Part II.—The effect of yeast on nutritional anæmia in rats

BY

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The literature upon experimental nutritional anæmia has assumed such proportions that a complete review of it is quite outside the scope of this paper; nevertheless there are certain outstanding and historical contributions which must be considered if a discussion of the problems raised in this and the succeeding papers are to be at all intelligible.

In 1889 Bunge found that the amount of iron present in the liver and spleen, and the total amount of iron in the body expressed as a percentage of the body weight, were highest at birth, and thereafter fell progressively until they reached a minimum at the end of the lactation period. He also held that iron was absorbed from the alimentary canal only when in organic combination in the food, and that the presence of an excess of inorganic iron produced an increase in the absorption of food iron. Ten years later his pupil, Abderhalden, produced nutritional anæmia in animals by limiting their diet to milk for a considerable period after the end of the lactation period, and found that the addition of inorganic iron to diet of these animals did not bring about an increase in haæmolobin. His comment on this result was as follows: The mere fact that the addition of iron to nutrient poor in iron does not have any distinct influence on the formation of haæmolobin ... indicates that other building material is wanted as well as the iron. He also proved the inaccuracy of the statement that inorganic iron could not be absorbed from the bowel.

From this time no experimental observations were made which had important bearing on nutritional anæmia until in 1920, Whipple and Robsheit-Robbins of Rochester, New York State, commenced an investigation on the rate of haæmolobin formation in dogs previously rendered anæmic by repeated bleedings and which were given a diet low in iron. These researches led to a renewal of interest in the effect of iron on haæmolobin formation, and in 1925, Hart, Steenbock and their colleagues at the University of Wisconsin started on what has proved to be the most important and extensive work on experimental nutritional anæmia. Amongst other things they found that impure salts of iron cured nutritional anæmia, but that pure iron salts were quite ineffective: further, that to obtain a cure the addition of copper to iron salts was essential. These results have been challenged along two main lines. First, Drabkin and Miller state that the exhibition of certain amino-acids which they claim to be copper-free will cure nutritional anæmia. Secondly, Beard and his co-workers, although agreeing that copper is the most effective curative supplement to iron, state that other metals, nickel, germanium, manganese, arsenic, vanadium, titanium, zinc, rubidium, chromium, selenium, mercury, can also act as curative supplements; and that the addition of pure iron alone to a milk diet will effect a cure. These criticisms have
been answered, apparently satisfactorily, by the Wisconsin workers, for the balance of opinion strongly supports them. Their latest contribution (Elvehjem and Sherman) shows that in the absence of copper, inorganic iron is readily assimilated by rats suffering from nutritional anæmia and stored in the liver and spleen, but that it cannot be used until copper is supplied, when the greater part of the iron in the liver is removed and built into hæmoglobin.

**Preliminary experiments with yeast.**—Some two years ago we decided to try the effect of adding yeast to the diet of black and white rats which had been rendered anæmic by prolonged milk feeding. One gramme of a dried preparation of brewer's yeast (Yestamin, of the English Grains Company) was therefore added to the daily milk ration of each rat, and it was found that thereafter the hæmoglobin rose steadily until a normal value was attained. Further, unlike control rats cured by copper and iron, these rats reproduced within a relatively short time after the anæmia was cured, although at the end of the lactation period (21 days) the progeny was found to be anæmic: in the individual members of the litter the hæmoglobin ranged from 12 to 25 per cent., and some also showed splenomegaly. These results were so surprising that we decided to repeat them, and to attempt to find what curative factor or factors were present in yeast.

The details of these preliminary experiments may now be given. The rats used were rendered anæmic by feeding them on liquid milk ad lib., and when two months old their hæmoglobin ranged from 10 to 45 per cent. These were then divided into four groups, which were fed as follows:

<table>
<thead>
<tr>
<th>Group</th>
<th>Diet.</th>
<th>Supplement (daily)</th>
<th>% Hb. in each member of group.</th>
</tr>
</thead>
<tbody>
<tr>
<td>A</td>
<td>Liquid milk.</td>
<td>1 grm. (about) dried yeast</td>
<td>12, 27, 34</td>
</tr>
<tr>
<td>B</td>
<td>Liquid milk.</td>
<td>Ditto +0·005 mgrm. Cu</td>
<td>10, 24, 34, 39</td>
</tr>
<tr>
<td>C</td>
<td>Liquid milk.</td>
<td>0·5 mgrm. Fe (pure FeCl₃)</td>
<td>25, 42, 45</td>
</tr>
</tbody>
</table>

The only group in which all the rats recovered was the yeast group (Group A), the hæmoglobin of these rats reaching over 80 per cent. in a period of from six to seven weeks. One rat in the pure iron group (Group C) achieved normal hæmoglobin figures, and two died; but all

![Graph I.—Rise of hæmoglobin in Groups A, B and C, on liquid milk with various supplements (see text).](http://adc.bmj.com/)
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the control rats (Group D) died in a few days. Comparisons of the rate of hæmoglobin formation in Groups A, B, and C, are shown in Graph 1.

Waddell, Steenbock and Hart\(^7\) found that growth and reproduction were below normal on a diet of whole milk and copper, although eventually they succeeded in obtaining some litters, very few of which were nursed; also that on milk, copper and iron, reproduction was still subnormal and there was poor rearing of the young. The doe in the Group A, i.e., on a diet of milk and yeast, when about seven months old produced a litter, which she consumed; eventually she had two further litters, the intervals between them, however, being about three months. An account of these two litters, here designated A and B, is appended.

