CLXXXI. THE RÔLE OF CERTAIN DIETARY FACTORS IN THE FORMATION OF ERYTHROCYTES.

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Our knowledge about the precursors and catalysts involved in the production of red blood cells is very meagre. The most important advances in this field during recent years have been the recognition of copper as an important element concerned in the formation of haemoglobin [Hart *et al.* 1928] and of the principles present in liver, which are curative of pernicious anaemia [Minot and Murphy, 1926] which are now considered to be a peptide composed of β-hydroxyglutamic acid and l-γ-hydroxyproline [Dakin, West and Howe, 1931] and the more recently discovered tribasic acid of the composition $C_{11}H_{15}O_7N$, H_2O which gives reactions of the pyrrole group [Dakin and West, 1931]. The observation that a commercial liver extract (Eli Lilly and Co., No. 343) was a very potent source of vitamin B_2 and cured symptoms of depilation in rats [Guha, 1931, 1, 2] and was at the same time a product highly effective in pernicious anaemia raised the question of a possible relationship between the two factors concerned. While the possibility of a chemical identity was excluded on several grounds [Guha, 1931, 2, 3], it was considered of interest to investigate whether vitamin B_2 had a similar haematopoietic effect. The recent observations of Bliss [1930] and of Bliss and Thomason [1931] on the relation of pellagra to the blood picture, lent further point to this question. The rôle of vitamin B_2 in the economy of the organism is at present not understood. Hassan and Drummond [1927] observed that a high protein diet required a larger allowance of the relatively heat-stable component of marmite, which was presumably vitamin B_2, for the good growth of rats. Kon [1929] observed an increase in the urinary C/N ratio in vitamin B_2 deficiency. But no conclusive evidence was obtained by Kon [1931] to show that vitamin B_2 has a specific influence on nitrogen metabolism. Leader's work [Leader, 1930], on the other hand, tends to show that sugar in the diet has a predisposing effect towards the production of experimental pellagra. In fact, although a considerable number of attempts have been made to correlate one or other of the vitamins with processes of general metabolism, such studies cannot be said to have yielded so far any significant results. It

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was desirable, therefore, to attempt to explore the mode of action of vitamin B_2 in a different direction.

While this work was in progress, Sure, Kik and Smith [1931] published a note, in which they reported a diminution in haemoglobin concentration and in the red blood cell count in vitamin B_2 deficiency, when it was accompanied by skin lesions. This type of anaemia was not observed in deficiency of vitamins A, D or E and it was considered that the secondary anaemias sometimes observed in keratomalacia in man were probably due to secondary disorders and not specifically to vitamin A deficiency [Sure, Kik and Walker, 1929, 2; Sure, Kik and Walker, 1931; Sure and Kik, 1931]. Sure, Kik and Walker [1929, 1] had, however, formerly observed fluctuations in the concentration of erythrocytes and haemoglobin in rats on diets deficient in the vitamin B complex. These results appear to indicate a certain degree of specificity in the diminution of the erythrocyte concentration in vitamin B_2 deficiency.

The effect of minute amounts of copper in nutritional anaemia has been shown by Hart *et al.* [1928]. While, however, these authors found no evidence for the existence of an organic substance necessary for haemoglobin regeneration in rats on a whole milk diet, Drabkin and Miller [1931] have recently reported the efficacy of certain amino-acids, glutamic acid and arginine in particular, in cases of nutritional anaemia in the rat. In view of these statements and of the suggestion of Bliss [1930] that pellagra might be due to simple iron deficiency, the combined effect of copper, iron and glutamic acid on vitamin B_2-deficient rats has been studied. The effect of vitamin B_1 deficiency has also been studied for purposes of comparison.

