CXIX. THE WATER-SOLUBLE B-VITAMINS

IX. PROPERTIES OF THE DIETARY FACTOR CONTAINED IN THE FULLER'S EARTH FILTRATE FROM AUTOCLAVED YEAST EXTRACTS

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IN a previous paper of this series [1937], dealing with the vitamin B₂ complex, we described the separation from autoclaved yeast extract of two heat-stable dietary factors essential for nutrition of the rat in addition to lactoflavin. One of these factors was adsorbed when yeast extract was treated with fuller's earth at pH 1.4 and was afterwards eluted from the fuller's earth with Ba(OH)₂; this we have called the yeast fuller's earth eluate factor. The second constituent was present in the filtrate after fuller's earth treatment of the yeast extract; this we have called the yeast fuller's earth filtrate factor.

The present paper contains the results of an investigation of the physical and chemical properties of the filtrate factor, and of an attempt to obtain it in a purified state. A comparison is also given of its properties with those of other heat-stable factors of the vitamin B complex described by other workers.

EXPERIMENTAL

Biological tests were the only possible method of tracing the active fractions through the various processes employed. The criterion used was the stimulation of growth in young rats on diets containing vitamin B₁ and lactoflavin. When the greater part of this work was carried out we had not succeeded in separating the eluate factor, and therefore the increases in weight following administration of the filtrate factor are probably not as striking as they would have been if the eluate factor had been supplied in the basal diet. They were, however, definite enough to enable us to trace the presence of the filtrate factor in the materials tested.

Young rats were prepared for the routine testing of the fuller's earth filtrate factor from yeast by the method described in the preceding papers [Edgar *et al.* 1937; Edgar & Macrae, 1937]. The same basal diet was used and the rats each received 0.2 ml. of cod liver oil and 10γ of vitamin B₁ hydrochloride daily or 0.05–0.1 ml. of Peters's vitamin B₁ concentrate. After being constant in weight for several days, they received the test doses supplemented by 12γ daily of lactoflavin; 3 rats were used for each test and the growths were observed for a period of 4 weeks. The results were generally taken as positive when the average weekly weight increase was 10 g. or more. As shown in the previous communication, 12γ daily of lactoflavin alone produces weight increases of approximately 6 g. weekly for a period of 3 weeks. Some variation has been noted occasionally in

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the growth responses of rats from different litters, but within a litter, growth responses were fairly regular, and therefore the different preparations obtained from one fractionation were tested on litter-mates, one rat of the litter being given lactoflavin alone as a control. More stress was laid on the relative growths shown by litter-mates dosed with the various fractions and the lactoflavin control rat than on the actual increases in weight obtained.

The fuller's earth filtrate was prepared as described by Edgar *et al.* [1937] by extraction of 1 l. of aqueous yeast extract (1 ml. = 0.5 g. yeast, dry wt.), autoclaved for 5 hr. at 120° at pH 5, adjusted to pH 1.4, with 2 portions of 50 g. each of fuller's earth.

The experimental details of all the processes investigated are given below, and the results of the animal tests are given in Table I. Full descriptions are given in the hope that they may be useful to other workers and prevent misunderstanding.

Exp. 1. Stability. (a) To acid. To 200 ml. of the fuller's earth filtrate 10 g. of concentrated H_2SO_4 were added and the mixture was immersed for 2 hr. in a vigorously boiling water-bath. The sulphuric acid was removed with $\text{Ba}(\text{OH})_2$. Some darkening occurred during the heating, but there was no loss of activity.

(b) To alkali. 20 g. of very finely ground $\text{Ba}(\text{OH})_2 \cdot 8\text{H}_2\text{O}$ were added to 200 ml. of fuller's earth filtrate previously adjusted to pH 8 by the addition of $\text{Ba}(\text{OH})_2$. The mixture was heated as above for 2 hr., after which the $\text{Ba}(\text{OH})_2$ was removed with H_2SO_4 . No darkening occurred, and the vitamin was unaffected.

Exp. 2. Stability to light. (a) Sunlight. Clear glass bottles containing fuller's earth filtrate adjusted to pH 3 and pH 10 were exposed daily during 3 summer months to direct sunlight, thereby receiving about 200 hr. of sunshine. Controls in dark glass bottles wrapped in brown paper were also kept under the same conditions. All solutions at the end of this period were fully active.

