CXCVIII. THE COMPOSITE NATURE OF THE WATER-SOLUBLE VITAMIN B[1].

III. DIETARY FACTORS IN ADDITION TO THE ANTI-NEURITIC VITAMIN B_1 AND THE ANTI-DERMATITIS VITAMIN B_2.

BY HARRIETTE CHICK AND ALICE MARY COPPING.

From the Department of Experimental Pathology, Lister Institute, London.

(*Received October 28th, 1930.*)

IN 1926 Goldberger and his colleagues [1926] published the discovery of a new constituent, more heat-stable than the antineuritic vitamin (vitamin B_1), in the complex previously known as "water-soluble B" [McCollum and Davis, 1915]. This constituent, now known as vitamin B_2, they called the P-P, or pellagra-preventive, vitamin, adducing much evidence that it was concerned in the prevention and cure of human pellagra.

During the four years that have elapsed since the publication of Goldberger's work, the discovery has been announced of at least three additional water-soluble B vitamins [Williams and Waterman, 1927, 1928; Hunt, 1928; Reader, 1929, 1930; Peters, 1929]. For the separate existence of two of these, the Williams and Waterman vitamin B_3, required for weight maintenance in the pigeon, and the Reader vitamin B_4, necessary for correct nutrition of the rat, there is now an accumulation of confirmatory evidence.

In the course of several years spent on this subject the workers in this laboratory have also become convinced that there is present in yeast at least one water-soluble dietary factor essential for the growth and nutrition of the rat in addition to vitamins B_1 and B_2 and the evidence for this statement is contained in this paper. It is, however, certain that this factor which is provisionally called "Factor Y" is not identical with the new vitamins described

[1] The following nomenclature is here adopted for the B group of water-soluble vitamins.

Vitamin B_1 for the more heat-labile, antineuritic, antiberiberi vitamin, discovered by Eijkman [1897].

Vitamin B_2 for the more heat-stable, "P-P" factor, discovered by Goldberger and others 1926, found necessary for growth and prevention of dermatitis in rats.

Vitamin B_3 for the most heat-labile factor of the group, described by Williams and Waterman [1927], present in yeast and whole wheat and necessary for weight maintenance in the pigeon.

Vitamin B_4, for the heat- and alkali-labile factor described by Reader [1929], present in yeast and necessary for the continued growth of the rat; this factor has been known previously as "vitamin B_3."

respectively by Williams and Waterman and by Reader, seeing that it has been found to be highly resistant to the action of heat and alkali, whereas vitamins B_3 and B_4 are described as relatively sensitive to these agents [Reader, 1929; Eddy, Gurin and Keresztesy, 1930].

Much difficulty and uncertainty in this field of work is due to the fact that, except in work on the antineuritic vitamin B_1 and to some extent in that on the antidermatitis vitamin B_2, the criterion for presence or absence of these various B vitamins has been the growth (increase in weight) of a rat or pigeon. The interpretation of growth observations is more complicated and more liable to error than that of tests involving the cure or prevention of an easily recognised pathological syndrome. Advance in this field of work would be much helped if the physiological rôle of the various members of the group were elucidated and the pathological condition following deprivation recognised.

An unknown dietary factor Y in yeast.

(i) *Evidence derived from long period growth trials on "complete" synthetic diets.*

The existence of an unknown water-soluble dietary factor in yeast was suspected in the course of attempts to rear and breed young rats on artificial diets containing all recognised essential constituents, vitamins B_1 and B_2 being supplied from different sources. When the former was given as Peters's antineuritic concentrate prepared from brewer's yeast [Peters, 1924; Kinnersley and Peters, 1925; Chick and Roscoe, 1929, 2] it was found that the nutritive value of the diet was greater if vitamin B_2 were provided as autoclaved yeast or as an autoclaved watery yeast extract than if it were derived from egg-white, a material which, while rich in vitamin B_2 is devoid of vitamin B_1 [Chick, Copping and Roscoe, 1930].

The two diets were composed as follows. Diet I was the ordinary "$-$ B" basal diet (P2L) of this laboratory. It consisted of purified caseinogen 100; rice starch 300; hardened cotton seed or arachis oil 75; salt mixture [McCollum and others, 1917; No. 185] 25; water 500; and was steamed for 2–3 hours before feeding in order to cook the starch grains and prevent refection. Vitamins A and D were given separately to each rat in daily doses of cod-liver oil, 0·05–0·10 g., according to the weight of the animal. Vitamin B_1 was provided as 0·1 cc. daily ($\equiv$ 0·6 g. dry yeast) of Peters's concentrate and vitamin B_2 by 0·4 g. daily of dried brewer's yeast which had been autoclaved for 5 hours at 120° or an equivalent amount of a watery yeast extract, similarly heated. Many of the observations lasted for 6–9 months and these vitamin rations were proportionately increased as the rats grew larger.

The composition of diet II was similar to that of diet I except that the 100 parts of purified caseinogen were replaced by an equivalent amount (800 g.) of ordinary fresh egg-white, which supplied both protein and vitamin B_2. The egg-white was coagulated by heating before mixing with the other constituents of the diet and as with diet I the mixture was steamed before feeding. Vitamins B_1, A and D were administered to the rats separately in daily doses as to the animals on diet I.

The observations were made on young rats just weaned, 21 days old and of 35–45 g. body weight. During the first week it was usual to give all rats diet I without any addition of B vitamins so that this short period of deprivation might make the rats more eager to take the daily rations of these vitamins when offered subsequently.

Growth on diet I. Out of 11 rats (5 ♂ and 6 ♀) on diet I, the growth of 5 (2 ♂ and 3 ♀) reached the standard of the normal Wistar curve [Greenman and Duhring, 1923] over periods of observation ranging from 18 to 30 weeks. Of the remaining 6 rats, 4 were maintained at a level only slightly below this standard.

