CXXV. EXPERIMENTS ON NUTRITION. VI.

BALANCE OF FOOD BY VITAMIN B.

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OUR previous experiments [1922, 1923] on the rearing of chicks have indicated that the quantity of vitamin B required by the birds must bear a relation to the quantity of food consumed. These observations were thus similar to those described by Braddon and Cooper [1914]. Braddon [1907] had previously pointed out that in the epidemics of beriberi in the East the well-nourished succumbed first and were more liable to the disease than the underfed. Men with their greater energy output and consuming more rice than women were more subject to the disease. If men and women were kept upon the same fixed ration, as in institutions, both were equally susceptible. If they were supplied with equal quantities of other foodstuffs, an increase in the amount of white rice was followed by more cases of beriberi. The time of the appearance of the typical paralysis has been seen also to vary with the climatic conditions. Cold and wet weather, as well as exercise, hastened the onset, whilst warmth and rest delayed the appearance of the typical symptoms.

A connection between the actual quantity of food eaten and the time of onset of paralysis in birds receiving no vitamin B was first pointed out by Maurer [1907]. Cooper [1913] also observed that the disease appeared sooner in pigeons on larger amounts of white rice than in those on smaller amounts. Funk [1914] showed clearly that the onset of polyneuritis in pigeons occurred much sooner on 20 g. of rice than on 5 g., and also that on a constant amount of food polyneuritis appeared most quickly with starch or sugar, more slowly with caseinogen and lastly with fat.

The metabolic connection between carbohydrate and vitamin B was investigated in some detail by Braddon and Cooper [1914]. From their experiments with fowls it appeared that the greater the amount of carbohydrate, the greater was the amount of vitamin B required. Their work was never completed. No other direct experiments upon these lines had been made at the time that our experiments were started. The question of a quantitative

relation between food intake and vitamin B has seemed to us of so much importance in nutrition that our work during the past 3 or 4 years has been to test it, using each kind of foodstuff, protein, fat and carbohydrate.

In the meantime several other papers have appeared which show that vitamin B is concerned in the metabolism of food. Karr [1920] and Cowgill [1922] pointed out a relation between quantity of vitamin B and appetite. Osborne and Mendel [1922] found a connection between body weight and quantity of vitamin B. This connection has been elaborated by Cowgill [1922], Cowgill, Deuel and Smith [1925], Cowgill, Smith and Beard [1925] and by Beard [1925]. Randoin and Simonnet [1924] found a direct relation between carbohydrate consumption and amount of vitamin B. Hartwell [1924, 1925] and Reader and Drummond [1926] found a relation between quantity of protein in the food and amount of vitamin B. Our results point to the need of balancing all classes of foodstuffs by vitamin B, *i.e.* the supply of vitamin B must be in proportion to the calorie value of the food. Various pathological symptoms are seen which appear to originate from too little, or a shortage, of vitamin B in the food over a long period.

EXPERIMENTAL.

The rearing of chickens from day-old to maturity has been taken as the test to ascertain the balance of the three foodstuffs, protein, fat and carbohydrate by vitamin B. The rearing of ducks has been employed in additional experiments on balance of protein. In the case of balance of carbohydrate, pigeons and rats have also been used.

The housing of the chickens has been the same as described previously [1923]. Day-old chicks and ducks have been started in a foster mother and transferred, according to the weather, in 3 to 5 weeks, to a small house and later to a larger house, all the houses being provided with small runs. The houses have been on the roof of the building and exposed to the weather. Light Sussex chicks have been available for most of the experiments. Leghorns were used in one experiment and in another there was a variety of breeds. The pigeons were of the ordinary homer variety and were kept in pairs in cages, measuring approximately $4' \times 2' \times 2'$. The cages were kept side by side on the roof of the building and were fitted with a convenient perch. The rats have been kept in wire cages, measuring approximately $2' \times 1' \times 1'$, generally in pairs and housed in a warm room of the laboratory.

The birds and rats have always been fed *ad lib.* They have been provided with food in a tray, and as soon as it was finished, it was replenished. The food consumption could thus be recorded. All animals have been weighed weekly.

I. THE BALANCE OF PROTEIN BY VITAMIN B.

In our previous experiments [1923] it was found that chicks, groups VIII and IX, could be successfully reared on a diet composed of:

White rice	Marmite	Fishmeal	Cod-liver oil	
90	9	5	1	parts.

This diet presumably contained just enough vitamin B in the marmite to balance the rice and the small quantities of fishmeal and cod-liver oil. It has formed the basis of these experiments, which were devised on the following supposition.

Without any change in the above diet except raising the fishmeal to 30 parts, failure to rear chicks would indicate insufficient vitamin B, or some harmful effect of high protein in the food, and that the extra protein needed balancing. Then, by keeping the fishmeal constant at 30 parts and increasing the quantity of marmite, the capability of rearing chicks would show that the extra protein needed balancing with vitamin B and that the high protein was not in itself injurious.

First series of experiments.

Two groups of chicks, XII 5 and XII 30 were started together, the former as a control with 5, the latter with 30 parts of fishmeal. To the diet of group XII 5 was added the ash obtained by burning 25 parts of fishmeal, to represent the extra mineral matter supplied with the 30 parts of fishmeal to group XII 30.

Shortly afterwards, as a few more chicks became available, two more groups, XIII and XIII A, were started, both on low protein but with the proportions divisible into third quantities in order to be able to make up smaller amounts of food. These acted as a further control.

The rearing of group XII 5 was not so good as previously with groups VIII and IX. Groups XIII and XIII A reached maturity. The birds of group XII 30, except two, were not reared and these two were abnormal. More details are given below.

Further groups, XIV, XV, XVI, were then started in succession with increasing quantities of marmite. The results were worse with group XVI than with group XV. It was then suspected that the marmite was not so good as that previously used. Another control group, without and with ash from 25 parts of fishmeal, was then again tried. The results with these groups, XVII and XVII A, confirmed our suspicion that there had been some change in the marmite. Several other samples of yeast extract have in the course of these experiments been tested on pigeons and have been found to vary considerably (see later).

The series of experiments and the general results are best seen and compared by reference to Table I.

Table I.

Group	White rice	Marmite	Cod-liver oil	Fishmeal	No. of birds	Losses
XII 5	90	9	1	5	8	4
XII 30	90	9	1	30	8	6
XIII	90	9	1.5	6	3	0
XIII A	90	9.9	1.5	9	2	1
XIV	90	12	1	30	10	4
XV	90	14	1	30	12	1
XVI	90	16	1	30	10	6
XVII	90	10	1	5	9	5
XVII A	90	10	1	5	11	5

Composition of diets %.

Group	Water	Salt	Other ash	Fibre	Protein	Fat	Carbo-hydrate	Marmite	Calories per 100 g.	Ratio marmite calories
XII 5	12.9	1.4	6.1	0.3	11.2	1.5	70.3	8.6	348	1 : 40
XII 30	13.2	1.7	5.7	0.2	20.8	1.8	56.9	6.9	335	1 : 49
XIII	12.9	1.4	2.7	0.3	11.6	2.0	69.3	8.4	350	1 : 42
XIII A	12.9	1.5	6.1	0.3	13.1	2.0	66.9	8.9	347	1 : 39
XIV	13.5	2.0	5.9	0.2	21.0	1.8	55.7	9.0	331	1 : 37
XV	13.7	2.1	6.0	0.2	21.3	1.8	55.0	10.4	330	1 : 32
XVI	13.9	2.3	6.1	0.2	21.4	1.8	54.4	11.7	328	1 : 28
XVII	12.9	1.5	2.6	0.3	11.5	1.5	69.7	9.4	347	1 : 37
XVII A	12.4	2.0	5.9	0.3	11.1	1.5	67.2	9.1	335	1 : 37

Although more birds were raised on the higher amounts of marmite in the food, the result in respect of balance of protein by vitamin B was not satisfactory and conclusive. The chickens which reached maturity were not normal in any of the high protein groups. They had bow legs, or were knock-kneed, and in some cases there were signs of leg weakness.

