REFECTION[1], A TRANSMISSIBLE CHANGE IN THE INTESTINAL CONTENT, ENABLING RATS TO GROW AND THRIVE WITHOUT VITAMIN B IN THE FOOD.

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(With 10 Charts.)

CONTENTS.

[1] New term, defined on p. 72.

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I. SYMPTOMS, ETIOLOGY AND PATHOLOGICAL ANATOMY OF REFECTION.

THE indispensability of vitamin B in an adequate diet is well established and the proposition that, under special conditions, young rats are able to grow, thrive and multiply without any vitamin B in their food will sound very improbable but, nevertheless, we feel justified in asserting this, owing to phenomena observed during the last 18 months, a short summary of which was communicated to the Twelfth International Physiological Congress at Stockholm, 1926 (Fridericia, 1926).

Everyone experimenting with vitamin B knows the regular course of events in young rats deprived of this vitamin. In our laboratory, as in others, the growth of the rats stops after 1 to 3 weeks; after 2 to 3 weeks weakness of the hind legs appears and the body temperature drops, and after 4 to 6 weeks death occurs.

In the summer of 1925 a hitherto unobserved change occurred, apparently spontaneously, in a few rats on a diet devoid of vitamin B. The record shown in Chart 1 affords an example of what happened. Three young rats were fed on the following food-mixture, devoid of vitamin B but adequate in all other respects, which was used for all the experiments recorded in this paper.

Caseinogen (prepared by 1 hour's extraction with boiling water acidulated with 0·5 per 1000 acetic acid and then by 1 hour's extraction with boiling 10 per cent. alcohol), 20 per cent.; pure rice-starch, 57 per cent.; pure butter-fat, 15 per cent.; agar prepared by extraction with boiling alcohol, 3 per cent. salt-mixture (after McCollum and Davis, 1915), 5 per cent.

Two of the rats behaved in the usual way and died in the third week (broken lines in Chart 1). The third rat behaved in a remarkable manner. In the third week, when weakness of the hind legs had already appeared, this rat suddenly started growing at a normal rate as shown in Chart I[1] continuous line curve, whilst its faeces became white and bulky. Identical changes appeared at about the same time in two other individuals of a group of rats which were fed upon the same food-mixture devoid of vitamin B.

The next observation was that the effects could be transmitted to other young rats by adding these white bulky faeces to their diet. The results were obtained only when the young rats were fed upon the diet devoid of vitamin B.

Thus, a method was available, by which it was possible to transmit artificially the capacity to dispense with vitamin B in the diet. Since then the phenomenon has been produced in some 80 rats.

All symptoms of vitamin B deficiency disappear. The weakness in the hind legs, usually present, vanishes. The body temperature of the rats,

[1] In all cases the growth-curves are given for half a year, even when the rats lived for a much longer period.

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regularly dropping in the later stages of vitamin B deficiency to 35° C. or less, keeps normal at 36·5–37·6° C.

It is important to note that in some refected rats growth is resumed at a normal rate only for a limited period, whilst others grow, often intermittently, to full size, bear and rear young, being throughout deprived of vitamin B in their food.

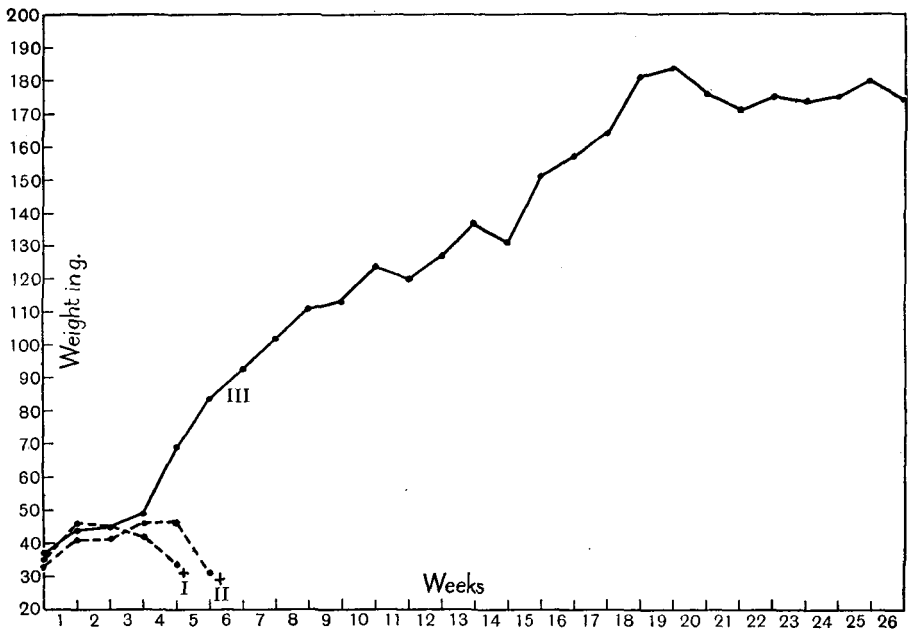

Chart 1. Growth curves of 3 young rats on a diet devoid of vitamin B, but complete in other respects.

Rats I and II exhibited typical behaviour and died in 4–5 weeks. Rat III became refected spontaneously at the end of the third week and grew to maturity.

The white faeces differ from the ordinary rat faeces not only in colour but the separate pellets are larger and the total quantity voided per day is much greater in bulk and weight than is observed with rats upon a normal diet or with usual rats on a diet deficient in vitamin B. The number of pellets, however, remains normal. Rats producing white faeces generally display a mild degree of meteorism.

The phenomenon above described has not been mentioned in the literature so far as we have been able to ascertain. Perhaps an observation by D. J. Hulshoff Pol (1912) points to a similar phenomenon in a cock fed upon damaged rice. The phenomenon needs a name. We propose to name this restoring change: *refection* (Latin: *reficere* = restore). To induce refection will be termed *to refect*: and agencies capable of refecting will be termed *refectious*.

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Spontaneous Occurrence of Refection.

As mentioned above, refection can be transmitted artificially, but young rats on a diet devoid of vitamin B may also become refected spontaneously. It was in this way that refection started among the rats of this Institute, and its origin is not known. Once started the refection is easily transmitted.

In this Institute the stock of rats for breeding purposes is kept in a different room from that of the experimental rats. The rats belong to two strains, albino rats and pied rats. The two strains behave similarly with regard to refection.

All experimental rats (for instance those on diet devoid of vitamin B) are kept in separate cages made wholly of galvanised iron wire gauze. The cages are raised about 5 cm. over the supporting trays. Thus the rats are prevented from eating faeces, which fall down through the meshes of the wire gauze at the bottom of the cages. These contain glass vessels for water and food, but no shavings or other bedding are provided. These are not needed because the room is kept at a constant temperature of 22° C.

In spite of the fact that the experimental animals are kept alone in separate cages, refection is often transmitted among the rats on a diet devoid of vitamin B, when their cases are placed in the same room as those containing refected rats. This means that the refection can be transmitted through indirect contact, and that experiments on the etiology of refection require the same precautions as those concerned with highly infectious diseases. The susceptibility of rats to refection varies during the different stages of B-avitaminosis. Among 20 cases of spontaneous refection 2 originated in the first week of feeding the deficient diet, 13 in the second week and 5 in the third. The susceptibility seems therefore to be greatest during the second week.

Characteristics of the Agent transmitting Refection.

Experiments on the etiology of refection could be made without risk of its spontaneous occurrence, when the rats' cages were placed in special compartments of a thermostat cupboard, which was cleansed and varnished after each experiment. The agent transmitting refection was found present in the white bulky faeces of the refected rats.

Experiment 1 (Chart 2). Eight young rats received the diet devoid of vitamin B when 28 days old. In Chart 2 the continuous curves represent the growth of 4 control rats who were not refected; the body weight decreases after a few weeks and death occurs. The dotted curves represent the growth of 4 other young rats, who were treated in every way as the control rats, but for a few days received fresh white faeces from refected rats in addition to their food (these days are represented in Chart 2 by the interval between two vertical lines). They became refected, grew and produced white faeces themselves. Three died after 3–6 months, one lives still after a year and has grown from 48 grm. to 158 grm. body weight, without receiving vitamin B in its food.

The phenomenon of refection resembles a chronic infection. The feeding of white faeces for a few days transmits the refection artificially, and this,

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when once started, maintains itself without further faeces-feeding and may last for months or years, if the rats continue to receive a diet devoid of vitamin B. This result does not indicate whether the white faeces contain vitamin B or not, but does prove that white faeces from refected rats contain some agent which enables rats to dispense with vitamin B in their food. It follows that this agent must be present in the contents of the intestinal tract of refected rats. Experiment 2 was carried out to investigate in which part.

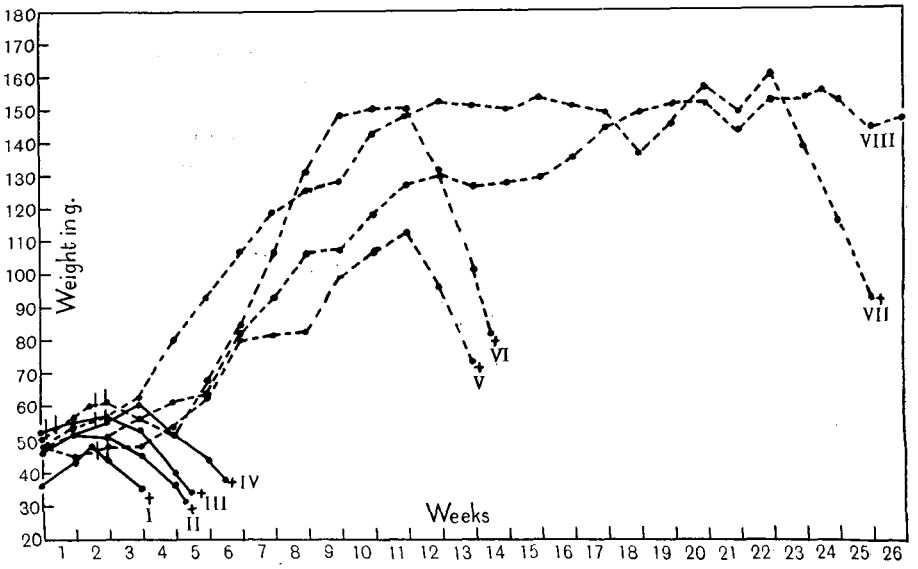

Chart 2. Experiment 1. Transmission of refection.

Dotted lines: growth curves of rats 5, 6, 7 and 8 on vitamin B-free diet, refected by temporary feeding of white faeces from other refected rats. Period of feeding indicated by interval between vertical lines. Continuous lines represent growth curves of control rats 1, 2, 3 and 4 on vitamin B-free diet.

Experiment 2. Refected rats were killed by ether, the abdomen quickly opened and the intestinal tract separated by ligatures into 4 parts, viz. (1) the stomach, (2) the upper 4/5 of the ileum, (3) the coecum, (4) the colon. Four groups of young rats on vitamin B-free diet were fed respectively with the contents of these 4 sections. Only one rat, fed upon the contents of the coecum, became refected. This result is not conclusive, but shows the agent of refection to be present, at least, in the coecum.

