LXXIII. THE WATER-SOLUBLE B-VITAMINS

XIII. ALLOXAZINE-ADENINE-DINUCLEOTIDE, ADENYLIC ACID, NICOTINAMIDE AND PIMELIC ACID IN THE NUTRITION OF THE RAT

By M. M. EL-SADR,¹ T. F. MACRAE AND C. ELIZABETH WORK²

From the Division of Nutrition, Lister Institute, London

(Received 1 March 1939)

RECENT investigations carried out in this laboratory have shown that the vitamin B requirements of the rat are satisfied only when the animals receive aneurin, riboflavin and three additional heat-stable factors belonging to what is known as the vitamin B₂ complex [Edgar *et al.* 1938, 3]. One of these factors, named yeast eluate factor, is contained in eluates prepared from fuller's earth adsorbates of autoclaved aqueous yeast extracts and has been identified [El-Sadr *et al.* 1939] with Lepkovsky's factor 1 [Lepkovsky, 1938] and György's vitamin B₆ [György, 1938, 1; Keresztesy & Stevens, 1938; Kuhn & Wendt, 1938]. A second factor, which so far has not been isolated in a pure state, is present in fuller's earth filtrates of autoclaved aqueous yeast extracts and in certain preparations from liver, and has been named yeast or liver filtrate factor according to the source. The existence of a third factor is indicated by the fact that rats receiving crude extracts of yeast or liver, as source of the vitamin B complex, increased in body-weight more rapidly than did rats receiving adequate amounts of aneurin, riboflavin, yeast eluate factor and yeast filtrate factor. Concentrates of this third factor have now been prepared by a method which will be described in a later paper.

A description is here given of experiments in which we studied the possibility of replacing for the nutrition of the rat certain of the above-recognized members of the vitamin B₂ complex by substances which, in other types of investigations, have been found to be biologically important. We have examined (A) alloxazine-adenine-dinucleotide, (B) a combination of yeast adenylic acid and nicotinamide and (C) pimelic acid.

A. *Alloxazine-adenine-dinucleotide.* Warburg & Christian [1938, 1] have recently isolated from liver and yeast a riboflavin-containing dinucleotide (alloxazine-adenine-dinucleotide), which is the coenzyme of *d*-amino-acid dehydrogenase, of xanthine dehydrogenase and of other enzymes [Warburg & Christian, 1938, 2; Ball, 1938; Haas, 1938]. It was to be expected that this important coenzyme would replace riboflavin in the diet of the rat, and it seemed possible that it might also possess the vitamin activity of some other member of the vitamin B complex. We have been able to investigate this problem through the kindness of Prof. Warburg, who put an adequate amount of the coenzyme at our disposal.

B. *Combination of yeast adenylic acid and nicotinamide.* Nicotinic acid or nicotinamide has been shown to be an essential nutrient for man [Fouts *et al.* 1937; Smith *et al.* 1937; Spies *et al.* 1938], the dog [Elvehjem *et al.* 1937], the pig [Chick *et al.* 1938, 1] and the monkey [Harris, 1938]. Frost & Elvehjem [1937]

¹ Mission Member of the Egyptian University.

² Formerly C. Elizabeth Edgar

reported that nicotinamide and adenylic acid from yeast or heart had growth-promoting properties when administered, either separately or simultaneously, to rats receiving a basal diet which contained 12 % of white corn. Later, however, Oleson *et al.* [1939] found no growth-stimulating action of nicotinic acid when given to rats on an entirely "synthetic" diet containing riboflavin. Euler & Malmberg [1936] stated that adenylic acid had no effect on the growth-rate of rats receiving a diet containing riboflavin, but that nicotinamide prolonged the life of rats receiving riboflavin and a yeast fuller's earth filtrate. More recently cozymase was found to have a growth-stimulating effect when administered to rats receiving riboflavin and a fuller's earth filtrate of an autoclaved acidulated yeast extract [Euler *et al.* 1938].