The most interesting and important details concerning these groups of birds were observed on *post mortem* examination. The details of the losses and *post mortem* examinations are given below.

Group XII 5. This experiment lasted for 27 weeks, from 15. viii. 1923 to 20. ii. 1924. The four losses occurred in the 4th, 5th, 7th and 18th weeks. A perforated gut was found in the first. In the second, the abdomen was full of liquid, the heart was enlarged, the gizzard small and gut empty. The third bird, before death, showed neck twist, which got better and then worse. It was chloroformed in the 27th week. Its caecum was very swollen. The fourth bird was pale and had drooping wings before death. The intestine was full of dark food material, the gizzard had thin walls and the kidneys seemed large. An extra bird introduced into this group at the age of 6 weeks showed leg weakness and bent legs in the 22nd week. It was chloroformed; its gizzard was thin-walled, intestines leathery and appendices swollen.

Slight leg weakness was noticeable in the 19th week and the group was kept longer, but distinct signs were not noticed in all the birds.

Group XII 30. This experiment was for 21 weeks, from 15. viii. 1923 to 9. i. 1924. The first loss in the 2nd week showed much liquid in the body cavity. The gut seemed normal. The second in the 4th week had an empty intestine: the bones seemed soft. The third in the 5th week had distinct leg weakness. The leg joints were large. The gut was almost empty. The fourth in

the 15th week was very wasted. The knee joints were swollen and legs twisted. There was very little muscle on the gizzard. The fifth and sixth died in the 20th week. There were hard lumps in the caeca with stoppage. The gizzards had thin walls. The two surviving birds were kept to the 26th week. No gut symptoms were noticed at autopsy. The ovaries were small. The birds were abnormal in appearance.

Group XIII. The experiment was for 25 weeks, from 11. ix. 1923 to 20. ii. 1924. One bird showed neck twist in the 11th week, but got better. There seemed signs of leg weakness in the 16th week, but it did not get worse. This group with the two remaining hens from group XII 5 were kept altogether for 12 months without any symptoms. The marmite was raised to 10 parts for the latter period.

Group XIII A consisted of only two chicks, one of which was very small and feeble at the start and should not be included. The surviving chick at the 8th week was put in group XIII.

Group XIV. This experiment was continued only for 10 weeks, from 6. ii. 1924 to 16. iv. 1924. Two birds showed badly twisted legs in the 4th week and were chloroformed. They had pale combs and swollen joints. The caeca contained masses of food which caused stoppage. Two further birds showed crooked legs in the 5th and 6th weeks and were chloroformed. The second had water in the body cavity; its intestines and appendices were swollen. All the birds at the 8th week were very weak on their legs. The marmite was raised to 16 parts, but they did not get better and were chloroformed at the 10th week.

Group XV. This experiment lasted for 9 weeks, from 26. iii. 1924 to 26. v. 1924. The loss in this group was in the 2nd week. The bird had a crooked leg and was chloroformed. It had an unabsorbed yolk sac. The other birds had bow legs, or were knock-kneed and weak on their legs. On this account the experiment was not continued longer.

Group XVI. These were Leghorns. The experiment was for 7 weeks, from 22. iv. 1924 to 18. vi. 1924. This was an unsatisfactory group as seven birds died in the 1st week from unabsorbed yolk sacs, which pointed to weakness in the other birds. One bird developed a crooked leg in the 5th week and was chloroformed. Another died in the 7th week. Its abdomen was full of water, but the intestines seemed normal; its heart was normal. All the birds were very ill and the experiment was discontinued.

Group XVII. This experiment lasted for 20 weeks, from 22. iv. 1924 to 10. ix. 1924. The first two losses occurred in the 3rd week. Both appeared to have stoppage of the gut. The third loss was in the 4th week. Both appendices of the bird were clogged with rice. At the 6th week the birds remaining from group XVII A were put in with this group. Two deaths occurred in the 19th week; no special symptoms were noticed. Another loss was in the 21st week. This bird had an enlarged heart with clot in the pericardium; the connective tissue in the abdomen was leathery.

Group XVII A was fed for 6 weeks, from 22. iv. 1924 to 28. v. 1924. The first loss was in the 2nd week. The liver was pale and the gall bladder large. The second and third losses were in the 3rd week. One showed a stoppage of the intestine and the other a distended gut with pale liver and gall bladder. The fourth and fifth died in the 4th week. In one the yolk sac was not completely absorbed and there was stoppage near this point. In the other, the gut was full of hard faecal matter, the liver and gall bladder were pale. The surviving birds were now put in with group *XVII*.

The general results of these experiments were not conclusive. On the whole the birds seemed to thrive better with the extra marmite in the food. The birds with the high protein diet were always abnormal. It was difficult to account for the abnormalities. Several possibilities could be suggested. They may have been due to the high salt content of the diet, derived from the marmite and from the fishmeal, or to the high amount of the protein, or possibly to too little cod-liver oil, or to a combination of several conditions. The extra mineral matter introduced with the higher amounts of fishmeal was of no value in preventing leg weakness and this does not support the claim that lack of mineral matter is the cause of deficiency diseases. In former experiments a diet had been fed to chicks with an equally high protein content, and no ill-results were observed. It was therefore difficult to attribute the errors to high protein. The amount of cod-liver oil though about 0.7 % of the diet would also appear adequate.

Experiments with ducks.

Some trials of a similar kind were made about the same time with groups of ducklings. As it was previously found that ducks required more marmite than chicks, the quantity of marmite was placed at a higher figure at the start. The diets and general results are given in Table II.

Table II.

Group	White rice	Marmite	Cod-liver oil	Fishmeal	No. of birds	Losses
D, III	90	12	1	30	12	4
D, IV	90	16	1	30	4	0
D, V	90	20	1	30	11	6
D, VI	90	40	1	30	7	0

Ducks III were not well and were shaky on their legs in the 2nd week. On this account, the marmite was slowly raised (every 2 weeks) to 20 parts. It seemed that it was only by these means that the ducks could be reared.

The first loss showed a pale fatty heart; the caecum had stoppage and the appendices were blown. There was stoppage of the gut with the cloaca distended with gas in the second loss. No cause could be found for the third death. The gut was normal in the fourth except that one appendix was full. The interior was pale.

Ducks IV were very shaky on their legs and were combined with ducks V at the 10th week.

Ducks V. All but one of the losses in this group occurred in the 3rd week. The only symptoms seemed to be swollen and pale kidneys. There was a loss in the 10th week apparently from tuberculosis.

As a final trial ducks VI were fed with a very high amount of marmite. They were all reared to the 10th week and were quite normal.

Altogether there was more evidence for balance of protein by vitamin B in these experiments than in the corresponding ones with chickens.

Second series of experiments with chickens.

Owing to the apparent deterioration of the marmite, this series of experiments was made with dried yeast as source of vitamin B. Its use would also get rid of the possibility of the high salt content of the previous diets. It had been found previously [1923] that dried yeast contained about half the amount of vitamin B present in the former marmite, so that 20 parts were taken as the equivalent of 10 parts of marmite as the minimum quantity to balance the basal diet. This series was carried out in the reverse order starting with high yeast and diminishing as shown in Table III.

Table III.

Group	White rice	Dried yeast	Cod-liver oil	Fishmeal	No. of birds	Losses
XIX	90	32	1	30	11	0
XX	90	28	1	30	11	0
XXI	90	24	1	30	10	0
XXII	90	20	1	30	10	2

Composition of diets %.

Group	Water	Ash	Fibre	Protein	Fat	Carbo-hydrate	Calories per 100 g.	Yeast	Ratio yeast calories
XIX	10.1	5.8	0.2	24.8	1.7	56.8	350	21	1 : 17
XX	10.3	5.7	0.2	24.3	1.7	57.1	349	19	1 : 18
XXI	10.5	5.6	0.2	23.7	1.8	57.5	350	17	1 : 21
XXII	10.8	5.6	0.2	23.2	1.7	57.9	348	14	1 : 25

All these experiments were made in succession for a duration of 10 weeks during the time 27. vi. 1924 to 16. ix. 1924.