Experiments upon Filtration.

Experiments 3 and 4 were carried out to investigate whether the agency of refection can pass the pores of Berkefeld or Chamberland filters.

Experiment 3 (Chart 3). One part of white faeces from refected rats was ground in a mortar with 9 parts of water, and the coarser particles removed by a slow centrifuge. One portion of the suspension was fed to two young rats on vitamin B-free diet in amounts of 1 c.c. daily to each for 10 days, in order

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to ascertain if the suspension was refectious. Both these rats became refected and one lived more than 6 months, growing from 45 grm. to 150 grm. in weight. The other portion was filtered through a sterile Berkefeld-filter and of the filtrate 1 c.c. daily was given for 10 days to each of three other young rats on the same diet. None of these rats became refected and all died in from 3 to 4 weeks.

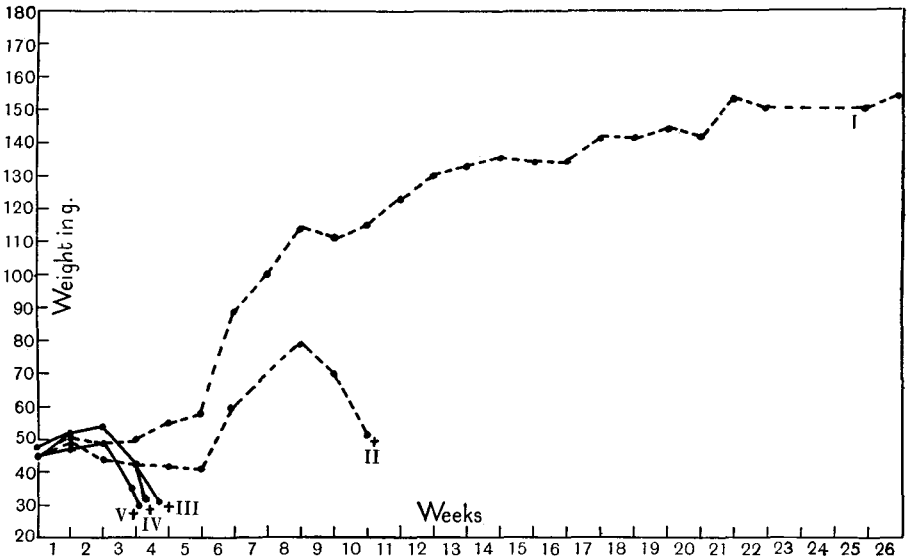

Chart 3. Experiment 3. Filtration through Berkefeld-filter of refectious faeces suspension.

Dotted lines represent growth curves of rats 1 and 2, refected by temporary feeding of unfiltered suspension of white faeces. Continuous lines represent growth curves of rats 3, 4 and 5 which received the Berkefeld-filtrate of the faeces suspension and did not become refected.

Experiment 4. A similar result was obtained after filtration through the L. 2 and L. 3 brands of Chamberland-filters, which are passed by filtrable viruses. Three young rats on vitamin B-free diet which received 2 c.c. of the filtrate daily for 6 days did not become refected.

These experiments show that the agency of refection cannot pass the pores of Berkefeld or coarse Chamberland-filters. Unless the active agent was entirely adherent to the particles of the suspension, this result shows that the agency cannot belong to the ultravisible microbes.

Resistance of the Agent of Refection to Drying and Heat.

The resistance of the agent of refection to drying and heat was investigated in Experiments 5 and 6.

Experiment 5 (Chart 4). White faeces from refected rats were dried at room temperature, kept in the dry condition for $5\frac{1}{2}$ months and fed to three young rats on the usual vitamin B-free diet. All three became refected.

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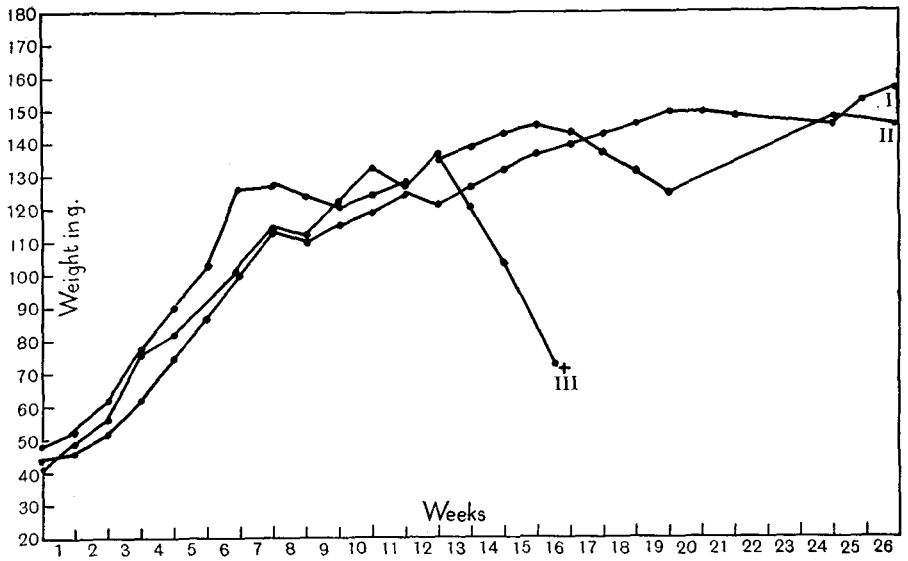

Chart 4. Experiment 5. Resistance of the agent of refection to drying.

Growth curves of rats 1, 2 and 3 refected by temporary feeding of white faeces, kept in a dry condition for $5\frac{1}{2}$ months.

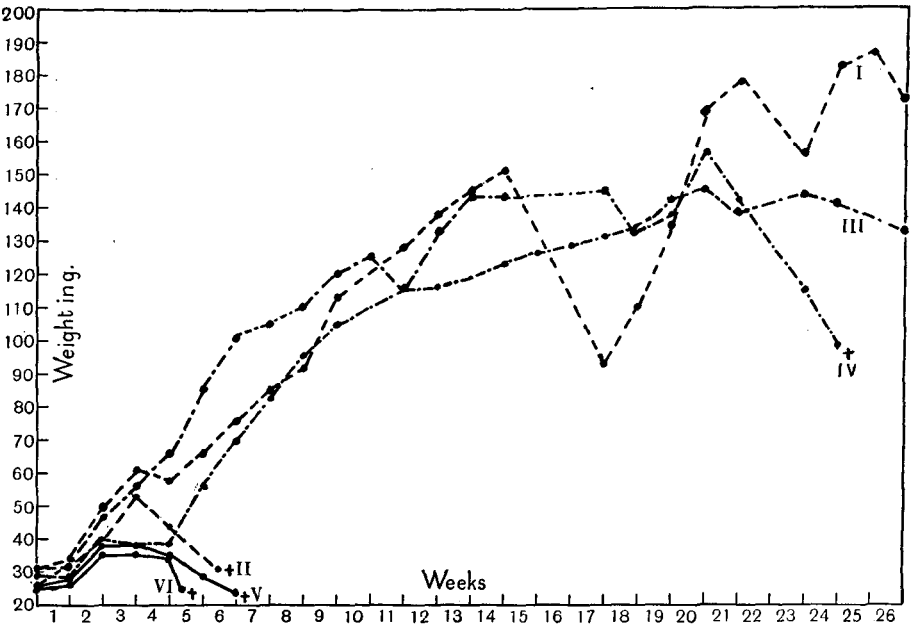

Chart 5. Experiment 6. Resistance of the agent of refection to heat.

Dotted lines represent growth curves of rats 1 and 2 refected by feeding of unheated faeces-suspension. Alternately dotted and pointed lines represent growth curves of rats 3 and 4, which received faeces-suspension after heating at 80° C. for 10 minutes and became refected. Continuous lines represent growth curves of rats 5 and 6, which received faeces-suspension after heating at 100° C. for 10 minutes and did not become refected.

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Experiment 6 (Chart 5). One part of white faeces from refected rats was ground and suspended in 9 parts of water. Part of the suspension was sealed up in glass tubes and heated to 80° and 100° C. respectively.

Six rats getting the diet devoid of vitamin B were divided into three pairs:

(1) Two rats received the unheated faeces-suspension as a control of its power to refect; both became refected, although the duration was very brief in one case. (Dotted curves, Chart 5.)

(2) Two rats got the content of tubes after heating at 80° C. for 10 minutes; both became refected. (Chart 5, the alternately dotted and pointed curves.)

(3) Two rats got the content of tubes after heating at 100° C. for 10 minutes; neither of these rats was refected. (Chart 5, the continuous curves.)

The above results show that the agent of refection can be kept in the dry state for half a year without losing its power to refect, and also that it retains its activity after heating at 80° C. for 10 minutes, but not after heating at 100° C. for 10 minutes.

Attempts to Grow and Isolate the Virus of Refection.

If sealed bulbs containing a suspension in water of white faeces from refected rats are heated at 80° C. for 10 minutes and then placed in a thermostat at 37° C. for 24 hours, an intense fermentation goes on with formation of butyric acid. The resulting product proved to be toxic, and young rats on vitamin B-free diet were killed when 1 c.c. daily of this fluid was administered, but survived the feeding of 0·12 c.c. daily. There was no refection, however, and it would appear as if the virus was killed by the anaerobic fermentation.

When, however, the white faeces suspension was mixed with powdered calcium carbonate in a flat Petri dish and incubated for 24 hours at 37° C., the fluid was found to be highly refectious. It is possible that the virus is able to multiply in this fluid because the generated acid is neutralised. While this result suggests that the growth of the virus is not hindered by exposure to the oxygen of the atmosphere, it does not exclude the possibility that it may also multiply under anaerobic conditions.

From heated suspensions of white faeces 9 strains of bacilli and 5 strains of cocci have been isolated and fed to rats on vitamin B-free diet. These experiments have not given definite results.

Autopsy of Refected Rats.

All the organs were macroscopically normal. The size of the spleen was normal and not atrophied as in other rats which had been fed on the diet devoid of vitamin B. (Cramer, Drew and Mottram, 1921.)

The whole digestive tract contained white material, half-fluid in the stomach and the ileum, coherent in the coecum and forming white faeces in

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the colon. The content of the stomach was strongly acid. The coecum was distended. The colon contained air bubbles in some cases.

The walls of the stomach (cardiac and pyloric part), duodenum, ileum and coecum, the tongue, the salivary glands, the liver, the pancreas, the spleen, the lungs, the kidneys, the testes and the thymus gland have been examined histologically. All the organs appeared healthy and unlike those of rats which had been deprived of vitamin B. In stained sections from the spleen lymphocytes occurred in abundance, and stained sections from testes showed active spermatogenesis, in contrast to the testes of other rats fed on a diet devoid of vitamin B (Drummond, 1918).

In no case were parasites, fungi or bacteria, observed in the tissues. In the lumen of the intestines many bacteria were seen as usual.

