CCIX. NOTE ON THE INCIDENCE OF DERMATITIS AMONG RATS DEPRIVED OF VITAMIN B_2.

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THE most consistent sign of a deficiency of vitamin B_2 in rats is failure of growth, but in addition to this an inflammatory dermatitis, considered by some to be analogous to human pellagra, may develop. This dermatitis, however, is irregular in occurrence; some authors have not observed it in any of their animals, others in only a proportion. Various reasons have been put forward to account for this irregularity. It has been suggested that in order to obtain symptoms constantly absolute deprivation of vitamin B_2 must be ensured [Chick and Roscoe, 1928], or, alternatively, that small amounts of vitamin B_2 must be supplied [Sherman and Sandels, 1931]; also that a seasonal variation in incidence of symptoms occurs [Leader, 1930]. Finally the theory has been advanced that the dermatitis is due to lack of some dietary factor, other than that promoting growth, the distribution of which is not as yet understood [Chick and Roscoe, 1928; Sure and Smith, 1930–31]. Kuhn *et al.* [1933] have in fact, stated that vitamin B_2 can be separated into two factors one of which is a skin factor (vitamin H).

In this paper data are given concerning the incidence of dermatitis among the vitamin B_2-deficient animals observed in this laboratory during the past seven years. In addition, results of experiments are reported, in which attempts were made by variations of the diet to influence the development of dermatitis.

EXPERIMENTAL.

Young rats, shortly after weaning, and weighing 30–40 g., were started on the deficient diet. This consisted of caseinogen 20 parts, rice starch 60, salt mixture[2] 5 and hardened cottonseed oil 15, mixed with an equal weight of water and steamed for 3–5 hours. Cod-liver oil was given separately each day. Vitamins B_1 and B_4 were given as a daily dose of a concentrate from yeast, prepared according to the method described by Kinnersley *et al.* [1933]. The presence of vitamin B_4 in this concentrate was assumed, as extraction of the charcoal was carried out with acid alcohol, in which process both vitamins B_1 and B_4 are stated to be eluted [Reader, 1929].

The type of dermatitis observed in this laboratory in rats deprived of vitamin B_2 has been described by Chick and Roscoe [1927] and is essentially the same as that originally described by Goldberger and Lillie [1926], with the exception that, whereas the lesions observed by them were stated to be bilaterally symmetrical, that has not always been the case with those observed here. Some authors report that the only lesions they obtain as a result of deprivation of

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vitamin B_2 are a scurfy condition with loss of hair. The latter condition is frequently observed among our vitamin B_2-deficient animals but for the purpose of the present study has not been counted as dermatitis.

In working out the proportion of rats developing dermatitis, only those that survived for more than 10 weeks without symptoms have been counted as negatives.

(1) *Number of rats developing dermatitis.* During the past seven years 108 out of the 191 rats on the vitamin B_2-deficient diets have developed dermatitis, an incidence of 57 %. The figures for each of the seven years were: 14/21, 16/26, 23/30, 6/20, 9/21, 16/24 and 24/49.

(2) *Time taken for dermatitis to develop.* The average time during which the rats received the deficient diet before symptoms developed was 10 weeks; in 32 % of the 108 cases the dermatitis occurred after 9–11 weeks of depletion.

(3) *Seasonal variation in the occurrence of symptoms.* The development of dermatitis in the above 108 rats has shown no significant relation to the time of year. Thus 55 % of the rats born in the quarter January-March developed dermatitis, 45 % of those born in April–June, 61 % of those born in July–September and 62 % of those born in October–December. Nor was the period of development of symptoms longer at any one time of year; during the same quarters the average time was 10, 9, 11 and 10 weeks respectively. The experiments were evenly distributed over these periods.

(4) *Weight increase of vitamin B_2-deficient rats.* Considerable variations in weight increase occurred among the animals receiving the same vitamin B_2-deficient diet. The degree of growth, however, did not appear to bear any relation to the occurrence of dermatitis. Thus the average growth during the first five weeks on the deficient diet was 17 g. for the animals which developed dermatitis, 16 g. for those which did not. Nor was a greater weight increase associated with delay in occurrence of symptoms. Rats developing dermatitis after 0–5, 5–10 or 10–15 weeks of depletion had increased in weight by an average of 23, 18 and 15 g. respectively in the first 5 weeks of the observation. This would seem to indicate that those which grew best were most liable to succumb, but the variations in growth were so great (− 3 g. to + 37 g. in 5 weeks) that the above differences in averages were not significant. After the animals had developed dermatitis, growth ceased, as was to be expected since they were then definitely sick.

