ON REFECTION IN RATS AND ON THE NATURE OF THE GROWTH PROMOTED BY THE ADDITION OF SMALL QUANTITIES OF MILK TO VITAMIN-FREE DIETS

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(With 9 Figures in the Text)

INTRODUCTORY NOTE

In a paper published by one of us thirty years ago (Hopkins, 1912), experiments were described showing the marked effects of very small quantities of milk in promoting growth in rats on diets which otherwise contained no vitamins.

At the time of these early experiments such factors as refection and the effects of coprophagy had not been recognized, while the large number of nutritional factors now known to contribute to normal growth was unsuspected. It seemed worth while, therefore, when the opportunity arose to attempt to define more closely the conditions necessary for the milk effect. As a matter of fact we have found little difficulty in reproducing the original results. Our experiments, however, have revealed some fresh facts and relations to which we feel attention should be called. Since Fridericia (1926) first described refection many workers have supplied abundant evidence for the reality of this nutritional condition. Even to-day, however, it cannot be said that its causal relations are clear.

It is remarkable that in dietaries capable of evoking the refected state the nature and condition of the carbohydrate supply should play so significant a part. Those who have had first-hand acquaintance with the facts all agree that refection does not occur except when the experimental diets contain raw starch. When the carbohydrate supply is starch dextrinized or cooked, or when soluble sugars replace the starch, refection is never observed. This fact was from the first emphasized by Fridericia himself. Our own experience is fully in accord with that of others. In this laboratory we have, moreover, always found it necessary to employ raw potato starch. When this has been replaced by raw rice starch we had never observed a case of refection on diets free from the vitamins of the B complex until a later date in circumstances which will eventually be discussed, but this has not been a universal experience. Indeed, Fridericia's pioneer studies of the phenomenon were made with rice starch though it would seem that relatively few of his animals developed refection. Miss Roscoe (1927)

also saw refection established on a rice starch diet, as also did Dr Harris at the Dunn Nutritional Laboratory at Cambridge. On the other hand, Kon & Watchorn, working in this laboratory so far back as 1928, could only be sure of establishing the condition by substituting potato starch for the rice starch then ordinarily used for the synthetic basal diet. During more recent years more than one worker has performed experiments in Cambridge involving the use of dietaries deficient in the B vitamins, but no one has reported the establishment of refection when the carbohydrate supplied was raw rice starch. It would be surprising if this were a wholly local circumstance, but Kon (1931) has reported that he found the same difference between these starches when working in three other laboratories, at Warsaw, Berkeley (California), and Reading. It is further noteworthy that all later workers on the subject in America have, for whatever reason, employed potato starch when wishing to obtain refection.

Factors involved in the production of refection

It seems to us that in their important paper of 1938, Kon, Kon & Mattick come nearer to presenting an acceptable view concerning the nature of refection, as based on the factors found necessary for its establishment, than any other authors. Summarizing their experience they remark: 'The results of this work show clearly that potato starch refection cannot be attributed to a single property of the reflective diets or to an infection with specific organisms.' They claim that the following conditions are essential for its occurrence: (1) raw potato starch in the diet, which is associated with (2) the presence of much undigested starch in the caecum. This leads to (3) vigorous bacterial growth in the caecum and (4) the establishment of an acid condition there. These factors are associated with (5) an enlarged caecum, which becomes the seat of active fermentation. On some available evidence these authors believe that acidity in the intestine encourages the production of B vitamins by bacteria and makes for better absorption of them. It would

seem then that in the opinion of these authors the initial stage in the establishment of refection is the passage of sufficient undigested starch into the caecum. The other necessary factors are for the most part consequential on this. The existence of one or another of the above conditions in relected animals has been confirmed by others, including Fridericia himself.*