Summary.

1. A few out of a number of young rats to which a food-mixture devoid of vitamin B was fed, grew, thrived and multiplied without receiving any vitamin B in their food.

2. All symptoms of vitamin B deficiency disappeared and simultaneously the faeces became white and bulky.

3. It is proposed to call this phenomenon *refection*.

4. When young rats, on a diet devoid of vitamin B, are fed for a few days with the white faeces from refected rats, the refection is transmitted to them and may last for months or years.

5. The faeces and the content of the coecum of refected rats contain the virus of refection.

6. The virus of refection does not pass the pores of Berkefeld- or Chamberland-filters.

7. The white faeces from refected rats may be kept in a dry state for $5\frac{1}{2}$ months without loss of power to refect.

8. The virus of refection is not killed by being heated to 80° C. for 10 minutes but is killed after heating at 100° C. for 10 minutes.

9. Attempts to isolate the virus of refection have been unsuccessful.

10. The organs of refected rats are macroscopically and microscopically normal as distinct from organs of other rats fed upon diets devoid of vitamin B.

REFERENCES (Section I).

Cramer, W., Drew, A. H. and Mottram, J. C. (1921). On the function of the lymphocyte and of lymphoid tissue in nutrition. *Lancet*, ii, 1202.

Drummond, J. C. (1918). A study of the water-soluble accessory growth promoting substance. II. Its influence upon the nutrition and nitrogen metabolism of the rat. *Biochem. Journ.* **12**, 25–40.

Fridericia, L. S. (1926). Refection, a transmissible change in the intestinal content, enabling rats to grow and thrive without B-vitamin in the food. *Skandinavisch. Arch. f. Physiol.* **2**. Abstracts of communications to the Twelfth Internat. Physiol. Congress, p. 55.

https://doi.org/10.1017/S002217240003182X Published online by Cambridge University Press

HULSHOFF POL, D. J. (1912). Iets over de aetiologie van Polyneuritis gallinarum, in verband met verzuurde-rijstvoeding. *Geneesk. Tijdschr. v. Nederlandsch-Indië*, **52**, afl. 1, pp. 11–18.

MCCOLLUM, E. V. and DAVIS, MARGUERITE (1915). The essential factors in the diet during growth. *J. Biol. Chem.* **23**, 231–246.

II. NATURE AND CAUSATION OF THE WHITE FAECES OF REFECTED RATS.

In the previous section the effects of refection in rats fed upon a diet devoid of vitamin B were shown to be firstly, the disappearance of the symptoms of deficiency in vitamin B, and secondly, the production of white faeces. In any attempt to elucidate the physiology of refection the first step would be to find out why the faeces of refected rats are white and bulky.

In human beings grey or whitish faeces are produced under certain pathological conditions, viz. in sprue and when the biliary duct is blocked; in these cases the faeces contain much fat and many soaps or the biliary pigments are absent. But in refected rats neither of these abnormalities are present. Their white faeces do not contain more fats or soaps than ordinary rat-faeces, see below, nor are biliary constituents wanting. Pettenkofer's test for bile salts, as modified by Brulé (1920), is positive. In the sublimate test of Schmidt (1905) a white precipitate is produced, but presence of urobiline or a similar substance was demonstrated by testing with an alcoholic solution of zinc acetate when conspicuous fluorescence was obtained. This test was made as described by Elman and McMaster (1925) with the precautions recommended by Hansen and Marcussen (1918) and by Adler and Schubert (1923).

The white colour of the faeces of refected rats is due to a peculiar and unusual abnormality, viz. a very large content of starch. When moistened with a solution of iodine in potassium iodide the white faeces turn dark blue and on microscopical examination look as if made up mostly of unchanged starch grains. Numerous bacteria, stained blue by iodine solution, are also seen. The food-mixture of the rats contains 57 per cent. rice starch (51·3 per cent. dry weight reckoned on the dry weight of the diet).

In the previous section mention was made of the mild degree of meteorism displayed by refected rats. Air bubbles often are seen in the colon at autopsy. Moreover a suspension of the white faeces when incubated at 37° C. undergoes butyric acid fermentation. All these phenomena are due to the high starch content of the faeces.

Chemical Analysis of the Faeces.

Methods. Dry matter was estimated by drying at 102° C. to constant weight.

Nitrogen was estimated by Kjeldahl's method as recommended by Andersen and Norman Jensen (1923).

The quantitative analyses of fats and soaps were made by the method of Gade (1919). The dry powdered faeces were extracted with pure alcohol for 2 hours. The alcohol-extract

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was evaporated and then extracted with ether. The residue was extracted with ether for 48 hours in a Soxhlet apparatus and this extract evaporated. The residue from the united ether extracts is reckoned as fat. The extracted faeces-residue was then saponified with 3 per cent. HCl in alcohol and the fatty acids extracted as described by Gade. The weight of these fatty acids is taken as the amount of soaps.

The starch in faeces is usually determined by hydrolising the starch with dilute acid and estimating the amount of glucose formed by some reduction method. This method has not been used because rat faeces contain reducing substances which make the starch estimations too high. Reliable results were obtained by the method of Mayerhofer (1910), which is generally used for controlling the amount of starch in sausages. In eight control experiments 93·3 to 99·9 per cent. of added starch were found by this method (0·1882 grm. to 1·0193 grm. of rice starch being used). In one experiment only 85·9 per cent. of the added starch was found, but this was due to a technical error in using too little KOH-solution for dissolving the starch.

Ash estimations have also been carried out, but the results are not of value because the rat-faeces were found rich in iron oxide, on account of the rats' habit of gnawing the iron wire of their cages.

All analyses were carried out in duplicate.

Results. The faeces were analysed from three different groups of young rats.

Group I contained normal rats on an adequate food-mixture made up of purified casein 20 per cent.; pure rice starch 52 per cent.; pure butter fat 15 per cent.; dried brewers' top yeast 5 per cent.; purified agar 3 per cent.; salt-mixture of McCollum and Davis (1921) 5 per cent.

The rats in Groups II and III received a diet devoid of vitamin B, *i.e.* the above food-mixture without dried yeast and containing 57 per cent. of rice starch. The rats in Group II showed symptoms of vitamin B deficiency and had normal brown faeces. Group III contained refected rats with white bulky faeces.

Table I. *Average values of results of the faeces analyses.*

		Adequate diet Group I Normal rats	Diet devoid of vitamin B: Group II Non-refected rats brown faeces	Diet devoid of vitamin B: Group III Refected rats white faeces
Dry matter in fresh faeces		38·0 (3)*	38·8 (12)	48·9 (6)
Per cent. in dry matter of faeces	Nitrogen	5·06 (15)	4·33 (9)	2·39 (10)
	Fats and soaps	11·02 (11)	13·65 (3)	5·56 (7)
	Starch	12·8 (9)	9·9 (4)	58·2 (10)

* Figures in brackets denote numbers of samples of faeces analysed.

Table I contains average values of the results of the faeces analyses, and shows that the white faeces of refected rats (Group III) contain less water, less nitrogen and less fats and soaps than those of rats in the two other groups. This is accounted for by the high starch content. The starch content of the white faeces varies between 47·2 per cent. and 64·7 per cent., average 58·2 per cent., as against an average of 9·9 per cent. and 12·8 per cent. of rats in Group II and I respectively (extreme values 6·3 and 21·9 in Group I and 7·0 and 13·0 in Group II). The starch in the white faeces is present as unchanged starch grains. In the faeces of rats in Groups I and II starch

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grains are not seen on microscopical examination, but often little amorphous lumps are present which stain blue with iodine.

Refected rats do not as a rule produce white faeces continuously but periods in which they are passed alternate with others in which brown or brownish bulky faeces are produced. These contain between 14 per cent. and 27 per cent. starch, which is more than is present in normal rat faeces but less than is contained in the white faeces. The starch is present as starch grains.

As far as we know, faeces containing 50–60 per cent. of starch have not been described previously.

Utilisation of Starch in the Intestinal Tract.

The starch digestion of animals on a diet deficient in vitamin B has been studied by Braddon and Cooper (1914) who worked with chickens receiving a diet of polished rice and sago with an inadequate allowance of yeast. When moderate amounts of starch were given, 95–100 per cent. was digested; when larger amounts, 90–100 per cent. Bickel (1922) states that his pupil Tsuji found the utilisation of starch to be normal in dogs upon a diet of polished rice, wheat protein, lard and salt.

Our experiments were carried out as follows:

Each rat was kept separately in a cage of iron wire gauze, containing nothing but the vessels for water and for the food, which was given as a dry powder. The food glass stood on a glass tray, so that scattered crumbs fell mostly on this tray and could be collected and added to the uneaten food. At a distance of 5 cm. under the wire-gauze bottom of the cage was a false bottom of narrow-meshed wire gauze, which retained the faeces and a few lost crumbs of the food, but let the urine pass into a receiver underneath. Several times a day the faeces were removed from the tray and the crumbs of food carefully picked up. In this way the amounts of food eaten and of faeces produced were measured.

During the whole time of each experiment, which included a series of periods lasting from 5 to 11 days, the rat was fed on the same food-mixture. During the first introductory period the faeces were not analysed and these periods are omitted from Table II, which summarises the results of the experiment.

The amount of dry matter and the content of nitrogen, fat and soaps, and starch was determined in average samples of the dry matter of food and of faeces for each period. All analyses were made in duplicate.

Table II. *Food-mixtures.*

	I Adequate diet	II Diet devoid of vitamin B	
Caseinogen	20	25	Per cent.
Rice starch	52	52	
Butter fat	15	15	
Dry yeast	5	0	
Agar	3	3	
Salt-mixture	5	5	
	100	100	

Table II B gives the apparent percentage utilisation of the different food constituents in several experimental periods for each of the three different

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groups of rats, viz. (1) rats on an adequate diet, (2) non-refected rats on the diet devoid of vitamin B, showing symptoms of deficiency and declining, and (3) refected rat growing and thriving on the deficient diet.

Table II A. *Analyses of food mixtures expressed as per cent. of dry weight.*

	I. Adequate diet	II. Diet devoid of vitamin B	III. Diet devoid of vitamin B fed to refected rat
Nitrogen	3·39	2·92	3·68
Fat	19·38	15·13	18·22 } per cent.
Starch	51·27	48·53	51·27

The refected rats were found to utilise about 65 per cent. of the starch consumed, as against a starch-utilisation of 98–99 per cent. in the other two groups. In spite of the high starch content of the white faeces (59–62 per cent.) the refected rat had utilised about two-thirds of the starch consumed. The ability of refected rats to digest rice starch is therefore not destroyed but only diminished.

