**LXXXVIII. THE PHYSIOLOGICAL RÔLE
OF VITAMIN B. PART III.
STUDY OF VITAMIN B DEFICIENCY IN PIGEONS¹.**

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THE recognition that there exists a close relationship between the consumption of food and the supply of vitamin B in the diet has greatly advanced attempts to determine the physiological rôle of this substance. It has, in the first place, provided a plausible explanation of many of the numerous divergencies of opinion that are recorded in the literature, whilst secondly it has, we believe, indicated the right line along which future research in this field should proceed.

It is obvious from a survey of the literature that the great majority of investigators have disregarded entirely or failed to appreciate fully the significance of the voluntary abstention from food that animals deprived of vitamin B usually exhibit. A few have borne this in mind, notably Funk [1924, p. 196].

OBJECT OF THE EXPERIMENTS.

In view of the conclusions reached by Drummond and Marrian [1926] concerning the part played by inanition in vitamin B deficiency in rats it seemed imperative that similar experiments should be carried out on pigeons. It was therefore planned to study the fate of pigeons fed on dietaries lacking vitamin B, but under conditions where the effects of inanition could be separated from those due more directly to the deficiency.

It is obvious that the experimental study of vitamin deficiency can give clear-cut results only when the vitamin under investigation is the sole limiting factor of a diet complete with regard to all other components and when all factors liable to affect the experiment are checked by the use of proper controls. In the present paper a new method of studying vitamin B deficiency is described, as well as the results obtained on pigeons with the aid of this method, which it is believed provides a control covering the disturbing effects of inanition.

Not less than six experimental animals are placed on the deficient diet. For each of them a control of the same weight and if possible the same age and sex is selected. The control animals are given the same amounts of the diet

¹ Recent work has clearly demonstrated the existence of at least two substances of the vitamin type in the yeast extracts employed by us. The name vitamin B used throughout this paper covers both these factors and possibly others yet undiscovered.

as are eaten voluntarily by the corresponding animals fed on the deficient diet, but in addition receive a liberal supply of the missing factor. Both lots are thus placed under identical conditions, except for the vitamin factor. It is therefore reasonable to assume that differences of behaviour exhibited by the deficient group as compared with the controls are attributable to a lack of the vitamin¹.

EXPERIMENTAL.

Pigeons were used as experimental animals, and were divided into four groups of eight birds, each kept in a separate cage. The individual initial weights (g.) of the pigeons are given in Table I.

Table I.

Lot I		Lot II		Lot III		Lot IV	
Pigeon	Weight	Pigeon	Weight	Pigeon	Weight	Pigeon	Weight
I <i>a</i>	414	II <i>a</i>	423	III <i>a</i>	337	IV <i>a</i>	353
I <i>b</i>	445	II <i>b</i>	443	III <i>b</i>	367	IV <i>b</i>	357
I <i>c</i>	445	II <i>c</i>	428	III <i>c</i>	398	IV <i>c</i>	400
I <i>d</i>	372	II <i>d</i>	380	III <i>d</i>	503	IV <i>d</i>	513
I <i>e</i>	457	II <i>e</i>	454	III <i>e</i>	443	IV <i>e</i>	447
I <i>f</i>	425	II <i>f</i>	433	III <i>f</i>	387	IV <i>f</i>	383
I <i>g</i>	395	II <i>g</i>	405	III <i>g</i>	411	IV <i>g</i>	410
I <i>h</i>	383	II <i>h</i>	355	III <i>h</i>	497	IV <i>h</i>	465
Average	417		415		418		416

Lot I received an artificial ration deficient in vitamin B. Each bird in Lot II received by the mouth the same quantity of food that the corresponding animal in Lot I had eaten in the previous 24 hours *plus* 1 g. of yeast extract (marmite). Lots III and IV were daily fed forcibly with amounts of the deficient ration calculated to maintain their weight if assimilated, Lot IV receiving in addition 1 g. of yeast extract every day.

Diet. A modified artificial diet of the type used by Randoin and Simonnet [1924, 1] was used. Polished rice, although much simpler to use, is by no means an adequate diet for the pigeon [McCollum and Davies, 1915; McCarrison, 1921], and in our opinion is unsatisfactory for use in such studies as these.

The ration had the following composition:

Rice starch	...	...	66 %	Butter fat	...	...	4 %
Caseinogen purified	...	...	16 %	Filter-paper pulp	...	...	2 %
Agar-agar	...	...	8 %	Salt-mixture (McCollum)	...	...	4 %

The foodstuffs were mixed with a sufficient quantity of water (4.5 litres for 6 kg. of the diet) to a stiff dough, the agar being added last, and placed in flat trays in a gas oven for 1–2 hours at 130°. The resulting loaves were cut in a bread-cutting machine into slices and then into cubes the size of small peas, and finally dried at 50–60°. The diet used for forcible feeding was not cut up, but the loaves were ground to a coarse powder after drying. The diet, when offered for voluntary consumption (Lot I), was placed in glass pots covered with a tin lid having a small hole in the middle permitting the pigeon

¹ After sending this paper to Press, we found that Gulick [1922] has described a similar technique in a short note published in the *Proceedings of the American Physiological Society*.

to pick up the diet without scattering. The quantities offered were always in excess of the daily voluntary intake and were replenished daily. For artificial feeding the powdered diet was mixed with a sufficient amount of water and placed by means of a glass syringe and a piece of rubber tubing in the pigeon's crop. The control lots (II and IV) were also given daily by means of a small funnel and a piece of rubber tubing 2.5 cc. of yeast extract solution (corresponding to 1 g. of marmite). All the birds were given once a fortnight five drops of cod-liver oil. The results obtained with Lots I and II, and III and IV will be dealt with separately.

LOTS I AND II.

Every day the birds were weighed and determinations of the food consumed by Lot I made. Each bird in Lot II received the same amount of food as consumed by the corresponding bird in Lot I during the previous 24 hours, together with the supplement of 1 g. of yeast extract in solution.

Fig. 1.

In Figs. 1, 2 and 3 are shown three typical records of body weights, food consumptions and, in the latter stages of the experiment, of body temperatures of birds in Lot I and their controls in Lot II. Fig. 4 shows the average curves for all birds in Lots I and II; this series covers a period of 28 days up to the death of pigeon *d* in Lot II. The average daily loss was 5.5 g. for Lot I and 5.0 g. for Lot II. The average daily food intake was 6.1 g. The average daily loss in complete starvation is about 9.3 g. (McCarrison). A general survey of the body weight and food intake curves demonstrates that the initial loss of weight is associated with insufficient food intake. In fact, during the first days of the experiment the pigeons, unaccustomed to be confined in individual cages, have no appetite. After a few days, however, they feed well and consume 20–30 g. of the diet, with corresponding rise in weight curves.

