70. RELATION OF SKIN LESIONS IN THE RAT TO DEFICIENCY IN THE DIET OF DIFFERENT B₂-VITAMINS

By HARRIETTE CHICK, T. F. MACRAE AND ALASTAIR N. WORDEN¹

*From the Division of Nutrition, Lister Institute, London, and
Roebuck House, Cambridge*

(Received 1 March 1940)

A NOTICEABLE feature about the earlier studies of the "dermatitis" occurring in rats when they are deprived of the heat-stable B-vitamins has been the irregularity of its incidence. The occurrence of two different types of skin lesions developing under these conditions was foreshadowed in earlier work [Chick & Roscoe, 1928, p. 795; Chick & Copping, 1930, p. 933] and has been more clearly demonstrated in recent investigations. As the members of the vitamin B₂ complex have gradually been separated from one another, and since vitamin B₁ has been available in the purified state, it has been possible to use basal diets supplemented with relatively pure preparations of one or other constituent [see György, 1934; Chick *et al.* 1935; Copping, 1936].

Type (a) is a florid dermatitis, roughly symmetrical, with swelling and redness of the digits of the paws and of the ears, "spreading over the limbs and trunk with cracking and desquamation of the skin", the so-called "rat pellagra" originally described by Goldberger & Lillie [1926] and called "rat acrodynia" by György [György *et al.* 1937]. In the present paper the expression "dermatitis" is reserved for this skin disease.

The lesions of type (b) occur more particularly in the skin over the head and chest with loss of fur and without swelling or inflammation. The fur becomes very scanty, especially over the head and forearms, and appears moist and matted, with bald patches of skin developing later. The eyelids and the nostrils become inflamed and the former are often stuck together with a serous, reddish fluid, which also exudes from the nostrils. The wrists become stained with this fluid from continual rubbing against the nose and eyes, and patches of fur round the vagina or penis also become stained by soiling with the urine.

With the discovery that riboflavin was a nutritionally active constituent of autoclaved yeast extracts and that the vitamin B₂ complex could be separated into riboflavin and a "supplementary substance", evidence was soon forthcoming that type (a) skin lesions developed on diets containing riboflavin and that riboflavin had curative value for skin lesions of type (b) [György, 1934; Copping, 1936].

Similarly, type (a) dermatitis yielded to treatment with the "supplementary factor" in the vitamin B₂ complex, which was called vitamin B₈ by György [1934]. This substance was found present in the "Peters's eluate" from charcoal as prepared from yeast extract. It was later found to be adsorbed from yeast on fuller's earth [Birch & György, 1936] and has since been separated in the pure crystalline state [Lepkovsky, 1938; György, 1938, 2; Kuhn & Wendt, 1938;

¹ Research Student in Animal Health, Ministry of Agriculture.

Keresztesy & Stevens, 1938; El Sadr *et al.* 1939, 2]. Its constitution has been investigated and its synthesis has also been accomplished [Harris & Folkers, 1939]. It has been called adermin but is usually known as vitamin B₆.

Chick *et al.* [1935] had also demonstrated the existence of a heat-stable substance which stimulated the growth of rats receiving vitamin B₁ and riboflavin, but this substance was present in the filtrate after adsorption of an autoclaved yeast extract with fuller's earth. At that time we assumed that this substance was identical with György's vitamin B₆ and we called it by that name [Chick *et al.* 1935; Copping, 1936]. This material, however, in contradistinction to György's, had no consistent curative effect on the florid dermatitis (type *a*); in fact in some cases the condition deteriorated after its administration [Copping, 1936].

Later investigations of the vitamin B₂ complex have demonstrated the existence of at least two heat-stable substances in yeast extract in addition to riboflavin, both of which are required to supplement vitamin B₁ in the diet of the rat [see Edgar & Macrae, 1937]. One, originally called the "yeast eluate" factor and now known to be identical with vitamin B₆ [El Sadr *et al.* 1939, 2] is adsorbed on fuller's earth together with riboflavin; the second, the "yeast filtrate factor" is in the filtrate. Similar fractions have been prepared by numerous workers from liver, yeast and rice polishings. Separation has been achieved by means of fuller's earth in almost all these cases, the one factor (vitamin B₆) being adsorbed and subsequently eluted and the second factor being found in the filtrate.

It is obvious, therefore, that the "supplementary substance" investigated by Chick *et al.* [1935] and by Copping [1936] contained not György's vitamin B₆, as was supposed, but the filtrate factor. Its failure to act upon rat dermatitis was therefore not inconsistent with the observations of György on "vitamin B₆".

It was later found that vitamin B₆ is present in relatively high concentrations in cereals and cereal products [Birch *et al.* 1935]. Copping [1936] found that rat dermatitis, which was untouched by her "supplementary factor" from yeast, was swiftly cured by an alcoholic extract of whole maize or wheat. These facts may be important in explaining the irregular incidence of dermatitis in the earlier work on this subject, since the basal diets almost invariably contained starch of cereal origin, which may well have been contaminated with vitamin B₆ (see also below, p. 591). Substitution of starch by sugar in the diet was found to favour the occurrence of dermatitis [Chick *et al.* 1935]. With a diet containing sugar as source of carbohydrate and with the purified "Peters's eluate" previously given as source of vitamin B₁ replaced by pure crystalline aneurin, as in the experiments recorded below, rat dermatitis is almost invariably developed in the absence of the yeast eluate factor (vitamin B₆).