The rats were mated when about 4 months old and litters of 6–8 normal young were cast by 3 of the 6 females, and one of these had two successive normal litters. The supply of B vitamins was increased to 3–4 times the usual dose during pregnancy and vitamin E given three times weekly as a small ration of wheat germ oil. Nevertheless, the diet proved inadequate to support lactation; none of the young was reared, all died within 24 hours or were eaten by the mothers.

Growth on diet II. Out of a large number of trials only 5 rats (2 ♂ and 3 ♀) showed sustained growth for periods long enough to be comparable with those on diet I. An example of the usual failure of rats on diet II compared with litter mates on diet I is shown in the curves in Fig. 1. The increase in weight of the two exceptional males which grew well and were observed for over 30 weeks was below the Wistar standard; that of the three females was also subnormal, though not markedly lower than that of some females on diet I. The two better grown of the three females were duly mated but without any successful pregnancy, although the observations lasted till the rats were 8 and 10 months old respectively. In both cases a sudden increase in weight occurred, followed by an unexpected fall, which was suggestive of a miscarriage, but no definite proof was obtained.

It seemed unlikely that the cause for the inferiority of diet II lay in the change of protein from caseinogen to egg-white, for the latter has repeatedly been found satisfactory for the rearing of rats receiving protein from no other source [Osborne and Mendel, 1913; Mitchell, 1925]. Nevertheless, to test this point two male rats were reared for 3 months on the caseinogen diet I with vitamin B_2 supplied as a concentrate prepared from egg-white [Chick, Copping and Roscoe, 1930]. The growth of both was subnormal and inferior to that of the males on diet II.

There was the further possibility that the rats on diet II might suffer from an inadequate ration of vitamin B_2 seeing that this was incorporated in the diet and the intake depended on the appetite. This diet was, however, well taken and the average daily intake was found to contain about 10–15 g. egg-white for rats up to 100 g. weight and about 14–18 g. for those heavier. These amounts would supply considerable excess of vitamin B_2 [Chick, Copping and Roscoe, 1930].

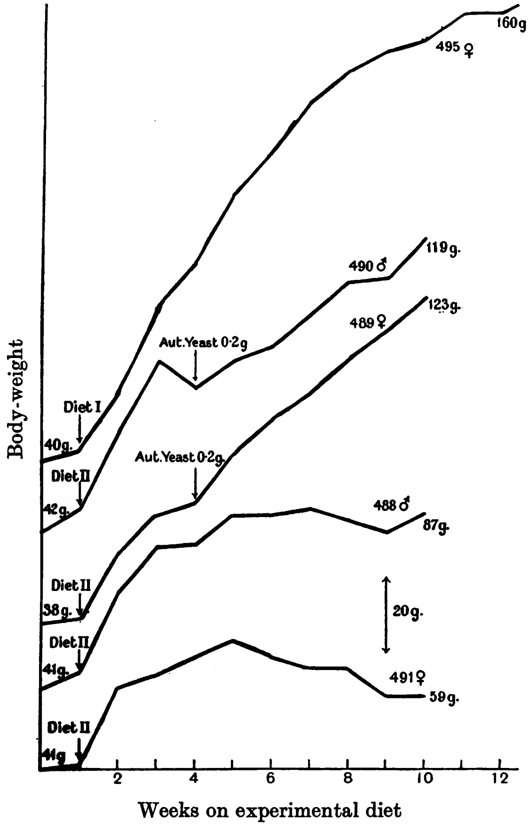

Fig. 1. Showing growth of young rats, litter mates, maintained for one week on a basa diet deprived of B vitamins and thereafter on

(1) Diet I (caseinogen diet) with vitamin B_2 as 0·4 g. daily (later 0·6–1 g.) yeast autoclaved at 120° for 5 hours and vitamin B_1 as Peters's concentrate, 0·1 cc. (≡0·6 g. yeast), later 0·2 cc.; rat 495 ♀.

Or (2) Diet II (egg-white as protein and source of vitamin B_2) with vitamin B_1 as Peters's concentrate: 0·05 cc. daily, rats 490 ♂ and 491 ♀; 0·1 cc. daily, rats 488 ♂ and 489 ♀.

Failure of growth of rats 490 and 489 restored by administration of 0·2 g. autoclaved yeast daily.

The conclusion we are led to adopt from these results and from those given in the following sections is that an essential, water-soluble, heat-stable, dietary factor hitherto unrecognised is present in autoclaved yeast and autoclaved watery yeast extracts, and deficient in egg-white. This dietary factor is provisionally called "Factor Y."

We have no explanation to offer of the fact that diet I, although superior to diet II, is itself unable to maintain normal development indefinitely or to support lactation. We have tried addition of iodine to the salt mixture; a change from purified to unpurified caseinogen ("light white casein", British

Drug Houses) and addition of antiscorbutic vitamin to the diet as a small daily ration of decitrated lemon juice. These changes, however, were without effect in raising the adult body weight of the males beyond about 300 g. or in enabling the females to rear their litters successfully.

Experiences similar to this are common among workers who have used "complete" artificial diets [Palmer and Kennedy, 1927, 1, 2, 1928; Evans and Burr, 1927, 1928; Guha, 1930]. Work is being continued in the hope of discovering the missing essential constituent or constituents and the natural foods in which these are contained.