It has been recorded before [Guha, 1931, 2] that an aqueous extract of liver suffers a great loss in vitamin B_2 on being autoclaved in an alkaline medium. As one of us (L. W. M.) has found, in course of an investigation shortly to be published on the rôle of liver in normal nutrition, that the feeding of fresh liver produces a marked increase in the red cell count of normal rats, it was of interest to enquire whether the feeding of an aqueous liver extract, in which vitamin B_2 was destroyed by autoclaving, was capable of raising the erythrocyte count of vitamin B_2-deficient animals. The results obtained indicate the existence of a heat-stable factor in liver, which has a most pronounced effect on the formation of red blood cells. That it is probably different from the factor responsible for the cure of pernicious anaemia appears from considerations, which will be discussed later.

The present work, which was begun to investigate the relation, if any, between vitamin B_2 and the erythrocyte count, has thus been extended to cover the following points.

1. The effect of vitamin B_2 deficiency, followed by administration of vitamin B_2, on the red cell count.

2. The influence of copper, iron and glutamic acid, if any, on the erythrocyte count in vitamin B_2 deficiency.

3. The question of the iron intake in vitamin B_2 deficiency.
4. Other dietary factors concerned in the production of erythrocytes.
5. The effect of vitamin B_1 deficiency on the red cell count.

Experimental.

Both albino and piebald rats, raised in this laboratory, were used. Blood for red cell counts was taken after clipping the end of the tail, and counts were taken with a Bürker haemocytometer. Haldane's method was used for estimations of haemoglobin, but as enough blood was not always obtainable for accurate estimations, these were carried out only in a small number of cases. Whether the haemoglobin concentration goes hand in hand with the red cell count or not, will, therefore, have to be settled by further work, which is in progress.

The following basal diets were fed in these experiments.

Diet 16	%	Diet 16 (*a*)	%
Rice starch	65	Rice starch	40
Palm kernel oil	10	Cane sugar	17
"Light white casein"	21	"Light white casein"	23
Salt mixture (Osborne and Mendel)	4	Palm kernel oil	15
		Salt mixture (Osborne and Mendel)	5

Diet 16 (*b*) was made up by incorporating 12 g. dried yeast per 100 g. of diet 16.
Diet 16 (*c*) was made up by incorporating 10 g. dried yeast per 100 g. of diet 16 (*a*).

All the animals received 2 drops of cod-liver oil daily. All other supplements were also fed separately from the basal diet unless otherwise stated. The vitamin B_1 preparation was obtained by the extraction of brewer's top yeast with boiling water and precipitation with neutral lead acetate, followed by adsorption with and elution from fuller's earth [Guha, 1931, 4]. Such a preparation provides an excellent source of vitamin B_1, practically free from vitamin B_2 [Guha, 1931, 2]. The vitamin B_2 preparation was obtained in the usual way by autoclaving marmite with baryta at p_H 9 for $1\frac{1}{2}$ hours at 18 lbs. pressure. Such a preparation, while it is a rich source of vitamin B_2, contains negligible quantities of vitamin B_1 [Guha, 1931, 2, 4]. The aqueous liver extract, free from vitamin B_2 was obtained by a modification of the method described before [Guha, 1931, 2]. Fresh ox-liver (100 g.) was finely minced, suspended in distilled water (300 cc.), brought to p_H 5 with dilute HCl, and boiled up for 5 minutes. The filtrate was brought to p_H 9 with dilute NaOH and autoclaved for $1\frac{1}{2}$–3 hours at 18 lbs. pressure. The resulting preparation (3 cc. = 1 g. of fresh liver) was almost entirely devoid of vitamin B_2 (Fig. 1).

The copper sulphate, ferric chloride and glutamic acid were administered in daily doses of 0·1 cc., 0·5 cc. and 1 cc. of aqueous solutions containing respectively 0·05 mg. copper sulphate, 0·5 mg. ferric chloride and 50 mg. glutamic acid.

The figures for the red blood cell count are given throughout in millions per mm.^{3} The leucocyte counts of a few vitamin B_2-deficient rats were compared

with those of normal animals but no marked differences were observed [see also Sure, Kik and Smith, 1931].