I. REFECTION

Diagnosis of refection

There can, of course, be little doubt that refection exists when an animal is growing steadily without pathological symptoms in the complete absence of the vitamin B complex from its diet. With regard to the large white starch-rich faeces which since Fridericia's publications have been looked on as the classical indication of refection, we are bound to say that our experience has been rather that of Kon (1931). We have had clear cases of the condition in which no such faeces were passed, or have seen them alternate with normal small excreta without any change in the rate of reffective growth. We should have remained certain except for a quite recent experience (see later) that no specific organism is responsible for refection. The three rats of Exp. 23 (Fig. 1), of which the caecal contents were examined, seemed to give strong evidence for this assumption. In two cases the dominant organisms were coliform, Gram-negative bacteria, while in the third a Gram-positive *Streptococcus* (almost certainly *S. faecalis*) was by far the most numerous. Yet these three rats had grown in adjacent cages in circumstances identical in all respects.

With regard to the possible influence of yeast Dr Marjorie Stephenson kindly made for us a number of plate cultures from the faeces of relected rats, using plates suitable for the encouragement of yeast growth. In some cases yeasts were numerous, and in one they seemed to be the dominant organism. Such results were not constant however; in some cases of typical refection yeast cells were very few or absent.

Prevention of refection; the influence of roughage

To avoid refection in biological studies of the B vitamins it has become customary to use diets containing a carbohydrate other than raw starch, but we have found that by giving roughage to the experimental rats in a particular way refection on the starch diets can nearly always be prevented. Whatever form of roughage is used it is not satis-

* Consideration of space prevents us surveying the literature on refection as a whole. In the above paper (Kon *et al.* 1938) complete up-to-date references, including important papers from the U.S.A., are given.

factory to mix it with the food. It should be of a kind and be so given that the animal tends to consume a large portion of it before eating more than a little of its daily food ration. The following procedure has been used in our experiments. An 18.5 cm. filter paper is cut into four strips roughly equal in weight. Each strip is folded lengthways so that the folded strip is about half an inch wide, and is then dipped in a very weak solution of cane

Fig. 1. Average growth curves of six rats, 3♀ 3♂, receiving alkali-acid-alcohol extracted potato starch. No vitamin B. No filter paper. Complete refection.

sugar. When this is presented to a rat through the bars of its cage, it is nearly always seized with avidity, and largely consumed immediately. About half a gram daily was eaten. We have ventured to mention these details in procedure, since they have consistently made for success. The statistics given in Table 1 illustrate the effect of roughage.

Table 1. Cases of well-defined refection on the various diets

Nature of starch used in basal diet	Whether filter paper present or absent	With or without milk supplement	No. of animals in group	No. cases of well-defined refection
Potato	Absent	With	26	19*
Potato	Absent	Without	51	34*
Potato	Present	With	84	4
Potato	Present	Without	71	7
Rice	Present	With	32	3†
Rice	Present	Without	31	1†

* 6 more died with diarrhoea, early in experiment, as described.

† See text.

It is to the point here that cultures taken from the caeca of rats receiving roughage have always shown remarkably few organisms. If roughage is first given to a rat after it has been long refected, its ability to prevent the continuation of the condition is somewhat less sure. Nevertheless, Fig. 2 shows an instance in which the giving of roughage on the 30th day of reffective growth immediately induced a sharp fall in weight and ultimately polyneuritic symptoms and death. Fig. 3 relates to an experiment (no. 28) in which the roughage was given from the first to four rats placed on the reffective diet with cod-liver oil, while six strictly comparable rats received no roughage. Three of the latter were given roughage on the 32nd day, and from then their growth curves commenced to decline.

Fig. 2. Growth curve of rat on potato starch diet. No vitamin B. ← indicates feeding filter paper as roughage.

Some authors have been led to consider the possibility that the potato starch preparations on the market might contain small amounts of vitamin B₁, but Kon, having thoroughly extracted his supply with alcohol, could find no evidence for the presence of B₁ in the extract. We have in certain experiments used potato starch which had been thoroughly extracted with dilute alkali and acid as well as alcohol.

The first experiment in which starch so treated was used in the synthetic basal diet showed that it was still capable of supporting refection.

