STUDIES ON THE VITAMIN B COMPLEX*

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New developments in the vitamin B complex have recently appeared in such rapid succession that any summary of its status would be incomplete almost before it had been printed. Without further introduction, this paper briefly records the results obtained in a series of experiments on the unknown factors in the hope that the observations will be useful to others studying the same subject as theirs have been to us.

EXPERIMENTAL

The growth of rats and their skin lesions were the methods of assay employed. The basal diet was designed to contain no water-soluble vitamins that are present in the common sources of protein and carbohydrate such as casein and starch.

The adequacy of purified casein as a source of protein was tested because earlier work (2) had shown that the nutritive value of commercial casein could be lowered by heating or by extraction with 95 per cent alcohol. Since this might possibly be due to an alteration of the protein itself as well as to the removal of accessory factors, the biological value (Mitchell) of casein after several types of treatment was determined. The biological value of crude (commercial) casein, 73, was not lowered by (*a*) heating for 2 hours at 120°, 76, (*b*) extraction for 4 days with hot 95 per cent alcohol, 71, (*c*) both extraction and heating, 74. A similar value, 72, was found for casein prepared from milk by isoelectric precipitation and purified by long extractions with acidulated water (2 weeks), alcohol (1 week), and ether (1 week). These values are the average figures obtained from eight to ten animals in alternating rota-

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tion on each kind of casein, and the complete data are available. Alteration of the protein apparently is not responsible for the nutritional inadequacy of various purified caseins.

Crude casein contains some of all of the factors necessary for the rat with the exception of thiamine chloride (vitamin B_1) (see Table I, *A*). Riboflavin is present in suboptimal amounts. After a time three of the five animals developed crusted lips and noses, indicating insufficient quantities of other factors.

TABLE I

Growth of Rats on Caseins with Various Supplements

Daily supplements*	No. of rats ♂ ♀	Initial weight	Final weight	Weight gain	Time
		gm.	*gm.*	*gm.*	*days*
A. Diet containing crude casein					
Flavin + B_1	2 3	49	88	39	40
B_1		88	111	23	90
None		111	94	−17	5
B. Diet containing purified casein					
B_1, flavin, physin concentrate	4 4	45	70	25	35
" " K-P B_1	3 4	48	55	7	30
" " K-P B_4	3 4	47	67	20	35
" " heat-treated yeast	4 4	46	132	86	40
" heat-treated yeast	2 3	92	125	33	15
" " " extract	3 3	67	99	32	15
" only	3 3	99	99	0	20

* The supplements were as follows: Flavin, 13 mg. (the flavin was Booher's flavin, prepared from whey powder as described in the text); B_1, crystalline thiamine chloride, 7.5 micrograms; physin equivalent to 2.0 gm. of fresh liver; K-P B_1 concentrate equivalent to 10 to 20 gm. of fresh yeast; K-P B_4 concentrate equivalent to 2.5 to 5.0 gm. of fresh yeast.

Purified isoelectric casein was first used in the diets but later commercial casein which had been extracted for 4 days with boiling alcohol in a percolator was substituted. Responses of animals on either casein seemed the same. Hydrogenated cottonseed oil[1] was used as the source of fat, since it also supplied vitamin E which has some influence on later growth. The possible presence of a water-soluble vitamin in this fat (3) must be considered in a final analysis of our results.

[1] Crisco.

The basal diets contained the following ingredients: casein, 18 per cent; cystine, 0.4; sucrose, 55.1; salts (Hawk and Oser (4)), 4.5; fat, 20; cod liver oil, 2. Cystine was added to avoid a possible deficiency in the casein. When thiamine chloride was supplied it was given in daily doses of 7.5 micrograms of Merck's crystalline preparation either by mouth or together with other daily supplements separate from the basal diet. In the earliest experiments riboflavin was supplied as a concentrate prepared from whey powder by the method of Booher (5). The concentration was carried only through the chloroform-ethyl alcohol extraction.

Three concentrates were first tested to see if any one of them alone would permit growth when fed along with thiamine chloride and riboflavin (Table I, *B*).

Physin—In view of the possible identity of physin (6) and the Coward factor (7) a liver concentrate was prepared according to Mapson's method and tested as a supplement to thiamine chloride and riboflavin. A fair growth rate was obtained.

