THE EFFECT OF LOW-PORPHYRIN DIET ON ERYTHROPOIESIS AND HAEMOGLOBIN REGENERATION

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A deficient iron intake has long been known to reduce the amount of haemoglobin in red blood cells of animals, whereas the effect of a diminished porphyrin intake is a matter of controversy. Drehmann [1930] and Patek [1936] both suggest that herbivora such as rabbits and oxen utilize chlorophyll as a source of porphyrin for haemoglobin formation, but Marchlewski & Urbanczyk [1933] deny this. Drehmann maintains that man must ingest large amounts of green stuff in order to absorb very small amounts of chlorophyll. Patek states that the early results of using chlorophyll preparations in both clinical and experimental work are confusing because some workers used unwittingly very small quantities of chlorophyll. Buergi [1932] has put forward the same suggestion.

In view of these differences of opinion it was decided to carry out further experiments to determine whether chlorophyll could be used by herbivora and carnivora for haemoglobin formation.

Rabbits were used as the herbivorous animals because chlorophyll in green stuffs, which forms their chief porphyrin supply, can be excluded from their diet. The effect of the diet alone and the effect of haemorrhage while on the diet were observed. As a contrast to the herbivora cats were used in a later series of experiments.

METHODS

Rabbits were placed on an artificial, low-porphyrin diet after a control period on a stock diet of bran and oats, 200 g. and cabbage 250 g. daily.
The artificial diet consisted of:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>g.</th>
</tr>
</thead>
<tbody>
<tr>
<td>White ashless casein</td>
<td>6-2</td>
</tr>
<tr>
<td>Sucrose</td>
<td>12-4</td>
</tr>
<tr>
<td>Starch</td>
<td>12-4</td>
</tr>
<tr>
<td>Swedes (Rutabaga)</td>
<td>29-0</td>
</tr>
<tr>
<td>Butter</td>
<td>5-0</td>
</tr>
<tr>
<td>White-paper foil</td>
<td>5-0</td>
</tr>
<tr>
<td>Salt mixture</td>
<td>0-16</td>
</tr>
</tbody>
</table>

Total 70-16

The white-paper foil was similar to that used for wrappings in chocolate boxes.

The salt mixture consisted of:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>mg.</th>
</tr>
</thead>
<tbody>
<tr>
<td>NaCl</td>
<td>45</td>
</tr>
<tr>
<td>NaHCO₃</td>
<td>25</td>
</tr>
<tr>
<td>Ca₃(PO₄)₂</td>
<td>45</td>
</tr>
<tr>
<td>KI</td>
<td>25</td>
</tr>
<tr>
<td>MgCO₃</td>
<td>20</td>
</tr>
</tbody>
</table>

Total 160

The constituents of the diet were mixed thoroughly and put through a mincing machine. The diet was stored in a refrigerator and not cooked. Each day a solution of 130 mg. iron ammonium citrate was given by means of a pipette.

The vitamins of the diet were supplemented by giving 20 g. carrots on alternate days to provide vitamin B₁ and B₂ and 4 c.c. orange juice daily to provide vitamin C. As the diet contained a small amount of butter no further supply of vitamins A and D was considered necessary.

The artificial and bran diets were subjected to Fischer's [1926] acetic acid-ether method of extraction for porphyrins. The extracts were examined spectroscopically. No chlorophyll or other porphyrin was found in the artificial diet, but it was present in quantity in the bran diet. Carotene was found in the artificial diet.

The low-porphyrin diet for cats consisted of milk. Rogers [1935] does not describe any porphyrin amongst the milk pigments, nor could any be found by acetic acid-ether extraction. Full cream milk was given, 350 c.c. daily, or more if this was finished before night.

Vitamins were administered in the form of 1-0 c.c. cod-liver oil for A and D, 4-0 c.c. orange juice for C and 100 μg. aneurin in distilled water for B₁, B₂ being present as lactoflavine in milk. The cats also received 7 mg. iron daily in the form of 130 mg. iron ammonium citrate. These solutions were given by means of a calibrated pipette.