Later, lack of appetite becomes manifest in those deprived of vitamin B and from that time these pigeons are in a state of partial or even total starvation. In the beginning of the fourth week they sometimes regain partially their appetite and quantities of 10–15 g. of the diet may be voluntarily ingested. A possible explanation of this phenomenon will be discussed later.

Fig. 2

Fig. 3.

The remarkable correspondence between the curves of Lots I and II and particularly between the averages in Fig. 4 demonstrates in a striking manner the part played by inanition as a consequence of vitamin B deficiency.

Attitude of the pigeons.

The birds of Lot I, at first very active, soon became apathetic, and spent most of the time sitting motionless on the perches with eyes shut and feathers ruffled. The controls behaved quite similarly, which suggests that these symptoms are most probably due to starvation. In the later stages a period of rest-

lessness was observed and the pigeons, apparently searching for a satisfying food, scattered a great part of the diet without, however, eating appreciably. Later, as already mentioned, the food intake sometimes increased, and there was less scattering. Nervous symptoms generally followed immediately afterwards.

Fig. 4. Average weight and food intake of deficient and control pigeons.

Faeces.

Changes of consistence and colour of faeces of pigeons on vitamin B-deficient diets have often been described [McCarrison, 1921; Funk, 1921-25; Collazo and Funk, 1924]; the last-named authors have even devised a rough method of determining the vitamin B content of a diet based on the presence or absence of the bright green colour of the faeces; it is said that the green colour disappears in the course of 24 hours after administration of the vitamin. This finding could not be confirmed by the present work, as far as a direct relationship between vitamin B and the consistency and colour of the faeces is concerned. Emerald green coloured liquid faeces were observed as frequently in Lot II as in Lot I and their appearance seemed obviously closely related to insufficient food intake, bacterial infection playing also possibly a rôle, as McCarrison has suggested. On the other hand, faeces of absolutely normal colour and consistency have always been passed by pigeons of Lot I, even in the last stage of the experiment, following the voluntary ingestion of larger quantities of the deficient diet. Green faeces were very rare in the forcibly fed group, appearing only after rejection of food by vomiting. The presence of large amounts of undigested starch in the faeces has been described in vitamin B deficiency by McCarrison [1921] and others. However, Braddon and Cooper [1914] point out that chickens fed one-tenth of their weight of polished rice and developing acute symptoms in the course of 10 days digested 93-98 % of the starch. Similar observations were reported by Eijkman and Hoogenhuyze [1916]. Randonin and Simonnet [1924, 2] consider that pigeons deprived of vitamin B are unable to digest starch. They find, however, also large quantities of starch—more than 60 % of dry weight—in the faeces of

normal pigeons fed on a complete artificial diet. This will be discussed more fully later, and only the results of the analysis of faeces of four lots of pigeons will be given here. The faeces after drying to constant weight were hydrolysed with hydrochloric acid for 5 hours, and the reducing substances determined by Bertrand's method and expressed as "starch" by multiplying the result by the factor 0.9. The faeces were collected between the third and fourth week of the experiment.

Table II.

Pigeons on deficient diets		Pigeons on complete diets	
Lot	% of starch	Lot	% of starch
Lot I (mixed sample)	18.5	Lot II (mixed sample)	18.1
Lot III* (one pigeon)	17.0	Lot IV (mixed sample)	19.6
Lot I† (two pigeons)	20.9	—	—
Average	18.8		18.8

* It was difficult to collect samples of faeces from this group owing to contamination with vomitus.

† Taken in the last stage of the experiment.

No difference in the digestion of starch can be traced from these figures, which are actually much lower than those recorded by Randoin and Simonnet [1924, 1]. It must be remembered that agar-agar yields reducing sugars on acid hydrolysis.

Nervous symptoms.

Five out of eight pigeons of Lot I developed acute symptoms: Nos. I *a*, I *b*, I *d*, I *e* and I *h* in the course of 26, 32, 25, 25, and 30 days respectively. Pigeon I *b* had emprostotonos, the remaining four typical opisthotonos associated with convulsions and "cartwheel" turning. I *a* and I *h* had leg weakness on the day previous to the outbreak of the acute symptoms, in the others the onset was more sudden. Of the three remaining pigeons, one, No. I *c*, received on the 33rd day of the experiment an injection of three units of insulin and was found dead on the following morning. I *f* and I *g* were killed on the 36th day of the experiment for histological examination, without having developed up to that time any acute symptoms. Pigeon I *h* was killed during the convulsions.

Spontaneous cures.

Pigeons I *a*, I *b*, I *d* and I *e* recovered without being treated, after unmistakable symptoms lasting several hours. Spontaneous cures have already been described by Theiler, Green and Viljoen [1915] and quite recently by Kartascheffsky [1926]. However, as such observations are scarce it seems worth while to give here a more detailed description of an interesting phenomenon. It must be emphasised that the temperature of the animal room being more or less constant at 65–68° F. the possibility of "heat cures" [Peters, 1924] is excluded. Nor was there any probability of a contamination of the diet with a vitamin B-containing substance as the symptoms cleared up at

a time when no food was being consumed by the pigeons. Pigeon I *a* looked quite normal on the day following the convulsions, when an X-ray picture of its gastro-intestinal tract was made. For that purpose a barium meal consisting of 5 g. BaSO₄, 5 g. of the synthetic diet and 10 cc. of water was administered to the pigeon. It must be pointed out that the barium meal was given after the spontaneous disappearance of the acute symptoms. The pigeon was then quite normal during 5 days and ingested voluntarily during that period 26 g. of the diet. On the sixth day it developed severe acute symptoms—opisthotonos and convulsions associated with impaired respiration—when the bird was killed. The control pigeon II *a* was killed on the following day. Pigeon I *b* developed convulsions and emprosthotonos on the 32nd day of the experiment; it was better on the following day; the symptoms reappeared, however, when the bird was taken out of the cage for weighing. Two days later the pigeon was found in convulsions during the morning inspection of the cages and was killed. The control was killed on the following day. No food had been taken during that period by pigeon I *b*. Pigeon I *d* developed acute symptoms on the 25th day; the pigeon was decidedly better on the following day, but head retraction and a fit of convulsions were provoked by mild disturbances of equilibrium. An X-ray picture was taken the next day and a fit of convulsions was also observed during the process. The following three days were uneventful, but the pigeon died suddenly on the 4th night, no food having been ingested after the first onset of convulsions. The control pigeon died a few hours earlier. Pigeon I *e* developed very severe symptoms on the 25th day of the experiment, “cartwheel” turning and convulsions lasting 7 hours with short intervals. The condition improved in the evening and the pigeon looked much better on the following day. An X-ray picture was taken on the next day. The following 7 days were uneventful. On the 5th day after the first attack the pigeon began to eat (no food having been taken for 6 days up to that time) and ingested 16, 13, 7, 12 and 10 g. daily respectively. On the 10th day after the first attack it developed acute symptoms and was killed. The control bird died on the 30th day of the experiment. The very interesting question of spontaneous cures is being now more fully investigated on a larger number of pigeons.