It should be noted that more recent work in this laboratory [El Sadr *et al.* 1939, 1], carried out since the present experiments were arranged and after most of them were completed, has shown that the "yeast filtrate factor" is itself complex and contains at least two at present unidentified nutrients essential for the rat: one, extractable with amyl alcohol, is the factor studied in this paper and called the "purified filtrate factor", the other is non-extractable with amyl alcohol. It has been shown further that at least one vitamin belonging to the vitamin B₂ group is contained in whole liver in addition to riboflavin and the nutrients present in the yeast eluate fraction (vitamin B₆) and in the yeast filtrate fractions. These facts must be remembered in drawing conclusions from the results of the present work, because there is the possibility that other deficiencies, in addition to those recognized, might have complicated the results observed.

The present experiments fall into two series. In the first, young newly weaned rats received a basal diet containing pure vitamin B₁ and at most one of the members of the vitamin B₂ complex; the symptoms developed were observed, and the effect on them of adding certain of the missing B₂-vitamins one by one. In the second series the effect was observed of feeding diets containing at least two of the B₂-vitamins, and of the result of restoration of certain of the missing factors.

EXPERIMENTAL

Series I. Methods used

The first series of observations was made on 32 young rats from 4 litters, of body weight ranging from 36 to 53 g. at weaning. The rats received the usual "W.G.L.-B" cooked diet¹ of this laboratory containing rice starch as carbohydrate and with 5 μ g. pure vitamin B₁ (aneurin chloride) hydrochloride and 0.08 g. cod liver oil given daily to each animal separately. After 3-4 days, the basal diet was changed to the following: purified casein (Glaxo ashless extracted) 22, sucrose 67, lard 5.5, salt mixture (McCollum's No. 185) 5.5 parts. Lard was given to ensure an adequate supply of the unsaturated fatty acid dietary factor, as it was obvious that the effect on the skin of any deficiency of this nutrient essential would seriously complicate the study of skin lesions which might be due to other defects in the diet. The food was kept as a dry powder and after weighing was mixed with water to the consistency of a thick cream in order to prevent loss by scattering.

The rats were divided into 4 groups, of which the members were distributed as evenly as possible over the 4 different litters. Group 1 (8 rats) received the basal diet with aneurin and cod liver oil only; group 2 (10 rats) received in addition 12 μ g. daily of pure riboflavin; group 3 (8 rats) received the purified yeast filtrate factor prepared by extraction with amyl alcohol, in a daily dose equivalent to 1 g. yeast (dry wt.); group 4 (6 rats) received the yeast eluate fraction (vitamin B₆) in amount equivalent to 2 g. yeast (dry wt.) daily.

The preparation of the purified filtrate factor from yeast extract was made by the method described by Edgar *et al.* [1938]. The eluate fraction was prepared from the fuller's earth adsorbate from yeast extract as described by Edgar & Macrae [1937]. In certain cases more highly purified preparations of the eluate fraction were employed.

The rats in the different groups were kept without any further addition to the diet for several weeks or until definite symptoms were observed. Careful watch was kept for the first occurrence of the dermatitis (type *a*) and also for the appearance of conjunctivitis and staining of the fur (type *b*). After the occurrence of dermatitis was noticed, addition was made of one of the missing constituents of the vitamin B₂ complex and the results noted, the other constituent or constituents of the complex being added later. As all the rats were deprived of two or more members of the vitamin B₂ complex growth was very poor, as might be expected. On the whole, those in group 4 receiving the eluate fraction remained in the best condition, at least for several weeks.

Results

No dermatitis occurred in the 6 rats in group 4 receiving the "yeast eluate" fraction but, with one or two exceptions mentioned below, it appeared regularly in the other 3 groups after periods varying from 6 to 13 weeks on the experimental diets.

¹ "Glaxo ashless extracted" casein 100, rice starch 300, cottonseed oil 60, lard 15, salt mixture (McCollum's No. 185) 25, water 500 parts; cooked by steaming for 3 hr.

Table 1. *Incidence of dermatitis in young rats maintained on basal diet with daily doses of 5 µg. aneurin and 0.08 g. cod liver oil together with the other supplement indicated*

Group	Supplement daily dose	No. of rats	No. developing (type a) dermatitis	Time on the diet before onset, weeks	No. showing (type b) skin lesions, conjunctivitis, exudation from eyes and nostrils	Time on the diet before onset, weeks
1	0	8	6	6-12	4	3-9
2	Riboflavin 8-17 µg.	10	9	6-12	7	5-12
3	Filtrate fraction ≡ 1 g. yeast (dry wt.)	8	7	8-13	4	6-10
4	Eluate fraction (vitamin B ₂) ≡ 2 g. yeast (dry wt.)	6	0	—	5	2-12

Only one rat (No. 4) in the group deprived of eluate fraction and receiving filtrate fraction failed to develop dermatitis in 10 weeks, but later this animal developed it after riboflavin was given. Two rats (Nos. 17 and 18) in group 1, receiving no supplement, showed no signs after 10-11 weeks, but of these one developed the symptoms also after receiving riboflavin for 9 days. In the riboflavin group, all the rats with the exception of one, No. 29, showed the usual symptoms. The digits of the fore paws were usually the first to be affected, becoming roughened, then red, swollen and inflamed, the inflammation spreading in time to the whole paw, the same sequence of events taking place later in the hind paws. The skin at the tip of the nose and at the corners of the mouth was later attacked, and also the tips of the ears. Often the characteristic thickening of the ears appeared only some weeks after the paws were affected and sometimes only after addition to the diet of riboflavin or the filtrate factor (see below, p. 584). Whether this fact was connected with these additions to the diet or depended only on the further lapse of time cannot be decided.