(ii) *Evidence derived from observations on previously deprived animals.*

(*a*) *Experiments with rats deprived of the antidermatitis vitamin* B_2. The inability of vitamin B_2 contained in egg-white or its products to supplement the antineuritic vitamin B_1 in maintaining steady growth is more strikingly demonstrated when it is given to young rats which have previously been deprived of vitamin B_2 and have received vitamin B_1 in a purified form (Peters's antineuritic concentrate). Whether the period of deprivation is only of a few weeks' duration or long enough for development of the dermatitis caused by vitamin B_2 deficiency, restoration of growth is immediate, and in the latter case rapid cure of dermatitis also takes place. But the rate of increase in body weight soon diminishes and is not restored by increasing the dose of egg-white [Chick, Copping and Roscoe, 1930].

In such experiments the deficiency in egg-white is more clearly demonstrated than in those described in Section (i) above. This is probably due to exhaustion of the young rats' reserves of "factor Y" during the previous period of deprivation, for the Peters's antineuritic concentrate given to provide vitamin B_1 appears to contain little if any of this factor (see below).

(*b*) *Experiments with rats previously deprived of the antineuritic vitamin* B_1. In a previous paper [Chick and Roscoe, 1929, 1] methods were described for estimation of vitamin B_1 in foodstuffs, in which the growth of young rats was taken as a criterion. Young rats were "run out" until the weight became stationary on diets deprived only of this vitamin, and comparative tests were made using the two basal vitamin B_2-containing diets, I and II, described in the previous section. The content of vitamin B_1 in the test material then administered was measured by the degree to which growth was restored during an observation period of 2–5 weeks. It was found that in testing some materials concordant results were obtained with the two different basal diets, with others the degree of growth restored on diet II (egg-white) was consistently inferior.

Further evidence of this discrepancy is contained in the work of Roscoe [1930] on the vitamin B_1 content of vegetables and in some additional data contained in Appendix I. These results may be summarised as follows.

(1) In growth tests with rats devised to estimate the vitamin B_1 contained in dried yeast and certain watery yeast extracts, in ox-liver, egg-yolk, and green

vegetables, watercress, lettuce, spinach, cabbage, it was found immaterial whether vitamin B_2 were supplied in the basal diet as autoclaved yeast products (diet I) or as egg-white (diet II).

(2) With meat (ox-muscle), wheat embryo and the etiolated inner leaves of cabbage, there was consistently inferior growth on diet II.

(3) In tests of onion and Peters's antineuritic "B_1" concentrate, concordant results were obtained if a large ration were given, but with small doses the growth on diet II was always inferior.

These results can be explained by the theory advanced in the previous section, that there is an essential dietary factor, deficient in egg-white but present in yeast and yeast extracts, and also present in adequate amount in the foods of group (1) and present in less amount or lacking in those of groups (2) and (3). The distribution of the unknown factor Y, as thus far studied, indicates its presence in foods known to possess the highest general nutritive qualities.

(iii) *Further study of factor Y as it occurs in yeast extracts.*

Reader [1929] in her work on the B group of vitamins found that antineuritic vitamin B_1 concentrates prepared from yeast by the Peters process were freer from admixture with her "vitamin B_4" if the final solution from the norite adsorbate were made with $N/10$ HCl instead of with 50 % acid alcohol. The vitamin B_1 preparations hitherto used in our work were prepared by the latter method and although the available evidence was opposed to the idea that "vitamin B_4" and factor Y might be identical, seeing that the former is relatively heat-labile, it seemed worth while, nevertheless, to investigate the two types of vitamin B_1 concentrate, to see if any difference could be detected in their ability to supplement vitamin B_2 when presented respectively as egg-white or as autoclaved yeast extract.

Extracts were, therefore, made from aliquot portions of the norite adsorbate derived from yeast XXVI with (1) $N/10$ HCl and (2) 50 % alcohol containing 1 % HCl. In each case the extractions were made by boiling with successive quantities of fresh reagent, these being afterwards combined and concentrated under reduced pressure until 1 cc. contained the equivalent of 6 g. yeast. The reaction was then adjusted to p_H 3·0 for storage.

Prof. Peters warned us that the vitamin B_1 content of extract (1) would probably be much lower than that of (2), and kindly estimated the antineuritic value of both by curative tests on pigeons suffering from polyneuritis. As was predicted, the alcoholic extract (2) was found to be about six times as potent as the hydrochloric acid extract. Accordingly, in the subsequent tests made to compare the power of these two preparations to supplement vitamin B_2 as provided by egg-white in diet II and by autoclaved yeast extract in diet I respectively, the doses given of (1) and (2) were as 5:1, these quantities being reckoned on the dry weight of the equivalent yeast.

The results of a series of such tests are given in Table I. Young rats,

Table I. *Effect of vitamin B_1 contained in extracts made (1) with N/10 HCl and (2) with acid 50 % alcohol in Peters's process as supplement for vitamin B_2 contained:—(a) in diet I as extract A (watery extract obtained from washed yeast XXIV autoclaved 5 hours at 120°, p_H 5·6, daily dose ≡ 0·5 g. yeast); (b) in diet II as fresh egg-white incorporated in the diet.*

Rat 698 A in Exp. 2 and rats 740, 742 and 739 in Exp. 3, received later a daily dose (≡ 0·5 g. yeast) of extract B (watery extract from yeast XXIV autoclaved in alkaline solution, 4 hours at 119° at p_H 10·3–9·6).