LITTER A.—The first surviving litter was not examined for three weeks after birth. The young rats were apparently normal in activity and growth as far as could be judged without handling them, but when examined on the 21st day they were found to be pale and slightly under normal weight; the average weight being 27.5 grm. against a normal weight at this age of 30 to 35 grm. One rat selected at random from the litter showed hæmoglobin of 15 per cent. only, and on post-mortem examination the organs were found to be anemic and the spleen somewhat enlarged. After removal of the intestinal tract and small portions of the organs for microscopical examination, the iron and copper content of the rat was estimated. The iron content was found to be low but the copper content was very similar to that of a stock rat of the same age. The figures are as follows:—

<table>
<thead>
<tr>
<th>Body weight</th>
<th>Anæmic rat 22·20 grm.</th>
<th>Stock rat 30·80 grm.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Percentage Fe (dried weight)</td>
<td>8·48 mgm.</td>
<td>16·80 mgm.</td>
</tr>
<tr>
<td>Cu</td>
<td>0·82</td>
<td>0·90</td>
</tr>
<tr>
<td>Total Fe per rat</td>
<td>0·66</td>
<td>1·67</td>
</tr>
<tr>
<td>Cu</td>
<td>0·06</td>
<td>0·09</td>
</tr>
</tbody>
</table>

Another rat, which had been noticed to be consuming some of the mother’s diet (yeast and milk), was killed on the 23rd day, and blood was taken from the heart immediately after death. The results of the examination of this blood compared with that of a stock rat of the same age were as follows:—

| Red cells | 3,340,000 | Control 5,625,000 |
| White cells | 14,600 | 6,000 |
| Hæmoglobin | 12 per cent. | 75 per cent. |
| Colour index | 0·2 | 0·67 |

At the same time a complete examination was made of the mother rat’s blood, and this was compared with the blood of a stock rat of the same age, fed on stock diet, two days after weaning her litter of eight. The results were as follows:—

| Red cells | 5,420,000 | Control 7,720,000 |
| Hæmoglobin | 95 per cent. | 98 per cent. |
| Colour index | 0·9 | 0·6 |
| Polymorphonuclears | 63 per cent. | 50 per cent. |
| Lymphocytes | 31 | 45 |
| Transitional | 5 | 2 |
| Eosinophil | 1 | 3 |
The conditions under which the stock rat was chosen make the results strictly comparable and if the details which are given above are consulted it is obvious that the blood in both instances is normal.

One week later for no obvious reason a third member of the litter died, aged 28 days, and in Table 1 are given the weights of the heart,

**TABLE 1.**

**WEIGHT OF ORGANS OF ANÆMIC RAT, WITH CONTROLS.**

<table>
<thead>
<tr>
<th></th>
<th>Anæmic rat Litter A</th>
<th>Normal rat of same age</th>
<th>Normal rat of same weight</th>
</tr>
</thead>
<tbody>
<tr>
<td>Heart</td>
<td>0·48 grm.</td>
<td>0·22 grm.</td>
<td>0·23 grm.</td>
</tr>
<tr>
<td>Liver</td>
<td>2·15 ''</td>
<td>1·98 ''</td>
<td>1·2 ''</td>
</tr>
<tr>
<td>Kidneys (2)</td>
<td>0·66 ''</td>
<td>0·54 ''</td>
<td>0·56 ''</td>
</tr>
<tr>
<td>Spleen</td>
<td>0·82 ''</td>
<td>0·09 ''</td>
<td>0·09 ''</td>
</tr>
<tr>
<td>Body weight</td>
<td>34·14 ''</td>
<td>43 ''</td>
<td>32 ''</td>
</tr>
</tbody>
</table>

liver, kidneys and spleen, and for comparison the weights of these organs in normal rats, fed on stock diet, of the same age and weight. From the results it is obvious that all these organs, and the spleen in particular, show enlargement, an enlargement which is all the more marked when it is considered in relation to the body weight. This rat had been taking yeast and milk for one week before death.

**LITTER B.**—The second surviving litter was born three months after litter A; in view of the results obtained in the former litter this one was watched more carefully and examined earlier. The members of the litter made excellent progress, and, although pale, were very active. One rat was killed when four days old, and blood immediately removed from the heart for examination. The blood picture showed a definite anæmia when compared with average figures from six stock rats of the same age:

Red cells ... ... ... ... 4,060,000 ... Control 3,162,000
White cells ... ... ... ... 8,000 ... '' 4,230
Hæmoglobin ... ... ... ... 52 per cent. ... '' 78 per cent.
Colour index ... ... ... ... 0·65 ... '' 1·2

Some of the organs of this rat were weighed and found to be of much the same weight as those of a stock rat of the same age, but relatively heavier in relation to the body weight. It is obvious, therefore, that the organs of the anæmic rat from litter B were larger than normal.

Another rat of this litter was killed at 21 days, and a pronounced anæmia was still present. The hæmoglobin and colour index were 25 per cent. and 0.3 respectively, as compared with 72 per cent. and 0.8 in controls. The anæmia was not quite so marked as in litter A. In this particular rat the viscera were slightly under normal weight.

The remaining rats of both litters A and B received various forms of treatment for their anæmia (p. 104).
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DOE OF LITTERS A AND B.—It is not possible to give accurate figures of the amount of yeast consumed by this doe in the course of these experiments, because much of it settled at the bottom of the dish of milk during the day and was not eaten. Presumably during her second pregnancy (litter A), which occurred whilst she was still growing, she managed to obtain a sufficient supply of iron to maintain a normal amount of hæmoglobin during growth, although insufficient to supply the foetuses with their full requirements. It is clear that she did not sacrifice her own iron storage to supply the foetuses. There were only two possible sources from which she could obtain iron, her diet and her first litter which she had consumed; the latter, judging from the results obtained in later litters, could not have been a very fertile source of iron. During the third pregnancy (litter B), when she was fully grown, she required less iron for her own maintenance, and the resulting slight excess over her own requirements was available for the foetuses; these, therefore, eventually showed a somewhat higher hæmoglobin than those of litter A.

In a later experiment we attempted to prove the truth of the foregoing explanation by adding dried yeast to the milk diet of a congenitally anaemic rat, together with pure iron, prepared according to the directions given by Steenbock, Waddell and Hart. This diet was given continuously from the time the rat was weaned and throughout pregnancy. The resulting litter did not show anaemia and actually at the end of lactation the young rats had hæmoglobin values which were greater than normal. This experiment, we maintain, bears out the view that yeast in the quantities given, and milk, do not during the periods of pregnancy and lactation contain the amount of iron necessary to produce normal litters.