In this laboratory we were unable to demonstrate that nicotinamide, nicotinic acid or codehydrogenase II were dietary essentials for the rat [Macrae & Edgar, 1937]. Cook *et al.* [1937] also failed to find that nicotinic acid alone or in combination with adenylic acid had any vitamin-like action in the rat. Chick *et al.* [1938, 2] noted no increased growth when rats fed on a maize diet received nicotinamide, and Chick (unpublished experiments) was also unable to demonstrate that nicotinamide had a growth-stimulating effect when fed to rats receiving a purified synthetic basal diet containing sucrose as source of carbohydrate. Helmer & Fouts [1938], on the other hand, reported that nicotinic acid did increase the growth-rate of rats receiving a maize diet containing riboflavin and liver filtrate fraction; György [1938, 2] has attributed an anaemia of rats, termed panmyelophthisis, to deficiency of nicotinic acid in the diet. Dann & Subbarow [1938] stated that nicotinic acid given to rats receiving a diet containing riboflavin had no growth-promoting action and did not prevent rat dermatitis, i.e. could not replace vitamin B₆.

In the present paper we report an extension of our former experiments [Macrae & Edgar, 1937] by investigating a combination of yeast adenylic acid and nicotinamide for vitamin activity.

C. *Pimelic acid*. Since the chemical behaviour of yeast filtrate factor proved it to be acidic in character [Edgar & Macrae, 1937, 2], it was thought possible that it might be identical with pimelic acid, a growth factor for *C. diphtheriae* [Mueller, 1937]. We have therefore tested pimelic acid for filtrate factor activity.

METHODS AND RESULTS

Growth of young rats was the criterion of vitamin activity employed; the basal diet and general experimental procedure have been described in previous papers from this laboratory [Chick *et al.* 1935; Edgar *et al.* 1937; 1938, 1]. All animals received 10 µg. aneurin and 0.08 ml. cod liver oil daily from the time of weaning until a body-weight of 100 g. was attained; the daily supplements were then increased to 15 µg. and 0.1 ml. respectively.

Synthetic aneurin and riboflavin were used throughout and the purified preparations of yeast and liver eluate factor and of yeast filtrate factor were those described in earlier papers [Edgar *et al.* 1937; Edgar & Macrae, 1937, 1; Edgar *et al.* 1938, 1, 2].

A. *Investigation of the vitamin B₂ activity of alloxazine-adenine-dinucleotide*

The sample of alloxazine-adenine-dinucleotide which we received from Prof. Warburg was a preparation of the monobarium salt obtained from yeast; it contained about 5 % of impurities. The material was fed to rats as the sodium salt, prepared by the addition of Na₂SO₄ to a solution of the barium salt.

Four separate experiments were carried out. Groups of rats received one of the following combinations of vitamin B₂ factors: (a) yeast eluate fraction and yeast filtrate fraction, (b) riboflavin and yeast eluate fraction, (c) riboflavin and yeast filtrate fraction, (d) riboflavin, yeast eluate and yeast filtrate fractions. The effect of the addition to the diets of alloxazine-adenine-dinucleotide on the growth-rate of the rats in these groups was observed.

(a) *Addition of alloxazine-adenine-dinucleotide to the diet containing yeast eluate factor and yeast filtrate factor (Table I A).*

These experiments were carried out to determine how efficiently the coenzyme replaced riboflavin in the diet of the rat.

Previous experiments had shown that rats receiving diets containing yeast filtrate and yeast eluate fractions, but no riboflavin, failed to grow, but gained rapidly in weight when riboflavin was subsequently added to the diet [cf. Edgar & Macrae, 1937, 1 (Fig. 2)]. The following experiment carried out in a similar manner with alloxazine-adenine-dinucleotide instead of riboflavin, indicated that this coenzyme could replace riboflavin in the diet of the rat. Two rats which received only yeast eluate and filtrate fractions as sources of the vitamin B₂ complex increased in weight at an average rate of 2.1 g. weekly for 3 weeks; after receiving an additional daily supplement of an amount of alloxazine-adenine-dinucleotide equivalent to 20 μ g. riboflavin, the average weekly gain in weight during two subsequent weeks was 15.0 g.

A quantitative comparison of the vitamin activities of riboflavin and the coenzyme was, however, thought desirable.

The curve of response of body-weight increase to riboflavin dosage in animals receiving yeast eluate and yeast filtrate fractions as sources of the supplementary factors of the vitamin B₂ complex is a logarithmic one, similar to that obtained when yeast filtrate fraction is the only supplement given [Edgar *et al.* 1937]. We found optimum growth only when 40 μ g. riboflavin daily were administered to each rat; the growth-rate, however, was most sensitive to small changes in the riboflavin content of the diet when the animals received only about one-quarter of the optimal amount.