Exp. 23 (Fig. 1). Six young rats, three males and three females, each weighing close to 35 g., were placed on the diet in July 1941. No B vitamins were supplied, but during the first 3 weeks each rat has three drops of cod-liver oil daily. All became refected, each animal growing at a uniform rate with a gain of 12 g. a week. On the 24th day the cod-liver oil was removed from the diet of one rat

without any effect on its growth rate. Three of the six while in perfect health were killed for examination of the caecal contents, one on the 50th day and the others on the 75th. After the 80th day the survivors were no longer systematically weighed, but they remained in full health and vigour till they reached maturity. It seemed at this stage of the investigation most unlikely that starch so extracted could retain any of the B-complex vitamins, unless in some exceptional kind of association. In the following section, however, it will be shown that another method of extraction has indicated that such an association may perhaps exist. During the

Fig. 3. A, Growth curve of three rats on potato starch diet. No vitamin B. Rats still growing steadily when experiments stopped. B, Growth curve of three rats on same diet. ← indicates feeding filter paper as roughage. C, Growth curve of four rats on same diet receiving filter paper from start of experiment.

course of our experiments seventy-seven rats were fed on the reffective diet containing raw potato starch. Fifty-three of these rats became fully refected. The growth curves of the remainder gave no indication of refection.

Experiments of refection

It will be of some use to what follows if we here briefly describe our general experience with regard to the onset of spontaneous refection in our rat community. The composition of the basal diet fed was as follows: 40 % potato starch, 23 % glaxo casein, 17 % cane sugar, 15 % arachis oil, 5 % salt mixture. We have observed that when young rats newly weaned, weighing 35–40 g., are put on the raw potato starch diet without roughage they sometimes display digestive disturbances with

distended caeca at about the 7th day. Some die shortly after this critical stage, while the survivors begin to show refection.

We have compared the effects of refection diets with and without the addition of cod-liver oil. With quite young animals the presence or absence of vitamins A and D makes little difference to their response to the absence of vitamin B complex. When other, usually older, rats are given cod-liver oil, some after about 20 days of maintenance, or very slow growth, start losing weight and soon die with symptoms of polyneuritis. Others, however, after varying periods of maintenance which may even extend to 40 days, suddenly start to grow rapidly as the result of established refection. Of the rats without cod-liver oil (vitamin-free diet) the majority of course grow little, and, after 15–20 days, decline in weight, develop polyneuritis and die. Some few exceptions to this have nevertheless occurred in our experiments. For example, three female rats on the raw potato starch diet with no vitamins displayed irregular but fairly quick growth, with occasional stationary periods, for no less than 50 days. Indeed, one of them then, after a long period of maintenance, started to grow again. We do not think coprophagy, although it is impossible to eliminate this habit, played any appreciable part in this behaviour, but rather that refection was present, though intermittent and never fully established. We have come to agree with Kon (1931) that the 'all or nothing' law does not apply to refection.

The interests of the phenomenon of refection must increase if the observations of Najjar & Emmet Holt (1943) are confirmed. Their experiments suggest that an analogous condition can occur in human subjects.*

II. ON THE EFFECT OF ADDING MINUTE RATIIONS OF MILK TO DIETS OTHERWISE DEVOID OF VITAMINS

As already pointed out, when the early experiments (Hopkins, 1912) were conducted refection had not been recognized as a definite nutritional condition.

* At the time when this paper was written the only evidence for the power of intestinal bacteria to supply the necessary vitamins was the phenomenon of refection, except for the paper quoted above. A large amount of work has since been done on the administration of a bactericidal drug of which a convenient and extensive summary will be found in *Lancet*, Dec. 30, 1944. Experiments on rats as well as on human beings have led to the obvious belief that the intestinal flora is to a large extent able to synthesize many of the recognized vitamins. The extent of the synthesis is greatly influenced by the diet. When drugs of the sulpha group are given they reduce the bacterial activity; therefore the vitamin requirement in the diet is much higher.

In any case evidence will be given later to show that refection and the milk effect are distinct phenomena.

