RELATION BETWEEN THE NATURE OF THE CARBOHYDRATE IN THE DIET AND REFECTION IN RATS.

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(With 3 Charts.)

Fridericia and co-workers have recently (1927) described in detail an important phenomenon in the rat, namely, occasional spontaneous growth with the production of bulky white faeces, when the animal is living on a vitamin B-free diet containing 57 per cent. rice starch. Other vitamin B-free rats when fed with these bulky faeces also resumed growth, and in many cases continued to grow for long periods. Fridericia has named the phenomenon "refection." It has also been described by Roscoe (1927).

The experimental work forming the basis of the present paper was carried out in the summer of 1927 before either of the writers had seen Fridericia's earlier communication (1926). Further confirmation of a matter so important to the study of rat nutrition would seem not to be without value, and furthermore, the problem here was approached from a different point of view, and has in this way brought out new points in relation to the incidence of refection.

Experimental.

Workers in this laboratory have had difficulty from time to time with the vitamin B-free[1] diet. Sometimes rats placed on it would decline for a short time only, and then resume growth, and continue to grow at a nearly or quite normal rate. The customary vitamin B-free diet used in this laboratory is the following:

Caseinogen (vitamin free)	23
Rice starch (B.D.H.)	40
Cane sugar	17
Salts (McCollum)	5
Palm-kernel oil	15

Cod-liver oil (3 drops) was fed daily by hand. The diet was mixed with a little water and fed uncooked.

In each case where the rats failed to show signs of vitamin B deficiency, the rice starch in the above diet had been replaced by potato starch. It was not until the present investigation was undertaken that the bulky white faeces

[1] In this paper, unless otherwise stated, vitamin B means the whole complex, including the anti-neuritic factor.

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were noticed. Refection does not seem to have occurred spontaneously in this laboratory when the rice starch has been used—at all events not since a careful watch for it has been kept. But apparently what is of occasional occurrence with rice starch becomes the rule when potato starch is used. The cages used by us had $\frac{1}{2}$-inch mesh, excluding the ingestion of faeces as far as is possible. Some rats on the potato starch diet grew from the start, but the majority first declined and showed symptoms of vitamin B deficiency —rough fur, weakness of the hind limbs, lowered temperature, etc. Improvement generally set in after 7–10 days (Chart 1). We have transferred rats

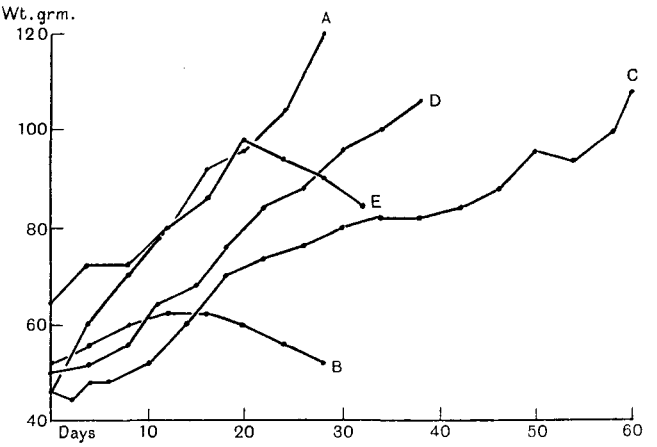

Chart 1. Curve A. Average curve for rats on normal mixed diet. Curve B. Average curve for rats on vitamin B-free rice starch diet. Curves C, D, E. Average curves for 3 sets of rats on potato starch diet.

which have been "run out" on the rice starch diet, to potato starch diet without the addition of any vitamin B. The condition of the animals remained stationary for about a week, and then improved, a normal healthy appearance being rapidly regained. Some few rats declined after a few weeks on the potato starch diet, the faeces at the same time becoming dark and small. We noticed, as did Fridericia, that rats that had been kept successfully on the diet for a long period often produced bulky brown faeces instead of white ones.

Our analysis of the starch content of the faeces and the estimation of the amount of starch digested agrees with the figures given by Fridericia, though his rats were refected by other means.

Both albino and piebald rats became refected, older ones not so readily as animals of 40–60 grm. Rats receiving more or less deficient amounts of marmite, Harris yeast vitamin concentrate, and alcoholic yeast extract also became refected when given potato starch. Roscoe (1927) also had spontaneous refection occurring in rats receiving either or both fractions of vitamin B, whereas Fridericia did not.

Washed potato starch.

The starch was thoroughly extracted three times with 70 per cent. alcohol. The grains were still unbroken after this treatment, and refection occurred in some rats to whom it was fed, but the initial period of decline seemed to last longer and the improvement was slower (Chart 2). In some cases no

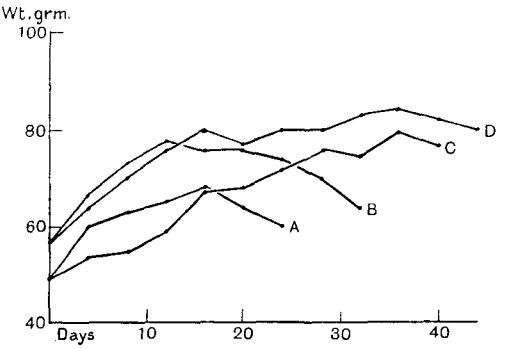

Chart 2. Curve A. Rats on cooked rice starch diet. Curve B. Rats on cooked potato starch diet. Curve C. Rats on washed potato starch diet. Curve D. Rats on arrowroot diet.

refection at all took place, the animals dying after 5–6 weeks on the diet. Sir Frederick Hopkins kindly allows us to say that he himself at one time had failed to produce vitamin B deficiency when using potato starch in place of the customary rice starch, but that after thorough washing of the starch only an occasional rat showed spontaneous growth.