Accordingly, the relative vitamin potencies of riboflavin and alloxazine-adenine-dinucleotide were determined by comparing the increase in body-weight of rats receiving only 6 or 12 μ g. of riboflavin with that of animals receiving amounts of coenzyme equivalent to 6 or 12 μ g. of riboflavin.

Animals from two litters of eight rats each received at weaning the basal diet supplemented by aneurin and cod liver oil. For the first few days the animals increased slightly in body-weight but this increase ceased after 7–10 days. Four of the animals from one of the litters each now received daily doses of yeast filtrate fraction purified by amyl alcohol extraction (=2 g. dry yeast), yeast eluate fraction (=2 g. dry yeast) and an amount of alloxazine-adenine-dinucleotide equivalent to 6 μ g. riboflavin. Their four litter-mates received the same doses of yeast filtrate and eluate fractions but, instead of the coenzyme, 6 μ g. of riboflavin daily. The rats of the second litter were similarly treated, but received double the amounts of coenzyme and riboflavin. The growth-rate of all rats was observed for 4 weeks after the supplements were given.

All rats showed immediate growth responses when the supplements were added (Table I A). The four animals receiving 6 μ g. of riboflavin daily maintained an average growth-rate of 8.3 g. weekly for the 4-week period, and those receiving an equivalent amount of coenzyme gained an average of 7.4 g. weekly. Similarly,

Table I. *Effect of alloxazine-adenine-dinucleotide on the growth-rate of rats receiving aneurin and various other factors of the vitamin B complex*

A						
No. of rats	Daily supplements of the vitamin B complex			Av. weekly wt. increases of group during 4 weeks after giving supplements g.	Mean weekly wt. increase g.	
	Yeast eluate factor = 2 g. dry yeast	Yeast filtrate factor = 2 g. dry yeast	6 μ g. riboflavin			
4	"	"	Alloxazine-adenine-dinucleotide = 6 μ g. riboflavin	11.2, 6, 8.2, 8	8.3	
4	"	"	12 μ g. riboflavin	9.5, 4, 8.5, 7.8	7.4	
4	"	"	Alloxazine-adenine-dinucleotide = 12 μ g. riboflavin	19, 14, 14.5, 12.5	15.0	
4	"	"	Alloxazine-adenine-dinucleotide = 12 μ g. riboflavin	18.5, 15.2, 12.2, 9.8	13.9	
B						
No. of rats	Daily supplements during preliminary period of 2 weeks	Av. weekly wt. increases during preliminary period g.	Mean weekly wt. increase g.	Additional supplement added daily (=S)	Av. weekly wt. increases during 2 weeks after giving S' g.	Mean weekly wt. increase g.
4	50 μ g. riboflavin + yeast eluate factor = 2 g. dry yeast	18.5, 9.8	—	Alloxazine-adenine-dinucleotide = 20 μ g. riboflavin	9.0, 5.5	7.2
2	"	21.5, 8.5	—	Alloxazine-adenine-dinucleotide = 40 μ g. riboflavin	11.5, 2.5	7.0
4	"	20.2, 10	—	None	9.5, 5	7.2
1	"	20, 12	—	Yeast filtrate factor = 1 g. dry yeast	17, 18	17.5
C						
3	50 μ g. riboflavin + yeast filtrate factor = 1 g. dry yeast	16, 13.3	14.6	Alloxazine-adenine-dinucleotide = 24 μ g. riboflavin	13, 13.7	13.3
2	"	14.5, 12.5	13.5	Liver eluate factor = 12 g. fresh liver	23.5, 22.5	23
D						
No. of rats	Daily supplements of the vitamin B ₂ complex	Alloxazine-adenine-dinucleotide given daily	Equivalent of 24 μ g. riboflavin	Av. weekly wt. increases of group during 4 weeks after giving supplement g.	Mean weekly wt. increase g.	
4	50 μ g. riboflavin + eluate factor = 2 g. dry yeast + yeast filtrate factor = 2 g. dry yeast	None	None	19.8, 20.5, 20.5, 21.2	20.5	
3	"	"	"	22, 19.3, 21, 22.7	21.2	

the two groups of animals receiving daily $12\mu\text{g.}$ of riboflavin or alloxazine-adenine-dinucleotide equivalent to that amount of riboflavin increased in body-weight at approximately the same rate, the average weekly weight increases being 15 g. and 13.9 g. respectively. It is therefore concluded that, when administered orally, alloxazine-adenine-dinucleotide replaces riboflavin in the diet of the rat, and that equimolecular amounts of the coenzyme and riboflavin have the same vitamin potency.