Temperature.

A progressive fall of the body temperature of pigeons on vitamin B-deficient diets has been observed by many investigators [Dutcher, 1918; McCarrison 1921; Farmer and Redenbaugh, 1925; Barlow, 1926, and others], and those findings are fully confirmed by the present work. Sub-normal steadily decreasing temperatures were also noted by McCarrison in starving birds. The same was found true for the control pigeons of Lot II. The decrease of temperature is roughly equal for both lots up to the onset of acute symptoms. It seems clear, however, that the thermal disturbances occurring during the acute nervous symptoms are of a more complicated character. Spontaneous cures

occurring in four pigeons gave a good opportunity for studying them in detail. Pigeon I *a* had a temperature of 102.2° (Fahr.) on the 23rd day of the experiment and some leg weakness could already be observed. The temperature was 100.4° on the 25th day and 99.5° on the 26th when convulsions occurred. The following day, when the symptoms cleared up, the temperature rose to 104°, *no food having been ingested*. In the morning of the day when the second attack occurred the temperature was 102.9°, it was 91° during the convulsions—a fall of 11.9° (Fahr.) in the course of a few hours. The temperature was unaltered during the first attack of pigeon I *b* (104°) and remained constant to the second. Pigeon I *d* had 104.3° on the 23rd day and 103.1° on the 25th during the convulsions. The temperature rose subsequently and attained 105.1° on the 29th day, on the 30th day it was only 102.5° and the pigeon died in the night (second attack?). Pigeon I *e* had a temperature of 101.4° before the first attack and 99.9° during it, the temperature was 103.2° on the following day, and 104.2° on the 32nd day and then began to drop in spite of a liberal food intake, falling to 101.8° on the 34th day and to 93.7° on the 35th, when the second attack occurred. It is obvious that the control of body temperature is greatly disturbed during the phase with which acute symptoms are associated. A comparison between both lots of pigeons is therefore scarcely justified, but a few average values have been calculated, the temperatures of pigeons having acute symptoms not being included. The data are presented in Table III.

Table III.

Day of Exp.	23	24	25	26	27	28	29	30	31	32	33	34	35
Lot I	103.5°	—	104.5°	104.2°	104.0°	104.3°	104.0°	103.2°	104.2°	104.0°	104.0°	104.3°	104.2° Fahr.
Lot II	—	105.2°	—	105.6°	104.7°	—	105.1°	105.0°	105.8°	105.2°	104.9°	105.0°	105.0° Fahr.

The average temperature is slightly higher in Lot II, but no stress can be laid upon this difference in view of the normal variations that may be encountered. Nevertheless, it seems fair to assume that the thermal changes occurring in pigeons during vitamin B deficiency are twofold; firstly, a progressive decrease which is most probably due to insufficient food intake, and secondly, a profound and rapid fall manifesting itself in the course of a few hours and associated with the onset and gravity of acute nervous symptoms. There seems a possibility that this phenomenon is more directly related to vitamin B. It will be remembered that Farmer and Redenbaugh [1925] observed during convulsions temperatures as low as 92.0° rising to 104° in the course of 6 hours after administration of 0.5 g. of yeast concentrate only. A temperature in the neighbourhood of 103–104° is probably normal for the state of partial starvation occurring in vitamin B-deficient pigeons or in similarly fed controls.

Examination of the alimentary tract by X-rays.

The important work of Cowgill and his colleagues [1921; 1923, 1, 2; 1925, 1926] demonstrates clearly that gastric atony is a characteristic symptom in dogs maintained on vitamin B-deficient diets. "Marked retardation and stasis" is reported from cases of human beriberi by Japanese clinicians [Ohomori *et al.*, 1922]. A study of the deficient pigeon in that respect seemed therefore important, and observations of the rapidity of the passage of a barium meal through the alimentary canal of the deficient birds and the controls were made. This was done on the 27th day of the experiment, almost simultaneously with the onset of acute symptoms in five of the pigeons of Lot I. A barium meal consisting of 5 g. BaSO_4 , 5 g. of the deficient diet and 10 g. of water was placed in the crop by means of a syringe.

An X-ray picture was then taken 1 hour 35 minutes after the meal; that period of time having been determined as the most suitable by a number of observations on normal birds. The control pigeons were treated similarly on the following day. The results were striking in that no appreciable deviation from the normal could be seen in the deficiently fed pigeons. It is impossible to reproduce here all the photographs, but a few typical ones are presented in Plates III and IV. The picture of pigeon Id ((4), Plate IV), deserves special attention, because acute symptoms (convulsions and opisthotonus) were marked whilst the photographs were being taken, so that it was necessary to hold the strongly retracted head in position in order to secure a satisfactory picture. Nevertheless, the food passed through the alimentary canal with normal rapidity, and the strong action of the gut, marked in the photograph, could be most readily followed on the screen. Faeces containing BaSO_4 were passed at the same time as by the control pigeons. Many photographs of normal pigeons were also taken but no appreciable differences between them and those of deficient birds or controls could be detected. Unfortunately the control II d behaved abnormally, in that no food passed in the gut after 1 hour 35 minutes. This bird died 2 days after the X-ray picture and was most probably ill when the picture was taken.

These results clearly seem to indicate that anorexia in the voluntarily feeding vitamin B-deficient pigeon is not necessarily connected with or accompanied by impaired motile function of the alimentary canal. The question of hunger contractions of the pigeon's stomach is naturally left untouched by the X-ray picture method of investigation. It will be seen later that these results are different from those obtained when the pigeons were fed forcibly on a B-deficient diet.

Histological examination of nervous system.