(5) *The influence of diet.* At first it was thought necessary to use a highly purified caseinogen in the diet in order to induce dermatitis [Chick and Roscoe, 1928]. Later, as the result of papers by Coward, Key and Morgan [1929] and Coward, Key, Morgan and Cambden [1929] showing that unpurified caseinogen did not contain appreciable amounts of either vitamin B_1 or B_2, diets were tested which contained the same (B.D.H. "light white casein") before purification. Substitution of this less pure material for the purified product did not cause any increase in the growth of rats receiving small amounts of either vitamin B_1 or B_2, nor was it found to influence the incidence of neuritis in vitamin B_1-deficient rats or of dermatitis in vitamin B_2-deficient rats.

The results obtained using three different kinds of caseinogen were as follows.

(i) *Glaxo purified caseinogen (physiological caseinogen, AB).* 13 out of 22 rats (64 %), developed dermatitis after an average depletion period of 8 weeks.

(ii) *Lister Institute purified caseinogen.* 61 out of 107 rats (57 %), developed dermatitis after an average depletion period of 11 weeks.

(iii) *"Light white casein" unpurified.* 36 out of 54 rats (67 %), developed dermatitis after an average depletion period of 9 weeks.

There was thus no significant difference between the effects of diets containing these different caseinogens upon the incidence of dermatitis. The increase in weight during the period of vitamin B_2 deprivation was, however, greater when the diet of the rats contained the unpurified caseinogen, the average being 20 g. in the first 5 weeks as against 8·5 g. on diets containing the "Lister Institute purified" caseinogen. This might be taken as an indication that, when unpurified, this caseinogen contained traces of vitamin B_2.

In this connection it may be noted that various authors have found that dermatitis occurred when the rats received some vitamin B_2 and not when complete deprivation was ensured. Sherman and Sandels [1931] obtained dermatitis in rats receiving very small doses of vitamin B_2, but Leader [1930] and Sure and Smith [1930–31] administered large doses of marmite extract or autoclaved yeast (10 % in diet) and obtained symptoms of dermatitis at the same time as good growth. These results are difficult to reconcile with those obtained here, where dermatitis has never developed in rats receiving doses of vitamin B_2, however small, and autoclaved yeast in as small quantities as 0·2 g. daily has been found to cure the symptoms [Chick and Roscoe, 1927].

In the present experiments, the use of unpurified caseinogen which may have contained small amounts of vitamin B_2 was not found to increase the incidence of dermatitis.

In addition to the above the following diets were tried, but no influence on the incidence of dermatitis was detected.

(*a*) A diet containing 17 % sucrose, with or without supplements of an alcoholic extract of marmite, containing small amounts of vitamins B_1 and B_2 [Leader, 1930].

(*b*) A diet containing traces only of fat. Evans and Lepkovsky [1929] found that dermatitis occurred more often in rats on a low fat diet.

(6) *Influence of initial reserve stores of vitamin B_2 possessed by the rat.* In observations of the incidence of dermatitis among members of the same litter, great variation has been found; frequently half of the litter succumbed and half did not. Thus it does not appear that some mothers pass on stores of the vitamin to their young while others fail to do so.

Discussion.

The present attempt to discover the factors influencing the incidence of dermatitis in vitamin B_2-deficient rats must be regarded as entirely unsuccessful. Purification of the protein of the diet, to remove all traces of vitamin B_2, was not found to increase the incidence of symptoms, nor was the presence of small amounts of vitamin B_2 in the diet. No seasonal variation in incidence was observed, and no support was obtained for the theory that the irregularity observed was connected with a hitherto unrecognised and uncontrolled dietary factor, differing from that responsible for growth. Further evidence on this last question is given in the following paper [Roscoe, 1933].

Summary.

1. Dermatitis has occurred in 108 out of the 191 rats fed on vitamin B_2-deficient diets in this laboratory during the past seven years. The average time taken for symptoms to develop was 10 weeks.

2. Time of year, weight increase and various alterations in the diet were without effect on the occurrence of symptoms.

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