The (b) type of skin lesions with conjunctivitis and serous secretion from eyes, nostrils etc. and matted fur was developed in some animals in all the groups, usually earlier than the dermatitis, if this occurred. It was particularly severe in some of the rats in group 4 receiving the eluate fraction, but deprived of riboflavin and filtrate factor, and in these cases was uncomplicated by simultaneous presence of dermatitis. Sometimes the whole fur was stained red, but the deepest stains were on the wrists, round the nostrils and eyes, and on the lower lip, as well as round the penis or vagina and on the tail. The red pigment could be removed with cotton wool moistened with water. The pink coloured solution gave none of the reactions for haemoglobin or blood pigments, the benzidine test being wholly negative. The red substance excreted was found by Mrs C. E. Work in this department to give the absorption bands of a porphyrin and was identified with protoporphyrin. Excretion of a porphyrin in the urine of rats deprived of B₂-vitamins was reported by Ellinger *et al.* [1935]; this was considered to be probably coproporphyrin.

Curative tests

i. *Effect of eluate fraction on dermatitis.* The action of the eluate fraction in preventing (type a) dermatitis was obvious in group 4 where none of the 6 rats which received it showed any symptoms. To test its curative action, 7 rats in groups 1, 2 and 3 with marked dermatitis received the various preparations of eluate fraction described above in daily amounts equivalent to 2 g. dry yeast.

Of the 4 rats which previously received riboflavin, good cures of the characteristic dermatitis occurred in about 3 weeks in 3 (Nos. 13, 14 and 22) after administration of the more highly purified preparations of the eluate fraction and a slower cure, taking $4\frac{1}{2}$ weeks, was observed in 1 (No. 21). Of 2 cases of dermatitis in group 3 receiving filtrate fraction, one (No. 11) was cured in 3 weeks with a highly purified eluate fraction; in the case of the other (No. 19) the cure was noted as "incomplete" after 2 weeks, and the fur grew better and became more normal after riboflavin was also given. One of the rats (No. 10) developing (type *a*) dermatitis in group 1, in which no supplement had been given, showed improvement with eluate fraction but died before this had progressed far.

ii. *Effect of riboflavin on (type a) dermatitis.* The effect upon dermatitis of giving riboflavin to rats previously deprived of it was tried in 9 cases. Growth was always stimulated by this addition and frequently there was a temporary remission in the swelling and inflammation of the paws, but the thickening and inflammation of the rims of the ears was never checked, and frequently developed after riboflavin had been given.

Of 3 rats with dermatitis in group 1 (no addition), treated with riboflavin, one (No. 9) became worse, but 2 (Nos. 1 and 3) showed temporary marked improvement of the swollen paws. In one of these (No. 3) there was a subsequent relapse and in the other (No. 1) the cure remained incomplete; in both cases the ears became inflamed only after riboflavin was given and subsequent addition of eluate fraction improved matters very greatly. Another rat in this group (No. 17) which had not developed dermatitis in 11 weeks showed symptoms 9 days after receiving riboflavin.

Of 4 rats with dermatitis in group 3 (receiving filtrate fraction) the symptoms improved after receiving riboflavin in 2 cases; one of these (No. 27), however, died shortly afterwards and the other (No. 12) relapsed subsequently. The remaining 2 rats (Nos. 2 and 20) showed no improvement when riboflavin was given, but these, as well as No. 12, were cured when eluate fraction was added. In the case of No. 20, a rapid cure was made with pure crystalline vitamin B₂ (20 μ g. daily). In addition to the above, one rat (No. 4) in this group, which showed no symptoms after 11 weeks, developed them in 2-3 weeks after riboflavin was given. With 4 of the above 5 rats it was noted that the ears showed the characteristic thickening, inflammation and hardening of the edges only after riboflavin had been given.

One rat (No. 30) developed dermatitis after 6 weeks on the basal diet with riboflavin but the skin trouble steadily improved during the subsequent 5 weeks; it then relapsed but again showed remission of the dermatitis. However, the fur remained very unhealthy and the animal finally died.

iii. *Effect of filtrate fraction upon dermatitis.* The effect of adding filtrate fraction to the diet of rats showing (type *a*) dermatitis was tried in 5 cases. In rat 26 (no supplement) the dermatitis of the paws became worse and the ears became affected. With rats 5 and 8 (receiving riboflavin) the ears also developed dermatitis after the filtrate fraction was given, but the inflammation of the paws subsided; there was, however, a subsequent relapse and final cure only after the eluate fraction was also given. In the riboflavin group rat 7 showed some improvement when receiving filtrate fraction but died, and rat 29 deteriorated after filtrate fraction was given, but improved when the eluate fraction was also added to the diet.

Occurrence of (type b) skin symptoms, with sticky exudation from conjunctiva and nostrils, staining of fur and later occurrence of bald patches

This condition, described in detail above (p. 580) as type *b*, was seen to a slight degree in 7 and in more marked degree in 20, out of the 32 rats. Of the latter 20, 13 belonged to the group which had been deprived of riboflavin, and 7 to the group which had received it from the beginning of the experiment. This result did not agree with our previous conclusion that this type of skin abnormality was produced in rats when riboflavin was absent from the diet and was cured promptly when it was given (see above, p. 580). In 8 of the above 13 cases deprived of riboflavin, the symptoms disappeared or showed improvement when riboflavin was given. In the other 5 cases they persisted to some degree in spite of administration of riboflavin and disappeared only when other B₂-vitamins were also added to the diet.

The results detailed in the preceding paragraphs may be summarized thus. In the 6 rats which received eluate fraction from the beginning of the experiment no (type *a*) dermatitis occurred, but it was noted in 25 of the 26 rats deprived of this fraction in periods varying from 6 to 12 weeks. Of these 25 rats, 7 were treated at once with preparations of the eluate fraction, and in all cases cures were observed, though the fur remained stringy and poor with bald patches of skin, and the rats did not usually recover a normal coat until the other missing constituents of the vitamin B₂ complex were supplied.