Pathological changes of the nerves in pigeons fed on vitamin B-deficient diets have been described by very many investigators. It was, however, very soon pointed out by Schnyder [1914] that the rapid disappearance of the characteristic symptoms under the influence of vitamin administration can

Pigeon *a*, Lot I, 1 hr. 35 mins. after BaSO₄ meal. Pigeon *a*, Lot II, 1 hr. 35 mins. after BaSO₄ meal.

Pigeon *b*, Lot I, 1 hr. 35 mins. after BaSO₄ meal. Pigeon *b*, Lot II, 1 hr. 35 mins. after BaSO₄ meal.

(1)

Pigeon *a*, Lot III, 1 hr. 35 mins. after BaSO₄ meal.

(2)

Pigeon *a*, Lot II, 1 hr. 35 mins. after BaSO₄ meal.
Given 1 g. yeast extract daily for 6 days
after showing acute symptoms.

(3)

Pigeon *a*, Lot IV, 1 hr. 35 mins. after BaSO₄ meal.

(4)

Pigeon *d*, Lot I, 1 hr. 35 mins. after BaSO₄ meal.
X-ray picture taken during convulsions.

scarcely be due to the repair of degenerated nervous tissue. A similar view is clearly expressed by McCarrison [1921]. Histological examination of pigeons I *f*, II *f*, II *g* and III *d* was kindly undertaken by Dr Woollard in the Department of Anatomy, University College, who reports as follows:

"An histological examination was made of a pigeon that had been on a deficient diet (*f*, Lot I) for 35 days. The bird had exhibited no nervous phenomena during life and an examination of the intermuscular nerves and nerve endings showed an abundance of normal nerves and endings. In fact no deviations from the normal could be recorded in this case. Two control pigeons (*g* and *f*, Lot II) which had received the same amount of food as the birds on the deficient diet voluntarily consumed were also examined. These birds had also been given yeast extract. The examination of the first bird of this group showed perfectly normal intermuscular nerves and nerve endings. The bird was fairly well nourished. The second bird of this series was extremely wasted, and in this case there could be observed considerable changes in the terminal nerves and endings in the muscles. In all cases the tissue examined came from the muscles of the leg. A pigeon from Lot III (*d*) was also examined, it having exhibited typical head retraction for some days. This forced movement can easily be overcome and the head made to assume a normal position. However, the slightest stimulus brings about a reproduction of the retraction. When the bird is held head downwards, the head under the influence of gravity will gradually sink, but again the slightest stimulus will restore the retraction. Such a position involves alteration of the tone in various muscle groups. There is no paralysis and the position of the head bears most resemblance to the forced movements that can be elicited from the labyrinth, from the mid-brain, and from the cortex. Indeed there is little neurological ground for supposing that head retraction depends on changes in the nerves of the neck muscles. The examination of intermuscular nerves in this case was unfortunately not entirely satisfactory, but the preparations were sufficient to show large numbers of normal fibres and endings. To what extent there were degenerative changes it is not possible to say from the specimens. Of the series studied it is to be remarked that the specimen which showed the most advanced changes was the bird that had received vitamin *plus* a quantity of food equal to the voluntary intake of the birds on the deficient diet."

It seems evident from this study that starvation and the wasting associated with it are largely if not entirely responsible for the pathological nerve changes observed in vitamin B deficiency in pigeons. This conclusion provides further support for the views recently put forward by Woollard [1927]. It cannot be sufficiently emphasised that no nerve regeneration is possible in the course of a few hours, and that the nervous symptoms, vanishing so rapidly under the action of vitamin B or even disappearing spontaneously must be due to a quite different cause. There is no logical impediment, however, in assuming that the paralytic form of pigeon's beriberi which is not curable or very hardly curable by vitamin administration may be associated with nerve degeneration.

Blood-sugar.

Funk and Schönborn [1914], Funk [1920], Randoin and Lelesz [1925], Collazo [1923], and others observed hyperglycaemia in pigeons on vitamin B-deficient diets. Funk [1914] considers vitamin B as specially connected with the carbohydrate metabolism. Control determinations on partially starving pigeons were done by Collazo [1923], who found in such pigeons much the same increase of blood-sugar as in pigeons on vitamin B-free diet. He also cites several papers dealing with starvation-hyperglycaemia.

The blood-sugar was estimated in four pigeons of Lot I and in four pigeons of Lot II by the reliable method of Hagedorn and Jensen and the following figures were obtained.

Table IV.

Lot I		Lot II	
Pigeon No.	Blood-sugar	Pigeon No.	Blood-sugar
I <i>e</i> *	0.214 %	II <i>b</i>	0.251 %
I <i>f</i>	0.222 %	II <i>f</i>	0.224 %
I <i>g</i>	0.222 %	II <i>g</i>	0.248 %
I <i>h</i> *	0.228 %	II <i>h</i>	0.186 %
Average	0.221 %	Average	0.227 %

* Blood taken during convulsions.

There is no difference between the two lots of pigeons. The blood-sugar content of the normal pigeon is 0.21 % according to Collazo [1923], and 0.185 % according to Honeywell [1922]. The slight increase observed in Lots I and II is most probably due to inanition.

Adrenaline content and weight of adrenals.

A hypertrophy of the adrenal glands in vitamin B-deficient pigeons was first observed by McCarrison [1921] and his observation has been confirmed by many investigators. McCarrison found also an increased output of adrenaline in vitamin B deficiency and considers it as playing a part in the production of oedema in cases of "wet" avian and human beriberi, whilst Funk [1924] ascribes to it a rôle in the causation of hyperglycaemia. It will be remembered that hypertrophy of the adrenals was also repeatedly observed in cases of inanition [Vincent and Hollenberg, 1920; McCarrison, 1921 and Findlay, 1921], but, on the other hand, pure starvation is always associated with vitamin B deficiency. Beznak [1923], while confirming the hypertrophy of the adrenals in beriberi pigeons, could find no increase in the size of adrenals in starving pigeons receiving only vitamin B (in the form of yeast). He therefore considers that hypertrophy of the adrenals is definitely caused by vitamin B deficiency, but points out, since he could find no increase in the adrenaline content, that probably the cortical tissue alone hypertrophies.

The results of a few determinations made on pigeons from Lots I and II (two determinations in each lot) are given in Table IV. The method of Baker and Marrian [1927] was used.

Table V.

