

## SPONTANEOUS CURES IN RATS REARED UPON A DIET DEVOID OF VITAMIN B AND ANTI- NEURITIC VITAMIN.

BY MARGARET HONORA ROSCOE.

(*From the Department of Experimental Pathology, Lister Institute.*)

THE preceding paper by Fridericia and his co-workers on "A Transmissible Change in the Intestinal Content, enabling Rats to Grow and Thrive without vitamin B in the Food" deals with results so novel and unexpected that a brief statement from another laboratory confirming them, is not redundant.

The phenomenon, which Prof. Fridericia has called "refection," was also observed at the Lister Institute in the autumn of last year and occasioned much perplexity. Hearing that Prof. Fridericia had made a communication on the subject to the Physiological Congress in Stockholm in August (1926), the writer's observations were sent to him and publication withheld until he should publish his results in full.

Recent researches at the Lister Institute (Chick and Roscoe, 1927) have confirmed the work of Goldberger and his colleagues (1926) and of previous workers, in showing that the water soluble vitamin B discovered by McCollum and Davis (1915, *a* and *b*) consists of at least two constituents: (i) the water soluble, anti-neuritic, vitamin discovered by Eijkman (1897), shown to be necessary to prevent and cure polyneuritis in birds and considered by him and others to be concerned in the prevention and cure of human beri-beri. (ii) a second water soluble dietary factor, usually present with the anti-neuritic vitamin in natural foodstuffs, in absence of which rats do not survive and often develop skin lesions. The lesions were described by Goldberger (1926) who considers that this vitamin may be concerned in the prevention and cure of human pellegra. The latter has been shown to be much more thermostable than the anti-neuritic vitamin. Both are contained in McCollum's water soluble B, since both are necessary in a diet to maintain health and growth. In this paper (i) will be alluded to as the anti-neuritic vitamin, and (ii) as vitamin B (*sensu strictu*) and the combination of both, as water soluble B vitamins.

### THE CONDITION OF REFECTION.

The spontaneous occurrence of refection was observed by us in September 1927 in 2 rats receiving a diet deprived of water soluble B vitamins<sup>1</sup>. No

<sup>1</sup> *Basal diet.* Purified extracted casein 100 gm., rice starch 300 gm., cottonseed oil 50 gm. salt mixture 25 gm., with cod-liver oil given daily by hand.

further cases developed till December, when in the course of 4 weeks, 11 rats out of 25, derived from 3 litters, became spontaneously refeeded.

The rats of the litters in which refection occurred spontaneously were all piebald. Albino rats were also in use in the laboratory at the time, but these were not affected. All rats were kept in cages with floors made of zinc wire netting of half-inch mesh to permit escape of faeces and hinder their ingestion. The age of the rat had no apparent bearing on the incidence of refection, which occurred in rats of ages varying from 6 to 14 weeks. The weight varied from 40 to 110 gm. The first two rats in which the phenomena was observed were receiving the basal diet deprived of water soluble B vitamins without any addition, but in the other cases various supplements had been added. Such were a yeast extract (Peters 1924) which contains the anti-neuritic vitamin, but not vitamin B *sensu strictu*, and autoclaved yeast or autoclaved wheat embryo which contains vitamin B, but is deprived by heating of the anti-neuritic vitamin. Refection was also observed in rats which were receiving daily supplements of dried whole yeast or marmite, each of which contains both the water soluble B vitamins. After refection was observed to be established these doses were stopped, but growth continued in spite of this.

The condition of refection was marked by the peculiar white faeces containing undigested starch described above by Fridericia; these were equal in size to those of rats on normal diets and large in comparison with the very small faeces usually excreted by rats on a diet deficient in the water soluble B vitamins. The total dry weight of faeces excreted in a day by a refeeded rat of 60 gm. weight was 2 to 3 gm. or 4 to 5 times the weight ordinarily excreted on the deficient diet. The colour was white instead of dark brown or black owing to the large content of undigested starch, the faeces were relatively hard and dry, and contained gas bubbles. As much as one-quarter of the total starch ingested in the diet has been recovered from them.

Autopsies on rats that had been refeeded for some time showed healthy, normally developed, organs. Undigested starch was present throughout the alimentary canal, the caecum especially being engorged with starch.

#### EFFECT OF REFECTION ON SYMPTOMS OF WATER SOLUBLE B VITAMINS DEFICIENCY.

The symptoms diagnostic of lack of the anti-neuritic vitamin in young rats are loss of appetite and weight, accompanied in some cases by paralysis of the hind legs; death follows after 3-4 weeks on the deficient diet. Two young rats became refeeded after 1 and 2 weeks respectively, of deprivation. Prevention of neuritic symptoms was complete and the rats grew normally.

In the other cases of spontaneous refection which have been observed, the anti-neuritic vitamin was being supplied at the onset of refection, vitamin B only being absent in the diet. Rats suffering from lack of vitamin B *sensu strictu*, cease to grow and after 5-10 weeks or even longer may develop typical skin lesions (see Chick and Roscoe, 1927). In one case these lesions were

well developed, and had been present for 2 weeks, when refection occurred. The skin healed spontaneously and normal growth began. In several other cases where insufficient vitamin B was being given and the growth was subnormal, it was maintained at the normal rate after refection had started.

The ability of refected rats to do without either of these vitamins is most clearly demonstrated by the normal growth and health immediately they become refected. In the case of rats receiving adequate supplements of the anti-neuritic vitamin or of both the water soluble B vitamins previous to refection, normal growth was maintained after these supplements were withdrawn.

