136. FURTHER INVESTIGATIONS ON VITAMIN B₆ AND RELATED FACTORS OF THE VITAMIN B₂ COMPLEX IN RATS. PARTS I AND II

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Rapid advance in the biochemical analysis of the vitamin B₂ complex has not only led to the recognition, isolation and identification of an increasing number of food constituents of vitamin-like character, but has at the same time opened the way for the experimental production of diseases, the nutritional basis of which was hitherto unknown, hidden or barely suspected. The first approach to this systematic analysis resulted in the isolation of lactoflavin [György et al. 1933; Kuhn et al. 1933], now called riboflavin. The work was stimulated by clinical interest in dermatological conditions in which a nutritional cause was, possibly, in the background. The so-called “rat pellagra” of Goldberger and his co-workers [1926] has been regarded as one of the best examples of this group.

In the investigations leading to the isolation of riboflavin, growth-promoting activity had been used as a guide. In the search for more specific manifestations, it was soon established [György, 1934] that rat pellagra has no relationship to a lack of riboflavin but must be attributed to a deficiency caused by a different member of the vitamin B₂ complex, for which the term vitamin B₆ was proposed. This term has since then been generally accepted. Circumstantial evidence was offered [Birch et al. 1935] to the effect that the aetiology of rat pellagra is essentially different from that of human pellagra. Following the identification of nicotinic acid (nicotinic acid amide) with the preventive factor for pellagra in man and for blacktongue in dogs [Elvehjem et al. 1937], it became possible to state definitely that vitamin B₆ is, indeed, a separate member of the vitamin B₂ complex [Dann, 1937; György, 1937–8]. In order to avoid any further confusion, the term rat acrodynia was substituted [Birch et al. 1935] for rat pellagra. The revised designation emphasizes the outstanding characteristics of the cutaneous lesions caused by deficiency of vitamin B₆, such as the symmetrical dermatitis with oedema and scaliness, mainly on the peripheral parts of the body, for example, the mouth, nose, paws, ears and tail. Vitamin B₆ was defined, accordingly, as “that part of the vitamin B₂ complex which is responsible for the cure of the specific dermatitis developed by young rats fed on a vitamin B-free diet supplemented with purified vitamin B₁ and lactoflavin” [Birch & György, 1936]. In bioassays for vitamin B₆, cure of the acrodynia was used as the criterion, independently of the growth-promoting activity of the food constituents or concentrates tested.

In rats kept on a diet consisting of purified casein, melted butter fat, rice-starch (or sucrose), salt mixture and cod liver oil, supplemented with vitamin B₁ and riboflavin, symptoms of vitamin B₆ deficiency appear in from 4 to 15 weeks.

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on an average in 7 weeks [György, 1935, 1]. These results have been confirmed in general by several authors who employed identical or similar diets. Dann [1939], however, reported that in an increasing number of his rats the specific cutaneous symptoms failed to develop and that he was unable, either by variation of the fat content of the diet or by using specially purified casein preparations, to increase the incidence of the rat acrodynia.

I. Use of dried heated egg white as a constituent of the basal diet for the production of rat acrodynia

Although in our laboratory the original diet proposed [György, 1935, 1] for the production of vitamin B₆ deficiency has proved, on the whole, very satisfactory, an attempt was made to improve the results with regard to frequency and time of appearance of rat acrodynia by the incorporation of dried egg white in the basal diet. In previous work [György, 1935, 1] it has been shown that egg white, although rich in riboflavin, is practically devoid of vitamin B₆. Thus, the addition of egg white could be expected to make the experimental diet more complete without furnishing it with vitamin B₆.

In view of the well-known injurious effect of raw egg white on rats, care was taken to add only relatively small amounts of egg white, detoxified by heat [Parsons & Kelly, 1933], to the experimental diet, in which casein remained the chief source of protein. In this connexion it should be pointed out, as a further safeguard against the possibly harmful effect of egg white, that casein contains the specific protective factor (vitamin H) for egg white injury in fairly high concentration [Boas, 1927].