(b) Ordinary electric light. Fuller's earth filtrate was exposed at pH 3 or 10 for 24 hr. in very thin layers at a distance of 21 cm. from an Osram 500 W. gas-filled clear lamp, the solutions being kept cool by a fan. A control solution at pH 10 was not irradiated. There was no destruction of vitamin by this irradiation either at pH 3 or 10.

(c) Ultraviolet light. Very thin layers of fuller's earth filtrate at pH 3 and 10 were placed 14 cm. distant from a quartz mercury arc lamp and irradiated for 10 hr., cooling being effected by standing the dishes in running water. Control solutions at pH 3 and 10 were also exposed with thick glass screens between the lamp and the solutions. There was no destruction of vitamin.

Exp. 3. Adsorption experiments. (a) Fuller's earth at pH 10 and 8. 100 ml. portions of fuller's earth filtrate were adjusted to pH 8 and approx. 10. Each portion was treated twice with 10 g. portions of fuller's earth (B.D.H. "for adsorption purposes"). Both filtrates were fully active, showing that little or no vitamin had been adsorbed.

(b) $\text{Al}(\text{OH})_3\text{C}\gamma$. The adsorbent was prepared according to the method of Willstätter *et al.* [1925]. 200 ml. of fuller's earth filtrate, adjusted to pH 4, were cooled in ice and treated with 27 ml. of a suspension of $\text{Al}(\text{OH})_3\text{C}\gamma$ (1 ml. = 18.5 mg. Al_2O_3). After shaking for 10 min. the $\text{Al}(\text{OH})_3$ was centrifuged out. The adsorption was repeated 4 times. The total adsorbate was washed with 50 ml. $M/10$ acetate buffer at pH 4 and suspended in 100 ml. $M/4$ Na_2HPO_4 . After 14 hr. the $\text{Al}(\text{OH})_3$ was centrifuged down and again eluted with an equal volume of Na_2HPO_4 . The eluates were mixed. No activity was detectable in the eluate whilst the filtrate appeared to have its full activity.

(c) *Norite charcoal*. 400 ml. of fuller's earth filtrate were adjusted to pH 2.5 with HCl and treated 4 times with 5 g. portions of norite. The norite was washed once with $N/10$ HCl and then eluted with 250 ml. of 0.25 N NaOH, being allowed to stand overnight. The elution was repeated with an equal volume of 0.25 N NaOH. In another experiment elution was accomplished by heating the norite on a water-bath with glacial acetic acid.

Further adsorptions with norite were carried out at pH 8.2, 7 and 1.2, also using 0.25 N NaOH as eluant. Adsorption was best at pH 2.5 and 1.2. At pH 8.2 and 7 little adsorption occurred. Good elution was obtained with NaOH or glacial acetic acid, but in one experiment using 0.1 N $\text{Ba}(\text{OH})_2$ as eluant, the eluate was inactive.

Exp. 4. Electrodialysis. The alcohol-soluble portion from 400 ml. of fuller's earth filtrate, dissolved in 800 ml. H_2O (pH 2.6), was put in the centre chamber of a 3-chambered cell divided by cellophane membranes which were permeable to the factor [Chick *et al.* 1935]. The anode and cathode chambers were filled with distilled water and all chambers were cooled by coils through which cold water flowed. The current was approximately 1–1.5 amp. and the voltage was 100–200 V. After $1\frac{1}{2}$ hr. fresh water was put in the anode and cathode chambers and the dialysis continued for a further $5\frac{1}{2}$ hr. No attempt was made to regulate the pH in the chambers. The solution in the centre chamber remained at approximately pH 2.6. The pH of the anode solution fell to 2.4 while that of the cathode solution rose to approximately 11 before the fluid in the chambers was changed and to pH 9.2 after the fresh water had been added. At the end of the dialysis approximately 65% of the nitrogen in the original material had migrated to the cathode, while 5% was in the anode solution. The remainder was in the centre chamber solution. The animal tests were not very satisfactory, but it appeared that most of the activity remained in the centre chamber.