Our first endeavours to repeat the early experiments were disturbed by the occurrence of refection among our control animals. After one of us (V.R.L.) had established the effect of roughage in preventing refection, we had no difficulty in reproducing the original results. The administration of roughage

Fig. 4. A, Typical growth curve of rats on potato starch diet and 3 ml. milk daily and filter paper as roughage. B, Growth curve of rats on same basal diet without milk.

Fig. 5. Of two sets of six rats on potato starch diet and filter paper as roughage, A received milk, B none; at ← milk removed from A and given to B.

has no effect at all on the growth with milk (Fig. 4).

The milk must be administered in such a manner that it is completely consumed. Before the animal receives its main daily food supply, it is placed in a cage by itself (if not already so housed) with a small vessel containing the measured milk which is usually very quickly consumed. The method of administration of the filter paper must also be rigorously observed.

Fig. 5 shows a curve typical of the result of

'cross-over' experiments of a kind described in the 1912 paper, and which are representative of many experiments.

In the early paper the remarkable ability of these small daily additions of milk to sustain long-continued growth on diets otherwise vitamin-free was not sufficiently stressed. Under conditions to be later discussed, we have had rats growing on such a diet plus the small milk ration for 6 or 7 weeks, and that without increasing the daily dose of milk. Such growth, while always slower than normal growth, is continuous. To make these experiments comparable with the early ones, the carbohydrate

stated in § I, in retractive animals it has proved to be always markedly acid (pH 5.1–5.8). While our pH values have usually been determined with indicators, a number have been checked with glass electrodes, always with good agreement.

(3) The following circumstance is perhaps still more definite as evidence for the absence of refection during the growth of milk. In a number of experiments with milk, the small ration was withdrawn at various stages of growth. In all cases this has been followed by an almost immediate fall of weight, often beginning as soon as the second day, when the

Fig. 6. Individual growth curves of rats on potato starch diet + 3 ml. milk daily + cod-liver oil + filter paper as roughage. ← indicates removal of milk ration. Third animal continuing on milk grew for further 10 days.

used in the diet was raw potato starch; the significance of this will later become clear.

The growth on milk not due to refection

The following facts seem to prove this independence satisfactorily:

(1) The milk growth is quite unaffected by giving roughage on the lines described in § I (Fig. 4). Plate cultures from the caecal contents show at most very few bacteria or yeasts, contrasting in this respect strongly with the caeca during the retractive growth.

(2) Doubtless as a consequence of this relative sterility, we have invariably found that at all stages of growth with the milk the caecal material is neutral or slightly alkaline (pH 7.7–5), whereas, as

Fig. 7. A, Average growth curve of group of rats on potato starch diet + 3 ml. milk daily. No filter paper as roughage. ← indicates removal of milk. Clearly retractive growth. B, Control group to curve A, receiving no milk. No filter paper. Also retracted. C, Rats receiving same diet as A + filter paper. ← indicates removal of milk. (No refection or growth would have continued after removal of milk.) D, Rats receiving same diet as B + filter paper. Both the growth and the fall in weight after removal of milk were so uniform as to justify an average curve.

rats are receiving roughage. This certainly would not occur if refection existed. The growth curves of Figs. 5–7 illustrate the effect of withdrawal of milk from animals growing with its aid, and correspondingly the effect of roughage on the controls.

(4) It may also be noted that the growth obtained on giving 3 ml. of milk is 9–10 g. weekly, whereas fully retracted rats grow 16–20 g. weekly.

The carbohydrate factor in the growth on milk

As the above evidence, consistently obtained, seems to prove satisfactorily that the two kinds of

growth promotion in rats on vitamin-free diets are distinct phenomena, it is surprising to find that so special a factor as a supply of raw potato starch rather than rice starch (we ourselves have not tried other starches) contributes to success as much in one case as in the other. It is possible indeed that this may even be a coincidence. In the one case any special quality peculiar to potato starch must be such as to promote the growth of bacteria. In the other, we must suppose that the effect is on the growth of the animal's own tissues. It is no less the fact that good growth on the milk, as in the case of refection, is not obtained with dextrinized potato starch or when the carbohydrate supply is in the form of a sugar.