Though no losses occurred in the first three groups, all the birds at the age of 10 weeks had either bow legs or were knock-kneed, but otherwise they were fairly normal.

In group XXII, in one of the birds lost, there was a twist in the intestine, probably an abnormality at the start as the bird died in the 2nd week. In the other bird the gut seemed normal, but the kidneys were large and uric acid was found by testing in the ureters and cloaca.

The results again were not clear. The high protein seemed to have a deleterious effect. The birds seemed to be distinctly better on the higher amounts of dried yeast.

Third series of experiments.

This series was made to ascertain the effect of increasing quantities of protein in the diet so as to find out whether the high protein was producing the bow legs and other symptoms. The amount of dried yeast was kept constant (Table IV).

Table IV.

Group	White rice	Dried yeast	Cod-liver oil	Fishmeal	No. of birds	Losses
XXIII	90	20	1	5	9	0
XXV	90	20	1	10	9	2
XXVI	90	20	1	15	7	0
XXVII	90	20	1	20	9	4
XXVIII	90	20	1	25	13	2
XXIX	90	20	1	30	8	2
XXX	110	20	1	5	12	4

Composition of diets %.

Group	Water	Ash	Fibre	Protein	Fat	Carbo-hydrate	Calories per 100 g.	Yeast	Ratio yeast calories
XXIII	9.9	2.8	0.2	15.1	1.5	66.9	350	17.2	1 : 20
XXV	10.1	3.4	0.2	17.0	1.5	64.1	346	16.5	1 : 21
XXVI	10.3	4.0	0.2	18.7	1.6	61.6	344	15.9	1 : 22
XXVII	10.5	4.6	0.2	20.3	1.7	59.2	342	15.3	1 : 22
XXVIII	10.6	5.1	0.2	21.8	1.7	57.1	339	14.7	1 : 23
XXIX	10.8	5.6	0.2	23.2	1.8	55.1	338	14.2	1 : 24
XXX	10.1	2.4	0.3	13.9	1.3	69.0	352	14.7	1 : 24

The last group acted as a control with extra white rice equal to 30 parts of fishmeal. All the groups were kept for 20 to 23 weeks, from 21. vii. 1924 to 4. v. 1925.

Group XXIII. All the birds were reared without any signs of illness. They were actually continued for another 10 weeks with one cock bird and laid eggs. They had a tendency to eat the eggs. On this account the cod-liver oil was raised to 2 parts for a further 7 weeks and then to 3 parts for a period of 5 weeks. This increase lessened the desire to eat eggs.

Group XXV. The *post mortem* examination of the two birds which died in this group did not reveal any particular symptoms. Almost the same diet was used again in group XXXIII and the birds were reared satisfactorily.

Group XXVI. This group was reared quite satisfactorily.

Group XXVII. This group turned out to be composed only of cock birds. The first loss was in the 4th week. The gut contained compact food masses and the appendices were swollen. The other three birds had crooked legs at the end of the period of experiment and were chloroformed. All three were found to have swollen appendices with dark food residues in them; the livers were large and fatty. The other birds seemed normal.

Group XXVIII. This group looked ill in the 7th week and the birds were shaky on their legs. Several showed only small increases in weight at the 13th week. The first loss was in the 19th week. The bird had enlarged kidneys, stopped gut and very swollen appendices. The other died in the 23rd week

and had also stopped gut and swollen appendices. The hen birds of this group showed bow legs or legs with slight twist.

Group XXIX. Like group XXVIII the birds showed signs of illness in the 7th week. All the birds were shaky on their legs and the legs of many were crooked. The first loss was in the 19th week; examination showed a flabby gizzard and one swollen appendix. The other bird died in the 20th week. Its appendices were swollen, the liver was fatty and there was much fat upon the heart.

Group XXX. This group, a control group to XXIX, showed its first loss in the 4th week. There was some stoppage of the large intestine and the appendices were full of black food residues. The second loss was in the 5th week. There was undigested rice in the large intestine and the appendices were large. The third bird died in the 6th week. The large intestine was full of food residues, but the appendices were empty. The fourth bird showed bent legs in the 9th week; it did not get better and was killed in the 17th week. Its abdomen was found full of water and gelatinous matter.

All birds of the group were more or less shaky on their legs at the end of the experiment.

It appears from these experiments that high protein affects the birds and may be the cause of the bow legs or knock knees. These symptoms do not appear until the fishmeal reaches 20 parts, or about 20 % of the food. A clear demonstration of the need of balancing the protein by vitamin B does not seem possible without producing ill-effects from too much protein. Excluding the leg abnormalities, the *post mortem* findings were very similar with the smaller amounts of vitamin B, and were generally concerned with the intestines and appendices and sometimes the heart.

Fourth series of experiments.

During the course of the previous experiments others were made with small amounts of cod-liver oil and it was being noticed that 0.4 to 0.6 % was too little and later that 0.5 to 0.7 % of cod-liver oil was enough to rear birds without any signs of illness. In the experiments with low fishmeal, the amount of cod-liver oil was 0.9 %. This amount became reduced to 0.8 and 0.7 % as the fishmeal increased in quantity. It was possible that too little cod-liver oil was the cause of the leg abnormalities. This series was tried with 2 % of cod-liver oil so as to avoid ill-effects from too little. The diets used in these groups were made to contain 2 % of cod-liver oil and the whole diet was made out to a total of 100 parts. The diets are shown in Table V.

Table V.

Group	White rice	Dried yeast	Cod-liver oil	Fishmeal	No. of birds	Losses
XXXIII	74	16	2	8	10	1
XXXV	78	12	2	8	10	2
XXXIV	56	12	2	30	10	2
XXXVII	52	16	2	30	12	3

Composition of the diets %.

Group	Water	Ash	Fibre	Protein	Fat	Carbo- hydrate	Calories per 100 g.	Ratio yeast calories
XXXIII	10.0	3.3	0.2	16.6	2.7	66.9	368	1 : 23
XXXV	10.4	3.0	0.2	15.1	2.7	68.4	367	1 : 31
XXXIV	11.1	6.8	0.2	27.0	3.3	50.6	349	1 : 29
XXXVII	10.7	7.3	0.2	28.5	3.3	49.1	349	1 : 22

The diet of group XXXIII was the same as that of group XXV except for the cod-liver oil. With group XXXV this group acted as control to the two groups with high protein.

Group XXXIII. The one loss in this group could be accounted for by an accident. All the birds were perfectly fit and this one showed one morning peculiar symptoms of turning somersaults. The *post mortem* examination showed normality in all the organs. The bird was probably pecked by another or had some injury to its head.

Group XXXV. The losses were in the 5th and 6th weeks. The first was shaky on its legs and showed slight leg weakness. At autopsy, decomposing food was found in the large intestine extending into the small intestine. The appendices were full and swollen. The second bird had excessive amounts of fat on the abdominal organs. The intestines were blocked and the heart was very large. The other birds of the group were quite normal.

Group XXXIV. All the birds in the group showed leg weakness and looked very ill. The experiment was consequently stopped in the 15th week.

The two losses were in the 5th and 7th weeks. Both birds were chloroformed on account of their leg weakness. The first had decomposing food in the large intestine and swollen appendices; the gizzard was flabby and full of water. There was gas in the appendices of the second bird.

Group XXXVII. The experiment was stopped at the 16th week as all the birds showed bow legs. The first loss was in the 3rd week. The intestines and appendices were full of dark food residues. The second died in the 13th week. There was ballooning of the gut near the gizzard. In both birds the hearts seemed small.

Group XXXVII. These birds were generally in better condition than those of group XXXIV. They were able to walk whereas those of group XXXIV could not do so. It would have been quite possible to continue group XXXVII, but no further information was expected.

As the cod-liver oil was raised in this series, the abnormalities in the legs thus appeared due to high protein in the diet. The birds were distinctly better with the higher quantity of yeast.

The definite proof of the need to balance the protein in the diet is not possible of attainment with chicks. The experiments, however, indicate in a general way that with high protein the birds thrive better with a higher quantity of vitamin B as supplied in dried yeast.