A. In these experiments animals, which had been previously undergoing partial vitamin B_2 deficiency, were subjected to relatively short periods of complete vitamin B_2 deficiency (3–4 weeks) and then their red cell counts

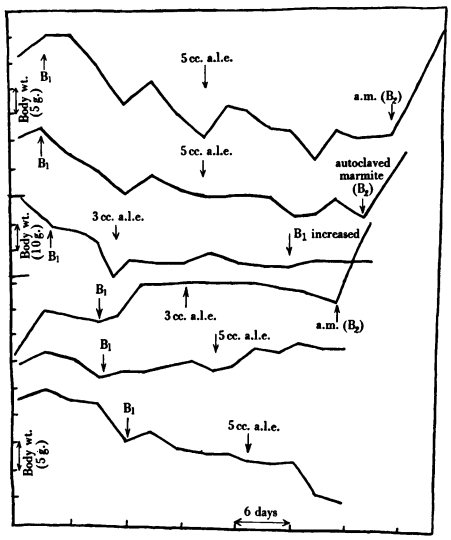

Fig. 1. Curves illustrating the ineffectiveness of autoclaved liver extract to supply vitamin B_2.
a.l.e. = autoclaved liver extract. a.m. = autoclaved marmite.

were taken. They were receiving throughout 1 cc. of the vitamin B_1 preparation, which was found from separate experiments to be ample. It was considered that the physiological rôle of vitamin B_2 should be more apparent under these circumstances than under drastic deprivation of vitamin B_2, as in this latter case secondary complications might mask the primary effect. The results are given in Table I.

Table I.

Rat No.	Colour	Sex	Age (weeks)	Period of complete vitamin B_2 deficiency (days)	Body wt. at the time of taking R.B.C. (g.)	R.B.C.	Average R.B.C.
106	Black and white	♀	25	25	129	8·0	
107	,,	♂	25	30	117	7·7	
108	,,	♂	25	30	129	7·8	
109	,,	♂	25	30	125	7·0	7·78
155	Albino	♀	20	30	105	7·9	
156	,,	♀	20	25	92	8·3	
157	,,	♀	20	30	112	7·5	
160	,,	♀	20	25	109	8·0	

The figures for normal rats, subsisting on milk, brown bread and whole wheat, of approximately the same age were 9·2, 9·2, 9·2 and 9·4, giving an

average of 9·25 millions/mm.^{3} The reduction in the red cell count in vitamin B_2 deficiency was thus relatively small but distinct, although symptoms of depilation or dermatitis had not yet supervened in these animals.

B. In these experiments, the effect of copper, iron, glutamic acid and autoclaved marmite on vitamin B_2-deficient animals was investigated. The vitamin B_1 preparation was fed in 1 cc. daily doses to all the rats. Copper sulphate, ferric chloride and glutamic acid, whenever fed, were administered in daily doses of 0·05 mg., 0·5 mg. and 50 mg. respectively. These three substances gave negative results. The effect of autoclaved marmite, in a daily dose equivalent to 0·5 g. of marmite, is shown in Fig. 2.

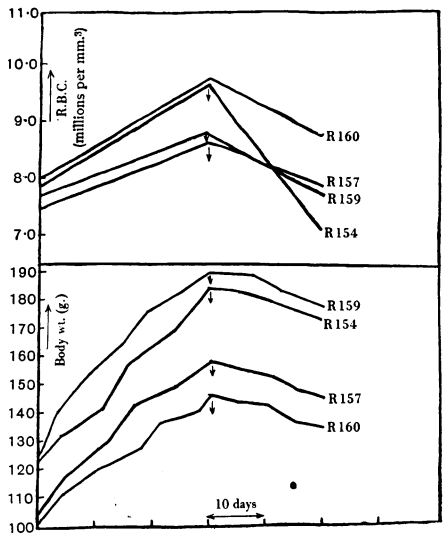

Fig. 2. Fuller's earth extract (B_1) *plus* autoclaved marmite (B_2) from beginning of exp.; ↓ autoclaved marmite withdrawn.

These results show that the diminution of the red cell count occurring in vitamin B_2 deficiency can be corrected by alkaline autoclaved marmite but not by copper, glutamic acid and additional iron.