Table II B. *Percentage utilisation of the food in rats.*

Rat No.	Duration of periods in days	Weight of rat in grammes	Weight in grammes Dry matter per day of: Food eaten	Faeces produced	Percentage utilisation ("absorbed") of: Dry matter	Nitrogen	Fats and soaps	Starch
			Rats on an adequate diet.					
Vs 83	11	70–103	9·11	0·50	94·5	91·2	97·3	98·5
	10	103–128	10·58	0·62	94·1	91·9	96·5	97·5
	11	128–155	11·17	0·69	93·8	91·7	96·7	97·8
Vs 84	11	65– 94	9·09	0·50	94·5	91·1	96·4	98·8
	10	94–121	11·20	0·68	94·0	91·3	98·0	99·3
	11	121–157	11·71	0·70	94·0	91·4	97·0	99·3
			Non-refected rats on the diet devoid of vitamin B.					
Bf 167	7	189–181	10·23	0·61	94·0	91·1	94·8	98·4
Bf 168	7	230–229	10·54	0·64	93·9	91·1	94·3	98·6
			Refected rat (white faeces) on the diet devoid of vitamin B.					
Bf 232	5	103–119	11·54	3·33	71·2	80·1	94·2	65·0
	5	119–129	12·39	3·67	70·4	80·7	95·5	65·7
	5	129–138	12·12	3·55	70·7	81·6	95·1	64·6

70–71 per cent. of the total dry matter of the food was utilised by the refected rat as against 93–94 per cent. by the other groups. This difference is mostly, but not exclusively, accounted for by the difference in starch utilisation. The apparent utilisation of the food nitrogen is also less (80–81 per cent.) in the refected rat than in the others (about 91 per cent.). It is impossible to say whether this indicates a poorer utilisation of the protein of the food or the production of a larger quantity of digestive juices which leave the body with the faeces. The utilisation of the fats is almost identical in all groups (94–98 per cent.).

In the periods when refected rats produce brown, bulky faeces (see p. 81), they utilise 82–90 per cent. of the starch of their food.

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Rate of Passage of the Food through the Intestinal Tract.

It might be supposed, that the defective utilisation of starch in refected rats was due to an abnormally quick rate of passage of the food through the digestive tract. This was investigated by adding carmine to the food of normal rats and refected rats and observing the time which elapsed before the stain appeared in the faeces. This time was very variable but the limits of variation (6–18 hours) were the same in the two groups. The average time was 12 hours for 14 refected rats and 15 hours for 5 normal rats. The difference in starch utilisation cannot therefore be explained by any difference of the rate of passage through the intestinal tract.

The Amylase of the Faeces.

Some investigators have stated that the amount of digestive enzymes—for instance pancreas amylase—is diminished in pigeons suffering from experimental polyneuritis (Tiger and Simonnet, Rothlin, *cit. a.* Funk (1924, p. 92) or in mammals suffering from deficiency in vitamin B. The experiments summarised in Table II show that the starch digestion is normal in ordinary rats suffering from deficiency in vitamin B. But it was possible that the production of amylase might be defective in refected rats.

A preliminary investigation has been made of the amylase present in white faeces from refected rats.

Method. The salt solution recommended by Willstätter, Waldschmidt-Leitz and Hesse (1923) has been used for extraction and dilution. This solution is buffered at *p*H 6·8 and contains the necessary amount of NaCl. In most experiments the amount of amylase was estimated by the method of Wohlgemuth (1910). This method is not very accurate and was modified. In all experiments but the first two the production of reducing sugar through the action of amylase upon starch was ascertained.

The results of Experiments 1 *and* 2 showed that an extract of 1 part of white faeces with 1200 parts of the salt solution contained considerably more amylase than an extract of 1 part of brown faeces from non-refected rats made with 400 parts of salt solution.

In view of these results the simultaneous presence of amylase and unchanged starch grains in the white faeces seems puzzling. These experiments, however, were made with dissolved starch. Some investigators (for example Fraenkel and Hamburg, 1906) have considered amylase to consist of two different enzymes, one dissolving the starch, the other hydrolysing it after solution. It was possible therefore that the amylase of the white faeces might be able to hydrolyse dissolved starch which was already in solution but might be incapable of dissolving the starch grains. To determine this Experiment 3 was carried out.

Experiment 3. The action of an extract of 1 part of white faeces from refected rats made with 10 parts of Willstätter's solution was tried on raw untreated rice starch. 1 c.c. of the faeces extract was mixed in little test

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tubes with different amounts of the raw rice starch and the volume of fluid made up to 4 c.c. with the Willstätter solution. The tubes were kept in a water-thermostat at 37° C. for 3½ hours and shaken intermittently. The solutions in the tubes were then tested for presence of reducing substances with Fehling solution. The results are tabulated below and show that the amylase of the white faeces is able both to dissolve and to hydrolyse raw rice starch.

Experiment 3.

Tube No.	1	2	3	4	5	6	7	8
Amount of rice starch in ctg.	9	4·5	2·25	1·13	0·57	0·29	0·15	0
Amount of 10 % faeces extract in c.c.	1	1	1	1	1	1	1	1
Amount of Willstätter fluid in c.c.	3	3	3	3	3	3	3	3
Reduction of Fehling solution after incubation	+	+	+	+	Faint	Faint	0	0

The next experiments, 4 and 5, were devised to show whether the amylase of the white faeces could act upon the undigested starch contained in the white faeces.

Experiment 4. 0·86 grm. of white faeces ground in a mortar with 8·6 c.c. Willstätter fluid. After centrifuging, three layers were formed: at the bottom a white layer of starch, then a layer of faeces particles and uppermost a finer suspension. The uppermost layer was removed to use as a "faeces extract." The middle layer was then washed away by means of Willstätter fluid, which was possible without removing the compact layer of starch at the bottom. This faeces starch was used for the experiment and was mixed in little test tubes with the "faeces extract" and Willstätter fluid. The tubes were placed in a water-thermostat at 37° C. for 3½ hours and shaken intermittently. The solutions in the tubes were then tested for presence of reducing substances by Fehling's solution.

Experiment 4.

Tube No.	1	2
Faeces starch	16 ctg.	1·6 ctg.
10 % faeces extract	1 c.c.	1 c.c.
Willstätter fluid	5 c.c.	5 c.c.
Reduction of Fehling solution after incubation	0	0

The result showed that the amylase of the white faeces was unable to act on the starch contained in the white faeces, although, according to Experiment 3, it is able to act on rice starch. The starch contained in the white faeces is also rice starch, but during its passage through the intestinal tract of refected rats it seems to have been changed and rendered insusceptible to the action of the faeces amylase.

Experiment 5 was a repetition of Experiments 3 and 4 but in addition the ptyalin of human saliva was tested for its ability to act upon the starch contained in the white faeces. The result shows that the starch of the white faeces is also very insusceptible to the action of human ptyalin. Tests were

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again made of the action of the faeces amylase and of the ptyalin upon common rice starch and positive results obtained.

Experiment 5.

	1	2	3	4
Faeces starch	2 ctg.	2 ctg.	0	0
Raw rice starch	0	0	2 ctg.	2 ctg.
10 % faeces extract	2 c.c.	0	2 c.c.	0
Filtered saliva	0	2 c.c.	0	2 c.c.
Willstätter fluid	2 c.c.	2 c.c.	2 c.c.	2 c.c.
Reduction of Fehling solution after incubation	0	Very faint	+	+

Experiment 6 was made in order to check this result and to see if the resistance of the faeces starch to the action of amylase could be removed by washing with different solutions. Among the solutions tested acid alcohol alone was successful.

The fluids and the faeces starch were prepared as in Experiments 4 and 5. One part of the faeces starch was suspended in 20 parts of cold alcohol containing 3 per cent. of hydrochloric acid. After removing the acid alcohol by centrifuge, the starch was washed with absolute alcohol and three times with Willstätter fluid and the final product is termed below "HCl-washed faeces starch."

The production of reducing sugar by action of the amylase was measured quantitatively by the method of Hagedorn and Norman-Jensen (1922).

Experiment 6.

Faeces starch	10 ctg.	0	10 ctg.	0
"HCl-washed faeces starch"	0	10 ctg.	0	10 ctg.
Filtered saliva	1 c.c.	1 c.c.	0	0
Boiled (inactivated) saliva	0	0	1 c.c.	1 c.c.
Willstätter fluid	5 c.c.	5 c.c.	5 c.c.	5 c.c.
Amount of sugar produced after incubation for 3 hours at 37° C.	0	1·5 ctg.	0	0

The result confirms that of Experiment 5 in showing that amylase (ptyalin) cannot act upon the starch as contained in the white faeces, but shows in addition that the faeces starch becomes susceptible after being washed with acid alcohol. The defective utilisation of starch in the intestinal tract of refected rats is therefore not due to any lack of amylase, but seems to be caused by the protective action of some substance adsorbed on the grains during their passage through the intestinal tract of refected rats. This substance can be removed from the starch grains by washing with acid alcohol.

SUMMARY.

1. The white, bulky faeces of refected rats are not devoid of bile constituents; reactions for the presence of bile salts and urobiline are positive.

2. The white colour of these faeces is due to their large content of starch, present as unchanged starch grains and accompanied by bacteria which are also stained blue by iodine.

3. Analyses of the white faeces of refected rats receiving a vitamin B-free diet are compared with those of faeces from non-refected rats on the same

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diet and from rats on an adequate diet. The white faeces contained 2·4 per cent. of nitrogen, 5·6 per cent. of fats and soaps and about 49 per cent. of dry matter, 60 per cent. of which consisted of starch. The brown faeces of the other two groups of rats contained 4·3–5·1 per cent. of nitrogen, 11–12 per cent. of fats and soaps and about 38 per cent. of dry matter, and of which about 10–13 per cent. consisted of starch.

4. Refected rats may at times produce brown, bulky faeces containing 14–27 per cent. of starch.

5. In the intestinal tract of refected rats about 65 per cent. of the starch, 80 per cent. of the nitrogen, 94–96 per cent. of the fats and 70 per cent. of the total dry matter ingested is utilised, as against 98–99 per cent. of the starch, 90–91 per cent. of the nitrogen, 94–98 per cent. of the fats and 93–94 per cent. of the total dry matter ingested in case of rats receiving a complete diet or non-refected rats on a diet devoid of vitamin B.

6. The rate of passage of food through the intestinal tract is the same in refected rats as in normal rats.

7. The white faeces of refected rats contain more amylase than brown faeces from non-refected rats.

8. The amylase of the white faeces is able to dissolve grains of rice starch.

9. Neither the amylase of the white faeces nor the ptyalin of human saliva is able to act to any appreciable degree upon the starch contained in the white faeces of refected rats.

10. The resistance of this starch to the action of amylase appears to be due to some protective substance, adsorbed upon the starch grains in these faeces. This substance is soluble in acid alcohol.

REFERENCES (Section II).

Adler, A. and Schubert, E. (1923). Ueber Urobilinbestimmung in den Faezes. *Biochem. Zeitschr.* **134**, 533–540.

Andersen, A. C. and Norman-Jensen, B. (1923). Om Kvaelstofbestemmelser. *Den kgl. Veterinaer- og Landbohoejskoles Laboratorium for landoekonomiske Forsoeg.* **113**, 46–83.

Bickel, A. (1922). Experimentelle Untersuchungen ueber den Einfluss der Vitamine auf Verdauung und Stoffwechsel und die Theorie der Vitaminwirkung. *Klin. Wochenschr.* 1 Jahrg. pp. 110–112.