Further experiments in production of anaemia of the new born in rats.—Since this preliminary experiment we have obtained a number of litters from rats fed on milk and yeast. The hæmoglobin percentages of the members of these litters at 21 days have varied from 12 to 75 with an average of 38; the highest figures may be due to the fact that some of the rats were consuming considerable amounts of their mother’s diet (yeast and milk) before they were 21 days old. The hæmoglobin values of different members of the same litter were found to vary a great deal, just as at times occurs with human twins or triplets. Estimations made on a few rats at the age of four days showed a range of hæmoglobin from 52 to 85 per cent., compared with a range of 65 to 85 per cent. in our stock rats at the same age. After the rats were a week old it was usually quite easy to see that they were definitely pale when compared with stock rats of a like age, and indeed the profound anaemia exhibited by these young rats is remarkable. Scott obtained from does which were fed on white bread and milk from puberty to ten months of age and which ‘showed practically no anaemia themselves’ litters which, when given the same diet, developed a hypochromic anaemia with a tendency
to spontaneous cure as they grew older. In contrast to these results, we have described instances of rats which suffered from nutritional anaemia, were cured by a yeast and milk diet, and then produced young with a pronounced anaemia although they themselves remained normal. Further, as will be shown later on in this paper, this anæmic progeny can be cured by the same diet, or a normal litter can be obtained from their mother by adding 0.5 mgm. of pure iron (as ferric chloride) daily to her diet of milk and yeast.

For the purpose of experimental work on the cure of nutritional anaemia we have made use of many rats bred from milk-and-yeast fed mothers, and we now always reduce their hæmoglobin to a very low value before commencing any experiment. We can reduce the hæmoglobin of such rats to 10 per cent. by feeding on milk only for fourteen days after weaning, whereas rats from parents fed on stock diets require six to eight weeks' milk feeding after weaning to reduce the hæmoglobin to 20 per cent.; indeed in some cases we have been unable to reduce the hæmoglobin below 30 to 40 per cent. after three months of feeding on a diet consisting only of milk. Since these preliminary experiments, we have not made use of rats for testing the curative value of the various methods of treating nutritional anaemia, unless their hæmoglobin had been reduced to less than 30 per cent.

Another interesting thing has been noted in the course of these experiments, namely, that when these piebald rats become anæmic the darkly pigmented hairs lose much of their colour, but that this is restored as the anæmia progresses towards cure, although the rate of progress is not the same in all cases. It has been suggested to us that the anaemia of new-born rats might be due to infection with Bartonella muris; search has therefore been made on several occasions for evidence of infection by this micro-organism but always with negative results.

These results appear to demonstrate quite definitely that the pre-natal storage of iron in the progeny of milk-and-yeast fed rats is deficient even in those cases where the hæmoglobin soon after birth is fairly high.

Curative factor or factors in yeast.

In view of the results of these preliminary experiments attempts have been made to find out what it is in yeast that is responsible for the cure of nutritional anaemia. This is a deficiency anaemia and the deficiency is in one of the substances, iron, copper, Vitamin C, thyroxin, which are necessary for the maturation of the normoblast in the red blood cell. For its cure two processes are essential: (1) the production of new red cells measured by the degree of reticulocytosis and leading to an increase in red cells; a process sometimes referred to as hematopoiesis; (2) the synthesis of hæmoglobin. It is clear that hæmoglobin cannot be put into red cells unless a supply of these cells is available; also that unless material for the synthesis of hæmoglobin is available, cure is impossible.
even if the supply of red cells is ample. It is conceivable that two factors are necessary for these two processes or that the same factor may initiate both; thus, in the case of iron, Vitamin C and yeast we have evidence showing that they are capable of initiating both these processes, whereas copper is of use only in haemoglobin synthesis.

**Composition of yeast.**—Yeast is rich in the Vitamin B complex, contains iron both in inorganic and organic form, copper, manganese, and in brewer’s yeast a trace of arsenic, also two proteins zymocasein and cerevisine. The organic iron in yeast is of considerable interest. According to Warburg respiratory in most living cells is due to a process catalysed by iron, this respiratory enzyme being a haematin compound. Actually more than forty years ago MacMunn of Wolverhampton found that a pigment related to haemoglobin (myohematin), which he believed to be respiratory in function, occurred in the cells of animals. This pigment has been extensively studied by Keilin during the last eight or ten years and has been renamed by him ‘cytochrome.’ He has shown that it is present in plants; that it is widely distributed in the cells of aerobic, but not anaerobic, micro-organisms; and that its highest concentration is found in cells capable of active metabolism, such as the thoracic wing muscles of flying insects, heart muscles of mammals and birds, pectoral muscles of flying birds, yeast and some bacteria, particularly the diphtheria bacillus. Cytochrome exists in two forms: (1) oxidized, from which (2) the reduced form is produced by the reducing action of the tissues in the absence of oxygen. In the reduced form it has a very characteristic absorption spectrum composed of four mains bands a, b, c, d. Cytochrome is not a single compound but is a mixture of three haematin compounds (haemochromogens) \( a^1, b^1, c^1 \), each of which shows two characteristic bands alpha and beta, the alpha bands of each corresponding respectively with the a, b and c bands of cytochrome, whereas the beta bands are the components of the d band of cytochrome. Cytochrome acts in the tissues as an oxygen carrier from which they obtain oxygen by reduction, whilst it spontaneously re-oxidizes by absorbing oxygen from its surroundings, that is, it is autoxidizable. Of the three components of cytochrome it is only the \( b^1 \) component which is autoxidizable; the components \( a^1 \) and \( c^1 \) are not. Keilin has further shown that the cells of aerobic organisms also contain an unbound haematin similar to the protohaematin of haemoglobin. This unbound haematin is also autoxidizable so that two out of the four haematin compounds in the cell are autoxidizable. Iron is therefore present in yeast, in four organic compounds, all of which are closely allied to haemoglobin. Recently Stone and Coulter have obtained from yeast and also from B. phosphorescens and C. diphtheriae by acetic acid-ether extraction haematin and a complex porphyrin which on disintegration yields coproporphyrin and the copper compound of porphyrin.

