

POTATO STARCH AND REFLECTION.

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(With one Text-figure and one Chart.)

SINCE Fridericia (1926) first reported the spontaneous appearance of reflection in rats of his colony receiving vitamin B-deficient diets the phenomenon has been observed by various investigators and the accuracy of Fridericia's findings amply confirmed (Fridericia, Freudenthal, Gudjonsson, Johansen and Schoubye, 1927; Roscoe, 1927; Kon and Watchorn, 1928; Scheunert, Schieblich and Rodenkirchen, 1929; Schieblich and Rodenkirchen, 1929, 1, 2).

Taylor and Thant (1929) have reported reflection in pigeons, while Bechdel, Eckles and Palmer (1926), Bechdel and Honeywell (1927) and Bechdel, Honeywell, Dutcher and Knutsen (1927, 1928) have shown that ruminants thrive on diets found to be deficient in vitamin B by experiments on rats and have isolated from the rumen of a cow subsisting on a vitamin B-deficient diet an organism, *Flavobacterium vitarumen*, considered by them to be responsible for the endogenous synthesis of vitamin B.

It is only fair to state that, as early as 1915, Theiler, Green and Viljoen (1915) advanced the hypothesis that vitamin B is synthesised in the alimentary tract of ruminants.

In 1929 Mendel and Vickery (1929) reported that they were unable to observe in their laboratories phenomena comparable to reflection either by using diets containing raw maize starch or dietaries similar to those used by Kon and Watchorn (1928) containing potato starch prepared in the laboratory from potatoes. The results obtained by the former authors when raw potato starch was used will be discussed in a later part of this paper.

The criticism by Mendel caused me to repeat the experiments with raw potato starch. This was done in the laboratory from which the present work is published. Previously I also produced reflection with diets containing raw potato starch in the laboratory of Prof. H. M. Evans during my visit to California in 1928. As the actual figures obtained there are not available this set of experiments will not be discussed further and only a photograph showing two littermate rats which had been kept for some time on the reflective diet deficient in vitamin B will be included. One of the animals became reflected while the other showed the usual signs of vitamin B deficiency.

As a result of my work I can fully confirm the previous findings of Kon and Watchorn (1928) that reflection becomes almost the rule when raw potato starch is used.

EXPERIMENTAL.

Raw potato starch diet. Seven young albino rats weighing from 49 to 77 gm. were placed on October 18th, 1929, on the following diet:

Casein (washed for a week with acidulated water)...	23 per cent.
Potato starch (commercial, moisture 14.2 per cent., N 0.018 per cent.)	57 ..
Hardened vegetable fat ("Ceres")	15 ..
Salt mixture (Steenbock's, No. 40)	5 ..
Cod-liver oil: 2 drops per rat per day.	

The rats were kept together in a cage (dimensions 18 × 10 × 9 in.) on a half-inch mesh screen. The diet and tap water were offered *ad lib*. The growth curves of the rats are given in Chart 1.

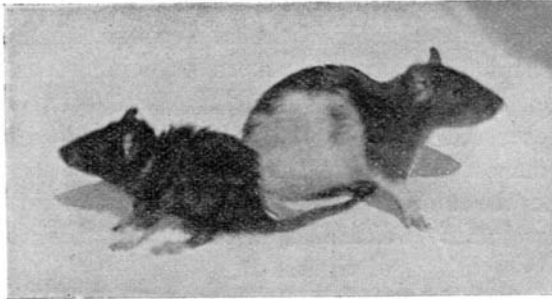


Fig. 1. Two littermate rats kept on raw potato starch diet. One became refeeded while the other showed the usual signs of vitamin B deficiency.

Appearance of the rats. Shortly after the beginning of the experiment all the rats were in bad condition: their fur was very ruffled, and for the first 10 days the weight was either stationary or dropped a little. One rat (1080) died after 5 days. On autopsy a very severe meteorism was found, the intestines were enormously distended with gas and the mesenteric vessels injected with blood. The lungs were atelectatic owing to the pressure of the abdominal contents. At the end of the second week the remaining rats started growing and their fur became smoother and better though by no means normal. From now on four rats (1077, 1078, 1081 and 1083) grew steadily at a subnormal rate. Two rats, 1079 and 1082, after a preliminary period of growth declined and died in the course of 82 and 81 days respectively without showing definite symptoms of vitamin B deficiency other than emaciation, weakness and roughness of coat.

