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CHLOROPHYLL AND HÆMOGLOBIN REGENERATION AFTER HÆMORRHAGE

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(*Received January* 24, 1936)

This communication forms part of a series of investigations into the problem of the synthesis of hæmoglobin in the animal body.

Very little is known about the actual chemical path along which the body proceeds in order to elaborate the pigment. The same remarks may be applied regarding the starting materials necessary for the synthesis. Does the body commence with simple amino acids or pyrrols, or must it

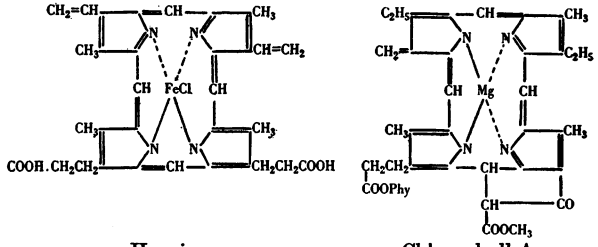

Fig. 1. Chemical structures of hæmin and chlorophyll.

first be supplied with the more complex porphyrins? Moreover, what factors are required for the synthesis of globin and what for the synthesis of the prosthetic grouping?

There seems to be much evidence for the fact that the amino acids required for the globin synthesis are normally present in practically all diets, and so the question of synthesis becomes part of the general question of protein synthesis in the animal body. This question has therefore not been dealt with at present.

The question of the synthesis of hæmoglobin from chlorophyll arises from their closely allied chemical structures shown in Fig. 1, and has

given rise to much controversy. According to some investigators [Buergi, 1916; Saunders, 1925; Hirasaw, 1923] the animal body can bring about the conversion; whereas others [Fischer and Hendschel, 1931, 1932, 1933; Marchlewski and Urbańczyk, 1933] state that the conversion does not take place.

The present communication is an attempt to settle this question.

Methods

Rabbits have been rendered anæmic by repeated hæmorrhages from an ear vein and then allowed to recover. During their recovery period the test animals were fed with chlorophyll dissolved in olive oil, and the controls received equal amounts of this oil.

Two weeks before commencing the bleeding, the rabbits were fed on the standard experimental diet, which was supplied throughout the experiment. This diet consists of a daily ration of 400 g. swedes; the vitamins are supplied in the form of 2 c.c. cod-liver oil, 2 c.c. orange juice, and a teaspoonful of bran and oats; mineral salts (NaCl 32 mg.; Pot. cit. 32 mg.; Pot. iod. 32 mg.; Mag. carb. pond. 32 mg.; Ca lactate, 97 mg.) were administered in admixture with the bran and oats; iron was given in the form of a solution of Ferri. et Ammon. cit. 130 mg. All liquids were administered to the animals by means of pipettes with rubber teats. The animals seemed to thrive on this diet and could be kept alive for many months without any unfavourable symptoms.

The diet has been carefully selected so as to be as poor as possible in porphyrin content. This illustrates the advantage of using herbivorous animals like the rabbit in contrast to the carnivorous dog used by Whipple and his co-workers and other investigators of this problem. The control of the porphyrin content of the diet of dogs is a very difficult matter indeed and is practically impossible.

The animals were bled daily by venesection of an ear vein, quantities of blood varying from 5 to 20 c.c. being removed each time. The hæmorrhages were continued over a period, so as to deplete the spleen and to use up as much liver factor or any storage substance as possible. About fourteen hæmorrhages were considered sufficient, owing to the fact that too much bleeding might produce an aplastic anæmia. The bleeding was controlled so that the animals finally attained approximately the same hæmoglobin levels at about two-fifths of their normal value. The hæmoglobin was estimated by means of the Newcomber plate.

Five series of experiments were performed, three with varying doses of pure chlorophyll, one with a large dose of crude chlorophyll, and one with a magnesium-free chlorophyll derivative.

The pure chlorophyll was obtained from Prof. Stoll of Basle, the other derivatives being obtained from B.D.H.

Experimental results

(1) *Large doses of chlorophyll*

0·33 g. chlorophyll dissolved in 4 c.c. olive oil was administered daily during the recovery period. Four control animals and three tests were used. The average results have been graphed out and are shown in Fig. 2.

This dosage of chlorophyll is therefore ineffective in aiding hæmoglobin recovery.