The proteins of yeast were shown by Thomas to consist of zymocasein a phospho-protein resembling casein, and cerevisine a yeast
albumin. Both these proteins have been broken up into their constituent amino-acids; zymocasein by Löwers and Nowak\(^5\) and cerevisine by Meisenheimer\(^6\) with the following results:

<table>
<thead>
<tr>
<th>Amino-acid.</th>
<th>% of total nitrogen of cerevisine.</th>
<th>% of total nitrogen of zymocasein.</th>
</tr>
</thead>
<tbody>
<tr>
<td>Glycine</td>
<td>10 to 15</td>
<td>7-4</td>
</tr>
<tr>
<td>Alanine</td>
<td>10 to 15</td>
<td>1-7</td>
</tr>
<tr>
<td>Valine</td>
<td>2-5</td>
<td>0</td>
</tr>
<tr>
<td>Leucine</td>
<td>5 to 10</td>
<td>3-2</td>
</tr>
<tr>
<td>Phenylalanine</td>
<td>8</td>
<td>0-9</td>
</tr>
<tr>
<td>Tyrosine</td>
<td>2</td>
<td>2-4</td>
</tr>
<tr>
<td>Cystine</td>
<td>2</td>
<td>0-7</td>
</tr>
<tr>
<td>Proline</td>
<td>2</td>
<td>4-0</td>
</tr>
<tr>
<td>Glutamic acid</td>
<td>3-5</td>
<td>present</td>
</tr>
<tr>
<td>Asparagine</td>
<td>3-5</td>
<td>1-5</td>
</tr>
<tr>
<td>Tryptophane</td>
<td>0-5</td>
<td>1-5</td>
</tr>
<tr>
<td>Histidine</td>
<td>0</td>
<td>3-4</td>
</tr>
<tr>
<td>Lysine</td>
<td>10</td>
<td>11-5</td>
</tr>
<tr>
<td>Arginine</td>
<td>10</td>
<td>8-4</td>
</tr>
<tr>
<td>Purine</td>
<td>12</td>
<td>0-6</td>
</tr>
<tr>
<td>Melanine</td>
<td>8</td>
<td>2-3</td>
</tr>
<tr>
<td>Ammonia</td>
<td>8</td>
<td>9-3</td>
</tr>
</tbody>
</table>

Drabkin and Miller\(^4\) have reported the cure of nutritional anemia in rats by the addition of certain amino-acids to a milk diet, the most effective amino-acids being arginine and glutamic acid and its salts. It is interesting to note that of all the amino-acids of yeast glutamic acid is present in the largest amount and that lysine and arginine follow in that order. In assessing whether or not these acids are of importance in haematopoiesis or haemoglobin-building, it must also be remembered that Elvehjem, Steenbock and Hart\(^17\) have demonstrated that if glutamic acid is properly purified it is completely inactive as a supplement to iron.

We thought at first that the curative action of yeast might be due to its copper and iron content, and therefore we attempted to obtain a preparation of yeast with the lowest possible iron, copper and manganese content.

Anaemic yeast.—A sample of ordinary brewer’s yeast was subcultured several times through wort, agar and peptone until a reasonably pure growth was obtained. From this growth a medium, made up of doubly distilled water containing sucrose and the following salts: potassium chloride, ammonium chloride, disodium hydrogen phosphate, calcium chloride, and magnesium sulphate (B.D.H., A.R. products), was inoculated. In this medium, which was aerated by the continual suction of filtered air through it, growth was very slow, but gradually the yeast was deposited at the bottom of the medium in a very fine white condition. The yeast was subcultured every three or four days and finally, after separating it from the medium by centrifugalization, was washed with doubly distilled water. Yeast so obtained differs from ordinary yeast in
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several ways. Its colour is white and not the normal brownish colour; indeed, as stated by Elvehjem who independently obtained a similar variety of yeast, this yeast may be regarded as itself anaemic and for the purpose of reference in this paper it is called 'anaemic' yeast. Its morphology is also altered and it takes on a mycelial form; indeed some people to whom we have shown this anaemic yeast are doubtful if it really is yeast. We have, however, succeeded in returning it to almost a normal appearance by subculturing five or six times in the same medium described above with the addition of copper and iron (Fig. 1 and 2). Elvehjem has shown that anaemic yeast, prepared by this method, is free from inorganic iron, the total iron content being only one-third to one-half of the normal; that the copper content is exceedingly low; and that the addition of a small amount of iron to the medium accelerates the growth of the yeast and increases the cytochrome content to normal. He also found that the addition of both iron and copper to the medium gave a further increase in cytochrome and caused the production of cytochrome containing a distinctly higher content of the $a'$ hæmochromogen showing that copper had a specific effect on this component.

Cure of anaemia by anaemic yeast.—Tests of this anaemic yeast were carried out on some members of litter A to which we have referred previously. The rats were kept in cages made entirely of glass to prevent

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**Fig. 1.**—Microphotograph of 'anaemic' yeast showing mycelial forms.

**Fig. 2.**—The same yeast after 6 sub-cultures into the same medium + Fe. 0.5 mgrm. and Cu. 0.05 mgrm. per 100 c.cm. (approx.).
the possible fallacy of their obtaining iron or other metal from the material of which the cages were made. In the earlier experiments many of the very young rats became edematous, but when a dried milk powder was substituted for liquid milk the oedema disappeared. Whether or not this was due to the alteration in the type of food cannot be stated because liquid milk has been used in some later experiments without the development of oedema. Distilled water has been given for drinking purposes.

In order to compare the effects of treatment with anaemic yeast with that of other forms of treatment the litters were divided into groups.

**Litter A.**—The anaemic rats of this litter, with haemoglobin reading of 12 to 25 per cent. at weaning, were divided into four groups and fed as follows:

- **Group (A)**: Dry milk powder + 1 grm. dried yeast per rat
- **Group (B)**: Dry milk powder + 1 grm. dried yeast + 0.5 mgrm. pure Fe. per rat
- **Group (C)**: Dry milk powder + about 0.3 grm. anaemic yeast per rat
- **Group (D)**: Dry milk powder + about 0.3 grm. anaemic yeast + 0.5 mgrm. pure Fe. per rat

**Litter B.**—Those in litter B were fed in two groups:

- **Group (E)**: Dry milk powder + liver extract
- **Group (F)**: Dry milk powder + liver extract + 0.5 mgrm. pure Fe. per rat

The progress made by the rats in litter A is shown in Graph II and Table 2, and by those in litter B in Graph III, and on the latter graph.