The growth curves of Figs. 8 and 9 are typical examples of our results obtained when sucrose, rice starch or cooked potato starch have formed the carbohydrate supplied. The curves are very similar. When potato starch is successively extracted with acid, alkali, and alcohol, a few experiments have

Fig. 8. Typical curves of rats on (A) cooked potato starch diet + 3 ml. milk. (B) same basal diet, no milk until 20th day. ← indicates addition of 3 ml. milk daily. (C) 57 % sucrose diet + 3 ml. milk.

shown that its power to promote milk growth is little, if any, minimized.

It seemed desirable to try the effect of the enzymatic treatment on our potato starch (compare Sakurai & Omori, 1940). Each kg. was placed in 1.5 l. of 0.2 % HCl containing 0.5 % of an active pepsin preparation. The mixture, continuously stirred by a mechanical stirrer, was kept at 37° C. for 8 hr. The starch was then filtered off and thoroughly washed with water. Under the microscope the grains showed no observable changes as a result of the treatment. In the following experiments rats were fed on our usual diet, but with potato starch thus treated replacing the original unextracted starch.

Four young rats (Exp. 41) were placed on the diet with pepsin digested starch, and four controls of the same sex and comparable in weight received a similar diet with unextracted starch. They were in adjacent cages, each had roughage, and all other conditions were the same, each being given 3 ml. of milk daily. At first their consumption of food was equal. The controls grew satisfactorily and remained

in perfect health. The others soon fell off in weight, and by the 14th day showed marked symptoms of polyneuritis, together with greasy matted fur.

The results of the above experiment were so dramatic that we were surprised no less than disappointed to find in a second experiment (no. 42) on these lines that, although the growth curve obtained was below that of the control on unextracted starch, no pathological symptoms were present.

In a further experiment (no. 43) we determined to digest with trypsin and pepsin. Of the six animals fed three grew only for 18–21 days and then steadily lost weight, dying between 39 and 42 days. The remaining three, however, grew at a fluctuating rate, much slower than normal. The growth of the last three in its irregularity with occasional pauses suggests a condition of intermittent or imperfect refection due doubtless to the fact that the starch did not satisfactorily support bacterial growth in the caecum. Unfortunately, this experiment was

Fig. 9. Typical curves of rats on (A) rice starch diet + 3 ml. milk daily + filter paper as roughage. (B) same diet, no filter paper. (C) same basal diet. No milk + filter paper.

carried out after refection had become more common owing to contact with the rats of another laboratory (see Discussion).

In a fourth experiment in which the starch was extracted with pepsin HCl, the result was negative, rats growing at about the same rate on the extracted and unextracted starch.

It must doubtless be admitted that the results of the above experiments were too inconstant to claim that they have done more than to offer a suggestion rather than complete evidence for the removal of a growth factor from the starch. It would seem at least that our results suggest that the pepsin treatment liberated the growth-promoting factor in a soluble form and further suggests that in its insoluble form it is in association with protein. It may be recalled that Neuberger & Sanger (1942), studying the distribution of nitrogen in potatoes, found that about 8 % of it is in some insoluble form. About half of this is liberated and becomes soluble after treatment with pepsin HCl.

The influence of coprophagy. We believe that the occurrence of this habit has been of no importance

in the experiments which this paper describes. In Miss Roscoe's study of the subject her rats were given a diet free from B vitamin containing cooked starch as the carbohydrate for the avoidance of refection. Each animal then received each day the whole of the faeces it had passed on the previous day. The amount was therefore large, and it was found that, unless about 70 % of it was consumed, it had little or no effect in promoting growth. Others have had the same experience. In our experiments in so far as there was faeces consumption it was out of all proportion smaller than in the experiments just referred to. With a group of animals growing steadily, in contrast with controls showing no growth, close observation made it clear that there was no appreciable difference in the amount of faeces consumed, which was very small in both groups.