Haemoglobin was estimated with a Klett colorimeter using a Newcomer plate as a standard, 20 c.mm. of blood being taken from the central
artery to the ear. In both rabbits and cats arterial blood was found to give less variable results than venous blood. Red blood cells were enumerated by the Thoma haemocytometer, arterial blood again being used. Estimations were made once a week in dietetic experiments until anaemia was observed, then once every 3 days. In the bleeding experiments they were carried out on alternate days.

Blood was removed by venesection from the marginal vein of the rabbit's ear, 10–20 c.c. being taken off daily until the haemoglobin content of the blood was reduced by at least 1/3 of its normal concentration. The cats were lightly anaesthetized with ether, and blood was taken from a femoral vein with aseptic precautions, 30–40 c.c. being removed on alternate days until the haemoglobin level was reduced to approximately 1/2 the normal.

Four series of experiments were performed, two series with the rabbits and two with the cats. Rabbits were placed on the artificial diet and some became anaemic. These were given chlorophyll (see below) to provide porphyrin, and they all recovered. Rabbits which did not develop anaemia on the artificial diet were bled and the rate of recovery of their haemoglobin and red blood cells was studied with and without the aid of chlorophyll.

The cats were treated similarly, but no anaemia occurred when they were placed on a low-porphyrin diet. The rate of regeneration of their haemoglobin and red blood cells after bleeding was noted.

The chlorophyll solution used was water-soluble chlorophyll (British Drug Houses). This substance was found spectroscopically to be a chlorophyll derivative, to contain magnesium and to give a spectrum closely related to the combined spectra of isochlorophyllin \( a \) and \( b \). One c.c. of the solution contained 0·5 g. total solids.

**Results**

*Dietetic experiment with rabbits*

Fourteen rabbits were placed on the artificial diet for 3 months. Five (36 %) became anaemic. The average normal haemoglobin level of 10·5 g. per 100 c.c. fell to 7·1 g. Red blood cells fell from 5,200,000 to 3,650,000 per c.mm. in approximately 100 days. These are all average figures. The majority of the rabbits (64 %) did not become anaemic but remained healthy and maintained their weight.

The anaemic rabbits were given 1·0 c.c. water-soluble chlorophyll. These animals required 28–32 days to recover their haemoglobin and red blood cells (Fig. 1).
PORPHYRINS AND HAEMOGLOBIN FORMATION

Fig. 1. Rabbits on the low-porphyrin diet. The vertical lines in this and subsequent figures show the range of values for each group. — Rabbits remaining normal. --- Rabbits developing anaemia.

Fig. 2. The effect of haemorrhage on rabbits on the low-porphyrin diet. --- Rabbits receiving chlorophyll. ---- Rabbits not receiving chlorophyll.

PH. XCV.
N. F. KIRKMAN

Haemorrhage experiments with rabbits

Rabbits on the artificial diet were bled and given chlorophyll. Control animals on the same diet were also bled and not given chlorophyll. Fig. 2 shows the results of these experiments. The first group of animals took 14 days to recover their haemoglobin and 15 days for their red blood cells. The second group took 23 and 20 days respectively. Rabbits on the stock diet were bled as well; it was found that they took an average of 13 days to recover their haemoglobin and 10 days to recover their red blood cells.

Cat experiments

Five cats were placed on the low-porphyrin diet but no anaemia was observed in any animal unless bled. Several animals were kept for periods of 60–90 days on the diet. They remained in good health with very little variation in their weight and blood constituents.

For the haemorrhage experiments three cats on the low-porphyrin diet were bled and it was found that their red blood cells and haemoglobin took 32 days to recover. One c.c. of water-soluble chlorophyll daily by mouth caused no acceleration in this rate. Cats on the normal stock diet when treated similarly took 14 days to regenerate their haemoglobin and 13 days for their red blood cells (Figs. 3 and 4).

Discussion

From the results of the rabbit experiments it can be seen that withholding porphyrins from their diet caused anaemia in 36 % of the animals. The majority, 64 %, lived and thrived on the same diet without becoming anaemic. The rabbits developing anaemia did so during the summer when white turnips replaced the rutabaga in the diet, as the latter was out of season. The possibility of this change being responsible for the anaemia has been borne in mind, but so far no evidence for it can be found. It was also thought that there might be some seasonal change in the blood picture of rabbits on normal stock diet, but no changes were discovered.