**Graph II.**—Litter A. Weight and haemoglobin curves of Groups (A) to (D).
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are added for comparison two graphs of the haemoglobin of rats (G) fed on a dry milk powder only. Since the dry milk powder used in this control was richer in iron than the undried milk used in the experiments described above (p. 96) this control was considered necessary to make sure that milk powder did not contain sufficient iron to account for the rise of haemoglobin.

TABLE 2.

BLOOD CHANGES IN LITTER A AFTER 7 AND 10 WEEKS ON VARIOUS DIETS (SEE TEXT).

<table>
<thead>
<tr>
<th>Group</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin per cent.</td>
<td>60</td>
<td>87</td>
<td>65</td>
<td>85</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>6,820,000</td>
<td>7,640,000</td>
<td>6,570,000</td>
<td>8,100,000</td>
</tr>
<tr>
<td>Size and shape</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
<td>normal</td>
</tr>
<tr>
<td>Polychromatophilia</td>
<td>present</td>
<td>very slight</td>
<td>present</td>
<td>slight</td>
</tr>
<tr>
<td>White blood cells</td>
<td>4,800</td>
<td>6,000</td>
<td>5,000</td>
<td>5,400</td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Group</th>
<th>a</th>
<th>b</th>
<th>c</th>
<th>d</th>
</tr>
</thead>
<tbody>
<tr>
<td>Haemoglobin per cent.</td>
<td>86</td>
<td>95</td>
<td>82</td>
<td>92</td>
</tr>
<tr>
<td>Red blood cells</td>
<td>7,080,000</td>
<td>8,100,000</td>
<td>5,250,000</td>
<td>7,210,000</td>
</tr>
<tr>
<td>White blood cells</td>
<td>4,500</td>
<td>6,000</td>
<td>7,000</td>
<td>4,500</td>
</tr>
<tr>
<td>Reticulocytes per cent.</td>
<td>1-5</td>
<td>10</td>
<td>12-15</td>
<td>5</td>
</tr>
</tbody>
</table>

GRAPH III.—Litter B. Haemoglobin curves: with curves (G) of two rats fed with dried milk powder only.

From a consideration of Graphs II and III it is clear that:

1. The addition of anemic yeast to milk leads to a cure of anemia although more slowly than by other methods used in this experiment (Group C).
2. Additional iron (0.5 mgm. per rat per day, not all of which was consumed) permitted a quicker formation of haemoglobin than anemic yeast (Group D).
3. Dry yeast stimulates haemoglobin formation more rapidly than anemic yeast (Group A).
(4) The addition of iron to yestamin increased the rate of hæmoglobin formation (Group B).

(5) Liver extract raises the hæmoglobin quickly and here also the rate is increased by the addition of iron (Groups E and F).

The facts that nutritional anæmia of rats can be cured by adding yestamin to the milk diet, and that it can be cured, though more slowly, by adding anæmic yeast to the milk diet, have now been confirmed on many animals.

The daily ration of each rat fed on milk and yestamin is 10.0 grm. of dried milk and 1.0 grm. of yestamin. The iron in different samples of yestamin varies from 20.5 to 43.1 mgrm. per cent., but 1.0 grm. of the yestamin used in these early experiments contained 0.25 mgrm., an amount which according to Beard and Myers\(^5\) probably represents the minimum daily iron requirement as an addition to a whole-milk diet for the cure of young growing rats suffering from nutritional anæmia.

The amount of copper in yestamin also varies, but within narrower limits. We have found values from 2.45 to 3.13 mgrm. per cent. In these experiments the amount of copper in 1.0 grm. of yestamin was 0.025 mgrm., which according to Beard and Myers is the minimum effective daily dose required as a supplement for 0.5 mgrm. of iron, but the amount of manganese present in this specimen of yestamin was 0.003 mgrm. which is considerably less than the minimum daily supplement (0.1 mgrm.). It is therefore clear that if all the yestamin and milk rations were consumed, which as a matter of fact did not happen, there was just sufficient iron and copper to cure the anæmia; nevertheless this is not the whole explanation because anæmic yeast in which the total iron content was reduced to 0.13 mgrm. per grm., and the daily dose of which was only 0.25 to 0.33 grm., also produced cure. If the whole of the ration of anæmic yeast were eaten the maximum amount of iron consumed in addition to that present in the milk would be only 0.0425 mgrm.

We have already mentioned that according to Elvehjem anæmic yeast is free from inorganic iron, so that if the cure of anæmia by anæmic yeast is due to its iron content it must be due to the hæmatin compounds of which a description has been given. Elvehjem\(^19\) has carried out a series of experiments to determine the effect of hæmatin on nutritional anæmia which showed that, in the absence of copper, hæmatin was as ineffective as pure ferric chloride, but that in the presence of copper it produced a partial cure of the anæmia, the regeneration being neither so rapid nor so complete as that obtained when inorganic iron (ferric chloride) was used as the source of iron. His results also suggested that all iron compounds must be broken down into iron salts before they can be assimilated, which led him to the important conclusion that the limitations of organs like the liver to act as a therapeutic source of iron, especially if the anæmia is a specific iron deficiency, must be borne in mind. The well-known inefficacy of liver preparations as the sole
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treatment in nutritional anæmias is thus explained, and is due to the fact that the hæmatin compounds which form their large organic iron content are not readily broken down in the alimentary canal. It is probable then that the curative effects of anæmic yeast cannot be due, except in part, to its hæmatin content.