	No. of pigeon	Weight at beginning g.	Weight at end g.	Weight of adrenals g.	Adrenaline $\times 10^{-2}$ mg.	Adrenaline in mg./g. gland
Lot I	I <i>h</i>	383	233	0.0691	17.8	2.57
	I <i>e</i>	457	281	0.0532	11.85	2.23
Lot II	II <i>h</i>	355	220	0.0395	17.6	4.45
	II <i>b</i>	443	298	0.0352	11.4	3.24

As far as these figures go they tend to confirm the observations of Beznak, but they are obviously inadequate and further work seems desirable before the conclusion is adopted that the hypertrophy of the adrenals is due specifically to a deficiency of vitamin B.

Blood-calcium.

A few estimations of blood-calcium were made with the view in mind that the convulsions might possibly be associated with a hypocalcaemia. Actually a slightly increased amount was found present in the blood of the pigeons lacking vitamin B. The blood-calcium was determined by the Clark-Collip [1925] modification of the excellent Kramer-Tisdall [1921] method. The blood was drawn from the wing vein.

Table VI.

No. of pigeon	Blood-calcium mg./100 c.c.	Remarks
Lot I	I <i>a</i> 12.1	Blood taken during convulsion On 36th day of "vitamin B"-free diet " " " "
	I <i>e</i> 11.4	
	I <i>f</i> 10.0	
	I <i>g</i> 10.0	
Average: 10.9		
Normal aver. 8.2	{ Normal 8.2	
	{ " 8.2	
Lot II	{ II <i>a</i> 8.3	
	{ II <i>b</i> 8.4	
	{ II <i>g</i> 8.3	
Average: 8.3		

Ungar [1927] reports no appreciable difference between the blood-calcium levels in normal pigeons and others during beriberi convulsions.

Starch-hydrolysing enzymes of the alimentary tract.

A complete loss of the amylolytic and lipolytic, and a marked decrease of the proteolytic enzymes in pigeons fed on vitamin B-deficient diets has been reported by Farmer and Redenbaugh [1925]. Previously various investigators tried to connect the outstanding features of vitamin B deficiency with changes in the quantity and activity of the digestive juices. That no direct relation exists between vitamin B and the secretory function of the digestive glands has been conclusively demonstrated by the work of Anrep and Drummond [1921] and Cowgill and colleagues [1921 to 1926]. On the other hand, it appeared to us that the almost identical behaviour of the pigeons in our Lots I and II was hardly compatible with the findings of Farmer and Redenbaugh. It seemed, therefore, important to repeat their work. This was done, using three normal pigeons kept previously in a large aviary on poultry mixture diet, four pigeons of the deficient Lot I, one pigeon of the deficient Lot III and four pigeons of the control Lot II. The technique of Farmer and

Redenbaugh was closely followed except that the pancreas and first 6 inches of the intestines were not minced in a meat mincer but ground with sand in a mortar. The glycerol extracts were centrifuged after 4 weeks' maceration and the amylolytic properties determined according to Long and Johnson [1913]. The results are given in Table VII.

Table VII. *Starch digestion.*

		Incubation at 37°.			
		<i>Normal pigeons.</i>			
Pigeon No.		After 10 mins.	After 45 mins.	After 80 mins.	After 120 mins.
Normal I		blue	blue	purple-red	red
" II		blue	blue	purple	purple-red
" III		blue	purple	pale pink	very pale pink
<i>Deficient pigeons (Lot I and Lot III).</i>					
All killed during convulsions with the exception of III <i>h</i> .					
I <i>a</i>		blue	purple	pale pink	very pale pink
I <i>b</i>		blue	faint yellow	faint yellow	faint yellow
I <i>c</i>		purple-blue	faint yellow	faint yellow	faint yellow
I <i>h</i>		blue	purple-red	faint yellow	faint yellow
III <i>h</i>		blue	purple-red	faint yellow	faint yellow
<i>Controls (Lot II).</i>					
II <i>a</i>		blue	blue	reddish purple	red
II <i>b</i>		blue	purple	pale purple-red	faint yellow
II <i>d</i>		blue	faint yellow	faint yellow	faint yellow
II <i>h</i>		blue	purple-blue	pink	very pale pink

Our observations are entirely different from those made by Farmer and Redenbaugh, but are in agreement with other findings reported in the present paper, all of which demonstrate clearly that the digestion, at any rate of starch, is normal in a vitamin B-deficient pigeon. The extracts of the organs of the normal pigeons are, if anything, rather less active than those of the deficient birds or of the controls. This somewhat unexpected result is probably explained when one takes into consideration the great wasting which the deficient birds have suffered, so that the extracts are simply more concentrated, as the wasting involves chiefly inactive material such as the fat of the pancreas and muscular wall of the gut. It must be remembered that in the experiment of Farmer and Redenbaugh the birds were fed forcibly and that they were eating voluntarily in the present experiment. A highly active extract was, however, obtained also in the case of pigeon III *h* belonging to a group that had been forcibly fed. It is not easy to account for the results of Farmer and Redenbaugh, but what is believed to be an inconsistency in their results may here be pointed out. It seems very difficult to understand how pigeons having, according to their opinion, completely lost the power of utilising starch and fats and to a marked extent that of splitting proteins could be kept for an average period of 27 days on a vitamin B-free diet of autoclaved grain with an average loss of only 3.7 g. daily. The average daily food intake was 25 g. Even leaving out of consideration the alleged partial impairment of the utilisation of proteins and total of fats, and only deducting from that figure 70 % for non-metabolised

starch it is easy to calculate that only 7.5 g. of the diet containing a considerable amount of roughage were in reality available for the pigeons. That quantity was furthermore greatly diminished by the fact that pigeons "frequently regurgitated a considerable amount" and was surely not sufficient to spare daily 5.6 g. of body tissue. A similar recalculation in the case of the lard diet fed in quantities of 15 g. daily and containing 39 % of starch and 21 % of lard would lead, if the assumptions of Farmer and Redenbaugh were correct, to an even more striking result as the daily loss in that group of pigeons was only 1.17 g. (McCarrison's figures for starvation are 9.3 g.). It will be remembered that the control pigeons of Lot II receiving a liberal supply of vitamin B in the form of marmite and with an average daily food consumption of 6.1 g., none of which was regurgitated, presented an average daily loss of 5 g. of weight. There is also another statement in the paper of Farmer and Redenbaugh with which we cannot agree, namely, that "the gastro-intestinal tract of the starved pigeon is normal and empty with little or no permanently decreased efficiency of the digestive enzymes. In the pigeons with polyneuritis . . . the gut usually shows degenerative changes." That the gastro-intestinal tract undergoes very marked changes in starvation and can by no means be considered as normal has been established beyond doubt by the researches of McCarrison on a great number of pigeons.