Experimental

Rats at weaning were placed on the experimental ration under conditions that are now sufficiently well standardized to need no repeated description here. The basal diet had the following composition:

<table>
<thead>
<tr>
<th>Ingredient</th>
<th>%</th>
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<tbody>
<tr>
<td>Casein</td>
<td>18</td>
</tr>
<tr>
<td>Sucrose</td>
<td>68</td>
</tr>
<tr>
<td>Butter fat</td>
<td>8</td>
</tr>
<tr>
<td>Salt mixture [Hubbell et al. 1937]</td>
<td>4</td>
</tr>
<tr>
<td>Cod liver oil</td>
<td>2</td>
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</table>

Freshly separated egg whites were dried, either directly or, as was regularly done in later experiments, after they had been heated for 3 hr. at 80°, by placing them in shallow pans over a simmering water bath. The dry mass was then ground in a mortar, put through a sieve and incorporated in the diet at a level of 10 %, replacing an equal quantity of sucrose. Two basal diets were thus obtained, differing only in that in one diet dried heated egg white replaced 10 % of the sucrose. These diets will subsequently be referred to as the "no egg white diet" and the "10 % egg white diet".

Each animal received a daily supplement of 20 μg. of synthetic vitamin B₁ and 20 μg. of synthetic riboflavin.¹ The temperature of the animal room was kept as constant as possible. The animals were weighed once a week.

Treatment with vitamin B₆ preparations of varying purity was begun when the symptoms became definite enough to warrant it. In reporting the results, correlations of weight and age with the incidence of acrodynia are made by comparison at the time of beginning the treatment rather than at the time of the

¹ Vitamin B₁ and riboflavin were supplied by Merck & Co. Inc., Rahway, New Jersey, and casein by General Biochemicals Inc., Cleveland, Ohio.
first appearance of the manifestations of acrodynia. In this manner questionable symptoms were obviated.

Post-mortem examinations were made on all rats that died without development of the symptoms of acrodynia, as soon after death as possible, in an attempt to discover intercurrent infections or concurrent deficiencies which might influence the incidence of acrodynia.

**Results**

A total of 519 rats from those kept on the two diets described was selected for the present investigation. Of this total, 328 were placed on the diet which contained no egg white, while 191 were placed on the diet containing 10% egg white.

In 232 of the 328 rats placed on the "no egg white diet" (70.7%), acrodynia developed of a severity intense enough to require treatment or to have been the cause of death before treatment was instituted. The average age of the rats in this group at the time treatment was begun was 53.1 days, the range being between 27 and 103 days. These figures are in good agreement with those previously reported [György, 1935, 1]. Of the 94 animals that died without exhibiting specific signs of acrodynia, post-mortem examination revealed that pneumonia developed in 21, purpura, anaemia, or adrenal haemorrhage in 10 [György et al. 1937], hepatic injury in 3 [György & Goldblatt, 1939], 9 had cutaneous lesions which recent investigations [György et al. 1939] have shown to be the result of a deficiency of pantothenic acid, and 51 showed no gross pathological changes to which death could be ascribed (Table 1).

Of the 191 rats fed on the diet containing 10% of egg white, acrodynia developed in 167, or 87.4%. In this group, however, the average age at which treatment was begun was 39.2 days, which is less by 2 weeks than that in the first group. The fluctuation of age at treatment in the second group was between 14 and 112 days. Of the animals that died without showing signs of acrodynia, 4 had pneumonia, in 2 kidney abscesses developed, 2 had symptoms of deficiency of pantothenic acid and 16 were found to be free from internal changes to which death could be attributed. In this group there were seen no cases of hepatic injury, panmyelophthisis (anaemia, purpura) or adrenal haemorrhage (Table 1).

In order to avoid any false conclusion it is important to note that in the rats kept on the "10% egg white diet" which were subsequently treated with vitamin B₆ (and pantothenic acid), panmyelophthisis was repeatedly observed at post-mortem examination, and even hepatic injury was seen in one animal. Thus the egg white diet seems to be devoid of the maturation factor of the bone marrow and probably does not contain the preventive factor for hepatic injury.

Definite correlations between the initial weight of the animals kept on the deficient diets and the incidence of acrodynia were not apparent (Table 2).