By way of contrast to these experiments we have tried the effect of the addition to the milk diet of a combination of iron, copper, and yestamin, and the results produced were astonishing. The amounts added were those used as standards in other experiments, viz.: 0.25 mgrm. of iron, 0.025 mgrm. of copper, and 1.0 grm. of yestamin. The amazing rapidity of the rise of hæmoglobin is shown in Graph IV; and in this graph are also shown the effects of the administration of Ferrofax powder (British Colloids, Ltd.) which contains these three ingredients, and, it will be observed, that this preparation also produces an extraordinarily rapid cure.

Anæmic yeast is somewhat lacking in the Vitamin B complex, particularly in B1. The two does in litter A which recovered on anæmic yeast were mated and continued on this diet. One rat had a litter of five, three being still-born. The two living rats were nursed but were very small and wasted, late in opening their eyes and in developing hair, and altogether very poor specimens. One was killed when 21 days old and blood taken immediately after death showed hæmoglobin 60 per cent., red cells 3,410,000, and colour index 0.9. This was not as low as we expected to find, probably because the rat was so small. (The weights of this rat with controls are shown in Table 3.) The mother's blood on
The same day showed that the haemoglobin was only 70 per cent.; this had fallen 10 or 20 per cent. during either pregnancy or lactation, or both. The surviving rat was fostered by a stock doe and lived about five weeks.

Since this time we have fed other rats on dry milk and anaemic yeast, and out of three litters only a very few individuals survived to nine or ten weeks and of these (at least) two developed severe oedema before death. These results suggest that there is a shortage of Vitamin B in anaemic yeast, but not sufficient to show symptoms until it has been fed through two generations.

The possibility that the milk used in these experiments with anaemic yeast contained sufficient iron for the cure of anaemia has been considered. Wallgren\textsuperscript{20} found that the iron content of milk increased when it was kept in tin vessels; thus, a specimen of cow's milk kept in glass vessels for a period showed an iron content of 0.266 mgrm. per litre, whereas a specimen of the same milk kept for a similar period in a tin vessel contained 0.483 mgrm. per litre. In another experiment milk kept for one week in a glass container contained 0.266 mgrm. and one kept for a week in a tin container 12.25 mgrm. of iron per litre. In his opinion this increase in iron content is chiefly due to the fact that the milk is always somewhat sour so that the lactic and citric acids thus present may attack and dissolve the ferric hydrate, but also there is a possibility of purely mechanical addition of particles of rust. It has been stated by Beard and Myers\textsuperscript{5} that when milk is dried by the roller process its iron content is raised by the absorption of iron from the rollers. We have asked the opinion of Mr. A. R. Jephcott, M.Sc., of the Glaxo Laboratories on this point, and he assures us that the amount of iron in dried milk obtained from the source must be infinitesimal, because after many years of use the rollers do not show any pitting. In our opinion the variation in different samples of milk is of greater importance than the possibility of an increase in the iron content brought about by the drying process. The amount of iron in milk taken from different cows may vary as well

<table>
<thead>
<tr>
<th>Mother's diet</th>
<th>Anæmic rat</th>
<th>Control same age</th>
<th>Control same weight</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Milk and anæmic yeast</td>
<td>Stock diet</td>
<td>Stock diet</td>
</tr>
<tr>
<td>Heart</td>
<td>0.08 grm.</td>
<td>0.18 grm.</td>
<td>0.08 grm.</td>
</tr>
<tr>
<td>Liver</td>
<td>1.45 ''</td>
<td>1.31 ''</td>
<td>0.50 ''</td>
</tr>
<tr>
<td>Kidneys (2)</td>
<td>0.15 ''</td>
<td>0.43 ''</td>
<td>0.18 ''</td>
</tr>
<tr>
<td>Spleen</td>
<td>0.01 ''</td>
<td>0.114 ''</td>
<td>0.05 ''</td>
</tr>
<tr>
<td>Body weight</td>
<td>13.3 ''</td>
<td>32 ''</td>
<td>15.4 ''</td>
</tr>
</tbody>
</table>

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as that in different samples from the same cow; for instance, Wallgren in milk from nine different cows found a variation of from 0.140 to 0.320 mgrm. of iron per litre, and exactly the same variation in different samples taken from the same cow. It is possible that different methods of assay account for some of the differing values in iron found by different workers; using the method of iron estimation described by Hart and Elvehjem our figures for the iron content of undried milk have agreed with theirs, and have been fairly constant, but our results with dried milk have varied. For instance, of three samples of milk dried by the roller process, one being produced in New Zealand and the other two in England, we found that the New Zealand milk and one variety of English milk showed higher iron values than the other English milk. This variation in different localities may explain some of the differences between the findings of Hart and his colleagues and those of some other American investigations.

Anæmic rats continued on a diet of undried milk do not live long. On dried milk powder they live considerably longer because this has a higher iron content and also because being a more concentrated diet proportionately more of it is consumed. Nevertheless these animals die before their hæmoglobin becomes normal. In our experience only two rats have ever attained to a normal hæmoglobin value on a milk diet. According to Elvehjem the explanation of this occurrence is that the copper stores have been insufficiently depleted before starting the cure of the anæmia.

Effect on anæmia of watery extracts of yeast containing vitamin B complex, amino-acids and salts.—The next step in the search for the curative factors in yeast was to determine the effect produced by giving a watery extract of yeast, since it would contain the vitamin B complex, amino-acids and certain salts, but no hematin. We have made use of two different extracts: (1) marmite, and (2) a preparation made for us by the English Grains Co., which was obtained by extracting brewer’s yeast with water and concentrating the extract at a low temperature. For the purpose of experiment 25 grm. of marmite and 25 grm. of yeast extract were each dissolved in 90 c.cm. of water and 5 or 10 c.cm. of the resulting solutions were used for the daily ration of a group of three rats. The amounts actually used were 5 c.cm. of the marmite solution and 5 c.cm. and 10 c.cm. of the yeast extract solution, which meant that each rat in the marmite group had approximately 0.5 grm. of marmite; each rat in the 5 c.cm. yeast-extract group approximately 0.5 grm. of yeast extract; and each rat in the 10 c.cm. yeast-extract group approximately 1.0 grm. of yeast extract. These values are, of course, maximal values because we found it impossible to ensure that all the ration was consumed and equally distributed between the rats, although from the appearance of the graphs it may be assumed that the ration was fairly equally shared.
In assessing results we may assume that a rise of haemoglobin to 80 or 100 per cent. in six to eight weeks is the normal rate of cure. From a consideration of the Graph V, it is clear that the daily addition of 1.0 grm. of yeast extract to the diet of dried milk powder brings about a satisfactory rise in haemoglobin and that the addition of 0.5 grm. of marmite produces almost but not quite as good results. The addition of 0.5 grm. of yeast extract eventually results in a cure, but the time required for cure is much longer than with the larger quantity of yeast extract or 0.5 grm. of marmite and obviously is too small a dose. From these observations it is also apparent that as a haemoglobin builder marmite is more potent than our yeast extract.