Exp. 5. Sublimation in high vacuum. 400 ml. of fuller's earth filtrate were adsorbed with norite charcoal at pH 2.5 and the activity eluted with 0.25 N NaOH as described in Exp. 3 (c). The eluate, adjusted to pH 7, was evaporated to dryness *in vacuo* and the residue extracted with boiling 96% alcohol. The alcohol was removed and the residue transferred to a vacuum tube, which was then kept in a vacuum desiccator over P_2O_5 for 2 days. A tube cooled by a rapid flow of cold water was inserted inside the vacuum tube, which was evacuated to 0.05 mm. The apparatus was heated in a metal-bath to 150° for 4 hr. The vacuum remained good during the heating. A partially crystalline acid sublimate was obtained on the surface of the cooled tube. This was biologically inactive, even when large doses were fed. There was some charring of the residue, but it retained some vitamin activity.

Exp. 6. Solubilities. (a) 96% Ethyl alcohol. 140 ml. of fuller's earth filtrate were evaporated to dryness on the water-bath and finally in a desiccator. The hard resin-like material was divided as finely as possible and extracted for 20 hr. with 96% ethyl alcohol in a Soxhlet extractor. The alcohol was removed from the extract *in vacuo* and the remaining gum was taken up in water. The greater part of the activity was found in the alcohol-soluble portion.

In a second experiment, the gum which separated from the alcohol extract as the extraction proceeded was removed and tested separately; it contained no activity.

(b) *Acetone*. Dried fuller's earth filtrate was extracted with purified acetone in a Soxhlet extractor for 30 hr. The extract was quite inactive even when fed in large amounts. The portion insoluble in acetone was active.

Table I. *Results of tests of fractions prepared from fuller's earth filtrate from yeast*

Each rat received 10 γ of vitamin B ₁ hydrochloride or 0.05-0.1 ml. of Peters's vitamin B ₁ concentrate and 12 γ lactoflavin daily								
Exp. no.	Treatment	Fraction	Equivalent of dry yeast given daily g.	Average weekly increase in wt. over 4 weeks in wt. g.	No. of rats	Average weekly increase in wt. over 3 weeks of litter control rat receiving lactoflavin only g.	Result of test	
1 (a)	Heating with 5% H ₂ SO ₄ for 2 hr.	—	0.5	11.5	3	7.6	+	
	Heating with 10% Ba(OH) ₂ , 8H ₂ O for 2 hr.	—	0.5	12.2	3		+	
2 (a)	Visible light (3 months in sun, pH 3)	—	0.5	13.3	2	5.3, 8.0	+	
	Control at pH 3	—	0.5	12.75	1		+	
	3 months in sun, pH 10	—	0.5	12.5	2		+	
	Control at pH 10	—	0.5	12.75	2		+	
(b)	24 hr. under 500 W. lamp, pH 3	—	0.5	10.75	3	5.6, 3.0	+	
24 hr. under 500 W. lamp, pH 10	—	0.5	12.3	3	+			
(c)	Ultraviolet light, 8 hr. at pH 3	—	0.5	11.4	3	8.6	+	
	Control at pH 3	—	0.5	11.4	3		+	
	8 hr. at pH 10	—	0.5	12.6	3		+	
	Control at pH 10	—	0.5	12.2	3		+	
3 (a)	Adsorption with fuller's earth at pH 8	Filtrate	0.5	12.5	4	7.7	+	
	Adsorption with fuller's earth at pH 10	Filtrate	0.5	10.2	4		+	
(b)	Adsorption with Al(OH) ₃ C γ at pH 4	Filtrate	0.5	11.7	3	7.4	+	
		Na ₂ HPO ₄ eluate	0.5	8.5	2		-	
(c)	Adsorption with norite charcoal, pH 2.5	Filtrate	1.0	12.4	3	6.3	+	
		NaOH eluate	1.0	11.5	3	5.6	+	
		Acetic acid eluate	1.0	11.9	3	6.3	+	
		Ba(OH) ₂ eluate	1.0	7.6	3	—	-	
	pH 1.2	Filtrate	1.0	11.4	3	5.0	+	
		NaOH eluate	1.0	13.2	3		+	
	pH 7.0	Filtrate	0.5	16.0	2	7.4	+	
		pH 8.2	Filtrate	1.0	10.8	3	7.3	+
	4	Electrodialysis pH 2.6	NaOH eluate	1.0	8.4	3		-
			Centre	1.0	8.6	3	6.3	±
Anode			1.0	5.4	3	-		
		Cathode	1.0	7.3	3	-		