Rat 1081 became pregnant 5 months after the beginning of the experiment and gave birth to one normal stillborn pup and seven fetuses partly macerated. At the same time a vaginal plug containing spermatozoa was found in female 1083, but no litter was born to her.

On January 15th, 1930, three new rats, Nos. 1146 and 1147, 22 days old,

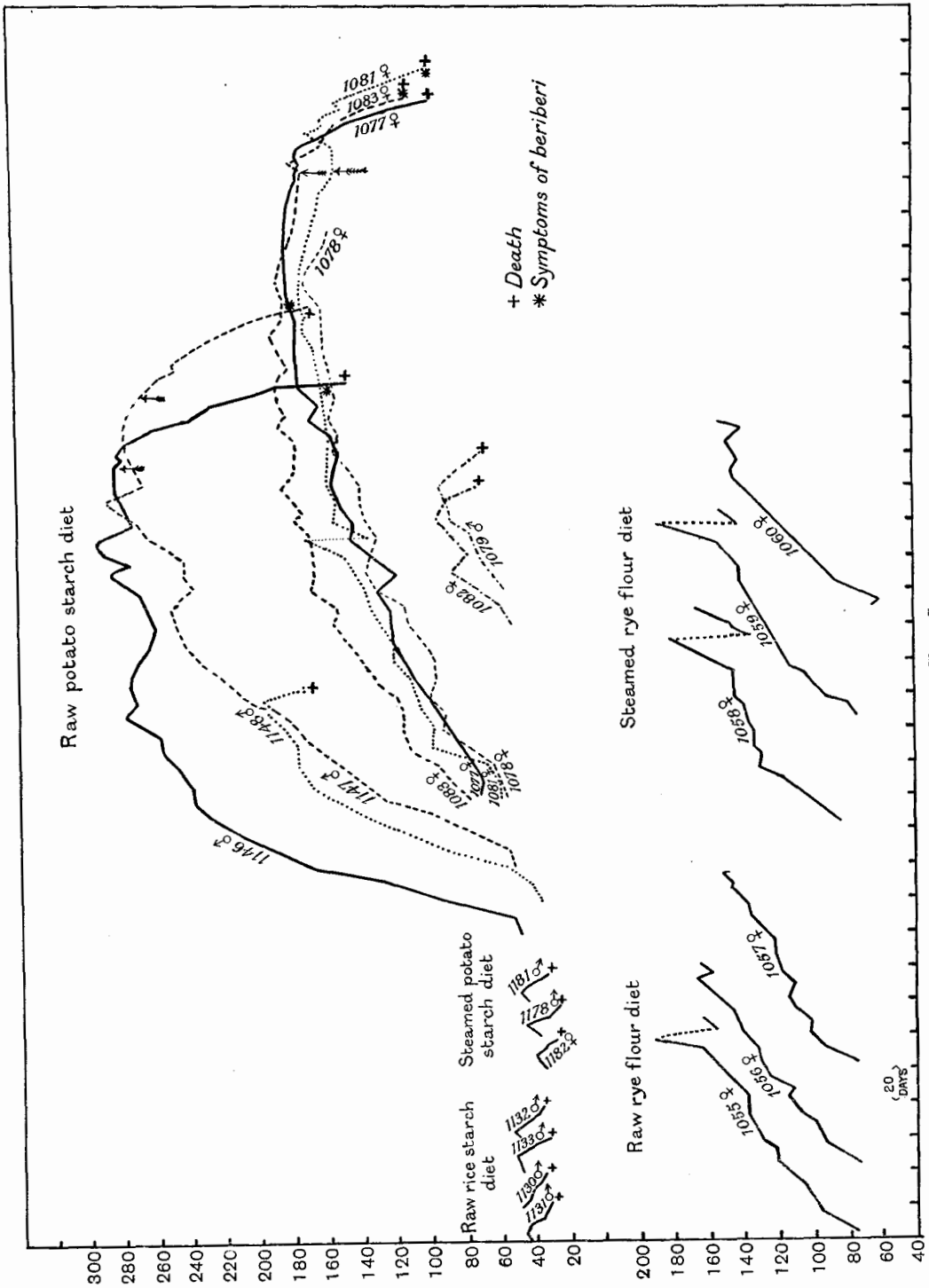


Chart I.