The results indicate that chlorophyll aided blood regeneration in those rabbits in which dietetic anaemia was induced. It was concluded that this supplied the porphyrin deficiency in their diet. Chlorophyll also shortened the recovery period after haemorrhage, when rabbits were placed on the artificial diet.

Now if porphyrin intake were essential for blood formation in rabbits in order to furnish the haem for their haemoglobin no regeneration would have occurred without it and its absence should have produced anaemia
in each case. Porphyrin-lack has thus not the same consistent effect as a deficiency of iron [Myers & Beard, 1931; Whitby & Britton, 1937; Davidson, Fullerton, Howie, Croll, Orr & Godden, 1933].

![Graph](image)

**Fig. 3.** The effect of haemorrhage on the haemoglobin level of cats on a low-porphyrin diet.

- - - Cats on low-porphyrin diet. --- - Cats on low-porphyrin diet + chlorophyll.

.... Cats on stock diet.

![Graph](image)

**Fig. 4.** The effect of haemorrhage on the red blood cells of cats on a low-porphyrin diet.

.... Stock diet. --- Low-porphyrin diet. --- - Low-porphyrin diet + chlorophyll.

The problem is however by no means simple. It can be shown chemically that the quantity of porphyrin required daily is only 10 mg. The average blood volume of a rabbit is 120 c.c. and this contains about
12 g. haemoglobin. Reckoning the average life of a red blood corpuscle to be 30 days, a rabbit must produce $\frac{12}{30} = 0.4$ g. haemoglobin daily. Since haem forms 4% of the haemoglobin molecule, 10 mg. haem is produced each day.

It may be that rabbits have a store of porphyrin on which they can draw for a long time when porphyrins are excluded from their diet. This may account for the fact that only 36% of the rabbits on the artificial diet became anaemic, possibly the porphyrin store of the rest was not exhausted. On the other hand it may be that porphyrins are not stored but formed freshly from pyrroles derived from cyclic amino-acids produced as decomposition products of diet and tissue protein as Rimington [1936] suggests. This would account for the fact that all the rabbits on the artificial diet when bled recovered their normal red blood cells and haemoglobin whether given chlorophyll or not. Extra porphyrin can undoubtedly be utilized as is shown by their accelerated recovery when chlorophyll is given.

As will be seen from the experiments on cats, deprivation of porphyrin only affected the rate of production of haemoglobin after haemorrhage. It may be that a cat in this condition utilizes the porphyrin of its diet, as the animals on a full stock diet regenerated their haemoglobin more quickly. Aetioporphyrin was thought to be the most suitable porphyrin to give, but unfortunately was not available at the time of the experiments. It is hoped to remedy this omission. Chlorophyll was tried but was found to have no effect, which is probably due to the inability of the cat to digest it.

It appears therefore that porphyrin derivatives are useful aids in the synthesis of haemoglobin but are not essential. It seems that haem is synthesized (a) from pyrroles freshly formed in the body from amino-acids, and (b) from preformed pyrroles derived from other sources such as animal tissues and green stuffs. Possibly there is a varying utilization of these two types of pyrroles in different species and in different animals of the same species, but most of an animal's total haemoglobin is synthesized from the former type of pyrrole.

**Summary**

1. Rabbits were fed on a low-porphyrin diet. A minority (36%) became anaemic after 3 months. They regenerated their haemoglobin and red blood cells in 31 days when given chlorophyll. The majority (64%) did not become anaemic on this diet.
2. After haemorrhage, rabbits on the low-porphyrin diet recovered their haemoglobin and red blood cells in 14 days when chlorophyll was given, and in 23 days without chlorophyll. When the rabbits on a stock diet were bled recovery took 13 days.

3. Cats on a low-porphyrin diet were bled. They took 32 days to regain their haemoglobin and red blood cells with the aid of chlorophyll and 33 days without it.

4. These results and their bearing on haemoglobin metabolism are discussed.

5. It is suggested that the rabbit can utilize preformed pyrroles and also manufacture them.

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REFERENCES

Fischer, H. [1926]. Hoppe-Seyl. Z. 155, 120.