(b) Addition of alloxazine-adenine-dinucleotide to the diet containing riboflavin and yeast eluate factor (Table IB).

Eleven rats received for 1 week from weaning the basal diet supplemented by aneurin and cod liver oil, and during a further preliminary period of 2 weeks each received daily, in addition, $50\mu\text{g.}$ riboflavin and yeast eluate fraction equivalent to 2 g. dry yeast. During the subsequent test period of 2 weeks, four of the animals received the additional supplement of an amount of alloxazine-adenine-dinucleotide equivalent to $20\mu\text{g.}$ riboflavin daily, two received an amount equivalent to $40\mu\text{g.}$ riboflavin, one serving as a positive control received an amount of yeast filtrate factor not purified by amyl alcohol extraction equivalent to 1 g. dry yeast, while four rats served as negative controls and received no further supplement. In this experiment the addition of alloxazine-adenine-nucleotide to the diet had no effect in restoring growth in the six rats receiving it (Table IB), the weekly weight increases being no greater than those of the negative controls. In the positive control rat, however, the growth-rate was restored.

(c) Addition of alloxazine-adenine-dinucleotide to the diet containing riboflavin and yeast filtrate factor (Table IC).

Five rats were maintained for 1 week from weaning on the basal diet supplemented by aneurin and cod liver oil, then for the further preliminary period of 2 weeks each received additional daily supplements of $50\mu\text{g.}$ riboflavin and of an amount of yeast filtrate factor not purified by amyl alcohol extraction equivalent to 1 g. dry yeast. During the subsequent test period of 2 weeks, daily supplements of the coenzyme equivalent to $24\mu\text{g.}$ riboflavin were given to three of the rats, and eluate fraction, equivalent to 12 g. fresh liver, to the other two animals which served as positive controls. The addition of the coenzyme caused no increase in the growth-rate, while the addition of the eluate fraction caused the usual marked increase (Table IC).

(d) Addition of alloxazine-adenine-dinucleotide to the diet containing riboflavin, yeast eluate factor and yeast filtrate factor (Table ID).

Seven male litter-mate rats at weaning received the basal diet supplemented by aneurin and cod liver oil for 1 week. Four of the animals then each received daily $50\mu\text{g.}$ riboflavin, yeast eluate fraction equivalent to 2 g. dry yeast, yeast filtrate fraction purified by amyl alcohol extraction equivalent to 2 g. dry yeast, and alloxazine-adenine-dinucleotide equivalent to $24\mu\text{g.}$ riboflavin. Three animals which served as negative controls received all these fractions except the coenzyme. There was no significant difference in the rates of growth of the two groups (Table ID), the average weekly gains in weight during a 4-week period being 20.5 and 21.2 g. respectively.

The usual weekly weight increase of similar male rats receiving unfractionated extracts of yeast or liver as sources of the whole vitamin B_2 complex is more than 30 g. The amounts of riboflavin and yeast filtrate and eluate factors administered were more than enough to supply the animals' needs of those factors; this experiment proves, therefore, (a) that at least one factor of the vitamin B_2 complex,

in addition to those factors, is required by the rat and (b) that alloxazine-adenine-dinucleotide cannot replace this additional factor.

The above experiments indicate that the only vitamin activity possessed by alloxazine-adenine-dinucleotide is that which it has by virtue of the riboflavin it contains bound in its molecule, and that when it is administered orally its vitamin potency is identical with that of an equimolecular amount of riboflavin.

Warburg & Christian [1938, 2] suggest that alloxazine-adenine-dinucleotide is probably the form in which riboflavin functions in the animal body, but it is also possible that riboflavin phosphoric acid or free riboflavin or other unknown compounds of riboflavin may be concerned with processes in the living cell. These experiments show, however, that riboflavin and alloxazine-adenine-dinucleotide, when administered orally to the rat, are converted with equal efficiency into the compound or compounds which function in the animal's tissues.