Braddon, W. L. and Cooper, E. A. (1914). The influence of metabolic factors in Beri-Beri. I. The effect of increasing the carbohydrate ration on the development of polyneuritis in birds fed on polished rice. *J. Hygiene,* **14**, 331–353.

Brulé, M. (1920). *Recherches récentes sur les ictères,* p. 51.

Elman, R. and McMaster, Ph.D. (1925). Studies on urobilin physiology and pathology. I. The quantitative determination of urobilin. *J. Exper. Med.* **41**, 503–512.

Fraenckel, S. and Hamburg, M. (1906). Ueber Diastase. I. Versuche zur Herstellung von Reindiastase und deren Eigenschaften. *Beitr. z. chem. Physiol. u. Pathol.* **8**, 389.

Funk, C. (1924). *Die Vitamine* (3rd ed. p. 92).

Gade, H. V. (1919). Bidrag til Kendskabet om Resorptionen af Fedtstoffer, de kvantitative Methoder til Bestemmelse af disse og den alimentaere Lipaemi. *Bibl. f. Laeg.* **111**, Suppl. pp. 1–160.

https://doi.org/10.1017/S002217240003182X Published online by Cambridge University Press

HAGEDORN, H. C. and NORMAN-JENSEN, B. (1923). Zur Mikrobestimmung des Blutzuckers mittels Ferricyanid. *Biochem. Zeitschr.* **135**, 46–58.

HANSEN, Sv. and MARCUSSEN, S. J. (1918). On the determination of urobilin in urine. *J. Biol. Chem.* **36**, 381–389.

MAYERHOFER, J. cit. a. KOENIG, J. (1910).. *Chemie der menschlichen Nahrungs- und Genussmittel*, **3**, part I, 441.

SCHMIDT, AD. and STRASSBURGER, J. (1903). *Die Faezes des Menschen*, p. 207.

WILLSTÄTTER, R., WALDSCHMIDT-LEITZ, E. and HESSE, A. R. H. (1923). Ueber Pancreasamylase. *Zeitschr. f. physiol. Chem.* **126**, 143–168.

WOHLGEMUTH, J. (1908). Ueber eine neue Methode zur quantitativen Bestimmung des diastatischen Fermentes. *Biochem. Zeitschr.* **9**, 1.

III. PRODUCTION OF VITAMIN B IN THE INTESTINAL TRACT OF REFECTED RATS.

One aspect of refection has been dealt with in the previous paper, viz. the production of white faeces, rich in starch. Another aspect is still more interesting, viz. the disappearance of the symptoms of vitamin B deficiency in refected rats fed on a diet devoid of this vitamin.

When refected, young rats start growing at a normal rate and continue to do so for a variable period. In some cases refection disappears spontaneously after an interval of some weeks or months. The growth stops, weakness of the hind legs and lowering of body-temperature occurs, the rats decline and die. In many refected rats, however, the growth is continued, often intermittently, until full size (250 to 280 grm.) is reached. Ten such rats have now lived for more than one year without receiving any vitamin B in their food. Three refected females have been impregnated by refected males and 4 litters have been born. Three of these litters were suckled and reared by the refected mothers. At the end of the lactation, a critical period sets in for the young ones who have access only to the same diet, devoid of vitamin B, as their mothers. Some display symptoms of vitamin B deficiency and die, but others get refected, presumably by means of the faeces of their mother, and start growing again.

Eleven of the 20 young in the 4 litters mentioned above became spontaneously refected in this way and grew to full size. Three of the refected females in this second generation were impregnated by refected males of the same generation and 3 litters (19 young ones) have been born. All the young of this third generation died at the end of the period of lactation, or a little later, without becoming refected.

The ability of refected rats to dispense with vitamin B in their diet is therefore not limited but may last for at least two generations.

The following Experiment (1) was made in order to ascertain whether adult rats, fed on a diet devoid of vitamin B, could become refected. Two rats about 1 year old (body weight: 215 and 275 grm.) previously fed upon the stock diet of the breeding rats (a mixture of grains and milk) received a

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diet devoid of vitamin B from July 20th. From July 26th until August 4th they received daily doses of white faeces from refected rats. These adults, unlike young ones, were rather unwilling to eat the white faeces. They did not become refected, but lost weight quickly and died on August 25th and 27th respectively. (Body weights at death: 112 and 176 grm.)

Experiment 2. The experiment was repeated with younger rats (body weight: 135 and 170 grm.) and with the same result. Death occurred after 5 to 6 weeks, body weight at death being 94 and 95 grm. respectively.

The results of Experiments 1 and 2 show that only young rats, weighing 40–60 grm., are easily refected, and attempts at refection have not succeeded with adults.

* * * * *

The most reasonable explanation of the observed phenomena of refection is that vitamin B is produced in the intestinal tract of refected rats by the agency of bacteria or other micro-organisms. Since no micro-organisms were observed in the tissues or intestinal walls of refected animals, the vitamin B-producing micro-organisms may be assumed to live in the lumen and in the contents of the tract. Such bacteria, if present, cannot be identical with the common intestinal organisms, for if these produced—or by their disintegration set free—appreciable amounts of vitamin B, the symptoms of vitamin B deficiency would not exist. Previous investigations dealing with the possible production of vitamin B by bacteria are of interest in this connection.

Several papers dealing with the feeding of cultures of *Bacillus coli* and other common intestinal bacteria or moulds to rice-fed pigeons with polyneuritic symptoms record neither recovery nor diminution of symptoms (Cooper (1914), Wollman (1921), Eijkman, van Hoogenhuijze and Derks (1922), Weill, Arloing and Dufourt (1922), Scheunert and Schieblich (1922), Bieling (1925)). Scheunert and Schieblich (1923) found later that large amounts of *B. vulgatus* lessened polyneuritic symptoms in pigeons, but the content of this vitamin in masses of this bacterium was less than in yeast.

Other researches deal with feeding of bacteria to young rats deprived of vitamin B. Cultures of *B. coli* and other faeces bacteria, of lactic acid bacteria, of *B. Friedlaender* and of *B. subtilis* etc. did not lessen the deficiency symptoms of the rats (Wollman and Vagliano (1922), Damon (1923), Bieling (1925)). A few species, however, when fed in big amounts, are reported to induce growth in rats deprived of vitamin B. Extracts of *B. typhosus* have been stated to possess this property (Pacini and Russell, 1918), but Damon (1921) obtained only negative results when feeding *B. paratyphosus*. Later Damon (1923, 1924) found the Pfeiffer bacillus and the Bacilli Pseudotuberculosis (*B. timothy*, *B. smegmatis*, *B. Moelleri*) to contain vitamin B. The same is reported to be true of some spore-bearing intestinal micro-organisms by Heller, McElroy and Garlock (1925).

More interesting are the following series of papers dealing with the vitamin B content of faeces, more especially of rat faeces.

The presence of vitamin B in the faeces of animals which receive it in their food is no proof of the production of this vitamin by the bacteria of the intestine. It may depend on defective absorption of the vitamin ingested. In this way Cooper (1914) explained his observation that an alcoholic extract of faeces from rabbits and chickens on adequate diets has a curative effect when fed to pigeons on a diet of polished rice. Braddon and

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Cooper (1914), however, could not reproduce this result. McCollum, Simmonds and Becker (1925) suggest that the results of some experiments of Steenbock, Sell and Nelson (1923) may be explained in the same way. Rats on a diet poor in vitamin B showed far inferior growth when kept on screens than when kept on wood shavings. McCollum and his collaborators suggested the explanation that the faeces corresponding to the diet taken before the beginning of the experiment, were allowed to remain among the shavings. It is doubtful, however, whether this objection could apply to the observation of Portier and Randoin (1920), that polyneuritic pigeons improve when fed on the faeces of rabbits receiving a diet of heated vegetables.

Heller, McElroy and Garlock (1925) and Salmon (1925) found that when rats on a diet devoid of vitamin B are fed on their own faeces they live for some weeks longer than is usual. Heller, McElroy and Garlock state, however, that the rats did not grow. These results afford no decisive proof of formation of vitamin B in the intestinal tract. Dutcher and Francis (1924) found that the faeces of rats on a diet devoid of vitamin B contain some vitamin B in the first two weeks of the defective feeding but not later. The vitamin B in the faeces may therefore originate in some way from the period before the defective feeding was started.

The papers just cited give some evidence in favour of the possibility that the common intestinal micro-organisms of rats on vitamin B-free diet sometimes produce vitamin B. But the amounts produced must be very small, because an appreciable amount, if absorbed, would prevent symptoms of deficiency. If, therefore, the ability of refected rats to grow, thrive and multiply without vitamin B in the food depends on the production of this vitamin in the intestinal tract, the amount produced must be considerable, and, if all is not absorbed, some will be present in the white faeces of such rats.

Presence of Vitamin B in the White Faeces of Refected Rats.

It is not possible to determine the content of vitamin B in the white faeces by feeding them to rats deprived of vitamin B. For this procedure causes refection and the disappearance of all symptoms of vitamin B deficiency, and it is impossible to tell whether the white faeces in addition contain pre-formed vitamin B. To decide this point the virus of refection must be killed before the white faeces are administered. This can be done by heating the faeces to 100° C. or more for 10 minutes.

In the first experiment the white faeces were heated in an autoclave at 120° C. for 20 minutes, which procedure could be trusted to destroy the virus of refection. The presence of vitamin B in appreciable amounts could not be demonstrated in these heated faeces, when fed to rats.

The following Experiment 3, however, proved that the above degree of heat had also destroyed the vitamin B present in the white faeces.

Experiment 3 (Chart 6). Fresh suspensions of white faeces (1 part in 9 parts of water) were prepared daily by grinding in a mortar. Sealed tubes containing 10 c.c. of the suspension (corresponding to 1 grm. of the faeces) were heated in a water-bath at 80° C. for 10 minutes, then placed in an ice box for 5 days and finally heated in a water-bath at 100° C. for 10 minutes.

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The preparation was so arranged that exactly similar material was always available for feeding.

Two rats on a diet devoid of vitamin B each received the content of one tube daily for 60 days (Chart 6, the dotted curves). Two control rats on the same diet received daily a suspension of 1 grm. of common brown faeces from non-refected rats on the vitamin B-free diet (Chart 6, the continuous curves). The suspensions of these brown faeces had been prepared exactly as those of the white faeces. Two other control rats (Chart 6, the alternately dotted and pointed curves) had no supplement to the diet.