DISCUSSION

In this laboratory during a period of several years we have always failed to obtain refection when raw potato starch was not the carbohydrate supplied in the synthetic diet. We have also found that giving roughage (filter paper) in the special manner described in the last section to a large extent prevents refection.

In the earlier experiments, showing the growth obtained by the addition of very small amounts of milk to vitamin-free diets, it happened that potato starch was the carbohydrate used though it was not then suspected that it possessed any growth-promoting properties. Early in the present research, however, it was found that the use of this starch was necessary for success. After recognizing this we have had no difficulty in repeating the original experiments in detail. It is remarkable that the milk growth, since it shows the same need for a special carbohydrate supply as does refection, should yet be independent of the latter. This, however, we believe to be the case from the following evidence:

Giving roughage has no effect upon the growth with milk. When animals receiving roughage and growing steadily on the milk are killed at different stages in the growth, the caeca are found to be normal in size and appearance with their contents showing an alkaline reaction; by plate culture they are shown to contain very few bacteria. This is in sharp contrast with the refected rat's enlarged caecum of which the contents contained very numerous and very active bacteria, with the contents showing always an acid reaction. If to a fully refected rat a minute dose of milk is administered for a period, neither its addition nor removal have any appreciable effect on the animal's growth, whereas in animals growing on milk its

removal is immediately followed by a sharp fall in weight.

It is generally held that the influence of potato starch in promoting refection is due to its relative indigestibility and its consequent accumulation in the caecum where it provides a suitable pabulum for the vigorous growth and activity of bacteria. Although in connexion with the growth on milk we have been led to suspect the presence of a growth-promoting factor in potato starch, the above view might still be true in the case of refection.

Certain experiments described in the last section, though the results are perhaps not conclusive owing to their lack of constancy, have led us to believe that a growth factor may be associated with potato starch. If so, it must be associated with protein as it only becomes soluble when the starch has been digested with pepsin HCl. Since milk is known to contain a very small amount of thiamine it is natural to suppose that it is this vitamin that is associated with protein in the potato starch. The amount of this vitamin in the starch would seem, however, to be so excessively small that it is difficult to believe that it could contribute to the effect in question.

Dr Wang, who has had a wide experience in estimating thiamine in the Dunn Nutritional Institute, under Dr L. J. Harris, has supplied us with the following figures:

Rice starch	0.01 i.u. per g.
Potato starch	0.01 i.u. per g.
Extracted potato starch	0.005 i.u. per g.
Milk	18 i.u. per 100 ml.

Concentrations so low as these seem to show that vitamin B₁ cannot alone be adequate to justify the above assumption. Moreover, the thiamine content of rice and potato starch is identical, whereas as we have shown, in this laboratory at least, the effect on milk growth is only obtained with potato starch. It is less likely in the case of refection that a small increase in the amount of vitamin B₁ in the food could appreciably affect the growth of the intestinal bacteria since they are themselves capable of producing it so readily, as well, doubtless, as other members of the B complex. If we assume in the case of milk that the factor is thiamine, we must recognize that in all experiments past and present the small quantity of that factor in the potato starch has been responsible for the familiar growth so consistently obtained when that starch is the carbohydrate employed. It is possible of course that the potato starch may also contain in protein association other members of the B complex or even other growth factors in which milk may be deficient. This circumstance, however, does not lessen the significance of the original experiments as they were intended in that pioneer period of vitamin research

only to show that the accessory growth factors quoted were active in very low concentration.* We felt at this stage it would be satisfactory if the results we obtained were confirmed on other stock rats with different laboratory conditions. Dr L. J. Harris, Director of the Dunn Nutritional Laboratory (M. R. C.) kindly consented that similar experiments should be conducted there. Our essential conclusions were confirmed, but the tendency to refection was undoubtedly greater at the Dunn Nutritional Laboratory. For instance, certain rats on rice starch diet became relected and on occasions the use of roughage failed to prevent the establishment of refection. In view of previous findings in the Dunn Nutritional Laboratory the fact that rice starch diet induced refection in this case was not surprising. It seems likely that this very real difference between the two Departments might be due to differences in the nature of organisms established in them. It is striking at least that while V. R. Leader was handling rats at the Dunn Nutritional Laboratory, four cases of refection on rice starch diet occurred in experiments run concurrently in our laboratory.