and 1148, 21 days old, were placed in the same cage. Within a week all three started growing at a rapid rate, rat 1146 for example making the following weekly gains:—2, 19, 28, 25, 25, 25, 23, 13, 10 and 15 grm., by which time he had reached a weight of 225 grm. It then progressed more slowly to a weight of almost 300 grm. These rats had good sleek fur, were very lively and seemed absolutely normal. They were much better in appearance than the rats first placed on experiment. Rat 1147, after having attained a weight of 190 grm. after 4 months on the diet, suddenly declined and died.

Rat 1078 developed a large abscess on the lower jaw, causing him much discomfort, and was killed on September 13th, 1930, after it had been almost 11 months on the raw potato starch diet.

Change of diet. On October 18th, 1930, exactly 1 year after the commencement of the experiment a change was made in the diet. From that date, instead of being offered raw, the diet was made up to a paste with water and steamed on the water bath with constant stirring for 3 to 5 minutes. The starch had then dextrinised and the whole mass set to a jelly. The change of diet is marked by arrows on the graphs. As a result all rats declined and died within 2 months (rat 1077 after 35 days, rat 1081 after 58 days, rat 1083 after 41 days, rat 1146 after 48 days and rat 1148 after 49 days). Rats 1081, 1083, 1146 and 1148 showed typical symptoms of beriberi: convulsions, incoordination and spasticity. After the change of diet rats 1077, 1081, 1083 and 1148 also showed progressive symptoms generally considered to be characteristic of vitamin B₂ deficiency, *i.e.* loss of hair, blood on the paws and vibrissae, and also urine-stained abdomens, while rat 1081 developed in addition swollen and reddened eyelids.

Steamed potato starch diet. Three young rats, Nos. 1178, 1181 and 1182, weighing 36, 42 and 31 grm. respectively, were placed on the steamed potato starch diet. They grew for a short time, then rapidly declined and died within 3 weeks without showing any definite symptoms. The growth curves are given in Chart I.

Rice starch diet. Four young rats, Nos. 1130, 1131, 1132 and 1133, weighing from 40 to 50 grm., were placed on the following diet:

Casein (washed for a week with acidulated water) ...	23 per cent.
Rice starch (commercial, moisture 13·1 per cent., N 0·073 per cent.)	57 ,,
Hardened vegetable fat ("Ceres")	15 ,,
Salt mixture (Steenbock's, No. 40)	5 ,,
Cod-liver oil: 2 drops per rat per day.	

The rats in this experiment (as well as in the preceding experiment) were kept together in a cage of the same type as used for the rats on the raw potato starch diet. They rapidly declined and died within 3 weeks. The growth curves (Chart I) are quite similar to the growth curves of rats on steamed potato starch diet.

Stability of the vitamin B complex in steamed diets. Three young female rats were placed on the following diet:

Bleached rye flour	88 per cent.
Butter fat	9 „
Salt mixture (Steenbock's, No. 40)	3 „

This diet had previously been shown (unpublished experiments) to contain inadequate amounts of the vitamin B complex permitting only subnormal growth at about the same rate as that observed in the first group of rats on the potato starch diet.

Three other females received this diet which had been steamed under precisely the same conditions as described previously for the potato starch diet. There was no noticeable difference between the two groups of animals. The reproductive cycles were very irregular in both groups. When exhibiting signs of oestrus the females were mated with normal males from the breeding colony. One female from the raw diet group and two from the steamed diet group had litters. A large percentage of the young were born dead. None were suckled and all were eventually destroyed by the mothers. The growth curves of both groups are given in Chart I.