Peters' Eluate—This vitamin B_1 concentrate was shown by György (8) to contain vitamin B_6. As prepared from fresh starch-free yeast (Fleischmann) by the method of Kinnersley, O'Brien, Peters, and Reader (9), and here designated K-P B_1, it produced very poor growth when supplemented with thiamine chloride and riboflavin.

Vitamin B_4—Reader's vitamin B_4 (10), here called K-P B_4, was obtained from yeast during the preparation of the K-P B_1 concentrate (9), and permitted rats to grow at a fair rate when supplemented with thiamine chloride and riboflavin. This growth was comparable with that obtained with physin but much better than that with K-P B_1 even though a smaller equivalent of yeast was given.

From these results it appears that some other factor (or factors) besides thiamine chloride, riboflavin, and vitamin B_6 is necessary to complete the diet. This is present in the K-P B_4 concentrate and in physin but not to any great extent in the K-P B_1 concentrate.

Heat-Treated Yeast—On the assumption that the unknown factor is possibly heat-stable, dried yeast[2] was autoclaved for 6 hours

[2] We are indebted to Northwestern Yeast Company for liberal supplies of dry yeast.

at 15 pounds pressure and then heated dry at 120° for 36 hours. When given in daily doses of 0.5 gm. to young rats receiving thiamine chloride and riboflavin concentrate, the growth rate was over 2 gm. per day (Table I and Fig. 1, Group IV at *A*). Apparently the unknown factor and vitamin B_6 are stable to moist and dry heating. The riboflavin is likewise, since animals receiving only thiamine chloride in addition to the heat-treated yeast grew as well as those receiving riboflavin also (Group II at *E* and Table I.) A concentrate prepared by extracting the heat-treated yeast four times with boiling 80 per cent alcohol was equally as effective (Group II at *B* and Table I) as the heat-treated yeast itself.

Fractionation of Heat-Treated Yeast Extract—Since the feeding trials and the fluorescence of the extract showed that the heat-treated yeast contained large amounts of riboflavin, an attempt was made to remove it by adsorption. Bringing a concentrated extract to pH 1.0 with hydrochloric acid preliminary to adsorption produced a heavy red-brown precipitate. Treating the clear filtrate at pH 1.0 with three portions of fullers' earth left a slightly yellow solution designated non-flavin extract. The washed fullers' earths were combined and eluted with a pyridine-methanol-water mixture. This fluorescent eluate was designated flavin extract. In all experiments with the heat-treated yeast and fractions thereof, except the acid precipitate, daily doses equivalent to 0.5 gm. of yeast were fed.

Animals fed the acid precipitate with thiamine chloride failed to grow (Fig. 1, Group I at *M* and *N*). The yeast residue remaining after the alcoholic extraction, when fed with thiamine chloride, permitted maintenance of weight (Group IV at *I*), thus indicating

FIG. 1. Growth rate of rats on the vitamin B complex. The roman numbers represent groups; the figures in parentheses, initial and final weights. The supplements are represented by letters as follows: *A*, vitamin B_1 + flavin (13 mg.) + treated yeast; *B*, B_1 + treated yeast extract; *C*, B_1; *D*, B_1 + flavin (13 mg.) + non-flavin extract; *E*, B_1 + treated yeast; *F*, B_1 + flavin (39 mg.) + non-flavin extract; *G*, B_1 + flavin extract + non-flavin extract; *H*, B_1 + non-flavin extract; *I*, B_1 + yeast residue; *J*, B_1 + flavin extract; *K*, B_1 + flavin extract + yeast residue; *L*, B_1 + non-flavin extract + yeast residue; *M*, B_1 + precipitate from extract (0.5 gm. of yeast); *N*, B_1 + precipitate from extract (1.0 gm. of yeast); *O*, B_1 + flavin (13 mg.) + yeast residue; *P*, B_1 + flavin (26 mg.) + yeast residue.