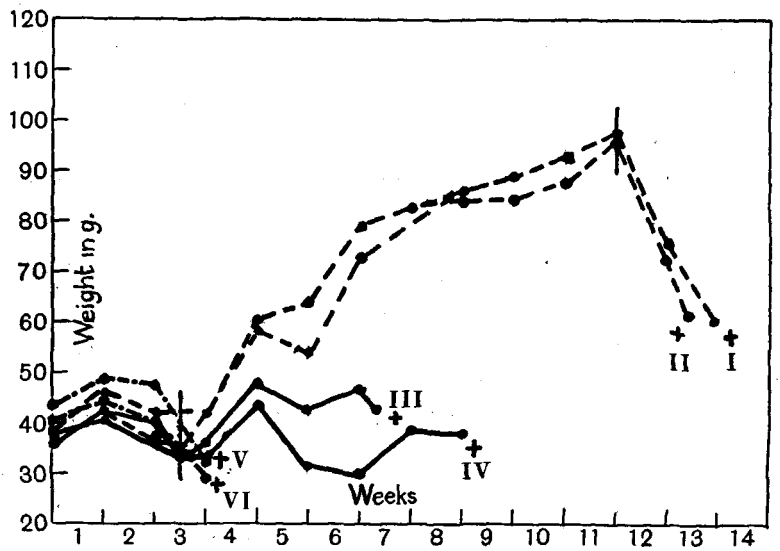

Chart 6. Experiment 3. Presence of vitamin B in heated white faeces of refected rats.

Dotted lines represent growth curves of rats 1 and 2 on a diet devoid of vitamin B, which received daily a suspension of white faeces previously heated (at 100°), for the period (60 days) between the vertical lines. Rats 1 and 2 did not become refected but grew until the faeces-feeding stopped. Continuous lines represent growth curves of rats 3 and 4 on a diet devoid of vitamin B, which received daily a suspension of heated brown faeces from non-refected rats on the same diet. Rats 3 and 4 did not grow appreciably, but lived for a longer time than rats 5 and 6. Alternately dotted and pointed lines represent growth curves of control rats 5 and 6 on a diet devoid of vitamin B.

The young rats which received the suspension of heated white faeces grew rather well; they did not become refected. Their faeces remained brown and normal, and as soon as the faeces-feeding stopped (marked in Chart 6 by a vertical line) the body weight decreased rapidly, and symptoms of deficiency in vitamin B were apparent. This result shows that the heating had destroyed the virus of refection in the white faeces, but that these contained considerable amounts of vitamin B.

The control rats receiving the heated suspension of brown faeces from non-refected rats grew a little more and lived for a longer time than the two rats

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which received no supplement to their diet. Traces of vitamin B are probably present in the brown faeces of non-refected rats.

The white faeces used in Experiment 3 were produced by refected rats, which for many months had been fed on a diet devoid of vitamin B. This vitamin B must therefore have been produced in these refected rats, probably in the intestinal tract. The amount produced must have been much greater than the amount present in the faeces, for part must have been absorbed and utilised by the refected rats themselves. And, further, the heating of the white faeces, although not excessive, had probably destroyed a certain fraction.

The ability of refected rats to dispense with vitamin B is thus probably due to their capacity to produce vitamin B in the intestinal tract.

Brown faeces of non-refected rats on a diet devoid of vitamin B contain but little vitamin B. This suggests that the normal intestinal bacteria of these rats can produce, at most, only trifling amounts of vitamin B. Small amounts were, however, demonstrated in these faeces, thus confirming the results of Steenbock, Sell and Nelson (1923), Heller, McElroy and Garlock (1925) and Salmon (1925). It is probable therefore that the vitamin B present in the white faeces of refected rats, is produced by some special micro-organisms in the intestinal tract, which may be the same as those which transmit the refection.

Bacteria and Chemical Processes in the Coecum.

Microscopical examination of the coecal content of refected rats shows a bacterial flora different from that of other rats. In gram-stained preparations gram-negative rods, curved as vibrio, are abundant and many large gram-positive cocci are also to be seen.

That the chemical processes taking place in the coecum of refected rats must also be different from those in the coecum of non-refected rats, is evident from the observed difference in the *p*H of the contents.

Table I. *p*H *of the coecal contents in rats.*

6 breeding rats (mixed diet)		5·25, 5·35, 5·50, 5·80, 6·10, 6·10
3 rats getting adequate artificial food mixture		6·25, 6·45, 6·75
Rats getting food-mixture devoid of vitamin B	5 non-refected rats (brown faeces)	7·65, 8·15, 8·50, 8·55, 8·65
	4 refected rats (white faeces)	5·90, 6·60, 6·70, 6·75

The *p*H of the coecal contents was measured electrometrically, shortly after the rat had been killed. The ninhydrin electrode of Biilman (1921) was used as described by Norgaard (1927). The results are given in Table I and show the coecal content of refected rats to be acid as in normal rats on an adequate diet. In non-refected rats, on a diet devoid of vitamin B, the coecal content was alkaline.

In this respect, as in others, the refected rats were shown to resemble normal rats and to differ from rats suffering from vitamin B deficiency. The change in bacterial flora and in chemical processes in the coecum of refected

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rats may be correlated with the production of vitamin B in the intestine of these rats.

Summary.

1. Refected rats are able to remain normal for more than two generations notwithstanding the absence of vitamin B in their food.

2. Attempts to cause refection in adult rats on a diet devoid of vitamin B were not successful.

3. White faeces of refected rats have been shown to contain considerable amounts of vitamin B. In this experiment the virus of refection in the white faeces had been killed by heating.

4. Brown faeces from non-refected rats on a diet devoid of vitamin B contain very little vitamin B.

5. The bacterial flora in the coecum of refected rats is different from that of other rats.

6. The coecal content of refected rats is acid as is that of normal rats. The coecal content of non-refected rats on a diet devoid of vitamin B is alkaline.

REFERENCES (Section III).

Bieling, R. (1925). Versuche ueber die Bildung von Vitamin durch Bakterien. *Zeitschr. f. Hygiene u. Infektionskr.* **104**, 347–357.

Billman, E. (1921). Sur l'Hydrogénisation des Quinhydrones. *Ann. de Chimie*, Sér. ix, **15**, 109.

Braddon, W. L. and Cooper, E. A. (1914). The influence of metabolic factors in Beri-Beri. I. The effect of increasing the carbohydrate ration on the development of polyneuritis in birds fed on polished rice. *J. of Hygiene*, **14**, 331–353.

Cooper, E. A. (1914). On the protective and curative properties of certain foodstuffs against polyneuritis induced in birds by a diet of polished rice. *Ibid.* pp. 12–22.

Damon, S. R. (1921). Bacteria as a source of the water-soluble B-vitamin. *J. Biol. Chem.* **48**, 379–384.

—— (1923). Some observations in regard to growth-promoting substances of bacterial origin. *J. Biol. Chem.* **56**, 895–902.

—— (1924). Acid-fast bacteria as a source of vitamin B. *J. Pathol. Bacteriol.* **27**, 163–169.

Dutcher, R. A. and Francis, Emma (1924). Vitamin studies. X. Feeding technique in vitamin studies. *Proc. Soc. exper. Biol. and Med.* **21**, 189–193.

Eijkman, C., van Hoogenhuijze, C. J. E. and Derks, T. J. G. (1922). The vitamine content of micro-organisms in relation to the composition of the culture medium. *J. Biol. Chem.* **50**, 311–314.

Heller, V. G., McElroy, C. H. and Garlock, Bertha (1925). The effect of the bacterial flora on the biological test for vitamin B. *Ibid.* **65**, 255–264.

McCollum, E. V., Simmonds, Nina and Becker, J. Ernestine (1925). Technique in the use of the rat for vitamin B studies. *Ibid.* **63**, 547–551.

Norgaard, A. (1927). On the hydrogen ion concentration in some food products and in faeces. Acta medica scandinavica. *Rapport et Compt. rend. du XII congrès de méd. des pays du nord*, Supplem. xvi.

Pacini, A. J. P. and Russell, Dorothy W. (1918). The presence of a growth-producing substance in cultures of typhoid bacilli. *J. Biol. Chem.* **34**, 43–49.

https://doi.org/10.1017/S002217240003182X Published online by Cambridge University Press

PORTIER, P. and RANDOIN, LUCIE (1920). Création de vitamines dans l'intestin des lapins recevant une nourriture stérilisée à haute température. *Compt. rend. de l'Acad. des Sci.* **170**, 478–480.

SALMON, W. D. (1925). Vitamin B in the excreta of rats on a diet low in this factor. *J. Biol. Chem.* **65**, 457–462.

SCHEUNERT, A. and SCHIEBLICH, M. (1922). Studien ueber die Magendarmflora polyneuritischer Tauben und die Bildung antineuritischen Vitamins durch Darmbakterien. *Centralb. f. Bakteriologie*, etc. I Abth. Originale, **88**, 290–298.

—— —— (1923). Zur Kenntnis der Vitamine. II. Ueber die Bildung von Vitamin B durch obligate Darmbakterien. *Biochem. Zeitschr.* **139**, 57–65.

STEENBOCK, H., SELL, MARIANA T. and NELSON, E. M. (1923). Vitamine B. I. A modified technique in the use of the rat for determination of vitamine B. *J. Biol. Chem.* **55**, 399–410.

WEILL, E., ARLOING, F. and DUFOURT, A. (1922). Essai de traitement de la carence du Pigeon par les cultures mortes ou vivantes de microbes intestinaux. *Compt. rend. de la Soc. de Biol.* **87**, Tome II, 49–52.

WOLLMAN, E. (1921). Sur le rôle des microorganismes dans la production des vitamines. *Ibid.* **85**, 801–803.

WOLLMAN, E. and VAGLIANO, M. (1922). Sur le rôle des microorganismes dans la production des vitamines. Recherches sur la production des vitamines de croissance par le Bacille bulgare et l'Amylomucor β. *Ibid.* **86**, 832–833.

IV. CONNECTION BETWEEN THE DIFFERENT EFFECTS OF REFECTION. ATTEMPTS AT REFECTION IN RATS ON A DIET DEVOID OF VITAMIN A AND IN OTHER ANIMALS THAN RATS.

Information regarding the two different effects of refection has been set forth in the preceding papers. The first, the ability to grow, thrive and multiply without vitamin B in the food seems to depend on the production of vitamin B in the intestinal tract of refected rats, while the second, the production of white, bulky faeces, is due to a defect in the digestion of starch. What connection may exist between these is not apparent. It is possible that the two effects are mutually independent. Attempts have been made to devise experiments in which one might be investigated apart from the other, *i.e.* to check the production of vitamin B in the intestinal tract by feeding vitamin B-containing food (yeast) to refected rats, or to eliminate the defect in starch digestion by giving a food-mixture devoid of starch.

Effect of feeding Yeast to Refected Rats.

Experiment 1. Three refected rats were fed upon the adequate food-mixture containing 5 per cent. of dried yeast (see Section I).

Rat Bf 80 received a vitamin B-free diet from Dec. 5th, 1925; it became refected on Dec. 18th, 1925. From July 8th, 1926, after having been refected $6\frac{1}{2}$ months (body weight 177 grm.), it received the yeast-containing diet. On July 9th the faeces had turned brown, but still contained plenty of starch grains. On July 15th there were very few starch grains, and after July 16th none at all to be seen in the faeces.

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Rat Bf 123, after being refected for 5½ months, received the yeast-containing diet on July 10th. On July 12th brown faeces appeared and after July 16th the faeces contained no starch grains.