Of the 14 rats with (type *a*) dermatitis, which were treated with riboflavin or filtrate factor or both, 8 showed an improvement or a temporary cure, especially of the skin lesions of the paws, but relapses occurred. Frequently the skin lesions were developed on the ears only after riboflavin or filtrate fraction had been given. In the above 8 and in the remaining 6 rats, cures took place later when the eluate fraction was supplied. In two cases this was given as pure crystalline vitamin B₆ kindly supplied by Dr Lepkovsky.

While the (type *a*) dermatitis in rats described above on p. 580 appears to be a specific result of deprivation of vitamin B₆ (eluate fraction), the development of the eczematous skin disease showing the (*b*) type of symptoms is evidently not to be similarly related to deprivation of riboflavin. The above results indicated that the maintenance of a healthy skin in rats depends on an adequate provision of all B₂-vitamins, a conclusion confirmed by the results of the second series of experiments described below.

Series II. Methods used

The general method used in the previous series of experiments is one that is open to criticism, in that the symptoms observed will probably have been the result of the multiple deficiency which was always present—seeing that in the earlier periods of the tests only one of the vitamin B₂ fractions was provided. It was therefore decided to observe over long periods of time the effects of diets arranged to provide the rats with at least two members of the vitamin B₂ complex in optimal amount and study the results of deprivation of one.

Accordingly, groups of young rats were observed which, from the time of weaning, received

(1) the yeast eluate fraction (vitamin B₆) and the purified yeast filtrate fraction, but no riboflavin;

(2) riboflavin and yeast eluate fraction, but no yeast filtrate fraction;

(3) riboflavin and purified filtrate fraction, but no yeast eluate fraction.

The rats, like those in Series I, were given the "W.G.L.-B" diet for 3-5 days from weaning, supplemented with pure vitamin B₁ and cod liver oil. Thereafter they received a diet constituted somewhat differently from that given in the first series. It contained purified¹ casein 22, sucrose 66, wheat germ oil 2.2, linseed oil 3.3, salt mixture (McCollum's No. 185) 5.5, parts. In this ration the lard of the previous diet was replaced by the mixture of linseed oil and wheat germ oil, the former to provide the essential unsaturated fatty acids which were previously supplied in the lard, and the latter to supply vitamin E which, regarding the long duration of the experiments, it was thought wise to include.

The following supplements were given separately to each rat daily: cod liver oil 0.08-0.16 g.; pure aneurin chloride hydrochloride (vitamin B₁) 5-20 μ g.; riboflavin 16-28 μ g.; purified filtrate fraction in amount corresponding to 1-3 g. yeast (dry wt.); eluate fraction in amount corresponding to 2 g. yeast (dry wt.), or crystalline vitamin B₆ 10-15 μ g. The amounts given varied with the body weight of the rats.

The carbohydrate in the diet was varied and the effect of the variation studied. In some instances the sucrose was replaced by an equal weight of purified starch, either rice starch or maize starch. The maize starch was the purest obtainable and contained 0.03-0.04 % nitrogen. The ingestion of raw starch introduced the danger of refectation and this complication occurred in 3 instances. In 2 cases (rats 33 and 52) it was cured by moving the rats to cages with wire floors of very wide mesh and thus hindering coprophagy; in one case the rat (No. 66) had to be discarded. The effect of cooking the starch-containing diet, as in the preparation of our usual "W.G.L.-B" diet (see above, p. 582), was also investigated. It has been frequently observed in this laboratory that if the cooked starch diet were replaced by one containing sucrose, there was a general deterioration in the condition of rats deprived of eluate fraction. If, however, the starch-containing diet was fed in the raw state there was much less difference. The results obtained in Exps. 3 and 4 below confirm this result and suggest that starches of cereal origin, even when purified, may be contaminated with traces of vitamin B₆ which are made more available after they have been cooked with water.

Exp. 1. 16 rats from 2 litters were divided at weaning evenly over 3 groups as follows: 2 groups of 4 rats were deprived respectively of riboflavin and of filtrate factor; these received the diet containing sucrose as carbohydrate. A group of 8 rats was deprived of eluate fraction (vitamin B₆); of these 4 received the sucrose diet, 2 the rice starch and 2 the maize starch diet. All the diets were given raw and were mixed with a little water to form a thick paste before being fed. The results are summarized in Table 2 and growth curves given in Fig. 1.

Riboflavin deprivation. Exp. 1 (a)

The group deprived of riboflavin made by far the poorest progress. There was no significant increase in weight; one rat (No. 41) of initial weight 42 g. which survived 20 weeks weighed only 54 g. at the end of the period (see Fig 1a). The skin was badly affected, showing the type (b) symptoms described above; there was erosion round the eyes which were sunken; the lids were stuck together with the serous exudate and there was dullness of the cornea; the fur was matted and eczematous, the skin in some places was exfoliating and in others nearly bare of fur, the wrists were badly stained with a reddish exudate. Two of the 4 rats,

¹ "Glaxo" extracted, ashless casein, further purified by washing 6 times with 2% NaCl solution followed by dilute alcohol and drying in a current of dry air.