There was no improvement in the condition of a vitamin B-free rat when treated with the potato washings, after they had been concentrated *in vacuo*.

This, and the easy destruction of the refective agent by low temperatures, and the fact that our unwashed potato starch was purer than the rice starch, containing only ·015 % N, whereas the rice starch contained ·053 %, all discredit the possibility that refection is merely due to the presence of vitamin B in the starch.

Cooked potato starch diet.

The diet was mixed with water and steamed in a double saucepan for 3 minutes, the temperature of the actual food never rising above 70° C. A few unbroken starch grains were still found in the faeces of rats fed on this diet. The animals grew slowly for 10 days only, and then declined with symptoms of vitamin B deficiency. The curves for these rats were similar to controls on cooked rice starch diet. The latter confirmed the findings of Baccharach and Hartwell (1927), namely that rats are slower in showing vitamin B deficiency when the diet is cooked than when it is fed raw (Chart 2).

Arrowroot starch diet.

Faeces of rats on this diet remained rather small and dark, but nevertheless contained many unbroken starch grains, and analysis showed that

about 25–30 per cent. of starch was present. The animals did not thrive so well as the rats on potato starch diet, but much better than those on uncooked rice starch diet. The arrowroot seemed to cause some degree of refection, but in no case did the animals continue to grow for more than 6 weeks (Chart 2). This, and the easy destruction of the refective agent by low temperatures, and the fact that our unwashed potato starch was purer than the rice starch, containing only ·015 % N, whereas the rice starch contained ·053 %, all discredit the possibility that refection is merely due to the presence of vitamin B in the starch.

Influence of potato starch on pellagra.

Only one rat was available for this experiment. It had been receiving 50 mg. daily of Harris yeast vitamin concentrate as its source of vitamin B, but after 7 weeks on the diet growth ceased and pellagra developed. There was loss of hair and marked dermatitis on mouth, paws, and feet, which were also oedematous. The ears were slightly involved. The starch in the diet was changed from rice to potato. Some improvement was noticed after 4 days and the condition was completely cured after 2 weeks. No gain in weight took place till the 13th day, when bulky white faeces were produced for the first time. Roscoe also reports that skin lesions were healed after refection. In the case of our rat, the lesion was healed before there was any visible sign of refection, as indicated by the change in faeces. This rat later declined, but there was no return of pellagra (Chart 3).

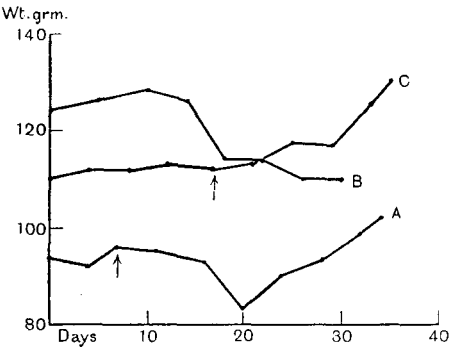

Chart 3. Curve A. Rat which developed pellagra after several weeks on rice starch diet plus the addition of 50 mg. daily of Harris yeast vitamin. Curve B. Control rat receiving rice starch and Harris yeast vitamin. Curve C. Rat which reached plateau on rice starch diet with the addition of 0·5 grm. marmite daily.

(Arrow indicates point at which rats A and C were transferred to potato starch diet.)

Discussion.

Apparently what is of occasional occurrence when the vitamin B-free diet contains rice starch, becomes almost the rule when potato starch is used. This is still further support for the contention of Fridericia that the phenomenon of refection is connected with undigested starch. Peculiar properties

in connection with potato starch have been noticed from time to time. As long ago as 1897 Eijkman discovered that it did not cause beri-beri in hens, as did polished rice. Randoin and her colleagues (1927, and other papers there referred to) have also noticed its apparent vitamin B sparing properties. They give the explanation that vitamin B is only required for the metabolism of carbohydrates, and that as potato starch is badly, or not at all digested, vitamin B is therefore not necessary when this is the carbohydrate fraction of the diet. But though the faeces of refected rats contain a high percentage of undigested starch, nevertheless 70 per cent. at least of the actual intake of starch (when fed at a 40 per cent. level of intake) is digested by these animals, while in addition our rats had 17 per cent. of sucrose. Sherman and Gloy (1927) have shown that varying the rice starch from 32 to 74 per cent. of the diet made no difference to the rate of decline of rats receiving an inadequate amount of, or no vitamin B. The hypothesis of Randoin and her collaborators is also contrary to the findings of Reader and Drummond (1926) and of Hartwell (1925), who found that the vitamin B requirements for successful growth were markedly raised when a very high protein diet was fed.