The photographs show the birds of groups XXXIII and XXXIV, the latter with the bow legs noticed with high protein in the diet (Plate VI, figs. 1 and 2).

1
Group XXXIII

2
Group XXXIV

3
Group XXXVI

4
Group XLIV

5
Group XLVII A

6
Group M VI p. 943).

II. THE BALANCE OF FAT BY VITAMIN B.

Our former observations that excess of cod-liver oil in the diet of chicks produced ill-effects and that these were counteracted by giving additional marmite were the starting-point of these experiments. Excess of cod-liver oil in the diet of chickens has also been found in agricultural experiments to be detrimental to their health and an endeavour was made to prove that vitamins were therefore of little value in nutrition. There was supposed to be an ample supply in the various foods given in agricultural practice.

In our experiments on high fat diets the use of a large quantity of cod-liver oil was avoided by working with cotton-seed oil. This oil does not contain any appreciable amount of vitamin A. Any ill-effect produced must therefore be attributed to the oil.

The diets for these experiments were planned on the same basis as those in the fourth series on protein balance. The experiments were carried out in succession as the results of each became clear. The series is shown in Table VI.

Table VI.

Group	White rice	Dried yeast	Cod-liver oil	Fishmeal	Cotton-seed oil	No. of birds	Losses
XXXVI	58	12	2	8	20	15	0
XXXVIII	54	16	2	8	20	10	1
XLI	50	20	2	8	20	10	1
XLIV	60	10	2	8	20	8	5

Composition of the diets %.

Group	Water	Ash	Fibre	Protein	Fat	Carbo-hydrate	Calories per 100 g.	Ratio yeast calories
XXXVI	8.1	2.8	0.2	13.8	22.6	52.2	481	1 : 40
XXXVIII	7.7	3.2	0.2	15.3	22.6	50.8	481	1 : 30
XLI	7.4	3.5	0.2	16.8	22.6	49.3	481	1 : 24
XLIV	8.3	2.7	0.2	13.1	22.6	52.9	481	1 : 48

Group XXXVI. Though there were no losses in this group, the birds looked abnormal throughout the time of the experiment, which lasted for 26 weeks, from 26. v. 1925 to 19. x. 1925. They were greasy to the touch and generally dirty in appearance. Slight leg weakness was noticeable in the 3rd week. It became more obvious in the 7th and 8th weeks and persisted until the end of the time. The birds were always sitting during the day. The chief feature of their appearance was the poor feathering; some birds were almost naked during the time of the trial.

Group XXXVIII. The birds of this group in contrast with those of the preceding one were always quite clean to look at and were not greasy to the touch. Their feathering was about as good as that of a normal bird. The one loss occurred in the 13th week. There was fluid in the body cavity and the intestines and appendices contained dark food material.

Group XLI. This group was tried to see if still more dried yeast would be of greater benefit in balancing the fat. No advantage was noticed. The loss in this group was in the 5th week and was due to pneumonia.

Group XLIV. The object of this experiment was to see the effect of less dried yeast than used in group XXXVI. The dirty and greasy appearance of the birds was again manifest. The first loss was in the 8th week. The heart was enlarged and covered with gelatinous matter. The appendices were swollen; the kidneys and liver pale. All the birds were very shaky at the 16th week and quivered as they stood. The second loss was in the 17th week; before death the bird showed distinct leg weakness. It was very fat in the abdominal region. The heart was also covered with fat. The appendices contained black food residues, and there was stoppage at their junction with the intestine.

The group was kept longer than the other groups to see the effect of long continuation of the diet. In the 23rd week the third loss occurred. The flesh of the bird was very wet, and there was fluid in the abdomen. It seemed as if there had been a burst blood vessel as blood clots were also noticed. The fourth bird died in the 26th week. It was also dropsical, its interior being full of gelatinous matter. The heart, kidneys and liver seemed very large and there were black food residues in the intestine. The fifth died in the 27th week. The inside was again very wet. Heart and liver were much enlarged. There was decomposing matter in the intestines and the appendices were swollen.

The other birds at the conclusion of the experiment on examination were more healthy in appearance. They showed a large amount of fat in the abdominal region and the heart of one was distinctly larger than normal.

There was a distinct difference in the rate of growth of the birds in these groups. It corresponded with the amount of dried yeast in the food, being greatest in group XLI and least in group XLIV. This could be attributed to the slightly greater amount of protein supplied in the food, but is more likely due to appetite which has so often been shown to depend on the quantity of vitamin B. The rate of growth is shown in Table VII.

The series of experiments clearly points to the need of balancing the fat in the food by vitamin B. The photographs of groups XXXVI and XLIV show the condition of the birds. The poor feathering of group XXXVI is not so clear in the picture as it was during the course of the experiment (Plate VI, figs. 3 and 4).

III. THE BALANCE OF CARBOHYDRATE BY VITAMIN B.

The experiment with group XXX in which extra white rice was included in the food in contrast with group XXIII had already shown that the whole of the carbohydrate needed balancing by dried yeast. In order to show the need of balancing more distinctly a series was started using the groups XXXIII and XXXV as the controls and feeding with diminishing quantities of dried yeast, until the amount was found which was not sufficient to rear the birds satisfactorily. The series is shown in Table VIII.

As stated above, group XXXIII was a perfectly normal group, and the losses in group XXXV have been described. It was not expected at the start that

Table VII. *Average weekly weights (g.) of birds in the balance of fat experiments.*

Group	Cocks				Hens			
	XXXVI	XXXVIII	XLI	XLIV	XXXVI	XXXVIII	XLI	XLIV
No. of birds	11	6	5	6	4	3	4	2
Week								
0	35	39	40	34	38	36	39	33
1	50	65	65	51	52	60	58	52
2	73	105	93	70	78	95	80	72
3	103	142	151	94	111	132	121	110
4	139	177	212	135	142	168	165	156
5	189	214	277	179	189	215	195	193
6	251	258	363	261	240	275	259	252
7	322	339	428	317	304	363	314	302
8	390	449	476	410	357	463	349	360
9	460	551	625	522	422	523	462	410
10	548	647	673	613	509	598	536	475
11	692	732	785	717	594	656	605	572
12	793	892	918	779	645	806	735	600
13	895	1058	1029	918	695	898	839	705
14	1030	1168	1260	999	811	972	999	762
15	1146	1210	1302	1092	900	1065	1054	785
16	1359	1515	1505	1208	1026	1233	1160	872
17	1462	1682	1723	1391	1114	1390	1317	715
18	1668	1852	1801	1581	1237	1497	1375	840
19	1880	1959	1986	1645	1341	1540	1477	850
20	2055	2111	2134	1802	1437	1605	1597	1000
21	2068	2215	2380	1870	1505	1735	1711	1030
22	—	—	2538	1993	—	—	1825	1100
23	—	—	2697	2136	—	—	1961	1200
24	—	—	—	2170	—	—	—	1245
25	—	—	—	2242	—	—	—	1360
26	—	—	—	2442	—	—	—	1435
27	—	—	—	2445	—	—	—	1585
28	—	—	—	2522	—	—	—	1655

Table VIII.

Group	White rice	Dried yeast	Cod-liver oil	Fishmeal	No. of birds	Losses
XXXIII	74	16	2	8	10	0
XXXV	78	12	2	8	10	2
XL	80	10	2	8	11	1
XLII	82	8	2	8	11	1
XLV	86	6	2	8	10	1
XLVI	86	4	2	8	13	0
XLVII A	88	2	2	8	12	2
XLVII B	88	2	2	8	9	6
L	89	1	2	8	5	5
XLIX	77	6	2	15	10	1

Composition of the diets %.