Rat Bf 138, after being refected for one month, received the yeast-containing diet from March 17th. On March 20th dark faeces appeared which after March 24th contained no starch grains.

These experiments agree in showing that the defect in starch digestion disappears when refected rats are fed on an adequate diet, containing yeast. The white faeces turned brown after 3 days and in about a week no longer contained starch grains. The growth of course continued.

Effect of feeding White Faeces to Rats upon an Adequate Diet.

Experiment 2. Two young rats (Vs 83 and Vs 84) received the adequate experimental diet from Nov. 11th, 1926 (body weight 41 grm. and 47 grm.). From Dec. 23rd (body weights 163 grm. and 174 grm.) until Jan. 3rd, 1927 fresh white faeces from the refected rat Bf 240 were given daily. From Dec. 23rd until Jan. 5th the faeces of Vs 83 and Vs 84, although dark, contained many starch grains, but after Jan. 6th these were no longer to be seen.

These starch grains were no doubt the starch of the consumed faeces, which in Section II, p. 84, were shown to be insusceptible to the action of amylase, and to pass the digestive tract of normal young rats in an unchanged state. When the faeces-feeding was stopped, the young rats ceased to pass starch grains in their own faeces, thus showing that the treatment had not altered their ability to digest starch.

When yeast is fed to refected rats, the defect in their starch digestion disappears; conversely it does not seem possible to induce this defect in rats upon an adequate, vitamin B-containing diet. These observations point to a connection between the vitamin B deficiency of the food and the defective digestion of starch in refected rats.

Effect of feeding Food-mixtures devoid of Starch or poor in Starch to Refected Rats.

The following food-mixture, known as Bfs, devoid of starch and of vitamin B was prepared.

Purified caseinogen	67 per cent.
Butter fat	15 ,,
Pure saccharose	10 ,,
Purified agar	3 ,,
McCollum and Davis' salt mixture	5 ,,

Experiment 3 (see Chart 7).

Rat Bf 221 was fed on a diet devoid of vitamin B from Aug. 23rd and became refected on Sept. 11th, 1926 (body weight 65 grm.).

Rat Bf 240 was fed on diet devoid of vitamin B from Oct. 8th and became refected about Oct. 28th, 1926 (body weight 42 grm.).

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Both rats were fed on the Bfs food-mixture from Jan. 3rd, 1927 (body weights 175 grm. and 201 grm.), after having been refected for 4 and 2 months respectively. The result is shown in Chart 7. In both, the body weight decreased rapidly after the change of diet (marked in Chart 7 by a vertical line), and the faeces turned brown. The rats lost weight for 3 months and died.

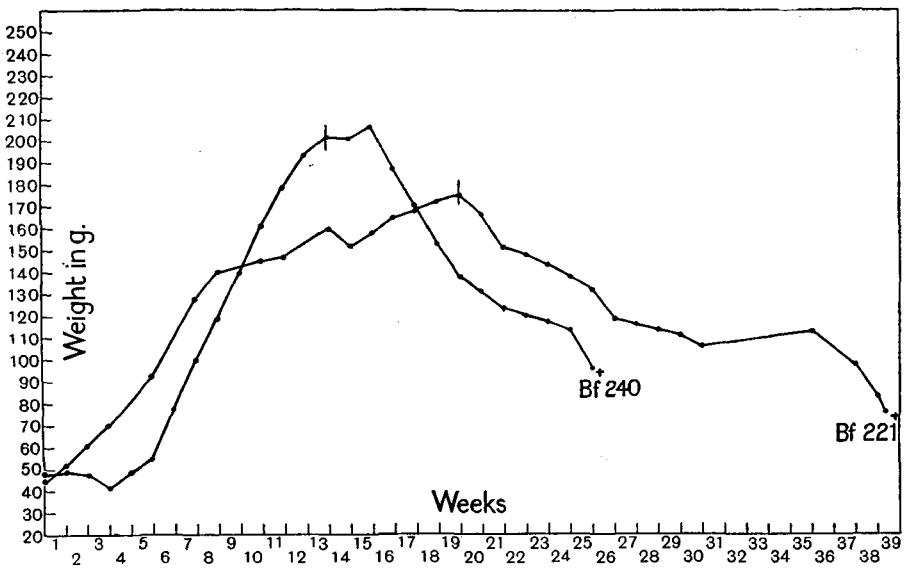

Chart 7. Experiment 3. The effect of feeding a food-mixture devoid of starch and of vitamin B to refected rats.

Growth curves of the refected rats Bf 221 and Bf 240. At the vertical lines the usual food-mixture devoid of vitamin B, was replaced by a food-mixture devoid of starch and of vitamin B, this producing rapid decrease in body weight of the rats.

Experiment 4 (Chart 8). Eleven young rats (body weights between 30 grm. and 40 grm.) were fed upon the Bfs food-mixture. Three died after a few days (not shown in Chart 8) and one control (Graph IV, Chart 8) died after 32 days. Five rats were fed daily with white faeces from refected rats. Two died after 5 and 10 days respectively (not shown in Chart 8). The faeces-feeding was continued for 2 weeks with two of the others and for 3 weeks with the third (see dotted curves in Chart 8). During the period of faeces-feeding (marked by vertical lines in the curve) the body weight increased by from 5 to 13 grm., but decreased rapidly after it was stopped, and the rats died 2 to 4 weeks later. Two rats were given 5 ctg. dried yeast daily (the continuous curves V and VI in Chart 8) to see if they could grow on the Bfs diet when thus supplemented. The rats grew at a normal rate and behaved normally.

The results of Experiment 3 show that the body weight of normally growing refected rats decreases rapidly when they receive a diet devoid of starch, suggesting an essential connection between the production of vitamin B

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in refected rats and the defective starch digestion. One must suppose that when such rats received starch-free food the production of vitamin B in their intestinal tract is very scanty. But it does not seem to stop completely, for the rats lived for a much longer time than other rats deprived of vitamin B.

The results of Experiment 4 show that young rats on the Bfs starch-free diet do not become refected when fed with white faeces from refected rats. During the period of faeces-feeding the rats grow on account of the vitamin B received with the white faeces (see Section III), but they decline and die as

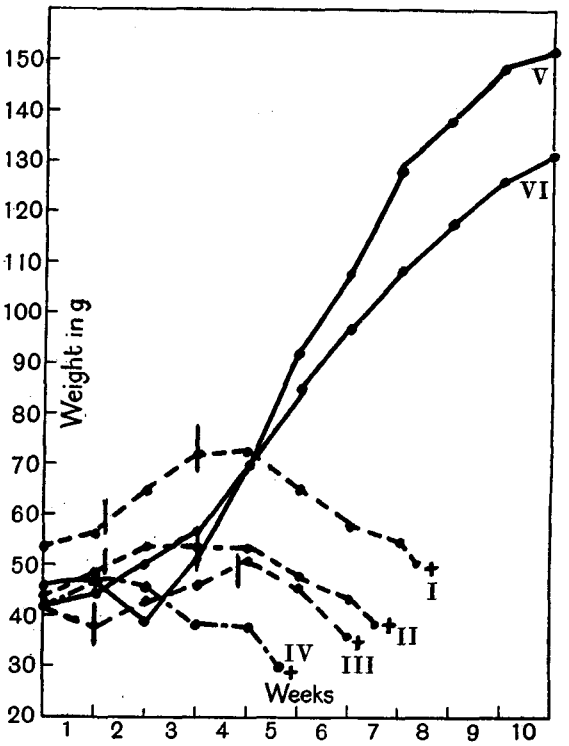

Chart 8. Experiment 4. Attempt to refect rats on a diet devoid of starch and of vitamin B. The dotted lines represent growth curves of rats 1, 2 and 3 on this diet, fed with white faeces from refected rats for the period between the vertical lines. These rats do not become refected and the growth during the period of faeces-feeding is due to the vitamin B received with the white faeces. The alternately dotted and pointed line represent the growth curve of a control rat 4 on the diet devoid of starch and vitamin B. The continuous lines represent growth curves of rats 5 and 6 on the same diet supplemented with 5 ctg. dried yeast daily, showing normal growth to be possible on a diet devoid of starch.

soon as this is stopped. The rapid growth of the two rats receiving a supplement of yeast in addition to the Bfs diet shows this diet is adequate for the growth of rats in spite of its lack in starch.

In Experiments 5 and 6 the starch in the usual food-mixture, devoid of vitamin B, was replaced by dextrin. The dextrin was not completely free of starch, but the small amount present could not be estimated, because no

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method exists for the estimation of starch in dextrin. This food-mixture is called Dx.

Experiment 5 (see Chart 9). Five young rats (body weights between 30 grm. and 40 grm.) received the Dx diet from June 28th. Three died after 6–14 days. The two remaining rats were fed daily from July 5th until July 16th (marked in the curves with vertical lines) with white faeces from refected rats. Their faeces did not turn white but contained some starch grains derived from the

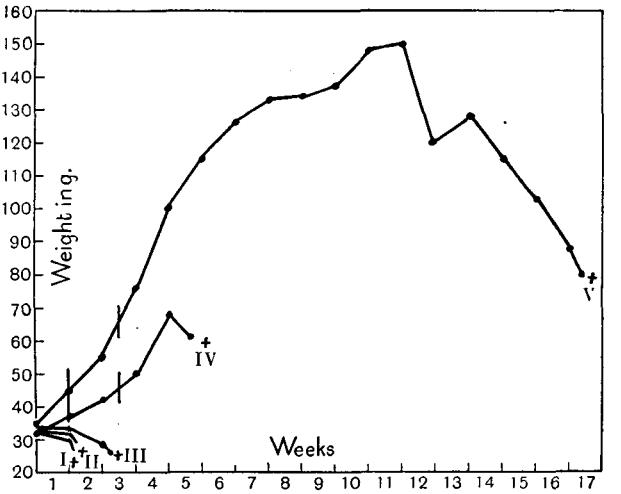

Chart 9. Experiment 5. Refection of rats on a diet devoid of vitamin B in which starch is replaced by dextrin.

Rats 1, 2 and 3 died after 6–14 days. Rats 4 and 5 were fed daily for the period between the vertical lines with white faeces and became refected.

small amount of starch present in the dextrin, and the rats grew very well. One died on August 8th. The diet of the second was changed on September 14th to the Bfs mixture, after which the rat declined rapidly and died on October 10.

Experiment 6. Rat Bf 88 received the usual food-mixture devoid of vitamin B from December 14th, 1925, and was refected on December 22nd, 1925. On June 23rd, 1926 the diet was changed to the Dx diet. The faeces turned brown but the rat continued to grow, although the Dx diet is devoid of vitamin B.

The results of Experiments 5 and 6 show that rats may be refected or may remain refected when their diet contains only a very small amount of starch. These rats produce brown faeces, containing starch-grains, but it was not possible to refect other rats by feeding them with these faeces; several attempts were made with negative results.

It is remarkable that these Dx-fed rats could become refected and grow without producing white faeces, although the brown faeces contained starch grains. The same phenomenon was observed twice in rats receiving the usual diet devoid of vitamin B.