The incidence of acrodynia has also been analysed in relation to the weight of the animals at the time treatment was begun. The rats were divided into 6 groups: (1) those which showed no weight response, (2) those which showed a gain in weight until treatment was begun (or a maximum gain in the case of death without development of acrodynia) of 0–5 g., (3) 5–10 g., (4) 10–15 g., (5) 15–20 g., and (6) those which showed a weight gain of 20 g. or over. The corresponding relationship is shown in Fig. 1. In rats placed on the diet that contained 10% egg white there was a greater tendency to gain weight before treatment, despite the fact that the average time until treatment was shorter by 2 weeks in this group than in the group placed on the diet without egg white.
Table 1. Incidence of acrodynia in rats fed on diets deficient in vitamin B\textsubscript{6} and cause of death in rats on same diets in which acrodynia did not develop

| Diet containing rats | Total no. of rats | Rats with acrodynia | No. | % | Age at which treatment began, days | No. | % | Purpura, anaemia, adrenal haemorrhage | No. | % | Hepatic injury | No. | % | Kidney abscess | No. | % | Filtrate factor deficiency | No. | % | Undetermined | No. | % | Still living | No. | % |
|----------------------|-------------------|---------------------|-----|---|----------------------------------|-----|---|--------------------------------------|-----|---|---------------------------------|-----|---|-----------------|-----|---|-----------------|-----|---|------------------|-----|---|-----------------|-----|---|-----------------|-----|---|
| No egg white         | 328               | 232                 | 70  | 232| 70                               | 2   | 9 | 51                                   | 2   | 9 | 51                   | 2   | 9 | 51                   | 2   | 9 | 51                   | 2   | 9 | 51                   |
| 10\% egg white       | 191               | 167                 | 87  | 167| 87                               | 4   | 0 | 0                                    | 2   | 2 | 16                   | 0   | 0 | 0                    | 2   | 2 | 16                   |

Table 2. Relation of incidence of acrodynia to weight of rats at the time they were placed on diets deficient in vitamin B\textsubscript{6}

<table>
<thead>
<tr>
<th>Diet containing rats</th>
<th>Wt. under 25 g.</th>
<th>Wt. 25-30 g.</th>
<th>Wt. 30-35 g.</th>
<th>Wt. 35 g. and over</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Rate with acrodynia</td>
<td>Rate without acrodynia</td>
<td>Rate with acrodynia</td>
<td>Rate without acrodynia</td>
</tr>
<tr>
<td>No egg white</td>
<td>60</td>
<td>69.8</td>
<td>26</td>
<td>73</td>
</tr>
<tr>
<td>10% egg white</td>
<td>53</td>
<td>91.4</td>
<td>5</td>
<td>67</td>
</tr>
</tbody>
</table>

Fig. 1. Histograms showing relationship of incidence of acrodynia to gain in weight of rats (A) on the “no egg white diet” and (B) on the “10\% egg white diet”.

Discussion

The ideal diet for the production of acrodynia in rats would be one which supplied all the vitamins required by the rats except vitamin B\textsubscript{6}. At present it is impossible to arrange such a diet because of the still fragmentary knowledge of the remaining factors of the vitamin B\textsubscript{6} complex.

In view of the data reported here, incorporation of egg white (up to 10\%) in the basal diet originally devised [György, 1935, 1] for the production of rat acrodynia seems to improve results considerably. In the first place, the greater...
gain in weight in the shorter time interval among the rats on the "10% egg white diet" indicates that this diet provides more completely the requirements of the rat than does the basal diet without egg white. This conclusion is also supported by the lower incidence of pneumonia seen in the rats on the egg white diet, as they are apparently better able to resist infection by reason of their better nutritional state.

With regard to the higher incidence and shorter time required to produce acrodynia in rats on the egg white diet, one is tempted to refer to the "concurrency of vitamin deficiency diseases" [György et al. 1937; György & Goldblatt, 1939]. This phenomenon is based on the observation that when an animal is placed on a multiple deficiency diet one of these deficiencies assumes the dominant role, relegating the other deficiencies to more latent positions. This has been the case with the original experimental diet used for the production of the vitamin B_6 deficiency disease. Rat acrodynia assumes the dominant role, suppressing the manifestation of the other deficiencies which are known to coexist. When a purified vitamin B_6 preparation or pure vitamin B_6 is given to a rat suffering from acrodynia, the acrodynia is cured and the other deficiencies may then become manifest. This is what occurs when panmyelophthisis develops in rats [György et al. 1937]. It was first observed in animals cured of acrodynia by the administration of relatively pure vitamin B_6 preparations. Occasionally, however, in the group of rats kept on the original basal diet without egg white here reported, acrodynia seemed to disappear spontaneously, without specific treatment, and the symptoms of panmyelophthisis became evident and assumed the dominant role over the acrodynia for reasons as yet obscure. The absence of these symptoms in the rats on the egg white diet tends to indicate that the function of the egg white is to supply some of the essential food factors, the addition of which seems to favour even more the suppression of panmyelophthisis. Inasmuch as the egg white diet does not definitely prevent the occurrence of panmyelophthisis in rats that have been cured of acrodynia by the addition to the diet of vitamin B_6, the conclusion is warranted that egg white does not supply the specific maturation factor for prevention of panmyelophthisis.