Group	Water	Ash	Fibre	Protein	Fat	Carbo-hydrate	Calories per 100 g.	Ratio yeast calories
XXXIII	10.0	3.3	0.2	16.6	2.7	66.9	368	1 : 23
XXXV	10.4	3.0	0.2	15.1	2.7	68.4	367	1 : 31
XL	10.6	2.8	0.2	14.4	2.8	69.1	368	1 : 37
XLII	10.7	2.7	0.3	13.7	2.7	69.8	368	1 : 46
XLV	10.9	2.5	0.3	12.9	2.7	70.5	367	1 : 61
XLVI	11.1	2.4	0.3	12.2	2.6	71.3	367	1 : 92
XLVII	11.3	2.2	0.3	11.4	2.6	72.0	366	1 : 183
L	11.4	2.1	0.3	11.1	2.6	72.4	366	1 : 366
XLIX	11.1	3.8	0.2	16.7	2.9	64.9	362	1 : 60

so many groups would be required before the balance would be shown by losses of birds.

Group XL. Twenty-one weeks, from 10. vii. 1925 to 5. xii. 1925. One bird in the 9th week showed distinct leg weakness. It was given 1 g. of marmite and was cured next day, which showed that the leg weakness was due to lack of vitamin B. It never showed the symptoms again and was quite well at the end. The bird which died had an abscess under the skin which caused peritonitis. The excess cocks were killed in the 22nd week. Examination showed them to be quite normal in their appearance. One cock and the hens were then kept altogether for 52 weeks. They laid eggs, which were mostly unfertile. One hen died in the 42nd week. There were masses of fat in the body cavity, every organ being covered with fat. The connective tissue was leathery and the heart pale.

The group was considered quite normal throughout the experiment.

Group XLII. Twenty-two weeks, from 10. viii. 1925 to 11. i. 1926. The loss in this group took place in the 5th week. The bird had leg weakness and died suddenly. All the birds in the 8th week showed poor feathering and one had a head twist. They scattered their food. At the 18th week the birds looked pale and seemed to have signs of leg weakness. The excess cocks were killed in the 23rd week. All were very fat in the abdominal region and the appendices were full of pasty black food residues. The connective tissue was leathery. One cock and the hens were kept on longer. The cock died suddenly in the 31st week. It had a pale liver and large heart. The hens were put in with those of group XLV. The eggs laid were mostly unfertile, and the fertile ones did not hatch.

The group could not be considered as a perfectly normal one.

Group XLV. Twenty-one weeks, from 11. xi. 1925 to 7. iv. 1926. There was one loss in the 5th week. Hard lumps of food were found in the intestine and the heart was pale. The birds generally did not look as well as those observed in the previous groups and also scattered their food. The excess cocks were killed in the 22nd week. In all these birds the gizzards were flabby, but only small amounts of fat were noticed around the gizzards and hearts. The hens and one cock were kept on to the 38th week. Most of the eggs laid were unfertile; the fertile ones gave a few hatches but the chicks soon died, being in very poor condition.

The group was not a perfectly normal one.

Group XLVI. Twenty-one weeks, from 5. v. 1926 to 29. ix. 1926. Though there were no losses in this group, the birds that reached maturity were not normal. From the 3rd week the birds always left the rice of their food, but were not given fresh food until it was eaten. One bird in the 8th week had leg weakness, but got better. At the 20th week the birds looked very ill. Five showed distinct drooping of the wings and three had weak legs and were distorted to one side. They were killed in the 21st week. At *post mortem* examination the chief feature of all the birds was the enormous amount of

fat around the necks and around all the internal organs. In many the hearts were enlarged. The intestines had pasty black food residues in them and the appendices were in some cases swollen.

The group was quite abnormal.

Group XLVII A. Twenty-one weeks, from 10. v. 1926 to 4. x. 1926. The first loss took place in the 14th week. The interior of the bird was very fatty and the appendices were slightly swollen. The heart, liver and kidneys were pale. The second loss was in the 17th week. The inside of the bird was very fat and the heart large. The birds in general did not feather well and were distinctly shaky on their legs. At *post mortem* examination the insides were found to be very fat, all the organs being more or less covered. In some the hearts were large and the intestines and appendices swollen. The birds were not by any means normal (see Plate VI, fig. 5).

Group XLVII B. Twenty-six weeks, from 29. x. 1926 to 29. iv. 1927. The survival of the birds of group XLVII A was so remarkable that a further experiment was made with the same diet. Six birds died during the course of 13 weeks. In each case the heart was large. In most the intestines had black food residues and the appendices in several were not normal. In these young birds there was not the visibly large amount of fat in the abdomen.

The three birds surviving to 26 weeks were hens. At *post mortem* examination they were found to be abnormally fat in the abdominal region. A mass of nearly 200 g. of fat could easily be removed by hand from one. They had large hearts and fluid was found in the pericardium of all three.

The losses and abnormality of the last three birds clearly show that 2 % of dried yeast is quite insufficient for balancing the food.

Group L. This group was started because the birds of group XLVII A lived so long. All birds except one died within 6 weeks. The single bird which survived lived for 20 weeks and then died suddenly. Its interior was not fat, but the small intestine was swollen and the heart large. The bird always ate very little and this may account for its long survival.

Group XLIX. Twenty-one weeks, from 6. viii. 1926 to 31. xii. 1926. This group was tried to see if more protein in the diet of the birds with 6 % of dried yeast, which were apparently normal in external appearance, would have any special effect. It was thought that high protein in the diet had a harmful effect. One bird died in the 3rd week from an unabsorbed yolk sac. The birds already in the 7th week looked ill and never seemed perfectly fit. They always panted after being caught for weighing. At *post mortem* examination some of the birds were found to be very fat. Several had crooked breast-bones. The appendices generally contained pasty food and black food residues were often observed in the intestines. In many the hearts were obviously large. The birds were not normal, though there were no distinctive abnormalities. High protein appears to have an influence upon the health and appearance.

These experiments show definitely that 2 and 4 % dried yeast are insufficient to balance the carbohydrate in the food. With 6 % dried yeast the

birds which reached maturity were not absolutely perfect. A really good result was only obtained with 10 % of dried yeast. The weights of the birds in the different groups as shown in Table IX also show quite definitely that 2 and 4 % are too little. The weights of the birds in the other groups, except possibly group XLII, are much the same each week. 6 % is the minimum of dried yeast and 10 % is a good quantity.

Table IX. *Average weekly weights of birds in the balance of carbohydrate experiments.*

		Cocks						
Group ...	XXXIII	XXXV	XL	XLII	XLV	XLVI	XLVII A	
% of yeast	16	12	10	8	6	4	2	
No. of birds	2	5	6	6	5	7	8	
Week								
0	42	39	39	33	35	40	36	
1	52	59	59	48	63	54	51	
2	101	95	102	76	99	88	69	
3	141	157	157	117	150	108	93	
4	174	248	225	185	221	146	126	
5	291	370	306	249	325	196	163	
6	447	451	368	329	405	249	209	
7	525	592	490	403	510	325	266	
8	777	707	637	489	666	438	368	
9	862	897	825	620	805	583	447	
10	1122	1039	1002	743	1000	684	528	
11	1317	1266	1156	963	1182	794	672	
12	1522	1435	1343	1144	1390	1023	818	
13	1730	1715	1533	1333	1479	1184	958	
14	1955	1982	1758	1537	1695	1381	1103	
15	2125	2140	1880	1779	1846	1584	1299	
16	2335	2332	2207	1930	2139	1785	1508	
17	2480	2589	2363	2119	2303	1979	1711	
18	2655	2799	2584	2234	2498	2119	1889	
19	2765	3074	2711	2412	2666	2240	2075	
20	3080	3215	2955	2553	2684	2492	2248	
21	3215	3225	3083	2778	2707	2359	2131	

		Hens						
Group ...	XXXIII	XXXV	XL	XLII	XLV	XLVI	XLVII A	
% of yeast	16	12	10	8	6	4	2	
No. of birds	7	6	5	4	4	6	4	
Week								
0	37	37	39	31	35	41	36	
1	54	59	57	49	61	54	54	
2	93	99	89	76	99	85	70	
3	137	165	130	117	147	102	93	
4	199	232	184	189	205	140	122	
5	305	347	251	249	294	176	158	
6	424	434	319	327	379	225	200	
7	546	546	406	405	472	285	262	
8	697	670	507	482	632	392	341	
9	791	809	642	631	750	496	396	
10	982	930	762	752	909	584	454	
11	1141	1087	898	937	1061	695	554	
12	1309	1202	1015	1076	1214	849	655	
13	1441	1397	1154	1210	1300	977	754	
14	1541	1532	1320	1387	1446	1133	848	
15	1649	1607	1388	1541	1528	1287	952	
16	1819	1678	1634	1624	1692	1444	1126	
17	1964	1838	1720	1724	1771	1569	1290	
18	2101	1944	1870	1844	1945	1681	1410	
19	2164	2111	1963	1977	2109	1775	1531	
20	2357	2217	2059	2045	2125	1932	1654	
21	2457	2213	2120	2180	2420	1877	1660	

EXPERIMENTS WITH PIGEONS.

