FEEDING AND BREEDING OF LABORATORY ANIMALS

III. OBSERVATIONS ON THE FEEDING OF GUINEA-PIGS

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(With 16 Figures in the Text)

Some problems of the feeding of laboratory herbivores are discussed in an earlier paper (Bruce & Parkes, 1946) where the successful elimination of fresh green food from the dietary of rabbits is described. With guinea-pigs the problem is much more complex. There is general agreement in the literature that, apart from vitamin C, the nutritional requirements of rabbits and guinea-pigs are similar, and there is considerable knowledge of the requirements of guinea-pigs for vitamin C under various conditions. Ecker & Pillemer (1940) have shown that serum complement does not reach maximum activity until the ascorbic acid of blood serum is at a level of 1 mg./100 ml. blood, which is only reached with a daily intake of 10 mg. ascorbic acid, an amount greater than that required to give full protection from scurvy or to give saturation of the tissues (Zilva, 1936), and double that required to prevent changes in the histological structure of the teeth (Cohen, 1938). The general level of requirement indicated by these experiments is vastly in excess of that of the rabbit, and the problem of meeting it, without feeding greenstuff, by a diet which can be made in bulk and stored under ordinary conditions, does not seem to have been attacked.

Definite knowledge of special dietary factors required by the guinea-pig, other than vitamin C, is less extensive. Work with simplified diets has suggested the existence of several such factors, the chemical nature and requirements of which are unknown.* The guinea-pig under optimum conditions will continue for very long periods to increase slowly in weight, and there is a danger of a chronic mild deficiency of one or other factor developing after prolonged maintenance on a diet.

* Goettsch & Pappenheimer (1931); Hogan & Hamilton (1942); Kohler, Elvehjem & Hart (1938); Kohler, Randle, Elvehjem & Hart (1939); Kuiken, McCoy, Schultze & King (1944); Sober, Mannering, Cannon, Elvehjem & Hart (1942); Woolley (1942); Wulzen & Bahrs (1941).

TECHNIQUE

Animals

The guinea-pigs used were bred at the National Institute for Medical Research Farm Laboratories at Mill Hill. Except in two breeding experiments, the animals were all males, and were used in groups of six. They were put on experiment at weaning, at a weight of about 200 g. They were weighed twice weekly, and were housed, not more than three to a cage, in all-metal cages $20 \times 15 \times 12$ in. high, with wire grid floors and without bedding. The cages were cleaned twice a week.

Feeding

The animals were fed daily. Hay was supplied from wire baskets half-circular in cross-section and tapered towards the bottom, with dimensions 8 in. deep $\times$ 7 in. wide $\times$ $3\frac{1}{2}$ in. in thickness at the centre. The back rests flat against the side of the cage. The basket is suspended from the top of the cage and is closed by the lid thereof. This method of giving hay was found preferable to the usual method of putting it loose in the cage, which results in it being used first as bedding and then as food. The animals kept much cleaner with the hay-basket, and there was no waste from fouling. Bedding was not found necessary in a warmed animal room, except for the pregnant females and very young litters. Water was given in 500 ml. bottles hung on to the outside of the cage, with a nozzle projecting inwards. As a rule one bottle supplied sufficient water for three animals for 24 hr., but in very hot weather and with some of the diets in meal form, particularly diet 13, the animals drank nearly twice this amount.

Diets

Stock diet. The original stock diet was a wet mash of bran and sugar-beet pulp, supplemented with hay and fresh greenstuff. The dried sugar-beet pulp was soaked in water overnight and partly dried off with

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bran the following morning. The mixture was freshly prepared every day. This diet had a very low protein content, about 7%. Growth of young guinea-pigs receiving stock diet was at the rate of about 5 g. per day.

Experimental pelleted diets. These were prepared by Messrs J. & H. Robinson Ltd., as has been described in an earlier paper (Parkes, 1946). The process involves exposure to slight heat, moisture, and pressure for short periods.

Institute, the ascorbic acid was added to a small portion of the mixed meal and this was then bulked with the remainder. In diet 10, one ton of which was prepared by Messrs Robinson, the ascorbic acid was premixed with the minerals and a small amount of bran or grass meal; in diet 19, also 1 ton, the premix of the ascorbic acid was made with a small amount of grass meal or barley meal.