It might also be assumed that a new protective factor is present in potato starch. The presence of such a factor correcting the dietary deficiency arising from the presence of dried egg white as the sole source of protein in an otherwise well-constituted artificial diet, has quite recently been postulated by Boas (1927). Her work is suggestive in view of the relation between potato starch and spontaneous refection. Her protective factor X has much the same distribution as vitamin B, the notable exceptions being that it is absent from marmite and present to a large extent in potato and arrowroot starch (and to a small extent in cooked potato starch and caseinogen). As far as we are aware the only proof of the absence of the protective factor X from marmite was the development of deficiency symptoms when marmite was present in the diet to the extent of 5 per cent., incorporated in the daily ration. It does not appear from the paper whether an increased amount of marmite was tried. The level at which Boas fed the marmite is certainly generally sufficient to supply the necessary amount of vitamin B, but results with this source of the vitamin seem to be very variable. For instance, Hartwell (1926) found that 2 per cent. was sufficient to give very good growth in rats, whereas Drummond (1917) found optimal growth only at 6 per cent. With one batch of marmite we ourselves found that 0·5 grm. daily (fed separately) was not sufficient to maintain growth, as the animals reached a plateau in the neighbourhood of 110 grm., and one rat developed a marked pellagra-like condition, and assumed a kangaroo-like posture. The skin lesions described by Boas were similar to the pellagra described by Goldberger and Lillie (1926) and it is interesting that this condition in her animals was cured by the addition of potato starch to the diet, while we were able to cure pellagra by the same means. It is evident then that the peculiar properties described by Boas can be equally well demonstrated when the protein used in the diet is caseinogen. Boas pointed out that when rats received potato starch or raw

potato in the diet they produced bulky white faeces due to the presence of undigested starch. She has thus evidently produced refection in her animals, and this confirms our point that refection is the usual result when potato starch is given. Her protective factor X and the refective agent of potato starch may not be identical, but they seem at least to have many points of resemblance.

If the endogenous production of vitamin B in the intestine is the explanation of this phenomenon, then it would seem as if differences in the power of absorbing the vitamin under different conditions must come into the problem, as it is well known that vitamin B is present in the faeces of rats suffering from a marked vitamin B deficiency. It seems possible that either the undigested starch causes greater absorption to take place, or that the starch favours the growth of some organism which produces much more vitamin than the usual intestinal bacteria, or that both these processes are involved. Fridericia found that the *p*H of the intestinal contents of refected rats was normal, whereas in the case of rats suffering from vitamin B deficiency it was alkaline. There is just a possibility that the acid *p*H favours absorption.

We carefully examined the faeces of our refected rats from a bacteriological point of view, but without any significant result. We can confirm Fridericia's statement that large cocci (Gram +) were frequently present to an unusual extent, but in many rats growing well on the vitamin B-deficient diet (containing potato starch) these were not seen, but there were large numbers of Gram + spore bearing bacilli. Others yet again showed large numbers of slender Gram + bacilli. The only respect in which the faeces of different rats seemed at all similar was that in all cases the Gram + bacilli were in excess of the Gram − varieties. In this connection it is interesting to note that Heller, McElroy and Garlock (1925) found that it is the Gram + spore bearing bacilli in the intestine which are able to synthesise vitamin B. Fridericia states that refection is transmissible through indirect contact, though it is difficult to see why he thinks that refection has been transmitted in some cases instead of being simply spontaneous in origin. We, however, have kept two rats living in the same cage, one of which became refected while the other did not.

Though refection seems to be due to the starch of the diet, evidently some starches are more favourable to its production than others. Potato and arrowroot starches are prepared by simple extraction with water, whereas rice, wheat and maize starches are all prepared by the action of weak acids or alkalis (König, 1920). This difference in preparation may account for the difference in refective power.

The phenomenon of refection may account in some degree for the very discrepant results obtained in testing the requirements of the rat for vitamin B. The composite nature of vitamin B (Mitchell, 1919; Funk and Dubin, 1922; Funk and Paton, 1922; Goldberger and collaborators, 1926; Chick and Roscoe, 1927) no doubt has led to confusion on this point, and experience in this laboratory seems to indicate that there are considerable irregularities in the commercial products of vitamin B. But it is surely possible that rats may be

refected to a mild degree, so that the addition of much smaller quantities of vitamin B to the diet are required than would otherwise be the case.

Fridericia has suggested the possibility of refection in the human subject and its relation to beri-beri. One thinks of this possibility also in relation to potato-eating populations such as the peasants of Ireland, Russia and Poland, but in the present stage of our knowledge it appears as if refection would only occur if the particular foods responsible for it were eaten raw.

SUMMARY.

1. The phenomenon of refection has been confirmed.

2. The incidence of spontaneous growth of rats on a vitamin B-free diet is very much greater when potato starch instead of rice starch is used in the diet.

3. Raw arrowroot starch gave similar but not such good results.

4. The protective action is largely destroyed by gentle cooking of the starch, and less so by extraction with alcohol.

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