XCVIII. THE RÔLE OF TRYPTOPHAN IN BLOOD DEVELOPMENT.

By ROBERT SAXELBY ALCOCK¹.

From the Biochemical Laboratory, Cambridge.

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THE importance of a complete knowledge of the nature of the various types of anaemia makes a settlement of the question of the precursors of the blood pigments desirable; indeed, the work of Fontés and Thivolle [1930] suggests that imperfect assimilation of certain amino-acids is the basis of at least some forms of anaemia.

This work was carried out on rabbits and dogs, in both cases without any previous treatment to reduce the haemoglobin content of the blood. A solution of tryptophan and histidine, in the proportion calculated from their supposed fates in the synthesis of the haematin and globin portions of haemoglobin respectively, was injected into the animals, and the changes in the red blood cell count, and in the haemoglobin percentages, were followed. In all cases there resulted from the injections increases in both figures.

Fontés and Thivolle next experimented to see whether lack of tryptophan in the diet would produce anaemia. Groups of rats were kept on a tryptophan-low diet, and after 10 weeks they were found to be anaemic. Finally these workers injected subcutaneously their tryptophan-histidine mixture into patients suffering from various forms of anaemia. In some cases cures were effected.

Later, Okagawa and Tatsui [1931], showed that animals recovering from anaemia, caused either by haemorrhage or by phenylhydrazine poisoning, recovered more rapidly when given injections of tryptophan. It was also found [Matsuoka and Nakao, 1931] that 2-methyltryptophan is effective in diminishing the time required for recovery from anaemia.

The results obtained by the injection of tryptophan are open to at least two explanations. One is that given by the above workers, namely that the tryptophan exerts its influence by actually forming part of the building material for the new blood. The other is that tryptophan has a stimulating influence on the blood-forming organs without necessarily being used up in the process.

The work of Fontés and Thivolle with rats, however, seems to suggest that the tryptophan does play more the part of building material than stimulator, and it is to be regretted, on this account, that these workers did not control their diets by similar ones to which tryptophan had been added. It seems likely that, had they done so, the results would have appeared less clear-cut, for the diet they were giving was inadequate in many respects.

Before making any study of the tryptophan metabolism of anaemics it seemed desirable to attempt to settle this fundamental question of the rôle of tryptophan with regard to blood formation.

¹ 1851 Senior Student.

Experiments have been carried out to determine whether the lack of tryptophan, and of tryptophan alone, does really produce anaemia, as Fontés and Thivolle state, and whether rats already anaemic can recover from their anaemia while on a diet lacking in tryptophan.

Preliminary experiments were also performed to see whether rats would respond to tryptophan and histidine injections, but it was not possible to show any effect. Rabbits similarly failed to respond.

EXPERIMENTAL.

Tryptophan-free diet.

The diet employed by Fontés and Thivolle in their experiments on rats is unsatisfactory in having gelatin as its sole protein. Rats do not digest gelatin well, and even predigested gelatin supplemented with the missing amino-acids does not give good growth.

Acid-hydrolysed caseinogen as sole protein failed to give satisfactory growth, nor did the animals thrive on a diet of which the protein consisted of zein supplemented with whole or acid-hydrolysed gelatin. The protein which has proved most successful is a mixture of equal parts of acid-hydrolysed caseinogen and zein. The last series of experiments was carried out, however, using only acid-hydrolysed caseinogen, as there is some possibility that all samples of zein contain some tryptophan.

The vitamins were given in the form of three drops of cod-liver oil per rat, and 0.75 cc. of an alcoholic extract of marmite, corresponding to 0.75 g. yeast per rat daily. This supplement provided the rats with less than 1 mg. of tryptophan daily.

After an initial period of rapid fall, there is little loss of weight on the tryptophan-free diet described. The animals get into low bodily condition and are liable to ulcers, but it has been possible to keep them alive for 5 months without tryptophan.

The composition of the tryptophan-free diet was as follows:

	%
Acid-hydrolysed caseinogen (autoclave) ...	9
Zein	9
Rice starch	40
Palm kernel oil	17
Sucrose	20
Salt mixture	5

The dry food was mixed with water to form a stiff paste, and was administered *ad lib*. The animals ate 6-8 g. daily.

The animals used were of a stock having red blood cell counts between 8 and 9 millions per mm.³, and haemoglobin values between 80 and 90 %. Blood was drawn by snipping a small piece off the end of the tail, and as it was expected that weekly or fortnightly readings would be necessary, not all the values were determined at the beginning, and in these cases the above limits may be accepted.