5	Sublimation <i>in vacuo</i> at 150°	Sublimate Residue	10.0 2.0	2.0 8.6	1 2	*	-
6 (a)	Extraction of dried fraction with 96% ethyl alcohol	Extract Residue	0.5 0.5	13.3 9.6	4 } 3 }	8.5	±
(b)	Extraction of dried fraction with acetone	Sparingly soluble fraction	1.0	5.9	2	5.7	-
(c)	Precipitation with 10 vol. of acetone	Extract Residue	2.0 0.5	6.5 9.1	2 } 3 }	4.0	+
7 (a)	Precipitation with picric acid	Filtrate Precipitate	2.0 2.0	10.9 9.7	2 } 3 }	7.0	±
(b)	Precipitation with picrolonic acid	Filtrate Precipitate	1.0 1.0	10.3 7.5	3 } 5 }	7.3	+
(c)	Precipitation with Reinecke acid	Filtrate Precipitate	1.0 1.0	12.3 4.4	3 } 3 }	7.0	-
(d)	Precipitation with flavianic acid	Precipitate	1.0	4.3	3	—	-
(e)	Precipitation with phosphotungstic acid	Filtrate Precipitate	1.0 1.0	13.8 6.4	3 } 3 }	7.7	+
8 (a)	Precipitation with copper acetate	Filtrate	0.5	13.1	3	8.7	+
(b)	Precipitation with lead acetate (pH 4)	Filtrate Precipitate	1.0 1.0	8.0 1.8	3 } 3 }	2.7	-
(c)	Precipitation with mercuric sulphate	Filtrate Precipitate	0.5 0.5	11.2 9.1	3 } 2 }	4.7	±
(d)	Precipitation with silver sulphate	Filtrate Precipitate	0.5 0.5	11.75 7.8	2 } 2 }	—	+
(e)	Precipitation with Ba(OH) ₂ in 90% alcohol	Filtrate Precipitate	0.5 0.5	10.1 7.2	2 } 3 }	8.7	-
9	Precipitation with Ba(OH) ₂ in 80% alcohol	Filtrate Precipitate	1.0 1.0	12.1 8.7	3 } 3 }	9.7	+
	Treatment with acetic anhydride	Water-insoluble fraction Water-soluble fraction	2.0 1.0	7.7 10.0	3 } 3 }	7.3	-
			1.0 1.0	9.3 9.0	3 } 3 }	3.7	+
			1.0 1.0	6.4 8.4	3 } 3 }	5.7	±

* This rat received 12.7 lactoflavin daily for 3 weeks and grew an average of 3 g. weekly; after receiving the sublimate it grew only 4 g. in 19 days.

(c) *Precipitation with acetone.* 100 ml. of a solution of the vitamin were prepared by elution with glacial acetic acid of a norite adsorbate obtained from 600 ml. of fuller's earth filtrate and were mixed with 1 l. of acetone. After standing overnight in the cold the acetone solution was decanted from the gummy precipitate. Acetone was removed from both fractions *in vacuo*. Some activity appeared to be present in the precipitate, but the greater part was found in the soluble portion.

Exp. 7. Precipitation with precipitants for bases. (a) *Picric acid.* 400 ml. of fuller's earth filtrate were adjusted to pH 7.2 and a hot solution of 7 g. of picric acid in 100 ml. H_2O added. After standing overnight in the cold the crystalline picrate precipitate was filtered off and the excess picric acid was extracted from the acidified filtrate with ether. The picrate precipitate was decomposed by suspending in dilute HCl and extracting with ether. There was no activity in the decomposed precipitate while the filtrate was active.

(b) *Picrolonic acid.* 400 ml. of fuller's earth filtrate were mixed with a solution of 5 g. of picrolonic acid in 100 ml. of 96 % alcohol (pH of the mixture was 4.5). The heavy precipitate was filtered after standing overnight in the cold, the filtrate freed from picrolonic acid with ether by the usual method and the picrolonate precipitate decomposed with acid and extracted with ether. All the vitamin activity was in the filtrate.