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Provisionally neglecting these exceptional cases which, though interesting, have not been investigated further, the results of the foregoing experiments suggest an essential connection between the defect in the starch-digestion and the production of vitamin B in the intestinal tract. The existence of this connection is impressed upon the clinical observer of the refected rats. The growth is often intermittent, periods of growth alternating with periods of cessation of growth or of decrease in body weight with corresponding production of white faeces and brown faeces respectively.

This phenomenon is shown in Chart 10. The white and black portions of the line at the bottom of the figure represent respectively periods in which

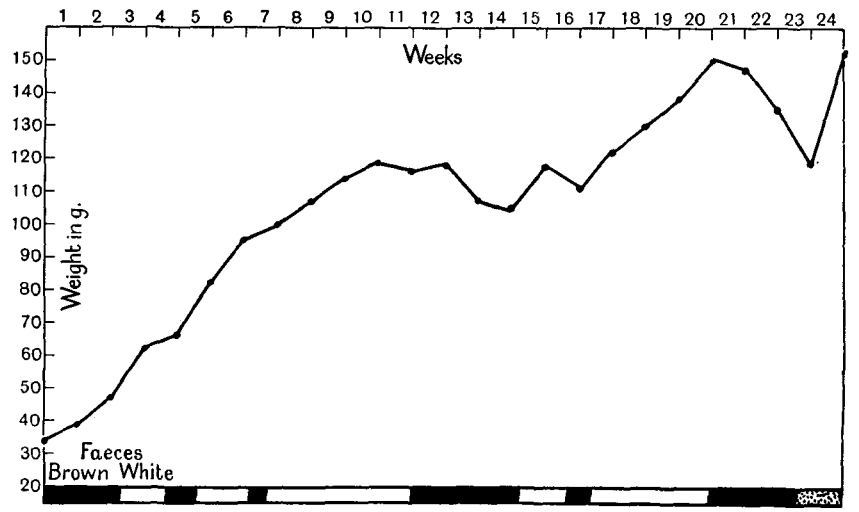

Chart 10. Coincidence of periods of growth with the periods when white faeces are produced. The continuous line represent the growth curve of a refected rat. The white and black portions of the line at the bottom of the figure represent respectively periods in which white and brown faeces were passed.

white and brown faeces were passed. The periods of growth coincide with the periods when white faeces are produced, and the weight of the rat decreases when the faeces turn brown.

Ability to grow and the defective digestion of starch are closely related, but the nature of this connection is not known.

Attempts at Refection of Rats upon a Diet devoid of Vitamin A, and either containing or devoid of Vitamin B.

Experiments were made with rats receiving the diet devoid of vitamin A generally used in this laboratory, to see whether such rats could also become refected.

The food-mixture was composed of: purified caseinogen 20 per cent.; pure rice starch 52 per cent.; oxidised lard 15 per cent.; agar 3 per cent.; yeast 5 per cent.; McCollum and Davis' salt mixture 5 per cent.

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Experiment 7. Three young rats (body weights 30–35 grm.) were fed on the diet devoid of vitamin A from May 20th. From June 17th the rats received white faeces daily from refected rats. No effects whatever were observed. The rats developed the usual eye symptoms, declined and died between July 8th and 17th. The diet of these rats contained vitamin B, and the results of Experiments 1 and 2 (see p. 93) showed that rats receiving vitamin B in their food cannot be refected. It was possible therefore that in order to induce refection on a diet devoid of vitamin A, the diet should also be deprived of vitamin B. This was tried in the next experiment.

Experiment 8. The food-mixture was the same as in Experiment 7 but without yeast and with 62 per cent. of rice starch. Six young rats (body weights between 30 grm. and 35 grm.) received this diet from June 26th. Two control rats receiving the diet without supplement died on July 22nd and 29th. Four experimental rats received white faeces from refected rats daily from July 3rd until July 16th, and all began to produce white faeces themselves and increased in weight by from 7 grm. to 10 grm. during this period. When the faeces feeding stopped, the body weight of all four rats decreased rapidly and they died between July 24th and 26th. These rats appeared to become refected for a limited period, but their lives were not prolonged and they died sooner than young rats receiving a diet deficient only in vitamin A.

Attempts at refection do not seem to have any beneficial influence upon rats suffering from deficiency in vitamin A, the phenomenon being only concerned with deficiency in vitamin B.

Attempts at Refection of Animals other than Rats.

As yet only preliminary experiments have been carried out with mice and pigeons.

Experiment 9. Ten albino mice about 3 weeks old were fed upon the usual diet devoid of vitamin B from February 2nd. Four control mice died between February 9th and 20th, two of which had paresis of the hind legs. Six experimental mice received white faeces from refected rats daily from February 8th to 23rd. During this period some of the mice produced white faeces, but after the faeces-feeding was stopped all produced dark faeces, decreased in weight and died between March 3rd and 5th.

Experiment 10. Seven young albino mice of body weight varying between 11 grm. and 17 grm., were fed on vitamin B-free diet from March 24th. After March 29th all received white faeces from refected rats daily. Five mice died between March 28th and April 7th without having been refected. Two mice became refected and produced white faeces. Of these one was killed April 4th, and at autopsy showed the intestinal tract stuffed with a copious white content. The gall-bladder was empty. The other refected mouse died on April 6th.

Experiment 11. Three young albino mice of body weight 11 grm. to 14 grm.

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were fed on the vitamin B-free diet from January 8th and received white faeces in addition from January 14th to 27th. Two of the mice died on January 27th without being refected. The third produced white faeces from January 17th and increased in body weight from 10·2 grm. to 12·4 grm. This mouse was killed on February 5th. The fluid in the gall-bladder gave positive test for bile pigments (Huppert's test).

Experiment 12 was a repetition of the former experiments but no mice became refected.

Experiment 13. Six pigeons were fed on polished rice from June 22nd. The 3 control pigeons had lowered body-temperature on July 17th (39·8°–39·5°–38·7° C.) and died on July 17th, 20th and 25th respectively, two exhibiting typical spasms. The 3 experimental pigeons were fed daily with white faeces from refected rats from July 2nd until July 17th. On July 17th the body-temperature was still normal (40·4°–40·7°–40·3° C.) in all three. The pigeons however became paralysed later and died on July 25th, 28th and August 1st respectively.

The results of Experiments 9–12 show that albino mice on vitamin B-free diet are not easily refected; in three cases refection succeeded but lasted only for a limited period. In one of these refected mice the presence of biliary pigments in the bile of the gall-bladder was demonstrated in spite of the faeces being white (compare the observations concerning this point in Section II).

Refection of pigeons, fed on polished rice, was not induced. During the period of faeces-feeding the pigeons were healthier than controls receiving no supplement to their diet, but this was probably due to the vitamin B contained in the white faeces. There was no indication that the pigeons themselves were refected.

Discussion.

The refection of rats, fed upon a diet devoid of vitamin B, arose spontaneously in this laboratory and was discovered accidentally. But the phenomenon seems to have considerably more interest than a common "laboratory accident."

In the first place refection has an important bearing upon the rôle of vitamin B in nutrition. In all animals investigated till now, vitamin B has been found to be an indispensable constituent of the diet. Several attempts have been made to determine the necessary minimum of this vitamin which must be present in the diet of different species, and last year Plimmer (1926) published experiments indicating that this minimum was higher than had been formerly supposed. In future these statements will need modification, because the existence of refection has shown that this minimum in certain special conditions may be zero.

The facts concerning refection may lead research upon vitamin B into new directions. When in 1896 Eijkman produced experimental polyneuritis

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in rice-fed chickens, he thought the symptoms were caused by poisoning due to intestinal fermentations produced by the starchy nature of the food. This theory was abandoned long ago, but since the first experiments of Eijkman, there have been many suggestions of a connection between the effects of deficiency in vitamin B and the starch of the food. In an unexpected way the phenomenon of refection corroborates these ideas.

The results of the foregoing experiments on refection point distinctly to processes in the intestinal tract being involved when there is a deficiency of vitamin B in the diet. Several investigators have suspected such a connection, for example McCarrison (1921). The results of investigations upon refection may serve as a spur to further work upon this problem.

In refected rats the digestion of starch is defective. Possibly the process of starch digestion is not so simple as is mostly supposed. The symptoms of the "intestinale Gaerungsdyspepsie" in human beings point to complications in the digestion of starch. In patients suffering from this disease the starch is incompletely digested, as in refected rats, but to a much lesser degree. In refected rats there are indications that the defective starch digestion depends in some way on bacterial processes in the intestinal tract.

Finally, studies in refection may possibly have a bearing upon problems connected with the spread and treatment of beri-beri. McCarrison (1924) has called attention to the endemic character of beri-beri in certain regions of India and has contrasted the immunity enjoyed in other regions. McCarrison does not consider that this can be explained by any significant differences in diet but suggests the existence of a specific infection in those places where beri-beri is common. One might perhaps imagine that some kind of refection is common in the regions exempt from beri-beri! But no records are known to us of anything suggesting refection in human beings. If such a thing should exist the future therapeutic use of refectious processes may not be beyond the limits of possibility.

SUMMARY.

1. When refected rats are fed on adequate diet, containing vitamin B in the form of yeast, the defect in their starch-digestion disappears, and their faeces become normal.

2. Attempts to cause refection in rats receiving an adequate diet by feeding white faeces from refected rats do not influence the starch digestion of the former.

3. When refected rats, growing normally, are fed upon a diet devoid of starch (as well as of vitamin B) their body weight decreases rapidly, but in spite of this decrease they live for a considerable time.

4. Refection cannot be induced in young rats fed upon a diet devoid of starch and of vitamin B.

5. Rats may become or may remain refected when the starch of their

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diet is replaced by dextrin containing a small amount of starch. The faeces of these rats do not seem able to refect other rats.

6. Rats may be refected without producing white faeces, but starch grains have always been present in the faeces of such rats.

7. The results of special experiments and the clinical observation of refected rats suggest an essential connection between the ability to grow and thrive without vitamin B and the defective digestion of starch.

8. Attempts at refection have no beneficial influence upon rats suffering from a deficiency of vitamin A.

9. Refection has been transmitted to albino mice receiving a diet devoid of vitamin B, but the refection lasted only for a short time. Attempts at refection of rice-fed pigeons have not succeeded.

10. The bearing of studies in refection upon several problems connected with vitamin B is discussed.

REFERENCES (SECTION IV).

EIJKMAN, C. (1897). Eine beriberi-ähnliche Krankheit der Hühner. *Virchow's Arch.* **148**, 523.

MCCARRISON, R. (1921). *Studies in Deficiency Diseases.*

—— (1924). Rice in relation to Beri-Beri in India. *Proc. Roy. Soc. Med.* **17**, No. 8. Section of Tropical Diseases and Parasitology, pp. 65–82.

PLIMMER, R. H. A. (6. II. 1926). The relation of quantity of vitamin B to quantity of food. *Brit. Med. J.* i, 239.

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https://doi.org/10.1017/S002217240003182X Published online by Cambridge University Press