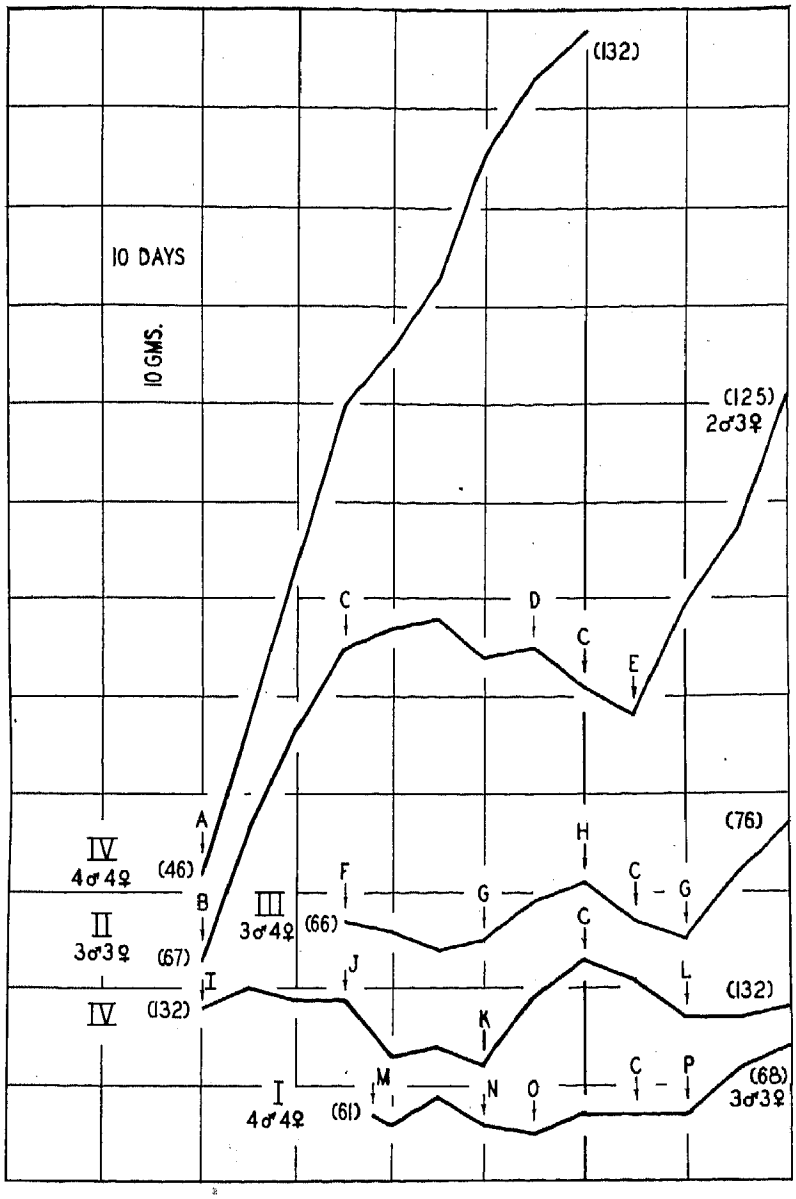
(132)
10 DAYS
10 GMS.
(125)
2♂3♀
C
D
C
E
A
(76)
H
IV
4♂4♀
(46)
F
G
C
G
B
III
3♂4♀
(66)
C
II
3♂3♀
(67)
J
L
I
(132)
IV
(132)
K
C
P
M
N
O
(68)
3♂3♀
I
4♂4♀
(61)

FIG. 1

that some activity still remained there. Apparently more of the adsorbable than of the non-adsorbable factors were extracted (Group I at *O* and *P*, Group IV at *K* and *L*).

The flavin extract when given with thiamine chloride produced loss in weight (Group IV at *J*). So did the non-flavin extract (Group III at *H*). However, when the two were fed together (Group III at *G*), growth was restored, but at a slower rate than with the unfractionated extract. This loss in total activity was probably through manipulation; the combined flavin and non-flavin portions had the qualitative properties of the whole extract.

Although animals grew when the flavin extract was fed together with the non-flavin extract (Group III at *G*), the Booher flavin preparation with the non-flavin extract resulted in a loss of weight even when 39 mg. of the Booher concentrate were supplied daily (Group II at *D* and Group III at *F*). In the adsorbable material some factor other than riboflavin was therefore present, and this factor was not present in the concentrate prepared from whey powder.

Effect of Light on Heated Yeast Extract—Hogan and Richardson (11) reported a differentiation between the antidenuding and the antidermatitic factors by means of irradiation. The former (riboflavin) was destroyed by visible or ultraviolet light, while the latter was destroyed only by the ultraviolet. In our hands this method failed to provide an identification or segregation of the factors in heat-treated yeast extract, perhaps because of inadequate exposure to visible light (two 200 watt lamps at 6 inches for 24 hours). Exposure to a mercury vapor light at 6 inches for 15 hours destroyed about half the growth-promoting activity. The factors for growth present in yeast extract are apparently not as sensitive to light as the reports in the literature would lead one to suspect. Further experience indicated that reasonable care in preventing too much exposure to light gave consistently reliable results.