Table 2

Exp. no.	Litter no.	Deficiency in diet	Nature of carbohydrate in diet	No. of rats	Av. body-weight* (g.) after weeks on diet					Incidence of type (a) dermatitis		Incidence of type (b) skin lesions		Remarks
					Initial	8	16	24	32	No. of cases	Time of onset, weeks	No. of cases	Time of onset, weeks	
1 (a)	5/6	Riboflavin	Sucrose	4	42	48	57 (1)	—	—	—	—	4	4-8	Eye lesions specially noticed in group (a); reddish staining of coat and greying of pigmented fur in group (b)
(b)	5/6	Filtrate fraction	"	4	44	78	108 (3)	114 (3)	116 (3)	—	—	4	5-11	
(c)	5/6	Eluate fraction (vitamin B ₆)	"	4	42	66	88 (1)	88 (1)	86 (1)	3	8-9	—	—	1 rat cured with vitamin B ₆ at 10 weeks
		"	Rice starch, raw	2	43	74	77 (1)	—	—	2 (slight)	12-20	—	—	
		"	Maize starch, raw	2	41	81	91 (1)	88 (1)	92 (1)	—	—	—	—	
2	7	"	Sucrose	2	54	88	111	—	—	2	10-11	—	—	1 rat with dermatitis died at 17 weeks. 1 was cured with vitamin B ₆ at 31 weeks
		"	Rice starch, raw	3	52	94	96	103	—	2	10-24	—	—	
		"	Maize starch, raw	2	54	88	100	110	—	2	10-25	—	—	
3	8/9	Eluate fraction (vitamin B ₆)	Sucrose	6	45	78	99 (5)	101 (3)	111 (2)	5	8-16	—	—	1 rat with severe dermatitis cured with vitamin B ₆ at 12 weeks. 1 rat died at 19 weeks
		"	Rice starch, cooked	5	45	139	160	158	164	—	—	—	—	Observed for 35 weeks
		"	Rice starch, raw	4	44	81	102 (3)	94 (2)	116 (1)	3	8-24	—	—	Dermatitis only slight, 1 rat remained free for 35 weeks
4 (a)	10	Eluate fraction (vitamin B ₆)	Rice starch, raw	2 ♂	50	101	108 (1)	122 (1)	123 (1)	4	8-30	—	—	Dermatitis slight, chiefly ears
		"	"	2 ♀	47	86	91	101	105					
(b)	10	Addition of vitamin B ₆ 10-15 µg. daily	"	2 ♂	50	172	221	262	291	—	—	—	—	All completely healthy after 34 weeks
		"	"	2 ♀	47	160	184	206	212					

* Nos. in brackets signify nos. of animals from which average is calculated.

after 8 and 10 weeks respectively, were killed to provide post-mortem material for histological study by Dr El Sadr of this department. The 4th rat (No. 44) after 9 weeks on the diet weighed only 45 g. and then received 11 μ g. riboflavin daily. Although its condition was very bad, it at once began to grow, gaining 15 g. in the following 9 days and there was an immediate improvement in the skin condition. The eyelids which had been stuck together with the serous exudate became wide open and the opaque condition of the cornea vanished, the coat

Fig. 1 (see Table 2, Exp. 1a, b, c). Growth curves of litter-mate rats on diet containing sucrose as carbohydrate, with supplements of cod liver oil, pure vitamin B₁ and the following B₂-vitamins: (a) yeast eluate fraction and purified yeast filtrate fraction; (b) riboflavin and yeast eluate fraction; (c) riboflavin and purified yeast filtrate fraction. *D* signifies onset of dermatitis. *F* signifies onset of fits. *R* signifies administration of riboflavin. *E* signifies administration of eluate fraction (vitamin B₆). *†d* signifies died; *†K* signifies killed, very ill. The divisions on the abscissa represent periods of 4 weeks.

was much improved and the staining of the fur and the abdomen almost disappeared. The condition of these rats deprived of riboflavin was as described elsewhere by El Sadr [1940]. Cataract was not observed, but only in the case of one rat was the duration of the deprivation sufficient for this to occur.

Filtrate fraction deprivation. Exp. 1(b)

The 4 rats deprived of filtrate factor remained in much better condition than those deprived of riboflavin. Three slowly attained a body weight of 100 g. and over during the first 16 weeks on the diet but, after this, little more increase occurred and 2 female rats which survived 12 months weighed only 127 and 126 g. respectively. The eyes were not affected, symptoms being confined to the skin; the fur became matted and stringy and stained with the reddish secretion containing protoporphyrin mentioned above (p. 583). The stain is soluble in water and the animals could be cleaned by washing and frequently they cleaned themselves, but in the course of a few days became stained again. Two of the 4 rats showed greying of their dark pigmented fur, especially over the head and shoulders. Although stained fur was noted in some cases of rats deprived of riboflavin when in a very bad state, and also in those deprived of eluate fraction (vitamin B₆) for long periods, this reddish staining of the fur, especially of the

abdomen in the earlier stages of deprivation, seems to be characteristic of filtrate fraction deficiency, as the eye lesions described above are specific for riboflavin deficiency. The 2 rats which survived a year without filtrate fraction became nervous and excitable but, in contrast to those deprived of eluate fraction, readily took their daily doses and no fits were observed (see below).

The rats in this series deprived of riboflavin and filtrate factor were few in number, but the symptoms observed were confirmed by more numerous observations by other workers in this department.

Deprivation of eluate fraction (vitamin B₆). Exps. 1(c), 2, 3, 4, Table 2

Of the 4 rats in Exp. 1 receiving sucrose as carbohydrate, 3 developed type (a) dermatitis in 8–9 weeks. The 2 rats receiving raw rice starch as carbohydrate behaved similarly but the dermatitis was less severe; the 2 rats receiving maize starch had shown no dermatitis after 13 and 33 weeks' observation respectively.

In Exp. 2 dermatitis occurred in the rats receiving maize starch in their diet and in 2 of the 3 rats having rice starch but later than in the 2 litter-mates receiving sucrose (see Table 2). It was noted that with these rats developing dermatitis and the others doing so in Exps. 3 and 4 the sequence in which the different sites of the skin showed inflammation was different from that observed in the experiments of Series I. In the experiments of Series II, the skin of the edges of the ears and that on the nose was usually the first to be affected, and the paws became inflamed later. Sometimes the paws remained without inflammation for a very long period; this was frequently the case when the diet contained starch as carbohydrate. The rats in Series I, deprived of riboflavin or filtrate factor as well as of vitamin B₆, invariably showed the earliest lesions in the paws, the ears becoming affected, with thickening and inflammation of the edges, much later and frequently only after riboflavin had been added to the diet (see Plate 2, fig. 5).

