting that the organism does not develop such functions until the 'spare' yolk is exhausted. This does not happen until about a week after the chick is hatched.

The problem remains of the fate of the iron which may be liberated by haemoglobin catabolism towards the end of incubation. Whatever may be the solution, it is certain that iron liberated in this way is not 'stored' in the sense in which that word is usually used in relation to iron metabolism.

SUMMARY

1. Total blood iron in thirteen newly hatched chicks was 35.5 (S.D. 2.0) mg./100 ml.

2. The livers of twenty-one newly hatched chicks contained only 45.4 (S.D. 12.5) μ g. total iron and 22.7 (S.D. 5.9) μ g. non-haem iron.

3. It is concluded that the liver of the newly hatched chick contains no appreciable store of iron.

The author is indebted to the Poultry Research Centre for the supply of eggs, to the Agricultural Research Council for a grant from which the expenses of the work were defrayed, and to Mr G. R. Wilson for technical assistance.

REFERENCES

- Barcroft, Sir Joseph (1946). *Researches in Pre-natal Life*. Oxford University Press.
- Brückmann, G. & Zondek, S. G. (1939). *Biochem. J.* **33**, 1845.
- Chapman, D. G., Maw, W. A. & Common, R. H. (1950). *Sci. Agric.* **30**, 194.
- Davidson, J. N. & Leslie, I. (1950). *Nature, Lond.*, **165**, 49.
- Entenman, C., Lorenz, F. W. & Chaikoff, I. L. (1940). *J. biol. Chem.* **133**, 231.
- Hahn, P. F. & Whipple, G. H. (1936). *Amer. J. med. Sci.* **191**, 24.
- Hamilton, W. J., Boyd, J. D. & Mossman, H. W. (1945). *Human Embryology*. Cambridge: Heffer.
- Hill, R. (1931). *Proc. roy. Soc. B*, **107**, 205.
- McCance, R. A. & Widdowson, E. M. (1940). *Spec. Rep. Ser. med. Res. Coun., Lond.*, no. 235.
- McFarlane, W. D. & Milne, H. I. (1934). *J. biol. Chem.* **107**, 309.
- Potter, V. R. & Elvehjem, C. A. (1936). *J. biol. Chem.* **114**, 495.
- Ramsay, W. N. M. (1950). *Biochem. J.* **46**, 168.
- Ramsay, W. N. M. (1951). *Biochem. J.* **49**, 494.
- Rostorfer, H. H. (1949). *J. biol. Chem.* **180**, 901.
- Scott, E. M. & McCoy, R. H. (1944). *Arch. Biochem.* **5**, 349.
- Vendrely, R. & Vendrely, C. (1949). *Experientia*, **5**, 327.
- Widdowson, E. M. & McCance, R. A. (1948). *Biochem. J.* **42**, 577.
- Zorn, C. M. & Dalton, A. J. (1937). *Amer. J. Physiol.* **119**, 627.