During the course of the various trials with chickens similar experiments upon balancing the carbohydrate of the food with vitamin B were made with pigeons. Their diet was composed of white rice, later white flour, fishmeal and marmite, or dried yeast. Cod-liver oil was omitted, as it had previously been found that pigeons did not need cod-liver oil in their food [1923].

First series.

Two pigeons were placed on 1. i. 1924 upon the same diet as group XII 5, *i.e.*

White rice	Marmite	Fishmeal
90	9	5

At the end of 12 weeks it was discovered that they were two hens. A cock was substituted for one of them and the pair kept on this diet for 10 weeks. During this time two lots of two eggs were laid by the hen. The first lot was unfertile, the second was fertile and squabs were hatched but soon died. In order to improve the hatching and rearing, the quantity of marmite in the food was raised to 10 parts for the next 8 weeks, and then to 12 parts for a further 7 weeks. The eggs that were laid during these periods were deserted by the birds, one lot later disappeared, possibly eaten by the birds. As probably some other food constituent was needed for reproduction, a further increase in the marmite was not tried.

Altogether one bird was kept for 23 weeks and the other for 10 weeks with 9 parts of marmite and the two birds at the end showed a slight increase in weight. This quantity could therefore be considered ample for maintenance.

In order to find out the least quantity of marmite that would suffice to maintain the birds, the quantity was reduced to 4 parts. At the end of 6 weeks both birds had lost over 100 g. in weight and looked very ill. They had lost their appetite and scattered their food.

The marmite was raised to 6 parts; the food was now eaten without scattering and both birds increased in weight. It seemed that 6 parts of marmite was the quantity to balance the rice.

Our former figure for marmite in the same diet was 3 parts. The marmite was thus not so rich in vitamin B as in the former year. This was already indicated in the experiments with chickens on the balance of protein.

Samples of other yeast extracts.

This pair of pigeons, as seen above, were able to discriminate by scattering of the food and loss of appetite with concomitant loss of weight whether the food contained enough yeast extract or not. They were used for a rough test of several other samples of yeast extract which we were able to investigate.

The first sample, designated by us as *A*, was started at the level of 3 %. The birds lost weight and did not like the food. On raising the proportion to 3.5 % the birds recovered their appetite and weight.

The second sample, designated by us as *B*, was started at 3.5 %. The birds scattered the food and declined in weight. The amount was first raised to 4 %, then to 4.5, then to 5 and to 6 %. At this level the birds ate the food without scattering, regained their appetite and increased in weight. On again reducing to 5 %, they showed loss of appetite and loss of weight.

The third sample, designated *C*, was started at 5 %. The birds scattered the food and lost weight; on raising to 8 parts they recovered. On reducing to 7 they lost weight again, but recovered on raising to 10 %. The hen now suddenly died and the experiment was stopped.

The fourth sample of yeast extract, designated by us as *V*, was tried on another pair of birds. On 3 % the birds were kept for 11 weeks, and then for another 4 weeks at 4 % without any appreciable loss in weight.

The fifth sample of yeast extract, designated by us as *R*, was tested on a further pair of birds, starting at 1.5 % and raising the quantity to 3 % as they were not well; they died in 9 weeks. Another pair started at 4 % died in 7 weeks even after raising to 6 %. This extract thus appeared to contain very little vitamin B. The several extracts of yeast which have been so tested have varied from 3 or 3.5 to 8 or 10 % for the amount to be incorporated in a vitamin B-free diet. One extract was very weak.

Second series.

White rice soaked in marmite was not considered as an altogether suitable method of testing the value of yeast extracts, partly on account of the scattering of the food, and partly on account of the unavoidable loss of some of the extract adhering to the sides of the food containers which we used. It was decided therefore to substitute white flour for white rice. By making the flour into a dough with a solution of the marmite and making small pills about the size of a dried pea, drying the pills in the air, or at 37°, and feeding with such pills, it was impossible for the pigeons to separate the components of the food. It had to be swallowed whole, or left.

The marmite was then tested carefully at levels of 1 to 4 or more % until a quantity was reached which sufficed to maintain the birds for a long period. As seen below, the standard time of 15 weeks employed formerly by Chick and Hume [1917] is really not long enough and we have taken 20 or 26 weeks as a more satisfactory time for a maintenance test.

These experiments were made in succession as the result of a test became known. The birds on the smaller quantities showed the typical signs of polyneuritis and were allowed to die, proving the quantity was insufficient. Table X shows the results.

Table X.

Diet			Result			
White flour	Fishmeal	Marmite	Birds died in	7th and 10th weeks		
90	5	2		14th	20th	
90	5	4	"	13th	16th	"
90	5	5	"	15th	18th	"
90	5	6	"	29th	36th	"
90	5	8	"			

On 2 parts of marmite the birds died with the typical symptoms of polyneuritis. One had an enlarged heart, the other showed no other special symptom.

On 4 parts of marmite, both birds showed polyneuritis. The second bird had these symptoms in the 16th week, but got better on being brought into a warm room. The symptoms appeared again in 4 weeks and the bird died. No special symptoms were noticed at autopsy.

On 5 % marmite, both birds showed polyneuritis. One bird had a distinctly large heart.

On 6 % marmite, polyneuritis was seen. The intestines were abnormal in appearance.

On 8 % marmite, the birds did not show the symptoms of polyneuritis. On *post mortem* examination the hearts were large and the intestines were abnormal.

The quantity of 8 % was taken as the minimum for long maintenance.

Third series.

It was also necessary to test the dried yeast used for the chickens as a source of vitamin B so as to compare it with the marmite.

It was being found that 6 % of dried yeast was a sufficient quantity to balance the carbohydrate for chickens. The first trial with pigeons was therefore made with 4 %.

The food was prepared as described above with white flour and made into pills.

On 4 % of dried yeast a pair of pigeons was kept for 37 weeks. During this time their weight kept constant and the hen laid six lots of eggs. The eggs were generally fertile, but only in two instances was a squab hatched; it died soon afterwards from unabsorbed yolk sac.

These birds were then given the diet with 2 % dried yeast.

They died in the 12th and 13th weeks afterwards. One bird showed the typical signs of polyneuritis; the other was paralysed. This latter bird had a distinctly large heart. The intestines of both birds were abnormal in appearance.

Other trials were made with 1 % and 3 % of dried yeast as these experiments were in progress. On less than 4 % the birds died. Still other experiments were made with larger amounts of dried yeast in the hope that rearing would be better. There was no great improvement in rearing,

but the actual hatching was better. The results of this series are seen in Table XI.

Table XI.

Diet			Results
White flour	Fish-meal	Dried yeast	
94	5	1	Both birds died in 6 weeks
93	5	2	Birds died in 12th and 13th weeks
92	5	3	Birds died in 9th and 15th weeks
91	5	4	Kept for 37 weeks. Six lots of eggs, no rearing
89	5	6	Kept 18 weeks. Five lots of eggs; 2 hatches, no rearing. Then given 8 % dried yeast
89	5	6	Kept for 48 weeks. Eleven lots of eggs; fertile, but no hatch
87	5	8	Kept 50 weeks. Five lots of eggs; fertile; no hatching
85	5	10	Kept 26 weeks. Eight lots of eggs; three hatches, but no rearing

It was clear that 4 % of dried yeast was sufficient for long maintenance and reproduction. There was no rearing. The increase of dried yeast to 10 % made some improvement in hatching. The experiment was not continued, as it was possible that some other factor than vitamin B might be concerned in the hatching of young that could be reared.