Whole Yeast Fractions as Supplements to Small Amounts of Heated Yeast[3]—If the factors in yeast are not equally sensitive to heat, supplementing the heat-treated yeast with concentrates

[3] One of us (H. W. S.) conducted these experiments during the summer of 1936 at Colorado College in Colorado Springs, Colorado. We are indebted to Professor R. J. Gilmore and to Colorado College for granting facilities.

of the various factors might yield semiquantitative information as to the extent to which each of them is inactivated. Elvehjem and Koehn (12), working with chicks, fractionated liver extract by adsorption into two components, riboflavin and a factor remaining in the filtrate which they called vitamin B_2 (designated "filtrate factor" by Lepkovsky and Jukes (13)). Later Elvehjem, Koehn, and Oleson (14) reported a principle necessary for the rat (precipitate factor) in an alcohol-ether precipitate obtained in the course of this separation. After an 80 per cent alcoholic extract of dried yeast was treated twice with fullers' earth at pH 1.0, the filtrate was fractionated into alcohol-ether insoluble (Y-4) and soluble (Y-9) portions by the method of these authors (12, 14). The

TABLE II

Growth of Rats with and without Supplements Added to 0.25 Gm. of Heat-Treated Yeast Daily

Daily supplements* (55th to 85th days)	Initial weight	Weight at 55 days	Weight at 85 days	Weight change (30 days)
	gm.	*gm.*	*gm.*	*gm.*
B_1 (thiamine chloride) 7.5 micrograms	40	99	121	22
" + 22.5 micrograms B_1	39	98	127	29
" + Y-4	43	101	130	29
" + Y-9	39	96	119	23
" + Y-3	42	100	145	45

* Yeast fractions fed were equivalent to 1 gm. of dry yeast.

soluble material was purified by successive treatments with acetone, ether, and amyl alcohol and should have contained the filtrate factor. The washed fullers' earths were eluted with pyridine-methanol-water mixture to give fraction Y-3.

Young rats were fed 0.25 gm. of heat-treated yeast and 7.5 micrograms of crystalline thiamine chloride daily for 55 days. Five groups were then compounded to give an even distribution as to sex and gain in weight during this period in which the animals had been supplied with suboptimal amounts of the growth factors. Each group contained six or seven animals.

During the succeeding 30 days, in which various further supplements were given (Table II), the differences in growth rates were not striking but the animals receiving fraction Y-3 showed the

greatest gains. From this it appears that the essential material most easily destroyed by heat is adsorbed on fullers' earth at pH 1.0. The alcohol-ether-soluble (Y-9) and insoluble (Y-4) materials are little affected by the heat treatment. The response to the adsorbate was not due to additional thiamine chloride, since this by itself did not measurably improve the growth rate.

There is an alternative explanation for these results. If the filtrate factor in yeast is thermolabile, as has been claimed by some (15), then it is not necessary for growth in the rat, for it is certainly not adsorbed by fullers' earth. If the precipitate factor is thermolabile, then the very slight activity of concentrate Y-4 may be due to the adsorption of that factor on fullers' earth at pH 1.0.

Experiments with Factor 1 and Factor 2—To substantiate one or the other of the above views concentrates of Factors 1 and 2 of Lepkovsky, Jukes, and Krause (16) were prepared from air-dried yeast essentially by the method of those authors for making preparations from liver and rice bran extracts. 2 kilos of yeast were extracted three times with 6 liter portions of boiling 80 per cent alcohol. The combined extracts were concentrated *in vacuo* to 550 cc. and the solids and the fats removed by centrifugation and ether extraction. When diluted to 800 cc., the pH was 4.8. The solution was then treated twice with 100 gm. portions of fullers' earth and three times with 80 gm. portions. The filtrate was concentrated *in vacuo* to 200 cc. for feeding as Factor 2. From the fullers' earth used in the second adsorption a concentrate of Factor 1 was prepared.