Material used as source of vitamin B_1	Dose cc.	Equivalent in dry yeast g.	Diet	Litter	Rat	Duration of preparatory period, days	Duration of test, days	Body weight: Initial g.	Body weight: Increase during period of test g.	Body weight: Weekly average increase g.	After administration of extract B: Duration of test, days	After administration of extract B: Body weight: Initial g.	After administration of extract B: Body weight: Increase during period of test g.	After administration of extract B: Body weight: Weekly average increase g.
Exp. 1.														
(1) HCl extract	0·25	1·5	I	1698	693 ♀	14	35	48	68	13·5	—	—	—	—
(2) Alc. "	0·05	0·3	"	"	691 ♂	37	"	69	61	12	—	—	—	—
Exp. 2.														
(1) HCl extract	0·25	1·5	"	1730	700 ♀	18	28	52	47	12	—	—	—	—
(2) Alc. "	0·05	0·3	"	"	702 ♂	21	"	55	34	8·5	—	—	—	—
(1) HCl "	0·25	1·5	II	"	695 A ♀	23	"	51	22	5	—	—	—	—
" "	0·5	3·0	"	"	697 A ♂	18	"	54	33	8	—	—	—	—
(2) Alc. "	0·1	0·6	"	"	696 A ♂	18	"	58	24 }	7·5	—	—	—	—
" "	0·1	"	"	"	698 A ♀	21	"	58	37 }		28	95	57	14
Exp. 3.														
(1) HCl extract	0·25	1·5	I	1783	736 ♂	16	35	41	58 }	12·5	—	—	—	—
" "	"	"	"	"	737 ♂	"	"	47	68 }		—	—	—	—
(2) Alc. "	0·05	0·3	"	"	735 ♀	19	"	44	67 }	12	—	—	—	—
" "	"	"	"	"	738 ♂	16	"	41	53 }		—	—	—	—
(1) HCl extract	0·5	3·0	II	"	740 ♂	"	"	46	51 }	11·5	14	97	39	—
" "	"	"	"	"	742 ♂	"	"	"	64 }		14	110	31	17·5
(2) Alc. "	0·1	0·6	"	"	739 ♀	14	"	42	37	7·5	14	83	39	19·5

21 days old, just weaned, of about 40 g. weight, were "run out" on diets I and II respectively, as in the preparatory period of the tests for estimation of vitamin B_1. When weight was stationary, usually after 2–3 weeks, vitamin B_1 was supplied as appropriate doses of extracts (1) and (2) respectively.

The results showed that rats maintained on the same diet, whether I or II, responded in a similar manner, as measured by the degree of restoration of growth, to each of the two "B_1" extracts. The difference, if any, was in favour of those receiving the concentrate prepared with hydrochloric acid (Table I, *cf.* Rats 693 and 691, Exp. 1; 700 and 702, Exp. 2; 740, 742 and 739, Exp. 3). It is possible that the dose of extract (1), as calculated from the pigeon trials, was overestimated and that excess of B_1 was administered.

The rats maintained on diet II, however, compared with their litter mates on diet I, showed a consistently lower response to both the vitamin B_1 preparations. In Exps. 2 and 3 it was found necessary to double the doses for rats on diet II, in order to get a comparable response. There was, however, no indication that the extracts prepared with alcohol had any supplementary power for egg-white greater than those made with hydrochloric acid.

Table II. *Capacity of extract B, a watery yeast extract (XXIV) deprived of vitamin B_2 by autoclaving (119° for 4 hours) in alkaline solution at* p_H *10·3–9·6 (daily dose ≡ 0·5 g. yeast) to supplement egg-white used as source of vitamin B_2.*

Vitamin B_1 provided as Peters's purified antineuritic concentrate (alcoholic extract from norite).

Diet I, caseinogen as protein and vitamin B_2 as extract A (see Table I).

Diet II, protein and vitamin B_2 as egg-white incorporated in the diet.

In Exps. 4 and 5, first period, the dose of vitamin B_1 was 0·045 cc. (≡0·27 g. yeast) and the period of the test 5 weeks; in Exp. 4, second period, the dose of vitamin B_1 was doubled and the test period reduced to 4 weeks.

Exp.	Diet	Litter	Rat	Preliminary period without vitamin B_1 days	Body weight: Initial g.	First period: Increase during period of test g.	First period: Average increase weekly g.	Second period: Increase during period of test g.	Second period: Average increase weekly g.
4	I	1770	727 ♀	25	56	35		33	
			728 ♂	28	63	40	7·5	33	8
	II	,,	730 ♂	14	40	34		27	
			731 ♀	14	43	42		37	
			734 ♂	25	45	16	6	24	7
	II + extract B	,,	729 ♀	14	39	41		41	
			732 ♀	25	42	37		43	
			733 ♂	14	47	66	9·5	56	12
5	I	1785	743 ♀	14	45	69			
			744 ♂	14	46	68	13·5		
	II	,,	750 ♂	23	58	48			
			751 ♂	14	40	50	10		
	II + extract B	,,	748 ♀	23	53	63			
			749 ♂	23	58	62	12·5		

(iv) *Supplementary effect of yeast extracts in which both vitamins B_1 and B_2 have been destroyed by autoclaving in alkaline solution.*

At the end of the observations described above certain rats in Exps. 2 and 3 (698A; 740, 742, 739) received a further addition to their egg-white diet, diet II, in the form of a small daily ration (equivalent to 0·5 g. original yeast) of extract B, a watery yeast extract, which had been autoclaved at alkaline reaction. It was prepared as follows. Washed, pressed brewer's yeast XXIV was boiled with twice its weight of 0·01 % acetic acid solution, and the filtrate concentrated until the equivalent of 1 g. yeast (dry weight) was contained in 1·5 cc. Sodium hydroxide was then added until the p_H was 10·3 (determined with a hydrogen electrode) and the material heated in an autoclave for 4 hours at 119°, after which operation the p_H was 9·6. It was immediately brought to p_H 3·0 for storage. This extract will be referred to as extract B. Proof that the vitamin B_2 originally richly present (see extract A, Tables I and II) had suffered destruction by this treatment was shown by the negative results of growth tests and curative trials of dermatitis made on rats suffering from vitamin B_2 deficiency [Chick and Copping, 1930].