LOTS III AND IV.

Pigeons fed forcibly with a vitamin B-deficient diet almost invariably vomit a great deal of it, so that accurate estimates of food intake are difficult to make. Similar rejection of food is sometimes seen in birds feeding voluntarily on the deficient diet, but was not observed in our Lot I. Such experiments are therefore much more liable to error than those carried out when the ingestion is voluntary. It is also, in our opinion, desirable that the abnormal method of feeding, particularly when applied to an animal that exhibits a distaste for the particular food, and its possible effects on the animal should be always borne in mind when the results of such experiments are being discussed. This applies to the results obtained in the present work with Lots III and IV so that they will be only briefly treated. Two interesting features must, however, receive attention. The two lots of pigeons were fed daily 20 g. of the finely ground diet mixed with a sufficient amount of water to permit passage through the syringe and rubber tube. Approximately 30 cc. of water were used. Pigeons of Lot IV were also given daily 1 g. of marmite. The pigeons of the deficient group began to regurgitate the food on the 5th to 9th day of the experiment. The vomiting was purely voluntary and was effected in the same manner as when feeding the young. Some birds vomited immediately after being fed, some only after a few hours had elapsed. Vomiting occurred also to a certain extent in the control Lot IV and was most probably due here to the discomfort caused by the large bulk of food being introduced at once in the crop. It must be emphasised here that vomiting never occurred in the

control Lot II—the quantities of food administered being much smaller than in the case of Lot IV. A marked retention of the food in the crop was always observed in the deficient pigeons in the later stages of the experiment and especially just prior to the onset of the acute symptoms when they were unable to execute the movements of the head and wings necessary to expel the contents of the crop.

Weight.

The initial average weights and the average weights on the 18th day of the experiment of both lots are given in Table VIII. After that date food retention associated with acute symptoms was occurring frequently and the weight figures were therefore unreliable.

Table VIII.

	Initial weight	Weight on the 18th day	Average daily loss
	g.	g.	g.
Lot III*	417	330	5.1
Lot IV	416	388	1.6

* Average of six pigeons—III *e* died on the 13th day of the experiment and III *c* developed acute symptoms and was given marmite on the 15th day.

The loss in weight of the pigeons of Lot IV was due to the occurrence of vomiting and not to an insufficient food intake, as gain in weight was observed in those pigeons which did not vomit.

It is very interesting to note that the average daily loss of Lot III is only slightly lower than that of Lot I—forcibly fed pigeons practically behave as if they were feeding voluntarily in the respect that only a certain definite amount of the food deposited in the crop is allowed to pass further down the alimentary canal and the remainder is expelled.

X-ray picture.

X-ray pictures of the gastro-intestinal tract of pigeons III *a*, III *b*, III *c* and their controls, and III *f*, III *g*, III *h* and their controls, were made under conditions quite similar to those already described on the 12th and 15th days of the experiment respectively. A marked difference between the deficient lot and the controls could be observed in all the cases. It is illustrated in two typical photographs of pigeons III *a* and IV *a* reproduced here ((1) and (3), Plate IV). 1 hour and 35 minutes after the meal the stomach alone is faintly visible in the deficient pigeon and no food is seen descending the oesophagus: in the control pigeon the oesophagus can be easily detected, the stomach and intestines are full and the barium meal is approaching the rectum. Pigeon III *a* was then given daily marmite, beginning with the 18th day of the experiment, and an X-ray photograph was taken on the 24th day showing normal passage of the food through the alimentary tract ((2), Plate IV). The retardation of the passage of food in the pigeons that have been forcibly fed might be due to an impairment of the mechanism for emptying the crop in a normal manner or

to a voluntary inhibition of this process. It might also be due to an absence of the stomach movements, and to decreased peristalsis of the intestines. We are inclined to favour the first view, for the reason that any food passing to the intestines was normally digested, and normal peristaltic movements could be seen on the screen when after a sufficient period of time the Ba meal was present in the intestines. If the crop was functioning normally a passive filling of the empty intestine a short time after administration of the Ba meal could be expected. It is believed that the impaired functioning of the crop is not due to muscle atrophy or nerve degeneration, because the condition improves rapidly as was observed by many investigators after administration of vitamin B in a suitable form.

Acute symptoms.

The acute symptoms occurred sooner in Lot III than in Lot I. Four pigeons, III *e*, III *d*, III *f*, III *g*, presented the typical picture after 15, 37, 19 and 22 days respectively with an average of 23 days as compared with 28 days in the case of those voluntarily feeding. Two other pigeons, III *a* and III *b*, developed paralysis and leg weakness, but no symptoms of head retraction after 17 days and 22 days. III *a* quickly recovered after administration of marmite, but III *b* died on the following day. Pigeon III *c* died from other reasons on the 13th day of the experiment. Pigeon III *h* was killed on the 26th day without showing acute symptoms.

DISCUSSION.

Reviewing the results of this investigation as a whole it seems apparent to us that many of the characteristic symptoms exhibited by pigeons fed on diets deficient in vitamin B are direct consequences of the failure to consume sufficient food to maintain the body weight. In this respect these experimental results are confirmatory of those reported by Drummond and Marrian [1926] derived from somewhat similar studies of the rat. In that paper evidence was presented to disprove a number of theories ascribing to vitamin B an essential influence on the production or functioning of the oxidative mechanisms of the tissues. It is believed that the present paper serves to throw doubt on another group of theories, namely, those which relate vitamin B to the digestive and assimilatory functions of the organism. In the first place, the view expressed by various authors that vitamin B is necessary for the proper utilisation of carbohydrates is unsatisfactory in that it ignores the fact that more than 50 % by weight of the molecule of proteins is metabolised along the path of carbohydrate breakdown. Such a view implies that the mechanism responsible for the utilisation of carbohydrates arising from proteins remains normal in vitamin B deficiency, whereas a distinct and independent mechanism involved in the metabolism of exogenous carbohydrates undergoes a profound disturbance. It is very difficult on that basis to account satisfactorily for the results of Randoin and Simonnet [1924, 1, 2], who reported that they were able to keep pigeons in perfect health for a period of 3½ months on a diet deficient in vitamin