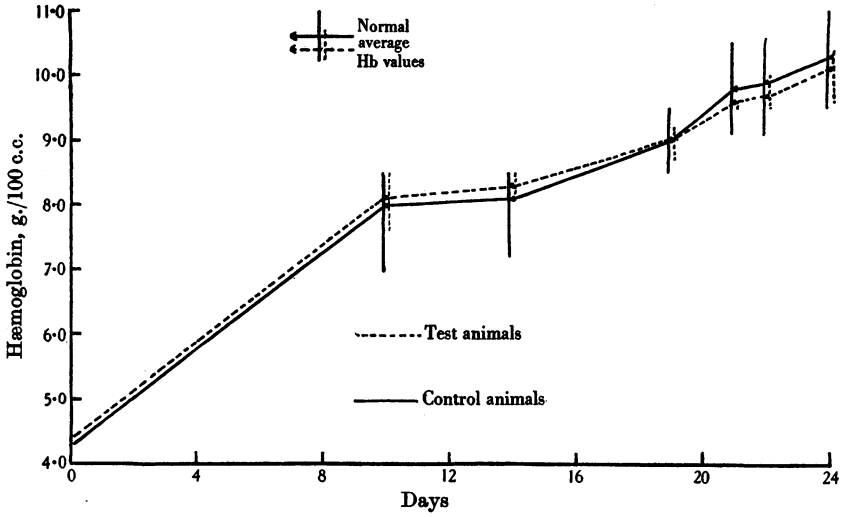

Fig. 2. Administration of large doses (0·33 g.) chlorophyll. The vertical lines, in this and subsequent figures, join the maximum and minimum values for each group.

(2) *Smaller doses of chlorophyll*

0·05 g. chlorophyll dissolved in 2 c.c. olive oil was administered daily. Two controls and two tests were used. The results are shown in Fig. 3.

Although there was not complete recovery in either case after 36 days, the chlorophyll seems to have been exerting some effect after the 14th day.

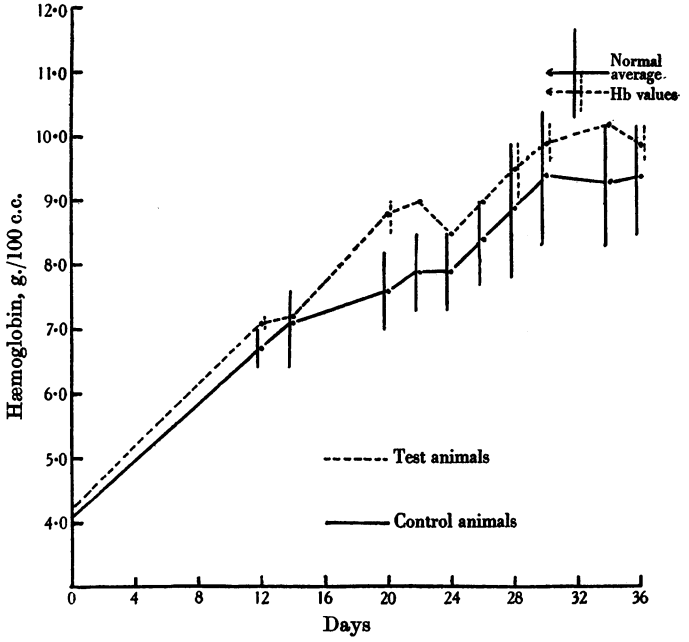

Fig. 3. Administration of smaller doses (0·05 g.) chlorophyll.

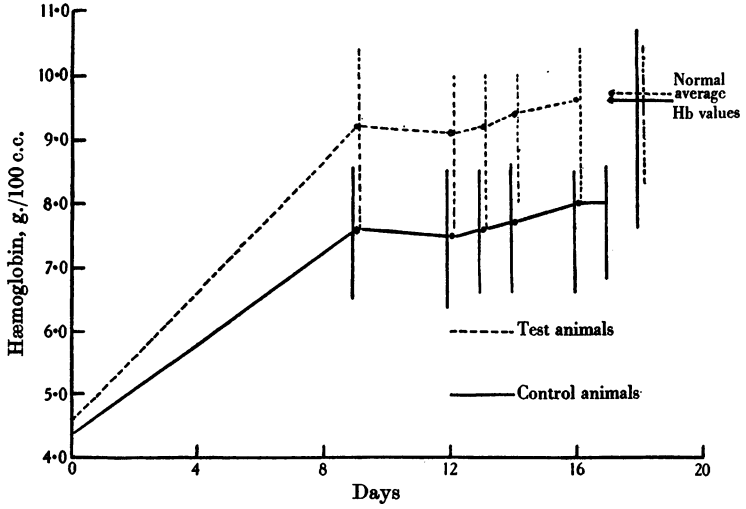

Fig. 4. Administration of very small doses (0·015 g.) chlorophyll.