Dr M. Stephenson kindly took cultures from the caeca of these rats growing on rice starch and in one she found a vigorously growing organism which had not previously appeared in the caeca of our rats. We have heard from more than one source that when a rat community is first started refection may not occur until a considerable time has elapsed. It would seem that only when a certain concentration of efficient organisms had become established in the laboratory did refection occur. While it is certain that a variety of organisms is able to support refection some are more capable of doing so than others. It would seem at least that systematic bacteriological study in cases of refection would be justified. It is remarkable that whereas all the caeca of rats relected on potato starch were acid, those of rats showing refection on rice starch were from neutral to alkaline.

We are tempted here to recall a remarkable observation reported by Sir Edward Mellanby (1944).

* It should of course be remembered that considerably more than 90 % of the solids contained in the milk administered are already contained in the synthetic diet. At the time my 1912 paper was published I had further evidence to show that the addition of other material in minute amounts could promote growth on synthetic diets. It is mentioned in the above paper that this is true of preparations from yeast. I had even then made a number of successful experiments with yeast. I had indeed obtained fractions from a large quantity of yeast, but while testing these I unfortunately replaced butter by lard in the synthetic diet. Failures then unexplained led to long delay and the research could not then be continued. (F. G. H.)

He found that the symptoms of inco-ordination in animals, described and explained by him as resulting from deprivation of vitamin A, only occurred at most to a slight degree if potato replaces the cereal (bread or oatmeal) in the vitamin A-free diet. He suggested that potato either contains some protective substance other than vitamin A or it does not make the same call on vitamin A reserves as is made by bread.

Any relation between these cases might seem to be remote, but conceivably an understanding of either might throw light upon the other.

SUMMARY

1. In the course of this investigation we have dealt with many cases of refection, but during a period of several years we never obtained the condition except when raw potato starch was the carbohydrate in the synthetic diet. Recently, in the exceptional circumstances described in the text, we have had a few cases with rice starch.

2. Like other authors, we have always found that in relected rats the caeca are always enlarged and their contents the seat of specially great bacterial activity. The nature of the dominant organisms present has varied from case to case, and in one it was apparently a yeast. Nevertheless, there is a suggestion that certain organisms are more potent in promoting refection than others. In rice starch refection, such organisms seem to be present.

3. We have found that the administration of roughage in the manner described can, to a large extent, prevent the establishment of refection. The number of organisms in the caeca is then greatly reduced and the contents indeed may become nearly sterile.

4. Using potato starch as the carbohydrate of the diet, we have had no difficulty in reproducing the results of the early experiments published by one of us (Hopkins, 1912), but we found that to obtain a growth induced by administering very small quantities of milk to vitamin-free diets, the presence of this starch is necessary. Indeed, the growth so promoted calls for the same particularity in the carbohydrate supply as does the establishment of refection.

5. Nevertheless, we have obtained what seems to be conclusive evidence showing that the growth with milk is wholly independent of refection.

6. We have obtained some evidence that potato starch contains growth factors in association with protein which only becomes soluble when liberated by treatment with pepsin HCl. As our experimental results bearing on this, for some reason at present unexplained, have lacked constancy, we can only claim that they are suggestive.

Our gratitude is due to Dr L. J. Harris for allowing us to repeat certain of our experiments in the laboratory under his charge and for the interest with which he followed the results. Our sincere thanks are due to Mr W. Mansfield, Director of

University Farm, who for a period of many months had us supplied with milk from his herd, on the quality of which we could always rely. We are also indebted to Dr E. Watchorn for help in the preparation of this paper.

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(MS. received for publication 9. VIII. 44.—Ed.)