It may here be noted that the connexion of nicotinic acid with panmyelophthisis assumed on the basis of preliminary experiments [György, 1937–8] has not been borne out by subsequent observations. As stated by one of us (P. G.) in a report given at the Symposium on the Vitamin B Complex at the Annual Meeting of the American Institute of Nutrition in Baltimore, Maryland, in 1938, nicotinic acid was devoid of any curative effect on panmyelophthisis, and even the prophylactic effect, at first suggested [György, 1937–8], was lacking, or at least uncertain, in later experiments.

II. Vitamin B_6 and cutaneous lesions in rats

In pursuing the isolation of vitamin B_6, the assumption has been followed that the cutaneous manifestations which occur in rats kept on a diet devoid of the vitamin B_6 complex with the exception of riboflavin are due to lack of vitamin B_6. On the other hand, the lack of parallelism between growth-promoting activity and prevention of dermatitis [György, 1935, 1, 2] appeared to be based on the multiple deficiency represented by the diet employed for the production of rat acrodynia.

Later, Lepkovsky et al. [1936] conclusively showed that rats need another factor in the diet, in addition to vitamin B_6, riboflavin and vitamin B_12, to supply the requirements for normal growth and development when they are
fed a diet deficient in the vitamin B complex. This additional factor was thought to be identical with the so-called chicken pellagra factor or the filtrate factor [Elvehjem & Koehn, 1935]. Addition of vitamin \( B_6 \) (called also Factor 1 [Lepkovsky et al. 1936]) alone did not permit growth in rats, and “some of the rats developed swollen eyelids which tended to stick together. The eyes were watery and the nose was inflamed”. Addition only of the filtrate factor (called also Factor 2) resulted in slow growth and in development of the specific acrodynia-like condition of the skin. Addition of both Factor 1 and Factor 2 assured normal growth and development. In the experiments of Lepkovsky et al. [1936] the separation of Factor 1 from Factor 2 was achieved by adsorption of the former on fuller’s earth and subsequent elution by \( \text{Ba(OH)}_2 \), the procedure first recommended in the purification of vitamin \( B_6 \) by Birch & György [1936]. Factor 2 remained in the filtrate. Both these preparations represented rather crude concentrates with no appreciable degree of purification.

It is of interest to note that in rats no cutaneous lesions other than sore nose and sticky eyes, and in some instances loss of hair around the eyes (“spectacled eyes” [Oleson et al. 1939, 1]), have been attributed to lack of the filtrate factor, whereas the supposedly equivalent deficiency in chicks is characterized by extensive dermatitis. In view of these observations and of the fact that the cutaneous manifestations in rats fed on a diet deficient in riboflavin are less conspicuous, the designation adermin has been proposed by Kuhn & Wendt [1938] for vitamin \( B_6 \).

After the combined effects of Factor 1 and Factor 2 on the growth of rats were known, it became possible to devise experimental conditions for bioassays in which the growth-promoting activity of Factor 1 (vitamin \( B_6 \)) could be utilized as the criterion. In their studies on vitamin \( B_6 \), Edgar et al. [1938] have relied mainly on this method. One should not overlook, however, the distinctly unspecific nature of the reaction of growth, and therefore the possibility that the promotion of growth, especially when crude concentrates are used, might result from the action of more than one constituent.

Working on similar lines to Edgar et al. [1938], Lepkovsky [1938, 1] and Kuhn & Wendt [1938] have also supplemented the basal diet containing vitamin \( B_6 \) and riboflavin with a crude concentrate of the filtrate factor, but, by giving equal significance to the curative effect on the acrodynia-like lesions in the skin, they have not relied solely on the growth-promoting activity of the vitamin \( B_6 \) concentrates tested.