B. Investigation of the vitamin B₂ activity of a combination of yeast adenylic acid and nicotinamide

The yeast adenylic acid was obtained from British Drug Houses, Ltd., and the nicotinamide from Messrs Hoffmann La Roche. These compounds were tested for vitamin activity by administration to rats receiving supplements of (a) riboflavin, (b) riboflavin and yeast eluate fraction and (c) riboflavin and yeast filtrate fraction.

(a) Addition of yeast adenylic acid and nicotinamide to the diet containing riboflavin (Table II A).

Six litter-mate rats received for 1 week from weaning the basal diet supplemented by aneurin and cod liver oil. Three animals which served as negative controls then each received a daily supplement of 50 μ g. riboflavin only, while the remaining three animals were each given daily 50 μ g. riboflavin, 2 mg. yeast adenylic acid and 2 mg. nicotinamide; the doses of the last two of these compounds after 1 week were changed to 1 mg. of each daily. The body-weights of the animals were observed for 6 weeks (Table II A). The average total weight increase during the 6 weeks subsequent to the animals receiving adenylic acid and nicotinamide was 25 g., while the corresponding average for the control rats was 33 g. This difference, however, cannot be regarded as significant and there was no apparent difference in the general condition of the rats of the two groups; all the animals were much undersized but were active. Two rats of the control group receiving only riboflavin developed the characteristic florid rat dermatitis [Copping, 1936] after 6 and 15 weeks respectively from the beginning of the experiment, while two of the animals receiving riboflavin, adenylic acid and nicotinamide developed the dermatitis after 8 and 15 weeks respectively. Addition of adenylic acid and nicotinamide therefore did not influence either the growth of the rats or the development of dermatitis.

(b) Addition of yeast adenylic acid and nicotinamide to the diet containing riboflavin and yeast eluate factor (Table II B).

Four litter-mate female rats received at weaning the basal diet supplemented by aneurin and cod liver oil and for a preliminary period of 2 weeks additional supplements were given of 50 μ g. riboflavin and yeast eluate fraction equivalent to 2 g. dry yeast. Three of the rats then each received for a further period of 3 weeks 2 mg. yeast adenylic acid and 2 mg. nicotinamide daily, while one animal received an amount of yeast filtrate fraction not purified by amyl alcohol extraction equivalent to 1 g. dry yeast. This last animal showed the usual growth

Table II. *Effect of a combination of yeast adenylic acid and nicotinamide on the growth-rate of rats receiving daily 10-15 μ g. aneurin, 50 μ g. riboflavin, and various other factors of the vitamin B₂ complex*

A		Av. weekly wt. increases of group during 6 weeks after dosing g.	
No. of rats	Daily supplements		
3	2 mg. adenylic acid + 2 mg. nicotinamide*	10.3, 4.7, 3.7, 2.3, 2.3, 1.7	
3	None	15, 6.7, 5.0, 4.0, 1.0, 1.3	
B		Av. wt. increase during week after giving S ₂ g.	
No. of rats	Daily supplements given during preliminary period of 2 or 3 weeks	Av. weekly increases during 2 or 3 weeks after giving S ₁ g.	Further supplement added (S ₂)
3	Yeast eluate factor = 2 g. dry yeast	14.0, 9.3	Yeast filtrate factor = 1 g. dry yeast
1	"	16.0, 7.0	—
C. Exp. 1		Av. wt. increase during week after giving S ₂ g.	
3	Yeast filtrate factor = 1 g. dry yeast	20.3, 21, 13.7	Yeast eluate factor = 2 g. dry yeast
4	Yeast filtrate factor = 1 g. dry yeast	19.2, 15.2	—
2	"	18.5, 16.0	—
2	"	21.5, 15.0	—

* After 1 week supplements of adenylic acid and nicotinamide reduced to 1 mg. of each daily.

response, but no increased growth-rate followed the administration to the test animals of adenylic acid and nicotinamide (Table IIB). At the end of the 3-week period addition of yeast filtrate fraction to the diet of these animals caused a striking increase in growth-rate, indicating that these amounts of adenylic acid and nicotinamide, although unable to stimulate growth, did not inhibit the growth of the animals when the missing nutrient was supplied. The increase in growth-rate caused by addition of filtrate factor, however, was not as great as that which follows administration of unfractionated yeast extracts to such animals; therefore, these compounds cannot replace that factor of the vitamin B₂ complex required in addition to riboflavin, eluate factor and filtrate factor.