In order to test the view of Bliss [1930], who considers vitamin B_2 deficiency as simply iron deficiency, the food intake records of the above animals were kept for 10 days, although the fact that rats Nos. 106, 155 and 156, which were receiving extra iron separately from the basal diet, showed no gain in weight, would by itself indicate that iron cannot replace vitamin B_2 for the growth of rats. The figures for the food consumption are given in Table II.

While these figures show that there is a distinct reduction in food intake in vitamin B_2 deficiency, it appears that 8·2 g. of the basal diet still carry enough iron to cover the iron requirements of the animals, as the salt mixture contained 3·2 % ferric citrate. The ineffectiveness of additional iron in the absence of vitamin B_2 also appears to confirm this view. In order to test this point further, rats Nos. 157 and 160, which were receiving autoclaved marmite,

were given restricted amounts of food (7 g. daily) for 10 days before the final erythrocyte count was taken, but in spite of this artificially lowered food consumption, they gave normal figures, which indicates that even 7 g. of the food provided a reasonable amount of iron. The vitamin B_2-deficient rats were normally consuming 8·2 g. of food daily on the average but still gave lower red cell counts.

Table II.

Rat No.	Daily food intakes for 10 days (g.)										Average daily food intake (g.)	
107	8	8	9	8	7	7	10	9	8	12	8·6	
108	6	6	9	7	7	7	10	6	10	9	7·7	8·2
109	10	8	8	8	8	9	8	8	7	9	8·3	
106	11	7	5	4	6	9	10	8	6	7	7·3	
155	4	5	6	6	8	7	8	7	6	6	6·3	6·3
156	3	5	3	6	5	6	6	7	6	6	5·3	
154	10	9	10	11	11	12	14	14	11	13	11·4	
159	7	10	11	13	11	10	12	12	12	13	11·1	10·9
157	11	10	9	11	11	11	11	12	9	11	10·6	
160	6	10	10	10	12	11	14	11	11	11	10·6	

C. The supplements administered to the animals in the above experiments were then interchanged in some cases and three of the animals were given a daily dose of 2 cc. of the autoclaved liver extract each—a preparation practically free from vitamin B_2 (Figs. 3 and 4). The striking effect of this

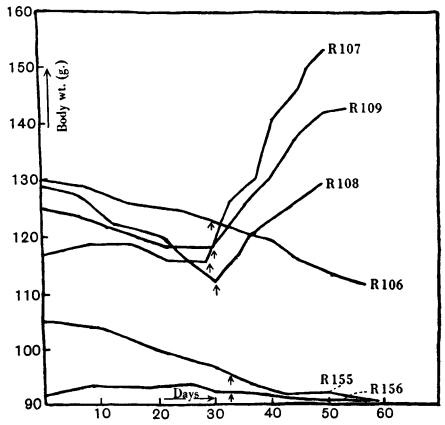

Fig. 3.

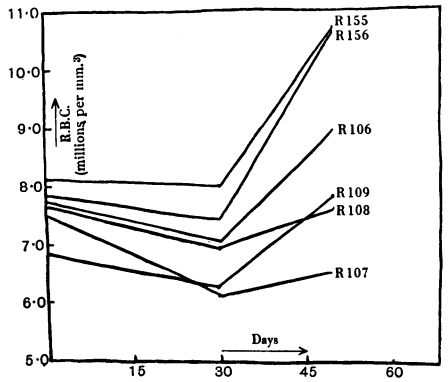

Fig. 4.

Fig. 3. R 107, R 108 and R 109, fed diet 16 + B_1, from beginning of exp.; ↑ 1 cc. autoclaved marmite in addition. R 106, R 155 and R 156, fed diet 16 + B_1 + Cu + Fe + glutamic acid from beginning of exp.; ↑ 2 cc. autoclaved liver extract in addition.

Fig. 4. Figures for red cell counts, corresponding to growth-curves shown in Fig. 3.

extract on the blood picture, in spite of the arrest of growth due to the absence of vitamin B_2 from the diet, is evident from the figures given in Table III.