(3) *Very small doses of chlorophyll*

0·015 g. chlorophyll dissolved in 2 c.c. olive oil was administered daily. Four controls and four tests were used. The results are shown in Fig. 4.

It is quite obvious that these very small doses of chlorophyll exerted a favourable effect on blood regeneration.

(4) *Large doses of crude chlorophyll*

The chlorophyll used was the commercial B.D.H. preparation. 1 g. dissolved in 10 c.c. olive oil was administered daily. Four tests and four controls were used.

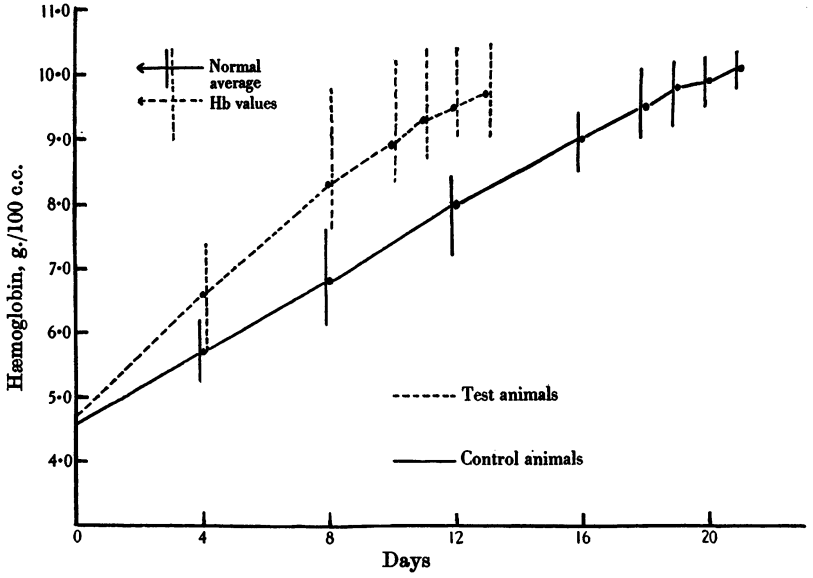

Fig. 5. Administration of large dose of crude chlorophyll.

This experiment, as well as the succeeding one, formed part of the M.D. Thesis of Dr Howell Hughes. The hæmoglobin was estimated with the Dare Hæmoglobinometer and is possibly not so accurate as in the above experiments. Nevertheless, a definite increased rate of regeneration, greater than the probable experimental error, was noted in the test animals (Fig. 5).

(5) *Magnesium-free chlorophyll derivative*

The derivative used was a water soluble chlorophyll supplied by B.D.H. and was shown spectroscopically to be free from magnesium. Seven test animals and four controls were used. Four of the tests received

1 g. daily dissolved in water and administered by mouth. The regeneration was obviously accelerated as shown in Fig. 6. The other three test animals received injections subcutaneously of 0·25 g. dissolved in 5 c.c. of mammalian Ringer. The injections exerted no effect on regeneration (Fig. 6).