(c) *Reinecke acid.* 15 g. of Reinecke acid in 300 ml. H_2O at 60° were added to 400 ml. of fuller's earth filtrate (pH 4.5). On standing, a heavy precipitate was deposited, which was filtered off, after being kept overnight in the cold, and dissolved in 100 ml. of 50 % acetone; 130 ml. of 1 % Ag_2SO_4 were added to the solution. The Ag reineckeate was filtered off, the acetone removed on a water-bath and the excess Ag precipitated with HCl. This decomposed precipitate was inactive when fed to animals.

(d) *Flavianic acid.* 25 g. of flavianic acid, dissolved in 125 ml. H_2O , were added to 400 ml. of fuller's earth filtrate, NaOH being added to pH 4.5. A heavy precipitate was obtained, which was filtered off after standing overnight in the cold. The filtrate was treated with 100 g. of basic lead acetate and the pH adjusted to 8, the lead salts were filtered off and excess lead removed from the filtrate with H_2S . The flavianic acid precipitate was decomposed by dissolving in H_2O and adding basic lead acetate at pH 8. The filtrate had full biological activity, whilst the decomposed precipitate was inactive. Filtrates from flavianic acid precipitations at more acid reactions were also active.

(e) *Phosphotungstic acid.* 200 ml. of fuller's earth filtrate were mixed with a solution of 40 g. of purified phosphotungstic acid in 200 ml. H_2O and conc. H_2SO_4 was added to pH 1. After standing 24 hr. with occasional stirring, the precipitate was filtered off. The filtrate, after removal of the excess phosphotungstic acid by the usual means, was found to have vitamin activity approximately equal to that of the original fuller's earth filtrate.

Exp. 8. Treatment with precipitants for acids. (a) *Copper acetate.* 30 g. of copper acetate dissolved in 300 ml. of hot water were added to 400 ml. of fuller's earth filtrate at pH 4. A heavy precipitate was obtained which was filtered off after standing overnight in the cold. The precipitate was decomposed with H_2S , but was found to contain no biological activity. The filtrate, freed from Cu with H_2S , was active.

(b) *Basic lead acetate.* 100 ml. of fuller's earth filtrate were adjusted to pH 8 and 110 ml. of a 10 % solution of basic lead acetate were added. After 16 hr. in the cold the precipitate was filtered off and, after washing, was decomposed with H_2S . The filtrate was also treated with H_2S . The activity was again found

in the filtrate. In earlier experiments treatment with lead acetate at pH 4 did not precipitate the activity.

(c) *Mercuric sulphate*. 200 ml. of fuller's earth filtrate were treated with 125 ml. of 10% mercuric sulphate in 3 N H_2SO_4 . After standing overnight in the cold, the precipitate was filtered off. The precipitate and filtrate were treated with H_2S and sulphate was removed from the filtrate. The decomposed precipitate was inactive and the filtrate active.

(d) *Silver sulphate at pH 7*. 400 ml. of fuller's earth filtrate at pH 7 were shaken with 20 g. of finely ground Ag_2SO_4 for 6 hr. at room temperature. The pH was maintained at 7 by the addition of small quantities of $NaOH$. After standing overnight in the cold a dark brown precipitate was filtered off; silver ions were present in the filtrate. The precipitate was decomposed with H_2S and the filtrate was also treated with H_2S . The activity was present in the filtrate.

(e) *Barium hydroxide in 90% ethyl alcohol*. The glacial acetic acid eluate, obtained by adsorbing 600 ml. of fuller's earth filtrate with norite and eluting as described under Exp. 3 (c), was freed from acetic acid and taken up in 100 ml. of H_2O . 900 ml. of absolute ethyl alcohol were added and then 0.3 N $Ba(OH)_2$ very gradually, the mixture being stirred vigorously and alcohol added to maintain the alcohol concentration at 90%. After the addition of approximately 140 ml. of the $Ba(OH)_2$ solution no further precipitate appeared on addition of more $Ba(OH)_2$; the pH was 8.5. After keeping overnight in the cold the precipitate was filtered off, washed with alcohol, dissolved in water and the barium precipitated with H_2SO_4 . The alcohol was removed from the filtrate *in vacuo* and the excess barium precipitated. The greater part of the activity was present in the decomposed precipitate, whilst the filtrate was inactive. In another experiment, the precipitation was carried out in 80% alcohol and the activity was shared about equally between the filtrate and precipitate.