(c) Addition of yeast adenylic acid and nicotinamide to the diet containing riboflavin and yeast filtrate fraction (Table IIC).

Three rats which had received the basal diet with aneurin and cod liver oil for 1 week from weaning were each given daily supplements of 50 μ g. riboflavin and an amount of yeast filtrate fraction not purified by amyl alcohol extraction equivalent to 1 g. dry yeast for a period of 3 weeks; 2 mg. yeast adenylic acid and 2 mg. nicotinamide were then given daily for a further period of 3 weeks, but there was no increase in the growth-rate; increased growth-rate, however, did occur when a daily ration of yeast eluate fraction equivalent to 2 g. dry yeast was added (Table IIC, Exp. 1).

In a second experiment eight litter-mate rats, treated as usual for the first week after weaning, each received daily 50 μ g. riboflavin and an amount of yeast filtrate fraction equivalent to 1 g. dry yeast for a period of 2 weeks. Four of the animals then each received the additional daily supplements of 2 mg. adenylic acid and 2 mg. nicotinamide for 3 weeks, two negative control animals received no additional supplement during that period and two positive control animals received yeast eluate fraction equivalent to 2 g. dry yeast daily. The animals receiving the adenylic acid and nicotinamide increased in body-weight at approximately the same rate as the negative controls while the growth-rates of the two positive control rats increased from an average of 18.2 g. weekly to 30 g. weekly on addition of the eluate factor (Table IIC, Exp. 2).

The results of the above experiments afford no evidence that yeast adenylic acid and nicotinamide administered simultaneously have any growth-promoting activity for rats deprived of the yeast eluate factor (vitamin B₆) or yeast filtrate factor of the vitamin B₂ complex; these compounds do not prevent rat dermatitis. We obtained no evidence that alloxazine-adenine-dinucleotide has any nutritional activity other than that due to the riboflavin it contains. Since this coenzyme contains also adenylic acid in its molecule, these experiments also suggest that adenylic acid is not an essential nutrient for the rat; additional support for this view was provided by experiments previously published which showed that codehydrogenase II (adenine-nicotinamide-dinucleotide) had no demonstrable nutritive value for rats [Macrae & Edgar, 1937]. It is of course possible that if nicotinamide and yeast adenylic acid were required by the rat in small amounts these might be contained in our purified yeast fractions or in our basal diet in such amounts that the rats were independent of a further supply, but it is at least certain that these compounds do not replace our yeast filtrate or yeast eluate factors in the diet of the rat. Recent experiments not reported and those described above also indicate that nicotinamide and adenylic acid cannot replace the additional unidentified factor which, with riboflavin, eluate

factor and filtrate factor, satisfies the vitamin B₂ requirements of the rat. The question whether nicotinamide and adenylic acid are essential nutrients for the rat will be decided only when all other factors are available in a pure state.

We have made no attempt to repeat the experiments of Frost & Elvehjem [1937], who found that a combination of yeast or heart adenylic acid and nicotinamide had very marked growth-promoting properties for rats receiving a diet which differed from that used by us principally in that it contained 12 % of white corn. The possibility cannot be excluded that white corn may have some property which renders the rat capable of benefiting from the administration of adenylic acid and nicotinamide; it appears to us, however, that the introduction of such complicated materials as cereals into basal diets used in the study of the different members of the vitamin B complex renders interpretation of the results difficult.

C. Investigation of pimelic acid for yeast filtrate factor activity

Pimelic acid was administered in the form of its sodium salt, which we received from Dr G. H. Jeffrey. The test for filtrate factor activity was carried out as previously described [Edgar *et al.* 1938, 1]. Three rats received none of the components of the vitamin B complex except aneurin for the first week after weaning and then riboflavin and eluate factor were added for a period of 2 weeks during which their weekly weight increases averaged 16.6 and 10 g. respectively. For a further period of 2 weeks the animals then received the additional supplement of 2 mg. pimelic acid, and the average growth-rates continued to fall to 4 and 1 g. weekly. Two positive controls which received an additional supplement of yeast filtrate factor when the other animals received the pimelic acid, increased in body-weight by approximately 20 g. weekly for the period of 2 weeks during which they were observed. Pimelic acid therefore does not replace filtrate factor in the diet of the rat.