Results obtained by supplementing the basal diet with various combinations are summarized in Table III. The animals on thiamine chloride, crystalline riboflavin, Factor 1, and Factor 2 (Lot 4) grew fairly well. Substituting heat-treated yeast for either Factor 1 or Factor 2 (Lots 7 and 8) produced slightly better growth, but when heat-treated yeast replaced the riboflavin (Lot 6) the growth rate was much slower. Best growth was shown by the animals fed heat-treated yeast in addition to thiamine chloride, riboflavin, Factor 1, and Factor 2 (Lot 5), suggesting that heat-treated yeast may contain yet another factor beyond those four. However, optimal amounts of Factors 1 and 2 may not have been fed in this series of experiments. The differences in

growth rates indicate that Factors 1 and 2 are present in the heat-treated yeast in rather large amounts; the riboflavin had been partially inactivated.

The various skin lesions provided further information as to the nature of these supplements. Rats which received no riboflavin (Lot 1) merely maintained their weights and all developed rough

TABLE III

Growth of Rats on Various Combinations of Thiamine Chloride, Riboflavin, Factor 1, Factor 2, and Heat-Treated Yeast

Lot No.	Supplements*	Initial: Average weight	Initial: No. of rats	40 days: Average weight	40 days: No. of rats	40 days: Weight gain	80 days: Average weight	80 days: No. of rats	80 days: Weight change
		gm.		gm.		gm.	gm.		gm.
1	B_1, Factor 1, Factor 2	39	6	46	6	7	36	1	−3
2	" riboflavin " 2	44	6	84	6	40	90	6	46
3	" " " 1	44	7	58	7	14	78	3	34
4	" " " 1, Factor 2	67	8	114	8	47	128	8	61
							50 days		
5	B_1, heat-treated yeast, riboflavin, Factor 1, Factor 2	65	8	128	8	63	143	8	78
6	B_1, heat-treated yeast, Factor 1, Factor 2	66	8	95	8	29	99	8	33
7	B_1, heat-treated yeast, riboflavin, Factor 2	66	9	126	9	60	136	9	70
8	B_1, heat-treated yeast, riboflavin, Factor 1	66	9	123	9	57	135	9	69

* Daily doses were as follows: B_1 (thiamine chloride) 7.5 micrograms; crystalline riboflavin, 10 micrograms; heat-treated yeast, 0.5 gm.; Factors 1 and 2 equivalent to 1 gm. of yeast per day.

and somewhat thinned coats. Some riboflavin was present in the concentrates of Factors 1 and 2 because weight was maintained and there was no denuding. The basal diet, when fed to young rats with crystalline thiamine chloride only, produces almost complete loss of hair in 2 weeks.

Rats which received no Factor 1 concentrate (Lot 2) grew fairly

well, but in 50 to 70 days four of the six animals developed vitamin B_6 lesions around the mouth, nose, and eyes. Those which received no Factor 2 concentrate (Lot 3) grew at a much slower rate and each of the surviving three rats developed "spectacled eyes." Although the growth of these two groups and the delayed production of symptoms show that neither concentrate contains one factor free from the other, there is an approximate separation of the two by fullers' earth adsorption at pH 4.8. The slower growth rate of Lot 2 and the faster rate of Lot 3 after the 40th day may be due to the use of new preparations of Factors 1 and 2 during that period. An English fullers' earth was used in the later experiments in place of one of unknown origin.

Except for absence of lesions on the ears, these observations suggest that Factor 1 is the vitamin B_6 of György as Lepkovsky and his coworkers believe. Since our experiments are concerned only with rats, they do not show conclusively whether Factor 2 is the filtrate factor as required by the chick or not. If the precipitate factor is also a necessary component of the vitamin B complex for the rat, as Elvehjem *et al.* believe, it must have been supplied with either Factor 1 or Factor 2.

DISCUSSION

These experiments have shown that at least four water-soluble vitamins are required by the rat. Two of these, thiamine chloride and riboflavin, were supplied as crystalline preparations. The other two, Factor 1 and Factor 2, were used in the form of fairly pure concentrates prepared from yeast. Factor 1 is apparently vitamin B_6 and Factor 2 is either the precipitate factor or the filtrate factor.

No evidence has been presented here to show that vitamin B_4 (Reader) is not required by the rat. If the dietary fat[1] contained this vitamin, as Kline, Bird, Elvehjem, and Hart (3) believe, then it was supplied to all of our animals. The growth of rats fed crystalline thiamine chloride and riboflavin (Booher) together with either the K-P B_4 concentrate or the physin concentrate may have been due to the vitamin B_4 in these latter two preparations. The experiments with Factors 1 and 2 more logically suggest that these preparations contained, in addition to vitamin B_6, the active constituent of Factor 2.