It will be observed that the administration and withdrawal of autoclaved marmite were followed by a perceptible rise and fall in the red cell count. It is, however, important to notice that autoclaved liver extract, which does

not contain vitamin B_2, produces a much more marked effect than autoclaved marmite.

Table III.

Rat No.	Nature of diet	Period of feeding on diet (days)	Initial wt. (g.)	Final wt. (g.)	Initial R.B.C.	Average initial R.B.C.	Final R.B.C.	Average final R.B.C.
107	$16+B_1+B_2$	20	119	152	6·3		6·6	
108	,,	20	111	128	7·1	6·6	7·8	7·5
109	,,	20	118	141	6·4		8·0	
106	$16+B_1$ + autoclaved liver extract	20	122	113	7·2		9·1	
155	,,	20	92	92	7·6	7·7	10·9	10·3
156	,,	20	98	92	8·2		10·8	
154	$16+B_1$	20	191	181	9·8		7·1	
159	,,	20	196	186	8·8	9·3	7·7	7·6
157	,,	20	166	153	8·7		7·9	
160	,,	20	154	143	9·8		7·8	

D. Somewhat similar experiments were carried out with a group of young animals in order to compare the effects produced by (*a*) autoclaved liver extract alone, (*b*) autoclaved liver extract *plus* autoclaved marmite, and

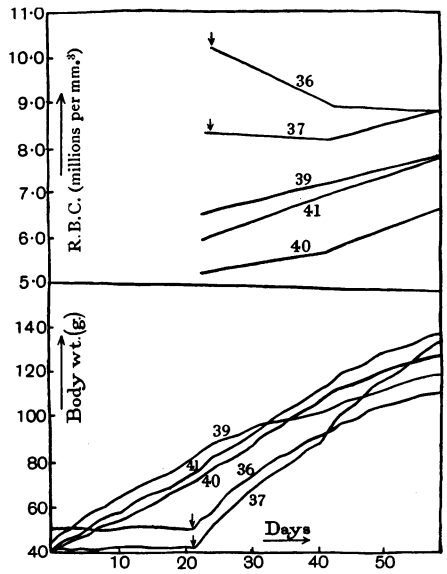

Fig. 5. Rats No. 39, 40 and 41 fed on diet 16 (*b*) throughout. Rats No. 36 and 37 fed on diet 16 + B_1 + autoclaved liver extract (3 cc.); ↓ 1 cc. autoclaved marmite in addition.

(*c*) copper, iron and glutamic acid together with a diet containing 12 % yeast (diet 16 (*c*)). The autoclaved liver extract and autoclaved marmite were fed in 3 cc. and 1 cc. daily doses respectively. In these experiments the effect of feeding the same or different supplements for different periods on the same rat was studied, the animals thus serving as their own controls. The results are given in Fig. 5.

These results indicate that the effect of 12 % yeast in the diet, supple-

mented by copper, glutamic acid and extra iron, on the red cell count, is not at all comparable with the pronounced effect of autoclaved liver extract. It also appears that the administration of autoclaved marmite in addition to autoclaved liver extract does not further stimulate the formation of erythrocytes (see also Section E). If anything it appears to have an inhibitory effect. This is in contrast to the stimulating effect of autoclaved marmite, when given by itself to rats, which had been previously undergoing vitamin B_2 deficiency (see Tables II and IV). The significance of these results is discussed later.

E. The results of experiments, similar to those described in the preceding section, on another group of rats, approximately 8 weeks old, are given in Table IV. The autoclaved liver extract and autoclaved marmite were fed in

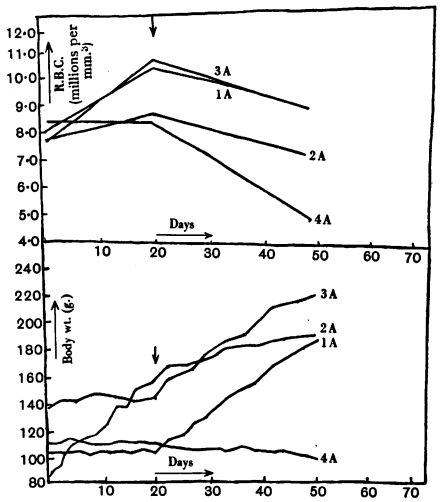

Fig. 6. 1 A and 3 A fed on diet 16 + B_1 + autoclaved liver extract; ↓ 1 cc. autoclaved marmite in addition, 2 A fed on diet 16 + B_1 + autoclaved marmite throughout, 4 A fed on diet 16 + B_1 + Cu + Fe + glutamic acid; ↓ 16 + B_1 + 0·5 cc. alkaline haematin.