The effect of feeding to anaemic rats the residue left after extracting brewer's yeast to make the special yeast extract was also tested. Again cure resulted, but not quite so rapidly as with the yeast extract (Graph V, D).

To determine what part vitamin B plays in the cure, yestamin and yeast extract were ashed to destroy Vitamin B, and the ash of each given to separate groups of rats. The ash given to each rat in each
instance is that obtained from the amounts, namely, 1 grm. of yeast extract or 1 grm. of yestamin, which had proved capable of restoring haemoglobin to normal in six to eight weeks. The ash was dissolved in a slightly acid solution. Actually the yeast extract ashed was from the same sample as that used in the previous experiment; a certain amount of drying had taken place so that the amount of ash given to each rat represents a somewhat larger amount than the extract. The results of feeding these two ashed residues are shown in Graph VI. It will be seen that the yeast extract was rendered rather less potent by ashing, but it cannot be said that ashing yestamin diminished its potency. Curiously enough, the yestamin ash and undried milk was more efficacious than yestamin ash and dried milk powder, a result the opposite to what has usually been found with yestamin itself.

This phase of the problem is now being approached from another aspect. In view of the observation of Wills that vitamin B in the form of marmite was efficacious in curing tropical macrocytic anaemia, and the probable identification by Strauss and Castle of their extrinsic factor as B₂, we have prepared a vitamin B₁ concentrate from baker's yeast by the method of Kinnersley and Peters, and vitamin B₂ from autoclaved alkaline marmite by the usual method. These are being used for feeding experiments with young stock rats who are placed on a vitamin B deficient diet and then divided into groups, some being given B₁ and others B₂, others again a combination of B₁ and B₂. In addition to

![Graph VII. Progress of rats fed on liquid milk and vitamin B₂.](http://adc.bmj.com/)

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**Graph VII.**—Progress of rats fed on liquid milk and vitamin B₂.
estimation of hæmoglobin and cell counts, Price-Jones curves are constructed, but the experiments have not yet been running sufficiently long to show whether $B_2$ has any effect on the size of the red cells. In another group of three rats who have been made anæmic (hemoglobin 10 to 20 per cent.) an arbitrary dose of $B_2$ prepared from liver extract by Guha's method has been added to the milk diet; in each case a high red cell count has resulted (11 to 14 millions) and the hæmoglobin has risen very slowly to nearly normal level (Graph VII).

Discussion.

From the evidence put forward it is clear that yeast has a curative effect on the nutritional anæmia of rats and allows reproduction to occur. The effect of a diet on growth and particularly on reproduction furnishes a much more stringent test of its completeness than its curative effect on nutritional anæmia, and judged by this standard yeast is a more effective addition to a milk diet than copper and iron, but it is not a complete supplement to the diet because the offspring are anæmic. The addition of yeast and iron to milk does, however, appear completely to rectify the diet in that reproduction occurs and the litter shows a hæmoglobin greater than normal. These examples of anæmia in the new-born rat

![Graph VII](image-url)

Fig. 3.—Spectrogram of various yeast preparations by H. Ramage, shewing intensity of lines as compared with standard solutions:—

1. 0.02 c.cm. standard solution.  
2. 0.05 yestamine.  
3. 0.1 yeast extract.  
4. 0.05 grm. marmite.  
5. 0.05 grm. yeast residue.  
6. 0.05 grm. yeast extract.  
7. 0.02 c.cm. standard solution.  
8. 0.02 grm. yeast extract.  
9. 0.02 grm. yeast extract.

The standard solutions used have the following percentage composition:—

Na. 0.15, K. 0.20, Ca. 0.02, Li. 0.004, Rb. 0.004, Mg. and Fe. 0.05, Cu. and Co. 0.005, Mn. and Ni. 0.002, Cd. 0.10, P. 0.50, Sr. 0.002, Ag. 0.0025 and Pb. 0.05.
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appear to us to be strictly comparable to nutritional anaemia of the new-born infant, because both show the characteristics of a nutritional anaemia and can be cured by the same therapeutic method as other forms of nutritional anaemia. Such conclusions seem to be clearly cut and beyond criticism, but when we strive to draw deductions from the experiments designed to discover the curative factors in yeast it is obvious that there are many difficulties.

The part played by iron and copper in these experiments is not easy to assess. We have used the method of Hart and Elvehjem for estimating iron and our results with regard to liquid milk and some samples of dried milk have been consistent with theirs. This method has, however, given values for iron, particularly in marmite and yeast extract, which are completely at variance with spectrographic estimations very kindly carried out for us by Dr. Hugh Ramage. Fig. 3 is a spectrogram of 0.05 grm. of the various preparations we have used, compared with three strengths of the ‘standard solution.’ The constitution of the ‘standard solution’ is indicated in the legend of Fig. 3, and the mineral content of the various preparations as estimated by Dr. Ramage are given in Table 4, where they are compared with the figures obtained by us by microchemical methods (Hart and Elvehjem’s method for iron).