When pure vitamin B₆, 15 μ g. daily, was given to the rats with these skin symptoms, swift cures took place. Similarly, those rats which, in addition to aneurin, riboflavin and purified filtrate factor, received also a supplement of pure vitamin B₆, 10–15 μ g. daily, from the beginning of the experiment, developed no dermatitis and grew into fine, apparently normal rats, the males reaching over 300 g. and the females over 200 g. weight in about 7 months (see Table 2, Exp. 4 (b)). The males and females were mated and normal pregnancies occurred, two healthy litters of 9 and 12 live young were born but the mothers were unable to lactate and the young died within 48 hr. The growth curves of these rats and of their deprived litter-mates are shown in Fig. 3 and a photograph is given in Plate 2, fig. 4.

After long periods of deprivation, 20–30 weeks and often sooner on the sucrose diet, changes other than those of (type a) dermatitis were noted in the skin and fur of rats deprived of vitamin B₆. The fur became scanty and matted, with bad areas especially on the chin and chest, over the forepaws and around the eyes. The spectacled state of the eyes was sometimes so marked as to resemble that resulting from deprivation of riboflavin. There was staining of the fur with the reddish pigment, found by Mrs C. E. Work to have the absorption bands of protoporphyrin; the colour of the fur was also affected, the white parts becoming yellowish and the pigmented parts rusty in colour, especially on the abdomen and chest.

The rats which survived deprivation of vitamin B₆ for long periods, usually not less than 4–5 months, also developed a tendency to fits. These formed the subject of special study and are dealt with in a separate paper [Chick *et al.* 1940].

Influence of carbohydrate in the diet

The 5 animals in Exp. 3 receiving the basal diet containing cooked starch had no dermatitis; they showed better growth and remained in much better general

Fig. 2. Growth curves of litter-mate rats on synthetic diet deprived of eluate fraction and containing carbohydrate. A, as rice starch (cooked); B, as rice starch (raw); C, as sucrose; N signifies supplement of nicotinic acid, 2 mg. daily; D signifies onset of dermatitis; F signifies onset of fits; †K = killed; †d = died; at CS rat 62 received the cooked diet; at B rat 71 received pure vitamin B₆. The divisions on the abscissa represent periods of 4 weeks. (Table 2, Exp. 3.)

Fig. 3. Growth curves of litter-mate rats on synthetic diet (raw) containing rice starch as carbohydrate and deprived of eluate fraction (vitamin B₆). Group A received pure crystalline vitamin B₆, 10–15 μ g. according to size. Group B received no supplement. D signifies onset of dermatitis; F signifies onset of fits; †d signifies died. The divisions on the abscissa represent periods of 4 weeks. (Table 2, Exp. 4 and Plate 2, fig. 4.)

condition than their 4 litter-mates receiving the same basal diet fed in the raw state (see Table 2 and Fig. 2). The latter behaved similarly to the groups having the

sucrose diet, except that the dermatitis was definitely less severe and usually took longer to develop. One explanation of this difference would be that the rats on the starch diet were receiving a small but inadequate amount of vitamin B₆. This vitamin is known to be present in cereals in fair amount (see above, p. 581) and has the property of being readily adsorbed on suitable materials. It is possible therefore that some small amount might remain attached to starches of cereal origin, even after purification by washing. Tange & Kaneko [1938] reported that vitamin B₆ was unnecessary in the diet of the rat when maize starch was given as carbohydrate and György *et al.* [1937] found that "acrodynia" was developed regularly in 6-20 weeks in rats deprived of vitamin B₆ when the rice starch in the diet was replaced by sucrose. Oleson *et al.* [1939, 1] observed better growth in rats on synthetic diets, apparently adequately supplied with B-vitamins, when the sucrose of the basal diet was replaced by dextrin.

The vitamin B₆ present in the raw rice starch used in the present work appeared, however, to be combined in some form in which it is relatively unavailable for the rat, but from which it could be freed and rendered more digestible by cooking with water. Even so, the amount appears to be small, for the rats in Exp. 3 receiving the cooked starch diet, although they developed no dermatitis, were much inferior to those in Exp. 4 which received an adequate daily dose of pure vitamin B₆ (see Figs. 2 and 3).

This effect of cooking has so far been investigated only with rice starch. Raw maize starch, however, also showed some protective power against the effect of vitamin B₆ deprivation (see Table 2, Exps. 1 (c) and 2) and experiments are now in progress to discover if this power is increased when the starch is cooked.

Influence of nicotinic acid

Of the 15 rats in Exp. 3, 2 of 5 receiving the cooked starch basal diet, 2 of 4 receiving raw starch and 3 of 6 receiving sucrose, were given 2 mg. nicotinic acid daily in addition to the supplements of aneurin, riboflavin and filtrate factor. In no way did these rats differ in behaviour from their litter-mates which received no supplement of nicotinic acid. There was no other source of nicotinic acid in the diet of these rats, seeing that all were deprived of the "yeast eluate fraction" which has sometimes been found to contain nicotinamide [Macrae & Edgar, 1937]. Fresh evidence is thus given for the conclusion that nicotinic acid is not required for the nutrition of the rat [see Chick *et al.* 1938; Macrae & Edgar, 1937; Cook *et al.* 1937].

As was pointed out above (p. 581), some of the diets used in the experiments of Series II were deficient in certain, at present unknown, members of the vitamin B₂ complex, in addition to the deficiency which was recognized. Traces of these factors may, on the other hand, have been provided in the purified casein, or in the purified starch, and have been absent when the carbohydrate was supplied as sucrose. When the basal diet contained starch, a partial protective action was certainly exercised against the effects of vitamin B₆ deficiency, and when it was adequately supplemented with aneurin, riboflavin, purified filtrate factor and vitamin B₆, the rats developed well, remained healthy and even reproduced (Table 2, Exp. 4b).