The maintenance with 4 % of dried yeast is less than the amount with chickens, which were found to need 6 % as the minimum. These results correspond with those that we have found previously, that chicks need more vitamin B than pigeons. The larger amount of dried yeast in the food is beneficial just as was noticed with the rearing of chickens. For perfect health it is advisable to give more vitamin B than the minimum required for maintenance.

EXPERIMENTS WITH RATS.

Having found that 8 % of marmite and 4 % of dried yeast were necessary to balance the food of pigeons, it was of importance to ascertain how much vitamin B in similar terms was required by rats.

Exp. 1. This experiment, though not actually the first to be started, was made partly to act as control and partly to find out how long adult rats could live on a diet free, or almost free, from vitamin B. There are numerous references to the short time of survival of young rats on such a diet, but it is difficult to find information about adult rats.

Four adult rats, one buck and three does, which had been kept previously on a diet of 90 maize, 5 marmite and 5 fishmeal for 20 weeks were given our diet of:

White flour	Fishmeal	Cod-liver oil
94	5	1

made up into pills dried at room temperature on 4. vi. 1925.

Each animal showed an increase in weight during the first 4 weeks. One of the does then had young, which died. Subsequently a decline in weight set in. The other does also had young, which were eaten by their mothers. At the 8th week, a doe again had young which were eaten. In the 9th week another doe had young during the birth of which she died. The first doe then

rapidly declined in weight and died in the 16th week. The third doe showed no signs of young.

The two remaining animals were now so ill that they were given a diet with 3 % of marmite. They showed improvement during the next few weeks, especially the buck, but they never seemed as well as our other rats. After 19 weeks on 3 % marmite in the food, both were chloroformed. The doe had seven young in her uterus, but her lungs were solid and she would have died of pneumonia. The buck was abnormal in appearance; the large intestine was yellow and contained gas; the appendices also contained gas. The animals did not appear to have recovered from the diet with no marmite.

During this period of diet with 3 % marmite, the buck was put with a doe also on 3 % marmite. This doe had been previously on 2 % marmite (see Exp. 2). At the 11th week on this diet this doe had young and she died of haemorrhage. On *post mortem* examination excess of fat in the abdominal region was the only abnormality observed.

Exp. 2. This was actually the first experiment on our white flour, fishmeal, marmite diet with 1 % of cod-liver oil. The amount of marmite was 4 %, half the amount that was found to be required by pigeons.

The object was to find out if a pair of rats could be maintained on this quantity of marmite and also reproduce. The experiment was started on 21. i. 1925 and the animals weighed 87 and 78 g. They increased in weight regularly every week. At the 6th week the doe had seven young which were eaten. At the 13th week she had a litter of nine. Five of these died, but the other four were reared by the mother and weaned in the usual time of 3 weeks (see Exp. 3).

At the 18th week the doe had another litter of nine; two of these were eaten.

As this doe had reared on 4 % marmite, the quantity was now reduced to 1 % to see if this quantity was sufficient for maintenance and rearing. The seven young gradually died, some showing distinct signs of paralysis. 1 % was thus not enough for rearing.

The two animals were then kept continuously on the diet with 1 % marmite. The doe had four more litters of young, which were at once eaten. The last litter was in the 34th week on this diet. During the birth of these young the doe died. Autopsy showed masses of fat in the abdominal region with black food residues in the large intestine extending into the small intestine. The buck rat did not look well and appeared very constipated; he passed only very few faecal pellets of very hard consistency daily. He lost weight, then gained about 40 g., then lost and then gained again. He was kept altogether 82 weeks on this diet and was then killed. Autopsy showed masses of fat in the abdominal region. Histological examination of the intestines of this rat has been made by Dr M. J. Rowlands who is publishing his results independently.

Exp. 3. The four young rats from the pair on 4 % marmite (Exp. 2) after

weaning were given the diet with 3 % marmite for the next 3 weeks. During this time they increased in weight from 110 to 303 g., average 48 g. They were one buck and three does.

The quantity of marmite was now reduced to 2 % to see if this amount was enough for maintenance and reproduction. It had been seen that 1 % was not enough.

In the 7th week on this diet, one of the does gave birth to a litter of seven. Two were eaten at once, the others gradually died in the next 2 weeks. During the next 15 weeks each of the does had several litters of young. In every case the young were eaten by the mother. One doe was then changed to 3 % marmite (see Exp. 2). The other two does were kept on the 2 % diet for altogether 25 weeks. Both had young which they immediately ate. It thus seemed that 2 and 3 % marmite were not enough for rearing the young. The diet was changed to 4 % marmite, upon which quantity young had been reared (Exp. 2), for a further trial of this quantity. One doe had seven young which she ate. The other had ten, nine of which she reared (Exp. 4). The rats were kept on this 4 % diet for 16 weeks. Both does had young, but in each case there was no rearing. One of the does then died and at autopsy was found to have a ballooned intestine.

The trials with pigeons were now showing the superiority of dried yeast over marmite in vitamin B content. The marmite was changed to dried yeast. The pair was kept on 4 % dried yeast for 17 weeks. The young that were born were eaten in every instance by the mother. The dried yeast was raised to 6 % to see if a further increase would improve rearing. The buck died of pneumonia in the 25th week. The doe was kept for another 13 weeks and was given the buck alternately with the remaining doe from Exp. 4. No young were born. The experiment was then discontinued, as it was possible that reserves of a possible vitamin E were exhausted.

Exp. 4. In order to test again the 3 % quantity of marmite in the food, the nine young rats from 4 % (Exp. 3) were placed on this food after weaning. They increased from 26 to 74 g. on the average during the first 4 weeks. There were four bucks and four does and the ninth unfortunately escaped. Only one buck was kept and he was placed alternately with the four does, which were kept two in a cage except at birth of young.

Those on 3 % did not thrive well, only increasing 50 to 60 g. in the next 5 weeks. They were found one morning accidentally injured and were chloroformed.

Those on 5 % marmite gained 70 g. in the first 5 weeks. One of these does also met with an accident during one night and was chloroformed. At this time the marmite was changed to dried yeast, as the marmite seemed less good than formerly.

A litter of five was born in the 18th week and two were reared. Though the pair was kept for another 40 weeks no more young were born. Altogether the pair was kept for 61 weeks on this diet.

Exp. 5. The two young rats from the 5 % dried yeast pair were two bucks. They were put upon a diet with 1 % dried yeast to compare with the diet of 1 % marmite. They were kept for 27 weeks and were then killed. On *post mortem* examination they were found abnormally fat in the abdominal area. Histological examination was carried out by Dr Rowlands who is recording his results separately.

Rats have thus been kept on diets containing 0, 1, 2, 3, 4 % of marmite. Adults were maintained for the long period of 34 and 82 weeks on 1 %, but young could not be reared. Young were not reared on 2 and 3 % marmite, but two litters were reared on 4 % marmite by different does. 4 % thus appears as the least possible quantity for proper maintenance.

Using dried yeast instead of marmite, young rats were kept for 27 weeks on 1 %, but on examination at autopsy they were abnormal. Reproduction was poor on 5 and 6 % dried yeast.

On comparison with pigeons, 4 % marmite for a rat corresponds with 8 % marmite for a pigeon and 2 % dried yeast with 4 % dried yeast, though rats could be maintained for a long period on 1 % dried yeast. Emmett and Peacock [1923] have also observed that rats have a smaller vitamin B requirement than pigeons.

A balance of food by vitamin B is thus also shown in the case of rats.

DISCUSSION OF RESULTS.