3 cc. and 1 cc. daily doses respectively. In this set of experiments the effect of different supplements on the same rat was studied. In previous communications [Guha, 1931, 2, 5] it was stated that haemin, administered in a fine aqueous suspension, was unable either to restore the growth or cure the symptoms of depilation of vitamin B_2-deficient rats. As much of the haemin fed was excreted and as Kollath's experiments [1929, 1930] were carried out with alkaline haematin, 0·5 cc. of a solution of haemin in dilute sodium hydroxide containing 2·5 mg. of haemin was fed daily to one of the rats of this group with entirely negative results. As this was not taken readily it had to be incorporated in the diet. The daily administration of this alkaline solution appeared to have a deleterious effect (Fig. 6).

These results again demonstrate the quite noticeable effect of autoclaved marmite on the red cell count, while the effect of autoclaved liver extract is

very marked. The results with copper, glutamic acid and alkaline haematin are negative. The curious fact is again noticed that on feeding autoclaved marmite in addition to autoclaved liver extract, the red cell count has a tendency to fall (rats 1 A and 3 A). This confirms the experiments described in the preceding section.

Table IV.

Rat No.	Colour	Sex	Nature of diet	Period of feeding on diet (days)	Initial wt. (g.)	Final wt. (g.)	Initial R.B.C.	Final R.B.C.	Final haemoglobin concentration (g. in 100 cc. blood)
1 A	Albino	♂	$16+B_1$	25	—	107	—	8·0	—
„	„	„	$16+B_1$ + autocl. liver ext.	20	107	108	8·0	10·3	—
„	„	„	$16+B_1$ + autocl. liver ext. + autocl. marmite	27	108	183	10·3	8·9	14·6
2 A	„	„	$16+B_1$	25	—	82	—	7·7	—
„	„	„	$16+B_1$ + autocl. marmite	20	82	157	7·7	8·6	—
„	„	„	„	27	157	187	8·6	7·0	14·0
3 A	„	„	$16+B_1$	25	—	136	—	7·6	—
	„	„	$16+B_1$ + autocl. liver ext.	20	136	146	7·6	10·6	—
„	„	„	$16+B_1$ + autocl. liver ext. + autocl. marmite	27	146	219	10·6	8·9	15·4
4 A	Black and white	„	$16+B_1$	25	—	113	—	8·3	—
„	„	„	$16+B_1+CuSO_4+FeCl_3$ + glutamic acid	20	113	104	8·3	8·4	—
„	„	„	$16+B_1$ + alk. haematin	27	104	98	8·4	4·6	10·6*

* On the day on which the last estimations were made this animal was extremely miserable and nearly dying.

F. Seven young black and white rats of the same litter, which were split up into three groups and were fed on basal diets (diets 16 (*a*) and 16 (*c*)), slightly different from the basal diets fed in the foregoing experiments, gave the following figures for the erythrocyte and haemoglobin concentrations (Table V). The autoclaved liver extract was fed in 2 cc. daily doses.

Table V.