**TABLE 4.**

**Comparison of estimation of metals in various yeast products by spectrographic and micro-chemical methods, parts per cent. in dry material.**

<table>
<thead>
<tr>
<th>Method</th>
<th>Spectrographic</th>
<th>Micro-chemical</th>
</tr>
</thead>
<tbody>
<tr>
<td>Marmite ...</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Yeast extract</td>
<td>high</td>
<td>high</td>
</tr>
<tr>
<td>Yeast residue</td>
<td>0.60</td>
<td>0.60</td>
</tr>
<tr>
<td>Yestamin ...</td>
<td>0.60</td>
<td>1.15</td>
</tr>
<tr>
<td>Anæmic yeast</td>
<td>trace</td>
<td>1.18</td>
</tr>
</tbody>
</table>

1 Another examination showed 'trace.'

Elvehjem states that anæmic yeast is poor in copper, and is free from inorganic iron, and that its total iron content is one-third to one-half that of ordinary yeast. Our estimates of the iron content of anæmic yeast agree with Elvehjem, and the figures quoted above show that the spectrogram is in agreement, but the spectrogram shows an increase of copper. Two points must, however, be considered. First, that the dose of anæmic yeast given was never more than one-third the amount of yestamin; this would make the actual amount of copper consumed less than in yestamin. Secondly, that the amount of copper present in the specimen of yestamin used in our ‘preliminary experiments with yeast,’
was the minimum effective dose required as a supplement to 0.5 mgrm. of iron and that the amount of iron present was the minimal daily iron required as an addition to a whole milk diet in the growing rat (Beard and Myers'). The assumption, therefore, that the cure of anæmia by anaemic yeast cannot be regarded as due solely to copper and iron is, we submit, abundantly justified.

With regard to marmite and the yeast extract the spectrogram and quantitative estimations are at variance. It is, of course, obvious that different specimens vary considerably in the mineral content, as we have seen yestamin itself does. As far as copper is concerned it is clear that both the extracts and especially the yeast extract are rich in copper when compared with the other preparations, and this is borne out by the quantitative estimations. The spectrogram, however, shows they are both free from iron, whereas quantitatively we have found that they have an amount of iron as great or greater than that in yestamin, and the actual daily amounts of iron available for each rat of which the progress is shown on Graph V were as follows:—

0.16 mgrm. of iron in rats receiving 0.5 grm. marmite.

0.247 mgrm. " " " " 1.0 grm. yeast extract.

0.124 mgrm. " " " " 0.5 grm. " " "

Whatever is the explanation of the discrepancy between the quantitative method of Hart and Elvehjem and the spectrogram, one thing is quite clear, namely, that the effective cure of the anæmia produced by 0.5 grm. of marmite and 0.5 grm. of yeast extract cannot be due, at any rate entirely, to the presence of iron because, to repeat, it is less than the minimal daily amount of iron (0.25 mgrm.) required as an addition to a milk diet to cure anæmia in the growing rat. The effective possibilities in these two extracts would therefore appear to be amino-acids, copper and vitamin B, and as the copper is ineffective except as a supplement to an adequate supply of iron it would appear that the cure is largely due to amino-acids or vitamin B, or both.

The residue remaining after the special yeast extract had been made is also comparatively rich in copper and according to the spectrogram contains more iron than yestamin, an observation which is borne out by quantitative estimations. The important difference between this preparation and the yeast extract and yestamin itself is probably that it is much poorer in vitamin B, and this may account for the fact that it appeared slightly less efficacious.

The results of ashing the extracts in an attempt to destroy vitamin B complex, and the few experiments we have so far carried out of feeding B₂ to anæmic rats, point to the conclusion that the B complex has some influence on hæmatopoiesis but that unaided it cannot be regarded as an effective curative agent. We have also quoted results which support the suggestion that the B complex is low in anaemic yeast and probably has little to do with its curative effect. The similarity to hæmoglobin of cytochrome which is composed of hæmatin compounds and the unbound
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hæmatin of the yeast cell naturally raises the hope that herein might be found an explanation of the action of yeast. This possibility has really been discussed under the heading of iron as actually these hæmatin compounds must be the source of iron in anæmic yeast if that material is free from inorganic iron. Moreover, as we have already stated, Elvehjem has produced definite evidence that hæmatin in the presence of copper promotes a partial cure of anæmia in the rat, but that recovery is neither so rapid nor so complete as when ferric chloride is used as the source of the iron.

There remain for discussion the amino-acids. We regret that we have no direct observations to offer as to their value except the action of marmite. Fontès and Thivolle found that the subcutaneous injection of tryptophane and histidine, or better still, of both these amino-acids, led to an increase in hæmoglobin and red cells in both dogs and rabbits. Drabkin and Miller⁴ produce the cure of nutritional anæmia in rats by the addition to milk of pure amino-acids which were copper free and contained only a trace of iron, and that they found the most effective were arginine and glutamic acid. It is interesting to note from the table of amino-acids in yeast given above that glutamic acid and arginine are present in large quantities, and that tryptophane and histidine do not form a large proportion. It will be remembered that Elvehjem repeated some of the experiments carried out by Drabkin and Miller, and was unable to confirm their results when the glutamic acid used was perfectly pure, and at the moment of writing this view of the ineffectiveness of amino-acids is usually accepted.

We have not achieved a complete answer to the question we have set ourselves. At present the partial answers we have been able to give appear like pieces in a jigsaw puzzle, some of which it has been possible to piece together, perhaps inaccurately, but the complete picture has so far eluded us. It is not improbable that all the factors, copper, inorganic iron, organic iron (cytochrome and hæmatin), vitamin B and possibly the amino-acids, are concerned in the cure and that the beneficial results of yeast are due to the summation of all of these. Of these factors it is probable that copper and iron are the most important. It is interesting to note, as will be pointed out in another paper, that yeast is nothing like so efficacious in the treatment of nutritional anæmia of infants as it is in rats, a fact for which we cannot advance any really adequate explanation. It does sometimes produce a slight reticulocytosis in infants and in two instances as an addition to iron produced a cure when iron alone had failed. This result may have been due to the copper of the yeast or, perhaps more likely, to all the factors mentioned above.

Summary.

Yeast has a curative effect on nutritional anæmia of the rat; animals so cured are capable of reproduction and the progeny show nutritional anæmia shortly after birth. Details are given of experiments planned to
determine what are the curative factors in yeast and evidence is brought forward showing that the beneficial effects are due in part to iron (organic and inorganic), copper, vitamin B complex, and possibly to amino-acids.

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