This material, however, was found to contain a factor supplementing diet II, for the rats in Exps. 2 and 3 to which it was given responded immediately and increased in weight at rates varying from 14 to 20 g. weekly, whereas previously the rate of increase had been subnormal, from 7·5 to 11·5 g. weekly. This result was confirmed by the results of two additional experiments (4 and 5, Table II), in which young rats were prepared similarly to those in Exps. 1–3 and maintained on diet II, but of these half received a daily ration of extract B from the time when vitamin B_1 was given.

Exp. 4 was divided into two periods, in the first 5 weeks the dose of vitamin B_1 given was sub-optimal and the growth of all the rats was subnormal. The increase in weight of those on diet II without addition of extract B was as usual inferior (average weekly increase 6 g.) to that of those on diet I, but the addition of extract B caused a growth rate (average weekly increase 9·5 g.) superior even to that of those on diet I (average weekly increase 7·5 g.). During a further period of 4 weeks' observation the dose of vitamin B_1 was doubled and growth in all three groups was enhanced, but showed a similar relationship, *viz.* average weekly increase of 8 g., 7 g. and 12 g. on diets I and on diet II without, and with, extract B respectively.

Exp. 5 included 6 rats, litter mates, divided into 3 groups as in Exp. 4. Extract B was given to two rats on diet II during the whole preparatory period, in order to prevent any possible depletion of the reserves of factor Y. After administration of vitamin B_1 growth was observed for 5 weeks. The result confirmed Exp. 4. The average weekly increase in weight of rats on diet I was 13·5 g., and of those on diet II without, and with, extract B, 10 g. and 12·5 g. respectively.

The increased gain in body weight observed when extract B was added to diet II was related to an increased appetite and intake of food. Thus, in Exp. 3, rats 740 and 742, during 5 weeks on diet II consumed an average of 14 g. food daily; during the subsequent 2 weeks, when extract B was given, the average daily consumption was 21 g. Similarly in Exp. 5 rats 750 and 751, during 5 weeks' observation on diet II, consumed a daily average of 17 g. food, while for their litter mates 748 and 749 which received extract B in addition the daily average was 22 g.

(v) *Nature of factor Y contained in alkaline-autoclaved yeast extract.*

To find an interpretation of the results in the previous section it might be argued that the inferior growth of the rats on the egg-white diet II was caused by insufficient intake of vitamin B_2, which might occur towards the end of tests when appetite flagged, seeing that this vitamin was incorporated in the diet. Yeast extract prepared as extract B by strong heating in alkaline solution has not, however, in our experience, been found to contain appreciable amounts of vitamin B_2. The negative results of the tests carried out in this laboratory with the actual extract B used in Exps. 2–5 have been already published [Chick and Roscoe, 1930; Chick and Copping, 1930], and curative tests on rat dermatitis with a similar preparation, extract BB (used in Exp. 6 Appendix II) have been kindly undertaken by Dr Reader at Oxford, who has confirmed our experience.

Another possible explanation of the beneficial effect of extract B when egg-white is the sole source of protein and nitrogen might be that it provides some nitrogenous material absent from egg-white but present in caseinogen or in the yeast extract A given in diet I to provide vitamin B_2. This nitrogenous material could hardly be of a protein nature, for the coagulable proteins of yeast with isoelectric point at *ca.* p_H 5·0 are removed in the preparation of these extracts by filtration after boiling in the 0·01 % acetic acid solution. It was possible, however, that some extremely stable, non-protein, nitrogenous substance essential for nutrition, might be present in these watery yeast extracts and perhaps also in caseinogen and lacking in egg-white. It has been found however that diets combining protein as caseinogen, vitamin B_2 as protein-free concentrates prepared from egg-white (extract E), with vitamin B_1 as Peters's purified concentrate are no more satisfactory for growth than those in which the two former are supplied as egg-white (see also Exp. 6 in Appendix II).

A remaining possible explanation and the one here adopted is that watery yeast extracts, in addition to vitamins B_1 and B_2, contain a further essential dietary factor, which is stable to severe heating in acid or alkaline solutions.

Stability to heat and alkali of the various B vitamins.

The evidence relating to the stability to high temperature, acid and alkali of the different B vitamins is in part conflicting. This has led to some confusion in the literature and to difficulty in the interpretation of experiments in which heating operations have been used to effect separations and to gain proof of the separate existence of the various factors. It is evident, from the available information which is collected in Table III, that there is room for more data based on accurate quantitative experiments.

Table III. *Stability to heat and alkali of B vitamins.*

Vitamin	Source	Temp. °C.	Duration of heating (hrs.)	p_H	Result	Nature of test. Experimental animal	Observer
Antineuritic or antiberiberi B_1	Wheat embryo	*ca.* 100	2	—	No significant loss	Cure of polyneuritis in pigeon	Chick and Hume [1917]
	"	113	0·66	—	50 % loss	"	"
	"	118–124	2	—	75–90 % loss	"	"
	Yeast extract	100	1	—	*ca.* 50 % loss	"	"
	(marmite)	122	2	—	*ca.* 70 % loss	"	"
Antidermatitis B_2	Yeast	120	2·5	—	Stable	—	Goldberger *et al.* [1926]
	"	120	5	—	50 % loss	Growth of rat	Chick and Roscoe [1927]
	Yeast extract	90–100	2	Acid, p_H 5·0	No significant loss	"	Chick and Roscoe [1930]
	"	90–100	2	Alk. p_H 8·3	50 % loss	"	"
	"	123	4	Acid, p_H 5·0 or 3·0	50 % loss	"	"
	"	122–125	4–5	Alk. p_H 8·3–10·0	75–100 % loss.	Growth and also cure of dermatitis of rat	Chick and Roscoe [1930]; Chick and Copping [1930]
	Yeast extract (marmite)	120	1	Alk. p_H 9·0	Stable	Growth of rat	Reader [1929]
	Yeast extract	110–120	1–3	Alk. 10–15 % $Ba(OH)_2$	"	"	Narayanan and Drummond [1930]
Vitamin B_4 (rat)	Yeast extract	120	1	Alk. p_H 9·0	Destroyed	"	Reader [1929]
Vitamin B_3 (pigeon)	Yeast	20	—	Alkaline	Slowly destroyed	Weight maintenance in pigeon	Eddy, Gurin and Keresztesy [1930]
	"	60	—	Natural reaction ? p_H *ca.* 5·0	Destroyed	"	"
	Malt extract	60		—	"	"	"
Factor Y	Yeast extract	120–125	4	Alk. p_H 9–10	Stable	Growth of rat	Chick and Copping [present work]