Composition of the diets. The constituents and the theoretical analyses of the diets, calculated from the

Table 1. *The composition and theoretical analysis of the diets*

Diet no. ...	1*	3	4	5	5	7	8	10	11	13	14	18	19
Ingredient	Cubes	Meal	Meal	Pellets	Meal	Pellets and meal	Meal	Pellets and meal	Meal	Meal	Pellets and meal	Pellets	Pellets and meal
Rolled or ground oats	30	30	20	20	20	—	20	—	20	20	—	—	—
Wholemeal flour	35	—	—	—	—	—	—	—	—	—	—	—	—
Bran	—	20	20	20	20	15	20	15	20	20	15	15	15
Barley meal	—	—	—	—	—	—	—	—	—	—	—	20	20
Sugar-beet pulp	—	25	10	—	—	20	—	20	—	—	20	—	—
Ground-nut cake	—	—	—	—	—	15	—	15	—	—	15	15	15
Linseed cake	—	—	—	—	—	10	—	10	—	—	10	10	10
Dried yeast	5	—	10	15	15	—	15	—	15	15	—	—	—
Dried skimmed milk	15	—	10	10	10	—	11	—	10	10	—	—	—
Dried meat and bone meal	10	—	—	—	—	8	—	8	—	—	8	8	9
Dried grass meal	—	20	25	30	30	30	30	30	30	—	—	30	30
Dried lucerne meal	—	—	—	—	—	—	—	—	—	30	30	—	—
Calcium carbonate	1	2	2	2	2	1	1	1	2	2	1	1	—
Sodium chloride	1	1	1	1	1	1	1	1	1	1	1	1	1
Nut oil	—	—	—	—	2	—	2	—	2	2	—	—	—
Calciferol in nut oil 5 mg./l.	—	—	—	2	—	—	—	—	—	—	—	—	—
Cod-liver oil	3	2	2	—	—	—	—	—	—	—	—	—	—
Ascorbic acid	—	—	—	—	—	—	1·5 g./ 10 kg.	11 oz./ ton	3 g./ 10 kg.	—	—	—	11 oz./ ton
	Theoretical analysis (%)												
Crude digestible protein	16·9	8·3	14·7	16·8	16·8	16·0	17·1	16·0	16·8	16·8	18·7	16·5	16·9
Fat	6·3	4·2	4·1	4·2	4·2	4·3	4·2	4·3	4·2	3·9	3·8	4·6	4·7
Soluble carbohydrate	44·4	41·2	38·0	35·8	35·8	32·8	36·4	32·8	35·8	33·0	29·6	33·7	33·7
Fibre	1·1	8·6	6·8	5·9	5·9	9·4	6·0	9·4	5·9	3·2	6·3	6·7	6·7

* Diet 1 also contained a trace of ferric citrate and potassium iodide.

Experimental meal diets. These were mainly prepared in small batches, as required, at the Institute. Some bulk preparations, however, were obtained as controls on pelleted preparations (diets 7, 10, 14 and 19). In these cases, 1 ton of diet was mixed and divided into halves, ½ ton being kept as meal and ½ ton pelleted.

Storage of the diets. Large batches of diets were stored in sacks or in concrete bins with wooden lids at room temperature. Over the period of test no trouble was experienced from pest infestation of the stored diets.

Fortification of the diets with ascorbic acid. In diets 8, 11 and 13, which were prepared at the Ministry of Agriculture and Fisheries *Bulletin* No. 124, are given in Table 1. The ascorbic acid content of the diets and of some of the constituents where figures are available are given in Table 2. In February 1945, sugar-beet pulp became unobtainable and was replaced by barley meal, thus deriving diet 18 from diet 7. This change was common to all animals on test at the time and has not been referred to in the text.

Supplements

Ascorbic acid. This was fed by hand from a pipette. The dosing solution was freshly prepared, as required, in recently boiled glass-distilled water.

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Only a few minutes elapsed between making the solution and completing the dosing.

Dehydrated cabbage. The cabbage, processed by Messrs Chivers & Sons, had been blanched in sulphur dioxide, dehydrated, and packed in nitrogen in sealed tins. One tin was opened at a time for soft and damp it was discarded. Thus the animals were never allowed to have stale cabbage uneaten for long periods. Little difficulty was experienced in persuading non-pregnant adult guinea-pigs to eat enough for growth and maintenance, though occasionally they showed a period of reduced appetite