Rat No.	Sex	Nature of diet	Period of feeding on diet (days)	Initial wt. (g.)	Final wt. (g.)	R.B.C.	Average R.B.C.	Hb (g. per 100 cc. blood)	Average Hb (g. per 100 cc. blood)
65	♀	16 (*c*)	30	67	130	7·3	7·1	12·5	13·0
66	„	„	30	57	117	6·9		13·5	
67	„	16 (*a*) + B_1	30	70	89	6·4	6·35	11·2	11·3
68	„	„	30	80	88	6·3		11·4	
69	„	16 (*a*) + B_1 + autocl. liver ext.	30	66	84	9·9	9·17	13·5	13·6
70	„	„	30	70	84	9·0		13·5	
71	„	„	30	60	79	8·6		13·8	

These results demonstrate that the presence of yeast in the diet (diet 16 (*c*)) gives somewhat higher figures for the red cell and haemoglobin concentrations

than when the basal diet (16 (*a*)) is supplemented only by vitamin B_1. But the addition of autoclaved liver extract to the diet gives a much higher figure for the red cell count than can be obtained with yeast, while the effect on the haemoglobin concentration is not so marked.

G. It was desirable to get a few figures for the red cell count in vitamin B_1 deficiency. Animals of approximately the same age were given synthetic diets with and without vitamin B_1 and the red cell concentrations of their peripheral blood estimated in the usual way (Table VI).

Table VI.

Rat No.	Colour	Sex	Nature of diet	Period of feeding on diet (days)	Body wt. at the time of taking R.B.C.	R.B.C.	Average R.B.C.
25	Albino	♂	$-B_1$	30	71	10·5	
27	,,	♂♀	$-B_1$	30	81	10·0	10·53
28	,,	♀	$-B_1$	30	67	11·1	
21	,,	♂♀	$+B_1$	30	115	9·8	
22	,,	♂♀	$+B_1$	30	130	10·4	10·33
23	,,	♂	$+B_1$	30	104	10·8	

While the number of these experiments is inadequate to justify definite conclusions, it appears that in vitamin B_1 deficiency, in contradistinction to vitamin B_2 deficiency, there is no reduction in the red cell count. Sure, Kik and Walker [1929,1] observed a slight rise in the red cell count in vitamin B_1 deficiency, which was accompanied by anhydraemia.

Discussion.

Whipple and his co-worker [1925], working chiefly with dogs, have shown in a series of important papers that certain food materials possess a very marked blood regenerative power in experimental secondary anaemia. The specific effect of copper in nutritional anaemia, observed by Hart *et al.* [1928], has been confirmed by Titus, Cave and Hughes [1928], who have also observed a specific effect of manganese. Beard and Myers [1929] have added nickel, cobalt and germanium to this list. It is necessary to stress the point that the factors, effective in one type of anaemia, may not be effective in a different type. Thus Robscheit-Robbins and Whipple [1929] found that Eli Lilly's commercial liver extract, No. 343, which is highly effective in cases of pernicious anaemia, was not nearly so effective in severe secondary anaemia in dogs. It is, therefore, not possible to say how far the factors with which we have been dealing in the present paper come into the picture of clinical and experimental anaemias.

The principle present in the aqueous extract of fresh ox-liver, which gives rise to supernormal concentration of erythrocytes in blood, even when the rats are in poor condition owing to vitamin B_2 deficiency, is apparently heat-stable. This response cannot be due to iron and copper, present in the liver extract, as these elements have been shown in this paper to be quite ineffective

in producing this unusual response when fed by themselves. The failure of glutamic acid, which, according to Drabkin and Miller [1931], is effective in nutritional anaemia in the rat, indicates that the principle in liver is of a different nature. Though we have not yet obtained conclusive evidence that this substance is different from the factors concerned in the cure of pernicious anaemia, an identity between the two appears to be improbable. Thus, as has been pointed out above, the factors curative of pernicious anaemia have been stated to be of relatively slight effect in cases of secondary anaemia, and it is, therefore, improbable that the factor in fresh liver, described in this paper, which exhibits its effect apparently even under normal conditions, is identical with them. Preliminary experiments with the E.L. liver extract No. 343 indicate that it is not nearly so effective under our conditions of experiment as autoclaved liver extract. Moreover, it would appear from the paper of Cohn, Minot, Alles and Salter [1928] that the factor curative of pernicious anaemia described there is not particularly heat-stable, likewise the acid described by Dakin and West [1931] appears to be unstable under the conditions in which the autoclaved liver extract described in this paper is made. Further work is being done in order to settle this question.