Though our experiments on the need of balancing the protein in the diet by vitamin B are not so conclusive as was hoped, there was no doubt that the chickens and ducks on the high amount of protein in the food were in better condition with additional marmite, or dried yeast. If our observations be taken in conjunction with those of Reader and Drummond [1926] and of Hartwell [1924, 1925] on rats, the conclusion may definitely be drawn that the protein of the food must be balanced by vitamin B. The ill-effect of the high protein on birds, not noticed on rats, is most likely due to the kind of protein. Reader and Drummond and Hartwell used caseinogen and in former experiments [1922] we have used a high proportion of milk proteins with which we reared first-class birds. It is probable that the flesh (fish) protein was the cause of the abnormalities in the birds.

The need for balancing the fat in the diet by vitamin B was made quite obvious in the miserable external appearance of the birds on the high fat diet with an insufficient amount of dried yeast.

The balance of the carbohydrate, though not so obviously affecting the external appearance, was shown by the abnormal *post mortem* condition of the birds at the end of the experiment.

Our results agree with those of Randoin and Simonnet [1924] who came to the idea that the ratio

$$\frac{\text{vitamin B}}{\text{carbohydrate}} = \text{a constant,}$$

but our results point to a wider relationship, such as

$$\frac{\text{vitamin B}}{\text{calories}} = \text{a constant.}$$

If reference be made to the composition of our diets (pp. 923, 925) and if the ratio which we propose be considered, then in the high fat experiments, the ratio

$$\frac{\text{dried yeast}}{\text{calories}} = \frac{1}{40}$$

and in the carbohydrate experiments the ratio is $\frac{1}{61}$, with 6 % of dried yeast, or $\frac{1}{37}$ with 10 % dried yeast. In the former case the yeast was scarcely sufficient, but the latter figure gave birds as good as those with 16 % dried yeast on the high fat diet.

A similar figure could not be arrived at from the protein experiments, as in all the experiments the ratio was greater than $\frac{1}{40}$.

In the case of pigeons the ratio is rather less, about $\frac{1}{80}$, and in the case of rats about $\frac{1}{160}$, if 2 % of dried yeast be taken as the minimum figure for their maintenance.

Cowgill, Smith and Beard [1925] and Beard [1925] have also found a relation between vitamin B and both food consumption and the body weight. They have proposed the relation

$$K = \frac{\text{vitamin B}}{\text{calories} \times \text{weight}^{\frac{5}{8}}}$$

and consider that during growth some additional factor affects the vitamin B requirement.

We have previously [1923] observed that young pigeons cannot be reared on the same diet as that on which their parents can be maintained. They need more vitamin B. The difference corresponded with the difference between adult pigeons and chicks. Young rats again could not be reared on the same diet as that on which their parents could be kept.

The function of vitamin B is more than that of simply balancing the food consumed and bearing a relation to the body weight. Drummond and Marrian [1926] have found that its rôle is not connected with the internal metabolism of the cell, for, in its absence from the food, the condition resembles the condition of the tissues in starvation.

Vitamin B appears to be of fundamental importance in life, having a rôle not only in general metabolism, but also a special rôle in relation to the endocrine organs. We believe that vitamin B is a definite and important chemical constituent of every cell, in fact a constituent of the nucleus. Without vitamin B the nucleus cannot exist. The greater abundance of vitamin B in nuclear tissues supports such a proposition, and, if Funk's original "vitamine" and other substances which have been isolated be accepted, the nuclear connection is emphasised by the fact that these substances belong to the pyrimidine or purine groups. The recent isolation of the antiberiberi vitamin

by Jansen and Donath [1927] again includes the vitamin in this group of compounds.

The function of protein in the food is to repair so-called wear and tear of the cell and for growth in young animals. Similarly, the function of vitamin B is to replace wear and tear of the nucleus and to aid the growth of nuclear material. Hence the greater need of vitamin B by young animals, for growth of the nuclei. The difference in requirements between birds and rats may be in connection with the amount of nuclear material in the whole body. Rats may have a relatively smaller amount of nuclear matter for repair and for growth.

Since it is clear that vitamin B must bear a relation to the total food consumed, it would follow that the general idea of a daily dose or quantity is not correct. Such a quantity might be determined for a small amount of daily food, but this quantity would not suffice for a larger amount. The quantity of vitamin B required daily must be in relation to the maximum daily food consumption.

Our experiments further make a distinction between absence of vitamin B, an insufficiency and a proper amount. The term deficiency has been generally used to describe the condition of total, or nearly complete, absence. The term partial deficiency has been used for amounts between absence and the proper amount. We propose the term *shortage* for the intermediate condition of too little, more particularly to a slight degree which produces no immediate ill-effects.

Very definite pathological symptoms have been noticed in our animals kept for long periods on a shortage of vitamin B. The very large amount of fat in the abdomen was the most striking condition. The large intestine was often blocked with dark residues of food, occasionally extending into the small intestine. Sometimes there was stoppage in the large intestine, as was noticed by the impossibility of pushing the food down the alimentary canal. The pigeons often had the cloaca distended with excreta. It has been noticed also that after evacuation pigeons with polyneuritis were distinctly better. The appendices were abnormal. The hearts in many cases were obviously enlarged. The tissues were sometimes wet. The gizzards in some cases had very thin walls. Unfortunately we have not been able to study the pathology of the organs, but Dr Rowlands is referring to the histology of the intestines in cases of shortage in rats, and Dr Pulvertaft has examined many of the hearts in our dead birds.

The pathological appearances which we have noticed are the same as described by McCarrison [1921] as occurring in the early stages of beriberi, in which vitamin B is absent from the diet. The early stages of beriberi have thus become a chronic condition on a diet with a continual slight shortage of vitamin B. They are the conditions commonly observed in the population at the present time. For this reason we have also made tests of the comparative vitamin B value of the common foods, some of which we hope to have ready for publication shortly. These tests indicate that the general diet of the people

is like that of the birds on a slight shortage of vitamin B, and that the symptoms produced by vitamin B shortage are the same in birds and man. The prevalence of gastro-intestinal trouble and heart affections may be traced to a continual slight shortage of vitamin B.

One other factor may be considered as concerned in the pathological condition of our animals, namely, the fat-soluble vitamin D. Though 2 % cod-liver oil was fed to all the chickens in the last series of experiments and rats to supply fat-soluble vitamins, it is possible that owing to faulty digestion on account of shortage of vitamin B the oil was not assimilated, so that there was at the same time a shortage of vitamin D. A full supply of vitamin B is essential for the introduction into the system of the vitamins A and D in the fat of the food.

Our results and conclusions are based upon the old idea that marmite and dried yeast contained only one vitamin B. Two vitamins, which may be termed B 1 and B 2, are now believed to be present in these foods. It is therefore possible that both B 1 and B 2 are concerned, or possibly B 2 only. The future will have to decide upon the separation and differentiation of these vitamins. For practical purposes, at any rate of human dieting, the existence of the two vitamins, closely associated in yeast and other foods, is in the same category as the existence of two fat-soluble vitamins, A and D in cod-liver oil, butter, other fats and eggs. The foods which supply the one most often contain the other also, so that they are consumed together.

SUMMARY.

Protein, fat and carbohydrate in the diet need balancing by vitamin B (B 1 + B 2). The quantity of dried yeast, which carries the vitamin B required, can be represented by

$$\frac{\text{dried yeast}}{\text{calories}} = \frac{1}{40} \text{ to } \frac{1}{60}$$

or from 6 to 10 % in a food containing no vitamin B in the case of chickens. The ratio is about $\frac{1}{80}$, or 4 % of the food, in the case of adult pigeons and $\frac{1}{160}$, or 2 %, in the case of adult rats.

8 % of marmite corresponded with 4 % of dried yeast as tested on pigeons. Samples of yeast extract have been found to vary considerably in their vitamin B content.

Young animals require more vitamin B than adult animals. It is therefore suggested that vitamin B is a constituent of the nucleus of the cell, and is required for growth and for replacement of wear and tear.

Various pathological conditions, obviously affecting the intestines and heart are noticed on diets containing too little, or a shortage, of vitamin B. These symptoms have been described as occurring in the early stages of beriberi. On a shortage of vitamin B these early symptoms become a chronic condition of ill-health.

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