In experiments on the isolation of vitamin \( B_6 \) we have repeatedly tried this procedure, except that the concentrates of the filtrate factor were added not at the beginning of the experiment but only after the first symptoms of acrodynia had become evident. We used several different preparations of the filtrate factor, some of which were kindly put at our disposal by Dr Lepkovsky and Dr Jukes, while others were prepared according to the methods described by Lepkovsky [1938, 1] and by Edgar et al. [1938]. A definite improvement in the cutaneous condition was almost always observed, together with some promotion of growth, even before any vitamin \( B_6 \) was added. In view of these results we suspected the presence of small, not completely removed amounts of vitamin \( B_6 \) in these crude concentrates of the filtrate factor. We therefore discontinued the use of these preparations as supplements and returned to the original basal diet, although it is admittedly incomplete in several respects, without or with \( 10\% \) dried egg white and with only vitamin \( B_1 \) and riboflavin to represent the vitamin B complex.
VITAMIN B₂ COMPLEX IN RATS

Isolation and chemical characteristics of vitamin B₂

With this method of bioassay vitamin B₂ was isolated [György, 1938, 1] from yeast, independently of and at about the same time as Lepkovsky [1938, 1], Keresztesy & Stevens [1938] and Kuhn & Wendt [1938]. In the preliminary report of György [1938, 1] the various steps in the analysis which led to the pure crystalline product were described. They were the same, in the main, as the procedure used independently by Lepkovsky [1938, 2].

Whereas the pure base was found to be very soluble even in an alcohol-ether mixture containing 90 vol. % of ether, the vitamin B₂ chloride readily crystallized out from a water-acetone solution acidulated with HCl or from alcohol-dioxane, in a similar way to vitamin B₁. The chemical properties of this natural vitamin B₂ were in complete accord with those described by Keresztesy & Stevens [1938] and by Kuhn & Wendt [1938]. Sublimation (molecular distillation) was successfully employed for final purification. The melting point of a preparation thus obtained was found to be 203–204° (corr.) and was accompanied by the appearance of a brown discoloration and by decomposition, again in complete accord with the data given by Keresztesy & Stevens [1938] and by Kuhn & Wendt [1938].

The chemical and biological properties of this natural vitamin B₂ have been compared with samples of vitamin B₂ received from Merck & Co. Inc. through the courtesy of Dr Molitor and Dr Keresztesy, and with samples received from Prof. Kuhn. All three preparations were found to be identical.

Biological effect of vitamin B₂ on rats

Of particular interest was the biological effect of these crystalline vitamin B₂ preparations when they were administered to rats under the experimental conditions chosen, namely, as a supplement to the “no egg white diet” or to the “10 % egg white diet”, both of which contained 20 μg. of aneurin and 20 μg. of riboflavin but lacked Factor 2 or any other member of the vitamin B₂ complex.

Therapeutic tests. In rats suffering from acrodynia, daily addition of 10 μg. of vitamin B₂ to the diet, and in many instances even 5 μg., brought about improvement of the specific dermatitis; in many rats it led to complete cure. A few rats, however, proved refractory and did not respond definitely to treatment with vitamin B₂. In general, promotion of growth was irregular and, as a rule, either slight and very transitory or lacking completely. Only exceptionally was the upturn of the weight curve well pronounced and sustained for several weeks. Weight curves of some of the rats are given in Fig. 2.

During the further course of the therapeutic experiments with the same rats the weight curves invariably became flat and even declined, a result which proves beyond doubt that despite the addition of vitamin B₂ the diet remained incomplete. Later, some of these rats died from different specific deficiency diseases, such as panmyelophthisis, adrenal haemorrhage with concomitant necrosis, and hepatic injury. In a large percentage of the rats that did not die, cutaneous manifestations persisted or developed anew after the specific acrodynia had been relieved or cured by treatment with pure natural vitamin B₂. As previously stated [György, 1938, 1], complete cure of rat acrodynia, with its effects of stunted growth and primary and secondary cutaneous manifestations, can be achieved only by the administration of a combination of vitamin B₂ and a second, unknown factor (or factors), which is (or are) present in the so-called filtrate factor.
As soon as synthetic vitamin B₆ became available,¹ it was possible to extend the foregoing observations by making tests on a larger scale and to confirm them in every detail. The use of very high doses (up to 100 µg. of synthetic vitamin B₆ daily) did not lead to materially better results than those previously obtained with daily doses up to 15 µg. of natural vitamin B₆.