The present work indicates that there is a diminution in the red cell concentration of blood in vitamin B_2 deficiency, which can be restored to normal by feeding autoclaved marmite, though, unlike the autoclaved liver extract, this was not found to push the erythrocyte concentration beyond the normal level. While the evidence is suggestive and prompts further investigation, it cannot yet be definitely stated that the factor concerned, which is present in autoclaved marmite, is vitamin B_2. Doubt is cast on this point chiefly by the consideration that the diminished red cell count occurring in vitamin B_2 deficiency can be increased above the normal by feeding autoclaved liver extract, which is practically free from vitamin B_2. While this experiment does not by any means show that vitamin B_2 has no haematopoietic effect, it is clear that it is possible to raise the red cell count in the almost complete absence of vitamin B_2. Whether the factor present in autoclaved marmite, which restores the red cell count to normal, is vitamin B_2 or is identical with the liver principle can be settled by obtaining a preparation of vitamin B_2, which is free from the liver factor. We have not yet succeeded in this objective, though we have been able to obtain a liver preparation, which is free from vitamin B_2.

The question of the aetiology of pellagra has been discussed elsewhere [Guha, 1931, 5]. It is obvious from the experiments described in this paper, in which food intake records were taken and additional iron was fed to some of the animals, that the arrest of growth due to vitamin B_2 deficiency cannot be due simply to iron deficiency. The reduction in the red cell count, occurring in vitamin B_2 deficiency can be corrected by autoclaved marmite but not by copper, glutamic acid or additional iron. Though we are not yet certain that this principle in autoclaved marmite is vitamin B_2, there appears to be enough

suggestive evidence to warrant the possibility that vitamin B_2 may be concerned in some phase of the mobilisation of ingested iron. Bliss and Thomason [1931] point out that, according to Elvehjem and Peterson [1927], hair is the richest source of iron in the body, and it does not appear impossible that the depilation, occurring in vitamin B_2 deficiency, might be at least partly due to a failure in this phase of iron metabolism.

Certain figures quoted by us indicate that there might be a question of balance between different factors concerned in the formation of erythrocytes. Thus the rate of erythrocyte formation due to the feeding of autoclaved liver extract appeared to be inhibited by the administration of autoclaved marmite. This inhibition was, of course, associated with increased growth produced by autoclaved marmite. Funk and Lejwa [1931], in a recent paper to which our attention was drawn after this work had been completed, mention that rapid growth induces a decrease in the number of red cells, and they consider that there are factors present in foodstuffs which stimulate, and others which inhibit, blood regeneration.

It may be interesting to state that some of the vitamin B_2-deficient animals, which were receiving vitamin B_1 and autoclaved liver extract, lost fur more readily than those receiving vitamin B_1 only, suggesting that the liver principle might hasten depilation by mobilising most of the available iron for the generation of blood, when the food intake and, therefore, the iron intake is limited.

It appears from the few measurements of haemoglobin, that we have made, that the influence of autoclaved liver extract on the concentration of red cells is relatively greater than on the concentration of haemoglobin. This might indicate a more specific effect of the liver principle on the formation of the stroma of erythrocytes.

Summary.

1. Rats under vitamin B_2 deficiency show a diminution in the red cell count, which can be restored to normal by autoclaved marmite but not by copper, glutamic acid, extra iron or alkaline haematin.

2. The arrest of growth in vitamin B_2 deficiency cannot be due to simple iron deficiency, as is shown by records of food consumption. Alkaline haematin cannot replace vitamin B_2 in the diet.

3. Vitamin B_1 deficiency does not result in a reduction of the red cell count.

4. The presence of a heat-stable factor in ox-liver is demonstrated, which exerts a powerful influence on the formation of erythrocytes.

5. This factor can be extracted with boiling water and can be freed from vitamin B_2 by autoclaving in an alkaline medium. Even when growth is arrested and the animals are in poor condition owing to vitamin B_2 deficiency, the administration of this autoclaved liver extract induces a supernormal

erythrocyte count. This effect cannot be produced by iron, copper, glutamic acid and yeast.

6. The significance of these results is discussed.

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