SUMMARY

1. Alloxazine-adenine-dinucleotide could replace riboflavin in the diet of the rat, equimolecular amounts of these compounds having the same nutritive potency. It had no demonstrable nutritive value other than its activity as a substitute for riboflavin.

2. A combination of adenylic acid and nicotinamide had no growth-promoting properties for rats receiving diets deficient in the yeast eluate factor (vitamin B₆) or the yeast filtrate factor of the vitamin B₂ complex, and with rats deprived of eluate factor did not influence the development of dermatitis.

3. Pimelic acid did not replace yeast filtrate factor in the diet of the rat.

We wish to thank Dr H. Chick for her interest and advice, Prof. O. Warburg who kindly presented the alloxazine-adenine-dinucleotide, Prof. A. R. Todd for the aneurin, Dr G. H. Jeffrey for the sodium pimelate, Dr M. Guggenheim of Messrs Hoffmann La Roche for the riboflavin and nicotinamide, and Dr B. K. Blount of Messrs Glaxo Laboratories, Ltd., for the liver extract used in the preparation of the liver eluate fraction. The yeast used in these experiments, as in previous experiments from this laboratory, was kindly given by Messrs Watney, Coombe and Reid.

REFERENCES

- Ball (1938). *Science*, **88**, 131.
Chick, Copping & Edgar (1935). *Biochem. J.* **29**, 722.
—— Macrae, Martin & Martin (1938, 1). *Biochem. J.* **32**, 10.
—— ——— (1938, 2). *Biochem. J.* **32**, 844.
Cook, Clark & Light (1937). *Proc. Soc. exp. Biol., N.Y.*, **37**, 514.
Copping (1936). *Biochem. J.* **30**, 845.
Dann & Subbarow (1938). *J. Nutrit.* **16**, 183.
Edgar & Macrae (1937, 1). *Biochem. J.* **31**, 886.
—— ——— (1937, 2). *Biochem. J.* **31**, 893.
—— El-Sadr & Macrae (1938, 1). *Biochem. J.* **32**, 2200.
—— ——— (1938, 2). *Biochem. J.* **32**, 2225.
—— ——— (1938, 3). *Chem. Ind.* **57**, 1111.
—— Macrae & Vivanco (1937). *Biochem. J.* **31**, 879.
El-Sadr, Macrae & Work (1939). *Biochem. J.* **33**, 611.
Elvehjem, Madden, Strong & Woolley (1937). *J. Amer. chem. Soc.* **59**, 1767.
Euler, Heiwinkel, Malmberg, Robeznicks & Schlenk (1938). *Ark. Kemi Min. Geol.* **12** A, no. 25.
—— & Malmberg (1936). *Biochem. Z.* **284**, 455.
Fouts, Helmer, Lepkovsky & Jukes (1937). *Proc. Soc. exp. Biol., N.Y.*, **37**, 405.
Frost & Elvehjem (1937). *J. biol. Chem.* **121**, 255.
György (1938, 1). *J. Amer. chem. Soc.* **60**, 983.
—— (1938, 2). *Proc. Soc. exp. Biol., N.Y.*, **37**, 732.
Haas (1938). *Biochem. Z.* **298**, 378.
Harris (1938). *Biochem. J.* **32**, 1479.
Helmer & Fouts (1938). *J. Nutrit.* **16**, 271.
Keresztesy & Stevens (1938). *Proc. Soc. exp. Biol., N.Y.*, **38**, 64.
Kuhn & Wendt (1938). *Ber. dtsh. chem. Ges.* **71**, 780.
Lepkovsky (1938). *J. biol. Chem.* **124**, 125.
Macrae & Edgar (1937). *Biochem. J.* **31**, 2225.
Mueller (1937). *J. Bact.* **34**, 163.
Oleson, Bird, Elvehjem & Hart (1939). *J. biol. Chem.* **127**, 23.
Smith, Ruffin & Smith (1937). *J. Amer. med. Ass.* **109**, 2054.
Spies, Cooper & Blankenhorn (1938). *J. Amer. med. Ass.* **110**, 622.
Warburg & Christian (1938, 1). *Biochem. Z.* **298**, 150.
—— ——— (1938, 2). *Biochem. Z.* **298**, 369.