Prophylactic tests. Synthetic vitamin B₆ has been tested not only in therapeutie but also in prophylactic experiments. A large series of rats just weaned were put on the basal ration supplemented daily with 20 µg. of aneurin, 20 µg. of riboflavin and with 10, 20, 50 or 100 µg. of vitamin B₆. Additional supplements of 2 mg. of choline were administered to smaller groups of rats, either from the beginning of the experiment or after the signs of acrodynia had become apparent and the treatment with vitamin B₆ had been initiated.

In the prophylactic experiments the “no egg white diet” was mainly used as the basal ration, whereas the therapeutic effect of vitamin B₆ was tested in rats kept either on the “no egg white diet” or on the “10% egg white diet”.

Later, in experiments with another group of rats, 500 mg. of choline were added per kg. of the diet. The addition of choline was considered necessary after severe renal injury had been observed in a relatively large number of young rats in the prophylactic experiments. This kidney lesion, details of which, together with a discussion of choline as a member of the vitamin B₆ complex, are given in a separate report [György & Goldblatt, 1940], is characterized by symmetrical haemorrhagic necrosis of the kidney cortex, of the type described by Griffith & Wade [1939] and Sure [1940] in choline deficiency, and by Hartwell [1928], Curtis & Newburgh [1927] and Cox et al. [1929] in cystine intoxication. It should be emphasized that the basal casein diet supplemented with vitamin B₁, riboflavin and vitamin B₆ is more liable to lead to lethal changes of this kind, visible to the naked eye at autopsy, than is the same diet without vitamin B₆. Addition of 2 mg. of choline daily or of 500 mg. of choline per kg. of the diet has invariably proved effective in the prevention of necrosis of the kidney cortex.

¹ Synthetic vitamin B₆ was generously put at our disposal by Merck and Co. Inc., Rahway, New Jersey.
These haemorrhagic renal lesions were observed only in very young rats, and therefore were absent from the older animals necessarily used in the therapeutic experiments.

In a few young rats of the choline-free group, flaccid paralysis of the hind legs was seen, which might be attributed also to choline deficiency, as recently described by Sure [1940].

A total of 507 rats was used in the therapeutic and prophylactic bioassays of vitamin B₆ (natural and synthetic). This number was distributed as follows:

(1) On the "no egg white diet":

(a) Therapeutic tests (5–10 μg. of vitamin B₆) with or without addition of choline ... ... ... ... 143 rats

(b) Prophylactic tests (10–100 μg. of vitamin B₆) with or without addition of choline ... ... ... ... 253 rats

(2) On the "10 % egg white diet":

Therapeutic tests mainly ... ... ... ... 111 rats

In this large group of animals the preliminary observations [György, 1938, 1] were fully confirmed. Addition of vitamin B₆ to the basal diet supplemented daily with 10 μg. of aneurin and 20 μg. of riboflavin was unable to assure permanent restoration of growth with lasting cure or prevention of cutaneous lesions in the rats which, in the therapeutic experiments, failed to react appreciably or which showed, as a rule, only transitory improvement.

Corresponding weight curves of a few of the rats are given in Fig. 2.

The animals in which cure or prevention of cutaneous lesions could be considered complete or almost complete succumbed during the further course of the experiments to secondary diseases, such as adrenal haemorrhage, with or without panmyelophthisis, hepatic injury, and, of course, to unspecific secondary infections, chiefly pneumonia.

In a fairly large group of these rats no cause of death could be determined, and only complete failure to gain weight gave any indication of the presence of a basic metabolic disturbance, probably of the character of a deficiency disease.

**Cutaneous lesions in rats treated with vitamin B₆**

In the majority of rats treated with vitamin B₆ lesions of the skin became apparent both in the therapeutic and prophylactic experiments. Three types could be distinguished [György & Eckardt, 1939]; they were different from the condition seen in specific acrodynia in rats, known as vitamin B₆ deficiency.

**Type I.** The hair over the abdomen is matted together, appears damp and is sticky to the touch. There is loss of hair around the chin, nose and eyes and over the forehead between the ears. The sides of the cheeks and also of the abdomen are often covered with scales which are generally brown in colour. These scales may sometimes become quite thick. In advanced cases, the lids of the eyes seem to be inflamed and are at times kept closed, while the brown scaliness may extend over the sides of the forehead. On account of irritation, the animal often rubs his forehead and cheeks with his forepaws and thus probably transfers a coating of the brown scaly matter to the paws. There is emaciation and inanition.