B and in carbohydrates but containing 54 % of protein and 32 % of fat, or on a diet almost identical to that used in the present work¹, but fed in amounts of 75 g. per pigeon daily, from which the pigeons were able to "pick out" the protein and other constituents, leaving the carbohydrate part untouched. Why a reduction of the daily intake of that diet to 20 g. should hasten the onset of acute symptoms—which then appeared after 2 months—remains obscure. A similar difficulty is encountered when discussing the work of Funk and his colleagues [Funk, 1914; Funk and Paton, 1922; Collazo and Funk, 1924] on the subject. In a recent paper Funk and Collazo [1925] report that pigeons receiving a constant amount of vitamin B and kept in weight equilibrium on a diet containing 12.5 g. protein showed an increase in body weight roughly proportional to the amount of protein ingested when offered dietaries containing 25, 50 and 75 % of protein, the other variable component being starch. It seems clear that in these cases the food values of the diets and their influence on the weight curve were not properly controlled. A review of our experimental observations makes it evident that no results corroborating these theories have been obtained. The actual food intake of a pigeon from which vitamin B is withheld, be it forcibly fed or partaking freely of the diet, is greatly diminished, but the ingested quantum is metabolised just as completely as in a pigeon receiving liberal amounts of vitamin B in the form of yeast extract; and this is true even in the stage of the deficiency disease where the characteristic convulsions appear. The similarity of the curves for food intake and body weight, the similar rates of passage of a meal through the intestines, the blood-sugar levels and composition of the faeces all support this view. Furthermore, it is believed that the diminished food intake and subsequent partial starvation are sufficient to explain any hyperglycaemia encountered in vitamin B-deficient pigeons. This confirms the findings of Collazo [1923], but the views expressed by Funk and Schönborn [1914], Funk [1920], Randoin and Lelesz [1925] and others cannot be shared. In fact, the starvation factor was not taken into account by these investigators. Passing now to the relation of vitamin B deficiency to the function of the digestive glands it is obvious that a normal digestion of starch observed in beriberi pigeons suggests the existence of a normally functioning digestive apparatus. This view is supported by the demonstration of the presence of a highly active amylolytic enzyme in the pancreas and intestines of beriberi pigeons, confirming the finding of Tiger and Simonnet [1921] and Rothlin [1922]. That the gastric secretion has a normal composition throughout the course of vitamin B avitaminosis has been conclusively demonstrated by Bickel [1922]. Anrep and Drummond [1921] and Cowgill and Mendel [1921] presented experimental data leading them to deny any stimulating influence of vitamin B on the digestive glands. It seems that the evidence weighs against the opinion and findings of Farmer and Redenbaugh [1925].

¹ Randoin and Simonnet used potato starch instead of rice starch and reach the curious conclusion that a vitamin B-deficient pigeon can digest the latter but not the former.

We have been greatly disturbed during this work by the number of spontaneous cures of the typical beriberi convulsions that have occurred. In our opinion such cures are much more frequently encountered in pigeons fed on an artificial diet such as we have employed than in those fed in the more customary manner on polished rice.

That a substance indistinguishable in all its characteristics from what we are accustomed to call vitamin B is present in the organism under normal conditions seems certain. Even after a long period of vitamin B deficiency the body stores are not completely exhausted as was shown by the interesting work of Funk [1914], since confirmed by various authorities [Theiler, Green and Viljoen, 1915; Hess and Takahashi, 1921; Abderhalden, 1922]. It will be remembered that Funk extracted the tissues of pigeons after death in beriberi convulsions in a manner similar to that used for extracting rice polishings and obtained extracts which had marked curative effects when administered to other pigeons in convulsions. That result recalls the observation of Baker, Dickens and Dodds [1924], that large quantities of insulin can be extracted from the organs of patients dying in diabetic coma.

There is a possibility that there may be a liberation of the reserves of vitamin B in the tissues during periods when there is none in the diet and when there is considerable tissue wasting; that such liberation may occur is suggested by the fact that pigeons and fowls do not exhibit in pure starvation (which is also obviously a vitamin B deficiency) the characteristic nervous symptoms of vitamin B deficiency unless they are "washed out" by administration of large quantities of water [Eijkman and Hoogenhuyze, 1916; Chamberlain, Bloombergh and Kilbourne, 1911; Chamberlain and Vedder, 1911]. The question immediately arises whether what is perhaps the most characteristic symptom of vitamin B deficiency, namely, the loss of the desire to partake of the deficient food, is to be looked upon merely as a morbid phenomenon or as an attempt of the organism to liberate by starvation and subsequent tissue wasting the body stores of the lacking vitamin bound in the tissues so as to be inaccessible to the animal under normal conditions and thus make available a substance essential for life. The occurrence of spontaneous cures on diets such as we employed suggests that there may be a liberation following a convulsive seizure which gives temporary relief and sometimes leads to a transient return of appetite. Further investigation must follow before an opinion on this question can be formed. It seems evident that almost all the pathological changes observed in beriberi pigeons are directly attributable to inanition. There are, however, certain differences between a vitamin B-deficient pigeon and one partially starving but receiving a liberal supply of the vitamin, namely, the occurrence in the former of two well-defined symptoms which in the order of their appearance are: (1) a diminished desire to partake of *the deficient food*, (2) symptoms known as opisthotonos, emprosthotonos, convulsive seizures, "cartwheel" turning. Leg weakness is also frequently present, but is not characteristic as a symptom. It yields only with difficulty to vitamin B treatment and is often observed in the premortal stage in control birds.

Regarding the fluctuations of temperature that are associated with the convulsion phase of vitamin B deficiency which seem of different origin from the fall that is a consequence of inanition, and the hypertrophy of the adrenals, we are not yet prepared to express an opinion. Much evidence is against associating the nervous symptoms described under (2) with the nerve lesions considered by many authorities as a characteristic consequence of vitamin B deficiency. Identical lesions have been found by Dr Woollard on histological examination of our control pigeons on restricted food but receiving a liberal supply of yeast extract, whereas they are often lacking in beriberi pigeons (McCarrison). Finally, the prompt recovery following administration of vitamin B excludes any possibility of nerve regeneration and strongly suggests that the mechanism responsible for the appearance of the symptoms or for their retrogression is of a different character.

It may be stated with some certainty that no existing theory is adequate satisfactorily to explain the origin of the nervous syndrome characteristic of vitamin B deficiency or the rôle played by vitamin B in the normal organism. As yet we wish to make no contribution to the theoretical side; we would, however, support the opinion of Karr [1920] that "some relationship exists between the desire to partake of food and the amount of the so-called water-soluble vitamin ingested."

SUMMARY.