With the reservations contained in the preceding paragraph it may be concluded: (1) that the nutritional dermatitis in rats showing the type (a) characteristics, formerly called "rat pellagra" and "rat acrodynia" by some authors, is the result of deprivation of vitamin B₆; (2) that an eczematous skin disease affecting chiefly eyes and nose is caused by riboflavin deficiency; (3) that a

similar affection, not including the eyes, but with greying of pigmented fur and reddish staining of the fur and abdomen, occurs with deficiency of filtrate factor. But loss of fur round the eyes with exudation of a reddish secretion, an unhealthy condition of the skin with matted stringy fur, often causing bald patches of skin, especially on the neck and chest and forearms, was seen with deprivation of each of the 3 above members of the vitamin B₂ complex, especially when the period of deprivation was prolonged.

The existence of a specific relation between vitamin B₆ and type (a) dermatitis or "rat acrodynia" has been confirmed by numerous workers in this field since it was first observed by György [1934]. As regards the other members of the vitamin B₂ complex, it is agreed that each is required for satisfactory growth, but there is at present little agreement as to the pathological effects of deprivation of the separate vitamins.

György *et al.* [1937] described a "haemorrhagic" condition with nose bleeding, bloody secretion from the eyes and haemorrhage in the adrenals, accompanied by anaemia of an aplastic type, in rats maintained on a synthetic diet containing sucrose as carbohydrate, with supplements of vitamin B₁ and riboflavin. The rats also developed "acrodynia", which was cured by pure vitamin B₆; the haemorrhagic condition, on the other hand, remained unchanged but yielded to crude preparations of vitamin B₆. In a later paper György [1938, 1] concluded that the anaemia-preventing substance was nicotinic acid. This conclusion is not confirmed by the present study and it must be noted that the filtrate factor was absent from György's diet. In a more recent communication György & Eckhardt [1939] describe three different types of skin disease of an eczematous nature with loss of hair and sticky secretion from the eyes, sore nose and mouth and scaliness of the skin, developed in rats maintained for long periods on a synthetic diet supplemented with purified vitamin B₁, pure vitamin B₆ and riboflavin. All these affections yielded to administration of the filtrates from wheat germ, yeast and rice polishings after treatment with fuller's earth.

In a recent paper Oleson *et al.* [1939, 1] have stated that, in addition to "acrodynia" which could be cured by vitamin B₆, three separate syndromes are developed in rats receiving aneurin, riboflavin and nicotinic acid: (1) paralysis, (2) a haemorrhagic condition, (3) a condition of "eye erosion". The "eye erosion" was cured by a "filtrate fraction" prepared from liver extract after adsorption in acid solution with fuller's earth; the same fraction was later [Oleson *et al.* 1939, 2] found preventive of "achromotrichia", presumably greying of the pigmented portions of the fur. The latter conclusion is confirmed by us, the former not, since in our experiments rats deprived of filtrate factor showed no marked changes in the skin or fur round the eyes. Greying of the fur in rats deprived of filtrate factor has also been reported by Morgan *et al.* [1938] and by Lunde & Kringstad [1939]. The haemorrhagic condition described by Oleson *et al.* [1939, 1] resembled that studied by György *et al.* [1937]. Both it and the paralysis were cured by the eluate fraction but not by pure vitamin B₆. We have not observed any paralysis in rats deprived of our yeast eluate fraction for periods up to a year. On the other hand we have found a tendency to fits invariably developed after deprivation for 4-5 months (see the following paper).

Such discrepancies between the conclusions of different workers are perhaps inevitable and may be explained by differences in the basal diets used and in the degrees of purity of the vitamin supplements and may only be resolved when all the latter are available in the pure state.

At the same time it is more than probable that many pathological affections, including those of the skin, occurring in animals suffering from faulty nutrition

are not related to any specific deprivation. It is generally agreed that the skin and mucous membrane of an animal are particularly sensitive to nutritional defects. The skin covering the external surface of the animal and the mucous membranes lining the alimentary tract and nasal passages and covering the eyes, in one respect exercise similar functions, viz. the protection of the organism from invasion by external bacteria, which are abundantly present on the "exterior" surfaces of these membranes. When the animal is deprived of nutritional essentials, the defensive mechanisms of these tissues are disturbed, and invasion by bacteria causes a diseased condition. The points of union of skin and mucous membrane appear to be sites of particular vulnerability; such are the edges of the lips and nostrils and the corners of the eyes and the margins of the skin round the vagina. The common occurrence of furunculosis, conjunctivitis and angular stomatitis in ill nourished human beings may be reactions to general, rather than to specific, dietary deficiencies. Angular stomatitis is frequent among poorly fed native races, especially among children, and although foods containing the vitamin B₂ complex have been found curative, no single member of the group has yet been found effective when administered in the purified state.

SUMMARY

1. Rats receiving purified synthetic diets with the addition of pure aneurin (vitamin B₁) and deprived of one or more of the B₂-vitamins were observed over long periods up to 1 year from weaning stage.

2. Rats deprived of riboflavin showed no significant increase in weight and developed an eczematous condition of the skin affecting specially nostrils and eyes; the rims of the latter became denuded of hair; there was dullness of the cornea, blepharitis and conjunctivitis, the eyelids being stuck together with a serous exudate. These symptoms cleared up rapidly when riboflavin was given.

3. Rats deprived of filtrate fraction showed slow subnormal growth up to a maximum weight of about 100–130 g. weight; they developed poor coats with matted and stringy fur which often became grey in the pigmented areas over the head and shoulders and was frequently stained with a reddish exudate containing protoporphyrin on the forearms and abdomen.