Exp. 9. Effect of heating with acetic anhydride. The alcohol-soluble portion from 400 ml. of fuller's earth filtrate was dried to a gummy residue; 5 g. of anhydrous sodium acetate and 100 g. of freshly distilled acetic anhydride were added, and the mixture was heated under anhydrous conditions for 3 hr. on a water-bath; the gum gradually dissolved. The acetic anhydride was then removed *in vacuo* on a water-bath. 150 ml. H_2O were added and the gum partially dissolved. The insoluble portion was thoroughly extracted with water and hydrolysed in the cold with 0.5 N $NaOH$. The water-insoluble acetyl fraction was not active, whilst the fraction which was water-soluble after treatment with acetic anhydride showed some activity, although it was difficult to test since it contained a great deal of acetate.

DISCUSSION

A summary of the results of this work and of those of other workers is given in Table II. Our yeast fuller's earth filtrate factor was not destroyed by visible or ultraviolet light or by heating with strong acid or alkali. It was adsorbed by relatively large amounts of norite charcoal, the adsorption being more complete from acid solution; no adsorption occurred by treatment with fuller's earth at various reactions or with aluminium hydroxide at pH 4. The activity dialysed freely through a cellophane membrane, but little or no activity migrated to the cathode when a solution at pH 2.6 was electrodialysed. The factor was readily soluble in ethyl alcohol, it did not sublime when the neutral gum left after

Table II. *Comparison of properties of factors supplementing lactoflavin, found by different workers*

Authors	Source of factor	Method of test	Effect of light	Electro-dialysis	Fuller's earth	Norite	Phospho-tungstic acid	Ba(OH) ₂ in alcohol	Basic lead acetate
György <i>et al.</i> [1934]	Yeast	Rat growth	—	—	Some ad-sorption	—	Inactivated	—	—
György [1935]	Wheat germ Fish muscle	Cure of rat dermatitis	Destroyed	Migration to cathode	Adsorbed	Not adsorbed	Precipitated	—	Not precipitated
Birch & György [1936]									
Jansen <i>et al.</i> [1935]	Yeast	Rat growth	Experiments differ	—	Not ad-sorbed	Adsorbed from alkaline solution	—	—	Precipitated
Hogan & Richardson [1936]	Yeast, Rice bran extract	Cure of rat dermatitis	Not destroyed	—	Adsorbed	—	—	—	—
Lepkovsky & Jukes [1936]	Liver	Chick growth	Not destroyed*	—	Not ad-sorbed	Not adsorbed	Not pre-cipitated	Not precipitated in 80% alcohol	Not pre-cipitated
Edgar & Macrae	Yeast	Rat growth	Not destroyed	No migration to cathode	Not ad-sorbed	Adsorbed	Not pre-cipitated	Partially precipitated in 80% alcohol. Precipitated in 90% alcohol	Not pre-cipitated

— = not tested.

* Elvehjem & Koehn [1935].

evaporation of the alcohol was heated in a high vacuum at 150°. It was precipitated by barium hydroxide from 90% alcoholic solution. It was insoluble in acetone, but addition of 10 vol. of acetone to an aqueous solution only partially precipitated the activity. No activity was precipitated by the following reagents: picric acid, picrolonic acid, Reinecke acid, flavianic acid, phosphotungstic acid and salts of copper, silver, lead and mercury. The factor did not give a water-insoluble derivative when treated with acetic anhydride.

The following is a short summary of the properties of the constituents of the vitamin B complex, other than vitamin B₁ and lactoflavin, described by other workers.