**Type II.** After complete cure or improvement of the acrodynia, new lesions start around the mouth, the groin and the axillae, in the form of inflammation and production of scales. Later, alopecia follows and extends to the neck and over the back. Hyperaemia may be seen also on the ears and the forepaws often
become excoriated. In several animals generalized scaliness (exfoliative dermatitis) has been observed.

Type III. This form seems to be identical with the condition described by Lepkovsky et al. [1936] in rats that received a rather crude concentrate of vitamin B₆ (Factor I) lacking Factor 2, or with that seen in similar circumstances by Oleson et al. [1939, 1], which they called "spectacled eye". This type is characterized by watery eyes, which stick together, by loss of hair (abrasion) around the eyes ("spectacle" appearance) and by sore mouth. In our own animals, progressive keratitis with final complete destruction of the eyeballs (panophthalmitis) would sometimes develop. The changes in the cornea were, as a rule, different from those seen in riboflavin-deficient rats [Eckardt & Johnson, 1939], but occasionally they resembled them closely. In this connexion it should be pointed out that rats with this type of lesion are commonly infested with pediculii and may show the same fine, creamy, almost almost dandruff-like generalized scaliness that has been described [György, 1934; 1938, 2] in rats suffering from riboflavin deficiency. A further analysis of this condition will be given in a later report, to be published separately.

Types I–III are progressive lesions and have a fatal outcome unless treatment is instituted in time (Fig. 2).

Fur alterations in rats treated with vitamin B₆

Apart from cutaneous lesions, fur alterations also can be observed in rats kept on a basal diet deficient in the vitamin B complex and supplemented with aneurin, riboflavin, vitamin B₆ and choline. The fur of these rats loses its pigment ("greying" effect) in piebald or black animals and shows "rusting" in albino rats. The fur changes appear to be identical with those seen by other investigators [Bakke et al. 1930; György, 1935, 1; Morgan et al. 1938; Lunde & Kringstad, 1939; Oleson et al. 1939, 2; György et al. 1940] in rats kept under similar conditions. They were found mainly in connexion with cutaneous lesions of Type II and somewhat less frequently with those of Type III. Their absence in connexion with Type I lesions might be explained by the fact that the rats in this group usually died very soon after development of the specific symptoms of Type I.

Discussion

From the observations reported it is evident that the routine use of crystalline vitamin B₆ in a diet devoid of the vitamin B complex supplemented with aneurin and riboflavin has had only a transitory effect in simplifying the problems encountered in the systematic analysis of the vitamin B₆ complex.

In prophylactic experiments the addition of vitamin B₆ to a diet deficient in the vitamin B complex supplemented with aneurin and riboflavin and containing casein as the source of protein has shed more light on the importance of choline as a necessary constituent, a fact which hitherto has not been sufficiently emphasized.

In further prophylactic and therapeutic experiments it has become clear that the problem of rat acrodinia, specifically that of the cutaneous lesions observed in rats fed on a diet lacking the vitamin B complex supplemented with vitamin B₁ and riboflavin, is not alone a question of vitamin B₆ deficiency. The three different types of cutaneous lesions seen in rats after the incorporation of vitamin B₆ in the experimental diets should be regarded as visible signs that the diets lack some other unknown members of the vitamin B₆ complex. This observation, that

1 It should be noted that "spectacled eye" has also been previously seen in rats suffering from lack of riboflavin [György, 1935, 1].
VITAMIN B2 COMPLEX IN RATS

vitamin B6 is only one of the factors which are influential in the cure or prevention of cutaneous manifestations in rats kept on the experimental diets heretofore used for the production of rat acrodynia, is well in accord with the results recently reported by Schneider et al. [1940].

SUMMARY

I. Incorporation of dried heated egg white in the experimental diet used for the production of rat acrodynia increased the incidence of the disease and shortened the time of its appearance.

II. 1. In young rats kept on a casein diet supplemented with aneurin, riboflavin and vitamin B6, necrosis of the kidney cortex occurred as a symptom of choline deficiency more often than in rats on the same diet not supplemented with vitamin B6.

2. Rats with acrodynia react only transitorily to vitamin B6. Cutaneous lesions, which can be classified into three different groups, and achromotrichia are regularly observed in the rats treated with vitamin B6 without the addition of other members of the vitamin B2 complex, apart from riboflavin and choline.

3. Attention is called to the severe lesions about the eyes (keratitis, panophthalmitis) observed in one group of these rats; in this group pediculosis was often observed.

REFERENCES

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