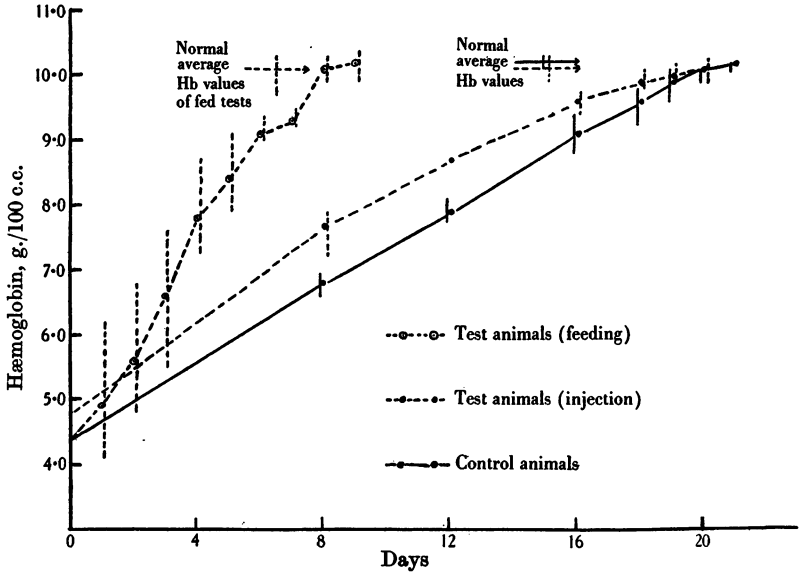

Fig. 6. Administration of magnesium-free chlorophyll derivative.

Discussion

It seems therefore that the animal body is capable of converting chlorophyll to hæmoglobin, if the former is given in small doses. This is in agreement with Zih [1930], who, however, showed the effect of chlorophyll injections on the red blood cell count of animals not rendered anæmic. As the normal count in rabbits shows great variations it is difficult to accept this work as final.

The failure of large doses to produce any effect is very remarkable. It seems that chlorophyll in large doses is toxic to the bone marrow. This may be due to increased absorption of magnesium either in combination with chlorophyll, or brought about in a manner similar to that in which the absorption of iron is increased on administration of bile pigments [Patek and Minot, 1934]. Minot has also demonstrated the increased absorption of iron on administration of chlorophyll. It is true that magnesium is supplied in the mineral salts of the diet, but the above possibility still holds as this element is absorbed only very slowly. The

fact that large doses of the magnesium-free chlorophyll derivative aid regeneration when administered by mouth seems to bear out this suggestion.

The quantity of chlorophyll in the very small doses is far in excess of that required for the increase in hæmoglobin regeneration.

This can be calculated from Fig. 4. According to the graph, the test animals recovered completely over a period of 16 days. By the end of this time, each test animal prepared 1·6 g./100 c.c. more hæmoglobin than each control. The average blood volume in a rabbit is 120 c.c. Therefore the test animals have made 1·9 g. ($1{\cdot}6 \times \frac{120}{100}$) more hæmoglobin than the controls. This excess hæmoglobin must have been prepared from the administered chlorophyll.

The total amount of chlorophyll administered to each rabbit over a period of 16 days was 0·24 g. On chemical reasoning, this amount of chlorophyll is equivalent to approximately 0·15 g. hæmin (hæmin≡3/5 chlorophyll approx.). Since hæmin forms 4 p.c. of the hæmoglobin molecule, this amount of hæmin is equivalent to 3·75 g. hæmoglobin, which is approximately twice the excess hæmoglobin made by the test animals.

Nevertheless, it is a distinct possibility that the chlorophyll is acting as a physiological stimulant of the bone marrow and is not really concerned with the actual chemistry of regeneration of the porphyrin grouping. This possibility is now being investigated.

In the case of the crude chlorophyll, large doses exert a favourable effect on hæmoglobin regeneration. It seems, therefore, that there is some substance in the crude chlorophyll which counteracts the toxic effect of the chlorophyll itself.

These various facts explain the confusion in the literature, as the result obtained depends on the dosage and purity of the sample of chlorophyll used.

It is important to note that the part played by intestinal bacteria might possibly be of some significance. Chlorophyll is known to be broken down by these bacteria, and it is quite likely that it is one of the breakdown products which is used for the subsequent synthesis of hæmoglobin. This possibility is borne out by the failure of subcutaneous injections of the chlorophyll-free derivative to produce any effect.

Summary

1. Chlorophyll in large doses has no effect on the speed of hæmoglobin regeneration after hæmorrhage.

2. Very small doses markedly increase the speed of hæmoglobin regeneration.

3. Crude chlorophyll is effective even in large doses.

4. A magnesium-free chlorophyll derivative also aids regeneration when given in large doses.

5. The possible explanations of these results are discussed.

In conclusion we should like to thank Prof. H. E. Roaf for much helpful criticism and advice.

Part of the expenses of this research were defrayed by a grant to one of us (J. H. H.) from the British Medical Association.

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