The antineuritic vitamin B_1 is generally acknowledged to be relatively labile to heat and alkali, and the antidermatitis vitamin B_2, to be relatively heat-stable at temperatures about 120° in neutral or acid solution. There is disagreement, however, among different observers as to the degree to which this vitamin is sensitive to alkali at this temperature. Vitamin B_3 (Williams and Waterman) is described as being the member of the group most sensitive to heat, being affected at 60°; vitamin B_4 (Reader) has been found to be heat-labile to an extent comparable with vitamin B_1.

The new water-soluble dietary factor Y dealt with in this communication, appears to be the most resistant of the group, for it can survive heating for 4 hours at 120–125° at a p_H of about 10.

SUMMARY.

1. Experiments with young rats are described which show the failure to sustain growth of a diet (diet II) which contains all hitherto recognised necessary constituents, and includes Peters's antineuritic concentrate as source of vitamin B_1 and hen's egg-white as source of protein and vitamin B_2.

2. A similar result was obtained with diets containing purified caseinogen as protein, vitamin B_1 as Peters's concentrate and egg-white, or concentrates prepared therefrom, as source of vitamin B_2.

3. Satisfactory development over long periods, including fertility and successful pregnancy, was attained on a similarly constituted diet, diet I, containing purified caseinogen as protein, Peters's antineuritic concentrate as vitamin B_1, and autoclaved yeast or autoclaved watery yeast extracts (120°; 5 hours; p_H *ca.* 5·0) as vitamin B_2.

4. Diet II was rendered satisfactory for growth by addition of a small daily ration of a watery yeast extract which had been autoclaved in alkaline solution (4 hours; 120–125°; p_H *ca.* 10). In this preparation vitamin B_2 was proved to have been destroyed.

5. It is concluded that yeast and watery yeast extracts contain, in addition to vitamins B_1 and B_2, a hitherto unrecognised dietary factor, referred to in this paper as factor Y, which can withstand prolonged heating in alkaline solution.

6. Comparative experiments with diets I and II carried out by Roscoe [1930] and others, published in an Appendix to this paper, indicate that factor Y accompanies vitamins B_1 and B_2 in nature, is present in relatively large amount in yeast, green leaf vegetables, egg-yolk and ox-liver and absent from, or present in relatively small amount in, egg-white, wheat embryo, meat (ox-muscle), etiolated leaves of green vegetables and onion. Small amounts appear to be present in Peters's antineuritic concentrate as prepared by the authors from brewer's yeast.

7. Factor Y differs from the new members of the vitamin B complex recently described by Williams and Waterman (vitamin B_3) and by Reader (vitamin B_4) in its stability to heat and alkali.

Appendix I.

Table IV. *Influence on growth of young rats, maintained on basal diets I and II respectively, of the addition of vitamin B_1 as contained in different foodstuffs.*

Diet I contained caseinogen as protein and autoclaved (120°, 5 hrs., p_H *ca.* 5·0) yeast or yeast extract as source of vitamin B_2.
Diet II contained egg-white as protein and source of vitamin B_2.