4. Rats deprived of eluate fraction (vitamin B₆) developed characteristic dermatitis, which was cured promptly when pure vitamin B₆ was given. After prolonged deprivation the rats became hypersensitive and developed a tendency to fits of an epileptic nature.

5. The relation of the skin symptoms described under 2, 3 and 4 to the known missing vitamin was not, however, always strictly specific. Dermatitis in rats deprived of vitamin B₆ and riboflavin showed temporary improvement when riboflavin was given. In prolonged deprivation of vitamin B₆ there occurred erosion of the fur round the eyes and frequently patches of eczematous skin with scanty matted fur and bald patches, resembling those occurring in deficiencies of shorter duration of riboflavin or of filtrate fraction. These facts may be explained by interference with the normal mutual interaction of the known members of the vitamin B₂ complex, or by absence from the diets of other unknown vitamins belonging to this group.

6. Starches, given as source of carbohydrate in the basal diet, exerted a protective action, as compared with sucrose, on animals deprived of vitamin B₆; this was greater when the diet was cooked. It is suggested that starches of cereal origin, even when purified, may retain traces of vitamin B₆ which are more readily available to the rat after the starch has been cooked with water.

In conclusion our thanks are due to other workers in this Department, including Dr L. Gravel of the Laval University of Quebec and especially Mr G. Flynn, for assistance in the care of the experimental animals. We are also indebted to the late Mr Walter Acton of Messrs Mackean, Paisley, for kindly arranging for a supply of pure maize starch and for information regarding its analysis; to Dr S. Lepkovsky, Berkeley, U.S.A., for a gift of pure vitamin B₆; to Dr S. Smith of Messrs Burroughs Wellcome & Co., for pure riboflavin, and to the firm of Roche Products, Ltd., for kindly providing pure aneurin (vitamin B₁) and riboflavin. The last stages of the experimental work were carried out at Roebuck House, Chesterton, Cambridge, in the house of Sir Charles Martin whom we desire to thank for his generous hospitality as well as for his help and advice.

REFERENCES

- Birch & György (1936). *Biochem. J.* **30**, 304.
 ——— & Harris (1935). *Biochem. J.* **29**, 2830.
 Chick & Copping (1930). *Biochem. J.* **24**, 932.
 ——— & Edgar (1935). *Biochem. J.* **29**, 722.
 ——— El Sadr & Worden (1940). *Biochem. J.* **34**, 595.
 ——— Macrae, Martin & Martin (1938). *Biochem. J.* **32**, 844.
 ——— & Roscoe (1928). *Biochem. J.* **22**, 790.
 Copping (1936). *Biochem. J.* **30**, 845.
 Cook, Clark & Light (1937). *Proc. Soc. exp. Biol., N.Y.*, **37**, 574.
 Edgar & Macrae (1937). *Biochem. J.* **31**, 879.
 ——— El Sadr & Macrae (1938). *Biochem. J.* **32**, 2200.
 El Sadr (1940). *J. Path. Bact.* **50**, 199, *Proc.*
 ——— Hind, Macrae, Work, Lythgoe & Todd (1939, 2). *Nature, Lond.*, **144**, 73.
 ——— Macrae & Work (1939, 1). *Biochem. J.* **33**, 611.
 Ellinger, Edgar & Lucas (1935). *Chem. Ind. Rev.* **54**, 269.
 Goldberger & Lillie (1926). *U.S. Pub. Hlth. Rep.* **41**, 1025.
 György (1934). *Nature, Lond.*, **133**, 498.
 ——— (1938, 1). *Proc. Soc. exp. Biol., N.Y.*, **37**, 732.
 ——— (1938, 2). *J. Amer. chem. Soc.* **60**, 983.
 ——— & Eckhardt (1939). *Nature, Lond.*, **144**, 512.
 ——— Goldblatt, Miller & Fulton (1937). *J. exp. Med.* **66**, 579.
 Harris & Folkers (1939). *J. Amer. chem. Soc.* **61**, 1245.
 Keresztesy & Stevens (1938). *J. Amer. chem. Soc.* **60**, 1267.
 Kuhn & Wendt (1938). *Ber. dtsch. chem. Ges.* **71**, 780.
 Lepkovsky (1938). *Science*, **87**, 169.
 Lunde & Kringstad (1939). *Hoppe-Seyl. Z.* **257**, 201.
 Macrae & Edgar (1937). *Biochem. J.* **31**, 2225.
 Morgan, Cook & Davison (1938). *J. Nutrit.* **15**, 27.
 Oleson, Bird, Elvehjem & Hart (1939, 1). *J. biol. Chem.* **127**, 23.
 ——— Elvehjem & Hart (1939, 2). *Proc. Soc. exp. Biol., N.Y.*, **42**, 283.
 Tange & Kaneko (1938). *Sci. Pap. Inst. phys. Res., Tokyo*, **35**, 64.

Fig. 4.

Fig. 5.

Fig. 4. (Left) Rat 76 ♂, body weight 115 g. after 7 months on a synthetic diet containing raw rice starch, with addition of cod liver oil, pure vitamin B₁, riboflavin and purified yeast filtrate factor; (right) litter-mate rat 72 ♂, body weight 344 g., after the same period on the same diet with addition of pure vitamin B₆ 10–15 μ g. daily.

Fig. 5. Rat 49, showing dermatitis of ears and nose after 19 weeks' deprivation of vitamin B₆.

(a)

(b)

(c)

(d)

Fig. 6. Stages in epileptiform fits developed after 20 weeks on a synthetic diet deprived of vitamin B₆. (a) Comatose stage, rat unconscious and flaccid. (b) Early stage in recovery of sensation and movement. (c) Later stage in recovery; sensation completely regained, while movement restricted to head and forelimbs. (d) Recovery nearly complete, rat beginning to move.