An active filtrate containing Factor 2 could be prepared from an extract of air-dried yeast adsorbed with fullers' earth at pH 4.8. When adsorption was carried out at pH 1.0, however, a purified concentrate of the filtrate factor was inactive; the alcohol-ether-insoluble material obtained during purification (which should contain the precipitate factor) was only slightly active. One might therefore conclude that the filtrate factor is not required by the rat and that most of the precipitate factor had been adsorbed at pH 1.0. Our method for demonstrating the inactivity of a purified concentrate of the filtrate factor is valid, because Keenan *et al.* (15) and Lepkovsky and Jukes (13) have shown that this factor *as required by the chick* is inactivated in yeast and dietary foodstuffs by dry heat.

A fullers' earth adsorbate prepared from yeast extract at pH 1.0 would therefore contain riboflavin, vitamin B_6, and the active constituent of Factor 2. Prepared at pH 4.8, it would contain only riboflavin and vitamin B_6. By analogy the same principle probably holds for adsorptions on charcoal; the greater activity of the K-P B_4 concentrate over that of the K-P B_1 concentrate in our first experiments appears to be due to its greater content of both vitamin B_6 and the constituent of Factor 2.

A summary, by no means complete, of reported adsorptions for the various factors of the vitamin B complex is given in Table IV. Obviously, success in the satisfactory adsorption of any one factor depends upon the physical and chemical state of the factor in the solution, the reaction of the solution, and the adsorbent (see Halliday and Evans (22) and Birch and György (21)).

Although riboflavin and vitamin B_6 are apparently adsorbed at any acid pH, no evidence is at hand for adsorption of either the filtrate factor or the precipitate factor on fullers' earth. Recently Edgar and Macrae (24) have reported that a rat factor remains in the filtrate after an autoclaved yeast extract is treated with fullers' earth at pH 1.4, 8, or 10. This confirms our observation that heat-treated yeast extract, after adsorption at pH 1.0, yields an active filtrate. Being unable, however, to prepare active concentrates of either the filtrate factor or the precipitate factor after adsorption (at pH 1.0) of an unheated yeast extract, we believe that the active constituent of Factor 2 essential to the rat shows a differential adsorption according to the reaction of the solution and

the state in which the factor exists in the heated and unheated sources. Differences in fullers' earths may also be concerned here.

Contamination of fullers' earth eluates with Factor 2, in the hands of Lepkovsky, Jukes, and Krause (16), may have been due to adsorption as they suggest; had they adsorbed from a more

TABLE IV

Adsorption of Vitamin B Complex

Factor	Adsorption on fullers' earth	Adsorption on charcoal	Authority
Thiamine chloride	pH 4.5 (yeast)		Guha (17)
	" 4.5 (rice polish)		Williams, Waterman, and Keresztesy (18)
		pH 7.0 (yeast)	Kinnersley, O'Brien, Peters, and Reader (9)
		Slightly, pH 1 (yeast)	" "
Riboflavin	pH 1.0 (milk, etc.)		Kuhn, György, and Wagner-Jauregg (19)
	pH 1–8 (liver)		Lepkovsky, Popper, and Evans (20)
Vitamin B_6 (Factor 1)	" 2.5 and 5.0 (wheat germ)		Birch and György (21)
	Not at pH 9.0 (wheat germ)		" "
	pH 4.0 (liver dialysate)		Halliday and Evans (22)
	pH ? (liver)		Lepkovsky and Jukes (23)
	" 5.2–5.4* (rice bran)		Lepkovsky, Jukes, and Krause (16)
		pH 7.0 (yeast)	György (8)
		Not at pH 6.0 (herring extract)	Birch and György (21)
Filtrate factor (chick)	Not in acid (liver)		Elvehjem and Koehn (12)
	Not at pH 5.6–5.8* (liver)		Lepkovsky, Jukes, and Krause (16)
		No, pH ? (liver)	Lepkovsky and Jukes (13)

TABLE IV—*Concluded*

Factor	Adsorption on fullers' earth	Adsorption on charcoal	Authority
Filtrate factor (rat)	Not at pH 5.6–5.8* (liver)		Lepkovsky, Jukes, and Krause (16)
	Not at pH 1.4, 8, 10 (heated yeast)		Edgar and Macrae (24)
		pH 1.2, 2.5 (heated yeast)	" "
		Slightly, pH 7, 8.2 (heated yeast)	" "
	Not at pH 1.0 (heated yeast)		Present report
	pH 1.0 (yeast)		" "
	Not at pH 4.8 (yeast)		" "
		pH 1.0 (yeast)	" "
		Slightly, pH 7.0 (yeast)	" "
Precipitate factor (rat)	No, pH ? (liver)		Frost and Elvehjem (25)
		pH ? (liver)	Elvehjem, Koehn, and Oleson (14)
Vitamin B_4		" 1.0 (yeast)	Kinnersley, O'Brien, Peters, and Reader (9)
		Slightly, pH 7.0 (yeast)	" "