		Daily dose		Basal diet I			Body weight			Basal diet II			Body weight			
Exp.	Foodstuff tested	Natural food g.	Dry weight g.	Rat No.	Duration of preparation period. Days	Duration of test. Days	Initial g.	Increase during period of test g.	Average increase g.	Rat No.	Duration of preparatory period. Days	Duration of test. Days	Initial g.	Increase during period of test g.	Average increase g.	Result
1	Dried egg-yolk A	0·95	0·5	461 ♀	21	21	51	9	—	464 ♀	21	21	40	5	—	Growth slightly better
		1·9	1·0	466 ♂	30	28	72	66	—	465 ♀	18	28	38	55	—	on diet I in Exps.
2	Do. B	2·0	1·0	629 ♂	25	35	56	77	—	626 ♂	16	35	56	64		1 and 2; no difference
		,,	,,	643 ♂	26	,,	65	69	—	641 ♂	23	35	54	54		in Exps. 3 and 4
		,,	,,	651 ♀	25	,,	53	69	72	649 ♂	23	35	50	49	56	
3	Do. F*	,,	,,	*643 ♂	—	14	134	31		*641 ♂	—	14	108	35		
		,,	,,	*651 ♀	—	,,	122	21	26	*649 ♂	—	14	99	16	25	
4	Do. B	3·0	1·5	610 ♀	39	28	80	79	—	620 ♀	16	28	51	79	—	
5	Dried ox-liver III	1·5	0·5	609 ♀	49	35	78	70		628 ♂	15	35	52	87		No significant differ-
				631 ♀	20	,,	54	88	79						87	ence
6	Do.	2·25	0·75	615 ♂	14	28	52	104		606 ♀	17	28	51	79		
									104	617 ♀	14	28	52	88	84	
7	Dried ox-muscle I and II	3·65	1·0	659 ♀	19	35	49	45	—	662 ♂	18	14	56	−11	—	
8	Do.	5·5	1·5	657 ♂	21	,,	60	100		662 ♂	—	35	45	80		Growth on diet II
		—	—	666 ♂	19	,,	55	87	93	660 ♀	19	,,	48	63		much inferior to that
										669 ♂	17	,,	56	33		on diet I
										671 ♀	17	,,	50	43	55	
9	Wheat embryo	—	0·1	607 ♀	49	,,	89	45		608 ♀	17	,,	53	19		
				621 ♀	42	,,	76	40	42·5	618 ♀	14	,,	47	32	25·5	,, ,,
10	Do.	—	0·2	633 ♂	33	,,	62	86		—	—	—	—	—	—	
				634 ♂	33	,,	67	92		632 ♂	19	,,	56	53		
				644 ♀	40	,,	78	60		642 ♀	23	,,	61	41		
				650 ♂	25	,,	47	85		647 ♂	21	,,	49	56		
				652 ♂	37	,,	75	75	80	—	—	—	—	—	50	
11	Peters's antineuritic concentrate Yeast XVIII	0·05 cc. = 0·3 g. dry yeast		550 ♀	47	,,	69	35	—	561 ♀	25	,,	70	28		
				551 ♀	39	,,	59	47		554 ♀	27	,,	64	27	27·5	,, ,,
				562 ♀	42	,,	87	39		553 ♀	25	14	44	15		
				563 ♀	37	,,	77	40	40	559 ♀	25	,,	73	14	14·5	
12	Do.	0·1 cc. = 0·6 g.		—	—	—	—	—	—	†553 ♀	—	35	59	32		
				—	—	—	—	—	—	†559 ♀	—	,,	87	46	39	
13	Do. Yeast XX	0·75	0·45	603 ♀	28	35	67	71		602 ♂	17	,,	51	46		
	Do. Yeast XXIII			630 ♂	14	,,	49	80	75·5	627 ♂	14	,,	53	57	51·5	,, ,
14	Do. Yeast XX	0·05	0·3	581 ♂	16	,,	55	44	44	585 ♂	20	—	67	24		
										587 ♀	17	—	61	23	23·5	,, ,,

* Tests with freshly prepared sample following the previous ones on same rats.
† Tests following previous ones on smaller dose.

Appendix II.

[Added December 21st, 1930.]

Exp. 6. (See Table V.)

The following additional experiment (Exp. 6) was devised to gain confirmatory evidence upon the various points concerning the existence of Factor Y which are discussed in Section v above. In this experiment comparison was made of the growth of young rats (litter mates) upon the following 6 diets.

1. Diet I (caseinogen) + vitamin B_2 as extract A, watery yeast extract XXVII, autoclaved for 5 hours at 123° at p_H *ca.* 5·0 (daily dose ≡ 0·5 g. yeast).
2. Diet I (caseinogen) + vitamin B_2 as extract E, a protein-free concentrate from egg-white (daily dose ≡ 10 g. egg-white).
3. As (2), but in addition Factor Y given as extract BB, watery yeast extract XXXIII autoclaved 4 hours at 125° at p_H 10·0–9·5 (daily dose ≡ 0·5 g. yeast).
4. Diet II (egg-white).
5. „ + extra vitamin B_2 as extract E, as in Group 2.
6. „ + extract BB as in Group 3.

These diets were distributed among 24 newly-weaned rats of body weight *ca.* 40 g., from 3 litters, as shown in Table V. The rats underwent a preparatory period on these diets without any addition, *i.e.* a period of deprivation of vitamin B_1, which varied from 12–17, 17–25, and 17–20 days, respectively, in the 3 litters. Vitamin B_1 was then given as Peters's purified concentrate and caused immediate resumption of growth which was observed for 5 weeks.

The dose of vitamin B_1 was small, 2 drops daily (0·045 cc. ≡ 0·27 g. yeast), to avoid giving any excess which might include an appreciable amount of Factor Y from this source (see p. 1769 above). Nevertheless, in Groups 1, 3, 5 and 6, satisfactory growth occurred during the 5 weeks of observation, the average increase in weight being from 63 to 73 g. or from 12·5 to 14·5 g. a week. In Groups 2 and 4, on the other hand, the increase in weight was markedly less.

In Group 4 the growth was so poor, the average weekly increase being only 7 g., that the observations were only continued for 4 weeks, after which the dose of vitamin B_1 was doubled. The average weekly increase then reached 11·5 g., but it was evident that growth was limited by the supply of Factor Y, for on receiving a daily dose of extract BB these rats showed a dramatic improvement and an average increase in weight of 40 g. in 2 weeks.

A comparison of the rats in Group 4 with those in Group 2 confirms the conclusion stated on p. 1773 above, that the inferior development on egg-white diets is not to be attributed to the nature of the protein given. The rats in Group 2 receiving caseinogen in the basal diet also showed a depressed

Table V. Exp. 6. *Comparison of growth of young rats on "complete" synthetic diets I and II (see p. 1765 above), with vitamin B_1 (? + B_4 Reader) provided as Peters's antineuritic concentrate from yeast and vitamin B_2 from various sources.*

All rats suffered a short preliminary period of vitamin B_1 deprivation after which they received daily:

(*a*) During Period I of 5 weeks, 2 drops daily (0·045 cc. ≡ 0·27 g. yeast) of Peters's concentrate.

(*b*) During Period II of 2 weeks, 4 drops daily (0·09 cc. ≡ 0·55 g. yeast) of Peters's concentrate.