(1) A method of studying vitamin B deficiency is described in which the effects of inanition are checked by a suitable control. The method, as applied to pigeons, consists in determining their voluntary food intake when kept on a vitamin B-deficient but otherwise complete synthetic diet and administering artificially the same amount of food to individual control birds of the same weight which receive in addition a liberal supply of vitamin B in concentrated form.

(2) As a result of such studies the decrease in body weight accompanying vitamin B deficiency in pigeons is considered as solely attributable to partial starvation.

(3) No difference could be observed in the utilisation of food between the deficient birds and the controls.

(4) Green faeces are considered to be due to insufficient food intake and possibly bacterial infection.

(5) The "ruffled feathers" attitude of the deficient birds is due to inanition.

(6) No difference was found between the blood-sugar levels of the two groups. The hyperglycaemia, if any, is due to inanition.

(7) A hypertrophy of the adrenals in vitamin B deficiency is considered as probable, but no difference in the adrenaline content is found.

(8) No difference could be observed in the rate of passage of a barium meal through the gastro-intestinal tract of birds feeding on the deficient diet voluntarily and of the controls receiving the vitamin.

(9) A marked slowing of the rate of passage of the barium observed in the case of artificially fed birds is attributed not to intestinal stasis but to a "barrier action" of the crop.

(10) The gradual fall of the body temperature observed in deficient pigeons is due to partial starvation.

(11) A loss of temperature control during the convulsive stage of beriberi is considered as probable.

(12) Highly active amylopsin preparations are obtained from the pancreas and intestines of deficient birds killed during convulsive seizures, as well as from controls.

(13) The view that vitamin B controls the carbohydrate metabolism is not corroborated.

(14) Lack of appetite for *the deficient food* and acute nervous symptoms are characteristic of vitamin B deficiency in the pigeon.

(15) No relation could be traced between the occurrence of acute nervous symptoms and degenerative changes in the nerves. Such changes were present in both groups of pigeons and are attributable to inanition.

(16) No existing theory is considered as giving a satisfactory explanation of the rôle of vitamin B in the normal organism, or of the true nature of the conditions induced by its deficiency.

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REFERENCES.

- Abderhalden (1922). *Arch. ges. Physiol.* **195**, 432.
Anrep and Drummond (1921). *J. Physiol.* **54**, 349.
Baker, Dickens and Dodds (1924). *Brit. J. Exp. Path.* **5**, 327.
Baker and Marrian (1927). (*Unpublished results.*)
Barlow (1926). *Amer. J. Physiol.* **78**, 322.
Beznak (1923). *Biochem. Z.* **141**, 1.
Bickel (1922). *Klin. Woch.* **1**, 110.
Braddon and Cooper (1914). *J. Hyg.* **14**, 331.
Chamberlain, Bloombergh and Kilbourne (1911). *Philippine J. Sci.* **6 B**, 177.
Chamberlain and Vedder (1911). *Philippine J. Sci.* **6 B**, 251, 395.
Clark and Collip (1925). *J. Biol. Chem.* **63**, 461.
Collazo (1923). *Biochem. Z.* **134**, 194.
Collazo and Funk (1924). *Med. Doswiadczalna i spol.* **3**, 359; *J. Metab. Res.* **5**, 187, 195.
Cowgill (1921). *Amer. J. Physiol.* **57**, 420.
—— (1923, 1). *J. Biol. Chem.* **56**, 725.
—— (1923, 2). *Amer. J. Physiol.* **66**, 164.
Cowgill, Duel and Smith (1925). *Amer. J. Physiol.* **73**, 106.
—— ——— (1926). *Amer. J. Physiol.* **77**, 389.
Cowgill and Mendel (1921). *Amer. J. Physiol.* **58**, 131.

- Drummond and Marrian (1926). *Biochem. J.* **20**, 1229.
- Dutcher (1918). *J. Biol. Chem.* **36**, 63.
- Eijkman and Hoogenhuyze (1916). *Proc. R. Akad. Wetensch., Amsterdam*, **18**, 1467.
- Farmer and Redenbaugh (1925). *Amer. J. Physiol.* **75**, 27, 45.
- Findlay (1921). *J. Path. Bact.* **24**, 175.
- Funk (1914). *Z. physiol. Chem.* **89**, 373, 378.
- (1920). *J. Physiol.* **53**, 247.
- (1921–25). Article on vitamins in *Handbuch der Bioch. Arbeitsmethoden*, **4**, ix.
- (1924). *Die Vitamine* (Dritte Auflage, Bergmann).
- Funk and Collazo (1925). *Chem. Zell. Gew.* **12**, 195.
- Funk and Paton (1922). *J. Metab. Res.* **1**, 737.
- Funk and Schönborn (1914). *J. Physiol.* **48**, 328.
- Gulick (1922). *Amer. J. Physiol.* **59**, *Proc. Amer. Physiol. Soc.* 483.
- Hess and Takahashi (1921). *Biochem. Z.* **122**, 193.
- Honeywell (1922). *Amer. J. Physiol.* **58**, 152.
- Karr (1920). *J. Biol. Chem.* **44**, 255.
- Kartascheffsky (1926). *Arch. ges. Physiol.* **215**, 197.
- Kramer and Tisdall (1921). *J. Biol. Chem.* **47**, 475.
- Long and Johnson (1913). *J. Amer. Chem. Soc.* **35**, 895.
- McCarrison (1921). *Studies in deficiency disease* (Oxford Med. Publ.).
- McCollum and Davies (1915). *J. Biol. Chem.* **23**, 181.
- Ohomori, Ohhashi, Nakanishi, Hara and Ota (1922). *Japan Med. World*, **2**, 128.
- Peters (1924). *Biochem. J.* **18**, 858.
- Randoin and Lelesz (1925). *Compt. Rend. Acad. Sci.* **180**, 1366.
- Randoin and Simonnet (1924, 1). *Bull. Soc. Chim. Biol.* **6**, 601.
- (1924, 2). *Compt. Rend. Acad. Sci.* **179**, 700.
- Rothlin (1922). *Z. physiol. Chem.* **121**, 300.
- Schnyder (1914). *Arch. Verdauungskrankh.* **20**, 147.
- Theiler, Green and Viljoen (1915). *3rd and 4th Director Veter. Res. Rep. Dept. Agric., Union S. Africa*, 9.
- Tiger and Simonnet (1921). *Bull. Soc. Chim. Biol.* **3**, 580.
- Ungar (1927). *Biochem. Z.* **180**, 357.
- Vincent and Hollenberg (1920). *Endocrin.* **4**, 408.
- Woollard (1927). *J. Anat.* **61**, 414.