György *et al.* [1934] reported that a rat growth factor supplementing lactoflavin was present in Peters's "B₁+B₄" concentrate and in aqueous yeast extracts which had been treated with fuller's earth. This factor was not precipitated by mercury salts but was precipitated by picric acid; it was partially adsorbed by fuller's earth, some activity being found in the pyridine eluate from the fuller's earth adsorbate. Wheat germ and fish muscle autolysates and other food materials were investigated by György [1935] and Birch & György [1936] as sources of "vitamin B₆", which was assayed by its power of curing the dermatitis which developed in rats receiving lactoflavin alone of the B₂-vitamin complex. The activities of the preparations were destroyed by light and the factor was adsorbed by fuller's earth at pH 2.5 and 5.0 but not by norite charcoal at pH 6. It was precipitated by phosphotungstic acid but not by picric acid or by salts of mercury, silver and lead; benzylation inactivated the factor; on electrodialysis it migrated to the cathode. Jansen *et al.* [1935] reported that a factor present in aqueous yeast extracts, necessary for growth of rats receiving vitamin B₁ and lactoflavin, was precipitated by silver nitrate or by lead acetate in neutral or alkaline solution, but not by mercury or copper salts; it was not adsorbed by fuller's earth, but was adsorbed by large quantities of supranorite from alkaline solution. Hogan & Richardson [1936] stated that the factor present in yeast or rice bran extracts which cured the dermatitis developing in rats receiving a fat-free diet supplemented by lactoflavin was adsorbed on fuller's earth and was not destroyed by light.

A dietary factor present in the fuller's earth filtrate from aqueous liver extracts was first described by Elvehjem & Koehn [1935] and later investigated by Lepkovsky & Jukes [1936]; Lepkovsky *et al.* [1936] and Jukes [1937]. This "filtrate factor" was identified by its growth-promoting action when fed to chicks receiving a heated grain diet [Kline *et al.* 1932] supplemented by lactoflavin; it also cured the dermatitis which chicks developed on this diet and had a growth-promoting action for rats receiving a synthetic diet supplemented by lactoflavin, but it did not cure rat dermatitis. This "filtrate factor" was not precipitated by phosphotungstic acid, by barium hydroxide in 80% alcohol or by basic lead acetate; it was not adsorbed by fuller's earth or norite; it was readily soluble in ethyl alcohol and was extracted from acid solution by butyl or amyl alcohol; it was comparatively stable to oxidizing agents and was not destroyed by nitrous acid or by treatment with benzoyl chloride.

Comparison of the properties of our yeast fuller's earth filtrate factor with those of "vitamin B₆" as described by Birch & György [1936] show the two to be distinct from each other. "Vitamin B₆" had basic properties, as shown by its migration to the cathode on electrodialysis and by its precipitation with phosphotungstic acid; our yeast filtrate factor, on the other hand, appears to have no basic properties, and its precipitation by barium hydroxide in alcohol suggests an acidic nature. The differences recorded in the behaviours of the two

factors on exposure to light and to treatment with fuller's earth also show that they are not identical. Similarly there are marked differences between our yeast fuller's earth filtrate factor and those described by Jansen *et al.* and Hogan & Richardson.

On the other hand a similarity exists between our factor contained in the fuller's earth filtrate from yeast and the liver "filtrate factor" of Lepkovsky & Jukes [1936]. The latter also had acidic properties, as indicated by the fact that it could be extracted from acid solution by amyl alcohol and removed from the amyl alcohol extract by dilute NaOH [Elvehjem & Koehn, 1935]. The behaviour of the two factors to phosphotungstic acid and other precipitants are also in agreement. Lepkovsky & Jukes [1936] did not observe adsorption of their "filtrate factor" by norite, while our yeast factor was adsorbed by norite, but under very different experimental conditions from those used by Lepkovsky *et al.* The filtrate factor from yeast was partially precipitated by barium hydroxide in 80 % alcohol, but that from liver was not precipitated by this treatment.

SUMMARY

1. A description is given of the behaviour to many physical and chemical agents of the heat-stable constituent of the vitamin B complex, necessary for the growth of rats, found in aqueous autoclaved yeast extracts after repeated adsorption with fuller's earth in acid solution.

2. This yeast extract filtrate factor is probably acidic in character since it is precipitated by barium hydroxide in 90 % alcohol.

3. Comparison of the properties of this factor suggest that it is distinct from "vitamin B₆" described by György, but perhaps identical with the "filtrate factor" from liver extracts described by Lepkovsky *et al.*

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