		Litter 1849 Body weight, g.					Litter 1852 Body weight, g.					Litter 1865 Body weight, g.					All litters Av. weight increase, g.			
				Increase during					Increase during					Increase during			Period I		Period II	
Group	Diet	Rat No.	At end of fore period	Period I	Period II	Final	Rat No.	At end of fore period	Period I	Period II	Final	Rat No.	At end of fore period	Period I	Period II	Final	Total	Weekly	Total	Av. final body weight
	Diet I (caseinogen)																			
1	+vitamin B_2 as	763 ♂	52	61	31	144	773 ♂	62	78	34	174	778 ♂	55	99	27	181	73	14·5	25	♂ 166
	Extract A	764 ♀	49	64	18	131	774 ♀	59	70	18	147	779 ♀	44	66	20	130				♀ 136
2	+vitamin B_2 as	761 ♂	48	31	19	98	769 ♂	66	54	38	158	—	—	—	—	—	45	9	24	♂ 128
	Extract E	762 ♀	49	49	22	119	770 ♀	64	47	18	129	—	—	—	—	—				♀ 124
3	+vitamin B_2 as	—	—	—	—	—	*771 ♂	75	74	46	195	—	—	—	—	—	69	14	31	♂ 195
	Extract E	—	—	—	—	—	772 ♀	67	64	17	146	—	—	—	—	—				♀ 146
	+Extract BB																			
	Diet II (egg-white)																			
4	No addition	—	—	—	—	—	775 ♂	54	13†	20	87	780 ♂	55	45†	23	123	30†	7	23	♂ 105‡
	(vitamin B_2 in	—	—	—	—	—	776 ♀	54	39†	23	116	781 ♀	52	22†	25	99				♀ 107‡
	diet)																			
5	+extra vitamin	—	—	—	—	—	777 ♂	61	85	30	176	782 ♂	52	70	47	169	70	14	27	♂ 172
	B_2 as Extract E	768 ♀	53	55	15	123	—	—	—	—	—	783 ♀	56	72	17	145				♀ 134
6	+Extract BB	765 ♂	60	66	17	143	—	—	—	—	—	784 ♂	52	67	43	162	63	12·5	25	♂ 152
		766 ♀	61	57	20	138	—	—	—	—	—	785 ♀	61	61	20	142				♀ 140

* Had loose stools throughout; was a "grower."

† Experimental period I of 4 weeks only owing to cessation of growth.

‡ These rats showed an average increase of 40 g. during a subsequent extra period of 2 weeks, during which extract BB was given to provide Factor Y.

rate of growth (average weekly increase 9 g.) when vitamin B_2 was provided from an egg-white concentrate. That this was due to lack of Factor Y is proved by the much improved growth when extract BB was given in addition, as in group 3. Here the average weekly increase was 14 g.

The results of Exp. 6 indicate further that egg-white is not devoid of Factor Y but contains it in relatively small amount. The rats in Group 5 which derived all their B vitamins, other than vitamin B_1, from egg-white showed optimal growth during the period of observation, the average weekly increase being 14 g. It is calculated, however, that they ingested daily about 10–15 g. egg-white in their basal diet and the equivalent of 10 g. from the supplementary extract E.

Thus 10 g. egg-white daily appears to be inadequate (Group 2) and 20–25 g. to be necessary (Group 5) to provide sufficient Factor Y to sustain normal growth, whereas an adequate daily dose of Factor Y is contained in 0·5 g. yeast.

REFERENCES.

Chick and Copping (1930). *Biochem. J.* **24**, 932.
—— —— and Roscoe (1930). *Biochem. J.* **24**, 1748.
—— and Hume (1917). *Proc. Roy. Soc. Lond.* B **90**, 60.
—— and Roscoe (1927). *Biochem. J.* **21**, 698.
—— —— (1929, 1). *Biochem. J.* **23**, 498.
—— —— (1929, 2). *Biochem. J.* **23**, 504.
—— —— (1930). *Biochem. J.* **24**, 105.
Eijkman (1897). *Virchow's Arch.* **148**, 523.
Evans and Burr (1927). *Proc. Soc. Exp. Biol. Med.* **24**, 740.
—— —— (1928). *J. Biol. Chem.* **77**, 231.
Eddy, Gurin and Keresztesy (1930). *J. Biol. Chem.* **87**, 729.
Goldberger, Wheeler, Lillie and Rogers (1926). *U.S. Public Health Reports*, **41**, 297.
Greenman and Duhring (1923). Breeding and care of the albino rat (Wistar Institute Publications, Philadelphia).
Guha (1930). *Chem. Ind.* **49**, 850.
Hunt (1928). *J. Biol. Chem.* **79**, 723.
Kinnersley and Peters (1925). *Biochem. J.* **19**, 820.
—— —— (1927). *Biochem. J.* **21**, 777.
McCollum and Davis (1915). *J. Biol. Chem.* **23**, 181.
—— Simmonds and Pitz (1917). *J. Biol. Chem.* **29**, 521.
Mitchell (1925). *Amer. J. Physiol.* **74**, 359.
Narayanan and Drummond (1930). *Biochem. J.* **24**, 19.
Osborne and Mendel (1913). *J. Biol. Chem.* **15**, 311; **16**, 423.
Palmer and Kennedy (1927, 1). *J. Biol. Chem.* **74**, 591.
—— —— (1927, 2). *J. Biol. Chem.* **75**, 619.
—— —— (1928). *J. Biol. Chem.* **76**, 591.
Peters (1924). *Biochem. J.* **18**, 858.
—— (1929). *Nature*, **124**, 411.
Reader (1929). *Biochem. J.* **23**, 689.
—— (1930). *Biochem. J.* **24**, 77.
Roscoe (1930). *Biochem. J.* **24**, 1754.
Williams and Waterman (1927). *Proc. Exp. Biol. Med.* **25**, 1.
—— —— (1928). *J Biol. Chem.* **78**, 311.