These observations are contrary to those described by Fridericia and his colleagues in Section IV (p. 93) of their paper; they were unable to observe refection in rats receiving vitamin B in their food. Their experiments, however, were somewhat different in type to those herein described.

#### ULTIMATE EFFECT OF REFECTION.

The normal growth of refected rats continued till the males reached an average weight of 210 grm., the females of 145 grm. In some cases the faeces of these older rats became darker in colour, though remaining large in size, in others they became small and dark for a while. When this happened there was temporary loss of weight, but recovery took place, and was accompanied by the presence of large, white, starchy, faeces.

In one case a spontaneously refected rat, No. 47, of 230 grm. weight, was placed in a cage constructed of wire of a wider mesh than was usual. This allowed its large faeces to fall through with greater readiness than occurred in the usual type of cage. After 2 days the faeces became dark and there was a loss of weight which continued as long as the rat remained in the cage. Recovery took place whenever it was restored to a cage constructed of smaller mesh wire. Finally after a spell of 3 weeks in the cage of large mesh, the rat declined in weight to 150 grm. and died with paralysis of the hind legs. Other rats placed in similar cages, at first declined but recovered afterwards. The inference drawn from these results was that to continue in the refected state it was necessary for the rats to eat some of their faeces. Whatever construction of cage is used, there are some rats that cannot be prevented from eating their own faeces, for they secure them and consume them as they leave the body. The drastic results of complete deprivation were only observed apparently in the one case of rat No. 47.

#### INDUCTION OF THE STATE OF REFECTION.

Seeing that the eating of their own faeces seemed necessary for the continuance of refection, it was thought probable that the state might be induced by the feeding of faeces from refected rats to others. This, as Fridericia and his colleagues found, proved to be the case. Immediately after feeding doses of 0.25 to 0.75 grm. dry weight daily of these faeces, rats on a diet deprived

of water soluble B vitamins passed white faeces themselves. They began to grow normally and usually continued to do so after the dose of white faeces was withdrawn. In a few cases, however, refection could not be well established in this way, the growth never became normal, and some weeks after the inducing dose of white faeces was withdrawn, the condition lapsed and the rats died from the effects of the deficiency in their diet.

As was the case with the spontaneously relected rats, so with those in which the condition was induced, refection was satisfactorily established both in rats on the basal diet and in those receiving additions of various fractions prepared from yeast and wheat embryo. Some of those products contained both, and some contained one only, of the water soluble B vitamins. Both piebald and albino rats (weight 35–100 gm.) were successfully affected.

#### CONDITIONS OF THE BASAL DIET NECESSARY FOR REFECTION.

Seeing that undigested starch was present in the faeces of all relected rats, it was thought advisable to try the effect of varying the form of carbohydrate in the basal diet deprived of water soluble B vitamins. Fridericia and his colleagues (Section IV) found refection impossible when the starch of their experimental diet was replaced by sugar, but possible when it was replaced by dextrine containing a small proportion of starch.

The results of the following experiments are in general agreement with these conclusions. Three relected rats were placed on diets in which the carbohydrate consisted of:

- (I) 50 per cent. rice starch, 50 per cent. cane sugar.
- (II) 25 per cent. rice starch, 75 per cent. corn dextrine.
- (III) 100 per cent. corn dextrine.

The rat on diet (I) continued to grow and had large grey faeces for 2 weeks, then refection ceased. It was permanently restored however, after the rat had received 1.0–0.5 gm. of white faeces from a relected rat daily for a fortnight. Refection would seem to have been disturbed by the reduction of starch in the diet, but was not made impossible.

The relected rats placed upon diets (II) and (III) rapidly declined in weight and their faeces became small and dark after the first few days. After 4 weeks both had attacks of paralysis. One week and three days, respectively, before these attacks occurred, a daily dose of 1.0 gm. dried white faeces from relected rats had been administered. These doses were without effect in preventing the symptoms of anti-neuritic vitamin deficiency, although a few white faeces were passed daily. In the case of the rat on diet (III) these could only contain the starch of the faeces dose. The paralysis was cured with a yeast extract containing the anti-neuritic vitamin only and devoid of vitamin B, and after this happened the rats proceeded to grow normally as long as the faeces dose was continued, in spite of the lack of this principle. This result might be explained either by presence of vitamin B in the white faeces administered, or by a mild degree of refection made possible by the amount of

starch in the diet. In case of the rat on diet (III), the only starch consumed was the very small amount taken with the faeces dose. From the relapse, which had taken place earlier in the history of these two rats, it would appear that refection is impossible when the total carbohydrate of the diet is soluble though the proportion of insoluble starch which makes it possible would seem to be variable. These experiments suggest that there is very little, if any, anti-neuritic vitamin in the faeces from refected rats. Doses of 1.0 gm. were given without prevention or alleviation of the symptoms of paralysis, due to lack of this vitamin.

Experiments were also undertaken to see whether refection would be possible if the starch was altered by cooking. The ordinary basal diet, after mixing with water, was steamed till all the starch grains had been broken. Refection ceased when refected animals were fed on this diet and on it no definite cases have occurred spontaneously. It is therefore now used by us as our standard diet free from water soluble B vitamins.

That refection does not occur when the diet is cooked or when the starch is replaced by dextrine, suggests that the presence of unchanged starch grains in the intestine is a necessary factor in refection.

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