Faeces of refected rats. At frequent intervals freshly voided faeces were collected separately from the rats on the potato starch diet, and observed for colour, size and presence of starch by iodine staining. The results confirm the opinion of Schieblich and Rodenkirchen (1929, 1) that refaction may be present without the faeces becoming white or especially bulky. In fact typical white faeces were observed only occasionally in this experiment and not always in those rats showing the best growth. As a rule they were already dark when voided or had a creamy colour immediately after which darkened rapidly on drying. The size also fluctuated quite markedly, the faeces being at times fairly small and of irregular shape. No check was noticed at such times in the growth or general appearance of the refected rats. Unbroken starch granules were always present.

Bacterial flora. Examinations of the bacterial flora have been made and it is hoped to report in a separate publication the results of a bacteriological examination of the faeces.

DISCUSSION.

Mendel and Vickery (1929) believe that they have failed to observe in their laboratories phenomena comparable to refaction. It is significant, however, that while all four of their rats placed on a maize starch diet declined and died in the usual way, two out of six rats fed on a potato starch diet behaved abnormally. One of them (rat B 6963) especially at first grew and then maintained its weight for almost 4 months. The explanation is advanced by Mendel and Vickery that potato starch at times may carry traces of vitamin B, while Dr Gudjonsson of Prof. Fridericia's laboratory who visited

those workers at the time of the experiment expressed the opinion that there was no evidence of refection in the case of rat B 6963 (Mendel and Vickery, 1929).

If vitamin B is present in potato starch one would expect the whole group of rats receiving the potato starch diet to behave similarly, especially as the starch was prepared in the laboratory under well-controlled conditions. On the other hand, if refection is caused by an endogenous bacterial agent (and there is at present no better, or simpler explanation of the phenomenon) then wide variations in the behaviour of rats submitted to conditions likely to produce refection would naturally be expected. Rats will thrive or die on refection diets according to the degree of susceptibility, exhibiting all stages of nutritional condition from a rapid decline and death, typical of vitamin B (complex) deficiency, through shorter or longer periods of maintenance, to instances of rapid growth, normal appearance and almost unlimited survival. An "all or nothing" law is certainly not applicable in the case of refection.

The present work shows an interesting example of variations in the degree of refection. The second group of rats did much better on the potato starch diet than the first group, while marked differences are noticeable within the groups. Most probably the better performance of the second group is due to a massive "infection" at an early stage of life contracted from refection individuals of the first group.

It is obvious that spontaneous refection is of quite common occurrence when potato starch is used. In my experience this is not the case with other starches. I have not observed spontaneous refection with rice starch, nor with diets containing varying proportions of raw wheat and rye flours. Growth and survival on diets containing raw potato starch are certainly not due to the presence of vitamin B (complex) in the starch. The refection ability of the starch is completely lost under conditions having no deleterious influence on the vitamin B complex present in other food materials. A comparison of the growth curves of rats receiving raw and cooked potato starch diets with those of rats fed on raw and cooked flour diets is sufficiently convincing. Moreover, even the presence of traces of vitamin B (complex) in the starch could not account for sustained growth at a rate of almost 4 gm. a day or for the survival of rats for periods covering more than one-third of the normal total span of life. The enormous variations within groups of rats receiving the raw potato starch diet also speaks against the presence of a fixed preformed amount of vitamin B (complex) in the diet and for an endogenous bacterial synthesis.

SUMMARY AND CONCLUSIONS.

1. Refection occurs as a rule in rats receiving diets containing raw potato starch.
2. Rats were maintained for one year on a diet deficient in the vitamin B complex and containing raw potato starch. Two of them attained a weight of almost 300 gm. and made sustained gains at a rate of almost 4 gm. daily.

3. Rapid decline, symptoms of vitamin B₁ and B₂ deficiency and death were observed in these rats after their diet had been steamed.

4. Steaming under identical conditions does not alter the vitamin B (complex) content of other diets.

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