There was a loss of weight of between 20 and 50 % of the original, but this bore no relationship to the blood picture or even to the duration of the experimental period.

The average final values (Table I) are, erythrocyte count, 8.7 mm.; haemoglobin value, 84 %.

Table I.

Rat	Sex	Duration of experiment days	Initial R.B.C. count millions/mm. ³	Final R.B.C. count millions/mm. ³	Final Hb %
9	F	40	9.7	9.4	—
10	M	40	7.8	8.2	—
11	M	40	9.1	9.3	—
30	F	52	—	8.9	80
12	F	60	—	6.6	74
13	F	60	—	10.5	90
8	F	100	8.5	6.8	80
21	F	100	—	9.5	100
22	F	110	—	8.9	82

Of the above animals, only Nos. 8 and 12 were in any way below the normal, and in both cases there had been ulcers on the tail, while the animals were in very poor bodily condition. In any case, the diminution in the haemoglobin figure for rat 12 is in no way comparable with that occurring in milk anaemia.

Otherwise, the blood figures were normal, in direct contrast with the finding of Fontés and Thivolle.

In the second series of experiments, the rats were made anaemic by the technique of Elvehjem and Kemmerer [1931], and 3–4 weeks after weaning the erythrocyte counts lay in the region of 2 millions per mm.³, and the haemoglobin percentage round 30.

Fig. 1. Continuous lines, deficient group 2. Broken lines, control group 2.

Four rats made anaemic in this way were divided into two groups of 2, the first being given the tryptophan-deficient diet without supplement, the latter, the same, with 0.5 % of tryptophan added. The red blood corpuscle counts were taken from the outset and the haemoglobin percentages in the later stages.

The deficient animals failed to put on any weight, and one succumbed at the end of 4 days. The survivor lived 17 days during which the erythrocyte count rose from 2.0 to 6.0 millions. The haemoglobin percentage at this stage was 90.

The animals receiving the supplement gained in weight, and the blood figures were nearly normal at the end of 3 weeks. The results are recorded in Table II.

Table II.

Group	No. of rats	Time in days	Weight		R.B.C. count millions/mm. ³		Haemoglobin %	
			Initial	Final	Initial	Final	Initial	Final
Deficient 1	1	17	52	44	2.0	6.0	—	90
Control 1	2	17	66	96	2.5	5.6	—	90
Deficient 2	5	11	50	40	—	—	32.7	69.2
Control 2	4	11	46	51	—	—	31.7	68.5

The experiment was repeated using 6 rats in the deficient group and 4 in the supplemented. Of the former, 5 survived for a fortnight—long enough for recovery from anaemia. Haemoglobin percentages alone were recorded in this case, and the results are shown in Table II and in Fig. 1. It is clear that the lack of tryptophan in the diet is having little influence upon the recovery from anaemia in these cases, and the very poor bodily condition of the animals almost precludes the possibility of drawing on reserves for the necessary building materials.

DISCUSSION.

The failure of tryptophan-deficient animals to develop anaemia, while not sufficient in itself to prove that blood haematin is not a tryptophan product, is more readily explained if this is the case. The loss of weight over the experimental period is so slight, in the later stages, as to demand an extraordinary economy of endogenous tryptophan if this is indeed the source of pyrrole-nitrogen throughout the months of dietary lack of tryptophan.

The recovery from anaemia of tryptophan-starved animals is difficult to fit in with the idea of the utilisation of this amino-acid in haematin building. The exact parallel in the recovery in the two groups of animals in these experiments makes it almost certain that haematin-pyrrole is not derived from tryptophan.

It remains to explain the results of the other workers. The anaemia developed by the rats used by Fontés and Thivolle may well have been due to deficiency of some other dietary constituent besides tryptophan. The results of work involving injections can be explained on the basis of a stimulating action of tryptophan on the blood-forming organs. Alimentary tryptophan could not be expected to exert such an effect, as excess of this amino-acid in the portal system is converted into kynurenic acid in the liver. If this hormone-like effect is real, it might be expected that compounds similar to tryptophan would exert a similar action. Matsuoka and Nakao [1931] were able to show that 2-methyl-tryptophan, while not able to replace tryptophan in the diet, will nevertheless accelerate the recovery from phenylhydrazine-induced anaemia, when injected.

SUMMARY.

Rats kept on a tryptophan-deficient diet over prolonged periods failed to develop anaemia.

Young rats suffering from milk anaemia recovered from this anaemia independently of the presence of tryptophan in their diet.

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