* Personal communication from T. H. Jukes.

acid solution, all of Factor 2 (the rat essential) might have been removed. Our experiments certainly show that separation of the two factors is incomplete by their method.

The filtrate factor as assayed with chicks is not adsorbed by charcoal (13). The precipitate factor, however, is adsorbed (14), and so is the factor discussed by Edgar and Macrae. This fact, together with our demonstration of the inactivity of a concentrate of the filtrate factor and variances in reports on heat stability, suggests that Factor 2 probably contains two essentials, one required by the rat, the other by the chick. It must be added, however, that the properties given by Edgar and Macrae for the rat factor conform quite well with those for the chick factor as given by Lepkovsky and Jukes (13).

Although we obtained very poor growth with the Peters' eluate (K-P B_1), György (8) was able to produce fairly good growth with thiamine chloride, riboflavin, and Peters' eluate when a vitamin B-free basal diet was used. Knowing, as we do now, that thiamine chloride, riboflavin, and vitamin B_6 are not the only factors necessary for the rat, the Peters' eluate used by György must have contained the active constituent of Factor 2 along with vitamin B_6; our own preparation contained small amounts. This factor could be present in the eluate because Edgar and Macrae have shown that it is not precipitated by lead acetate and that it is adsorbed slightly on charcoal at pH 7.0.

György (8) has also found that the Bourquin-Sherman diet (containing wheat extract) supplemented with riboflavin will not permit rats to grow. However, if either the Peters' eluate or a yeast extract from which the riboflavin has been removed by fullers' earth was also supplied, growth did result. These experiments indicate that some factor is adsorbed on charcoal but not on fullers' earth. The limiting factor here seems not to be vitamin B_6, which is probably present in the wheat extract, but rather the constituent of Factor 2.

According to a recent report by Booher (26), her whey powder concentrate (vitamin H) contains the combined growth and antidermatitis factors other than thiamine chloride and riboflavin. When we fed this preparation with thiamine chloride and K-P B_1 or with thiamine chloride and the non-flavin extract of heat-treated yeast, there was little or no growth. No suitable explanation of this discrepancy can be offered at present.

SUMMARY

1. By careful adsorption of an 80 per cent alcohol extract of yeast it has been demonstrated that besides thiamine chloride and riboflavin at least two additional factors are necessary for growth and well being in the rat.

2. One of these, Factor 1, is the "vitamin B_6" of György and is necessary for growth and prevention of dermatitis. It is adsorbed on fullers' earth at pH 4.8 and 1.0 from a yeast extract.

3. The second of these, Factor 2, is probably the "precipitate factor" of Elvehjem, Koehn, and Oleson, and is essential for growth. It is adsorbed from an unheated yeast extract by fullers'

earth at pH 1.0 but not completely at pH 4.8. As present in an extract of heat-treated yeast it appears not to be adsorbed by fullers' earth. With charcoal it is adsorbed at pH 1.0 and slightly at pH 7.0.

4. "Vitamin B_2" (filtrate factor) of Elvehjem and Koehn is not necessary for growth in the rat. A concentrate of Factor 2 probably contains it.

5. Both Factor 1 (vitamin B_6) and Factor 2 (precipitate factor), as present in dried yeast, are stable to autoclaving for 6 hours at 15 pounds pressure followed by dry heating for 36 hours at 120°.

6. Riboflavin, as present in dried yeast, is less stable to the same heat treatment.

7. A summary and discussion of the adsorption characteristics of the components of the vitamin B complex are given.

8. By various methods of purification commercial casein can be made more suitable for use in basal diets for studies on the vitamin B complex without altering its nutritive value as a protein. The biological value was not lowered by (*a*) extraction for 4 days with hot 95 per cent alcohol, (*b*) heating for 2 hours at 120°, (*c*) extraction and heating. The same biological value was found for casein prepared from milk by isoelectric precipitation and purified by long extractions with acidulated water, alcohol, and ether.

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