Table 2. *Ascorbic acid values*

Constituent or diet	Approx. date of analysis	Ascorbic acid mg./100 g. Theoretical	Analytical (corrected for reductones)		
Grass meal:					
Used in diets 3, 4, 5, 8, 10, 11	June 1944	—	5	—	—
Used in diet 7 batch 1	July 1944	—	6	—	—
Used in diet 7 batch 2	Oct. 1944	—	19	—	—
Used in diet 19	June 1945	—	52	—	—
Lucerne meal (used in diets 13, 14):					
Sample from a single sack	Sept. 1944	—	41	—	—
Bulked sample from several sacks	Jan. 1945	—	60	—	—
Dehydrated cabbage:					
Standard	Aug. 1945	—	250	—	—
Substandard 'scorched'	Mar. 1945	—	178	—	—
Substandard 'high SO_2'	Mar. 1945	—	256	—	—
Diet 7 batch 2 pellets	Oct. 1944	5·7	3	—	—
Diet 10 batch 2 meal	June 1944	31·5	31	—	—
After 4½ months' storage	—	—	—	In air 8	—
Diet 10 batch 2 pellets	June 1944	31·5	8	—	—
After 4½ months' storage	—	—	—	3	—
Diet 19 meal	June 1945	45·6	43	—	—
Diet 19 pellets	June 1945	45·6	9	—	—
Diet 14 meal	Nov. 1944	18	22	—	—
2nd sample	Dec. 1944		22		
After 1½ months' storage	—	—	—	In air 19	In N_2 17
After 3 months' storage	—	—	—	12	14
After 7 months' storage	—	—	—	3	4
Diet 14 pellets	Nov. 1944	18	10	—	—
2nd sample	Dec. 1944		9		
After 1½ months' storage	—	—	—	6	7
After 3 months' storage	—	—	—	3	3
After 7 months' storage	—	—	—	1·5	1·6
Diet 3	—	1	—	—	—
Diet 4	—	1·25	—	—	—
Diet 5 meal	—	1·5	—	—	—
Diet 5 pellets	—	1·5	—	—	—
Diet 7 batch 1 pellets	—	1·8	—	—	—
Diet 8	—	16·5	—	—	—
Diet 11	—	31·5	—	—	—
Diet 13	—	18	—	—	—
Diet 18. No values available	—	—	—	—	—

feeding, and the contents, 3 lb., lasted about 10 days, during which time it was assumed that little or no deterioration would have taken place. It was fed separately as a weighed dose in the dry state. Any of a day's dose which remained uneaten when the next dose was given was weighed and if still crisp was left to make up part of the next dose. If it was and would not eat the full daily allowance. The high ascorbic acid content of the dried cabbage, however, was sufficient to satisfy their needs in spite of this, for there was no break in the growth responses of the individuals concerned. Young guinea-pigs, at weaning, did not readily eat the dried cabbage at first, and it was necessary to remove the diet overnight for

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several days; all learnt to eat it eventually. In pregnancy, when the vitamin C requirement rises greatly, the maximum intake as determined by individual appetite was not always adequate.

At first, both the ascorbic acid and the dried cabbage were fed daily. Later, when it had been established that the consumption of the latter was satisfactory, dosing was reduced to three times a week, the amount given at each dose being adjusted so as to leave unchanged the total weekly intake. Zilva (1941) showed that ascorbic acid fed daily, every second day or every third day, was equally effective in protecting guinea-pigs receiving a scorbutic diet.

Cod-liver oil. Cod-liver oil, fed by pipette directly into the animal's mouth, was given at a dose level of 1 ml. weekly in two equal doses.

RESULTS

Food consumption

Diets in meal form were spilt to such an extent that it was sometimes necessary to fill the pots twice a day, and it proved impossible to obtain a reliable estimate of food eaten. On the other hand, pelleted diets were spilt but little, unless the pots were overfilled, and food consumption could be measured fairly accurately. Observations were made on several groups of animals at different ages and in different nutritional states. The combined results are shown in Table 3. Food intake is related to body weight, as can be seen from the table, but too few figures are available to calculate the exact relationship. The failure of appetite which accompanies severe vitamin C deficiency is illustrated by the two animals receiving unsupplemented diet 7, and the rapid recovery of appetite when the deficiency is relieved is shown by the same two guinea-pigs after receiving ascorbic acid and by the four guinea-pigs receiving diet 14. For normal adult guinea-pigs weighing from 800–900 g. the average daily consumption of dry diets of this type is about 35 g. per animal.

Growth of guinea-pigs on dry diets without supplement

In the first experiment an attempt was made to rear guinea-pigs on a dry diet which contained dried grass, supplemented only with hay and water (diet 3). The sample of dried grass used was said to have an ascorbic acid content of 60 mg./100 g.; it was added to the diet at a level of 20 % which was expected to supply 3–4 mg. of vitamin C per guinea-pig per day. The results are shown in Fig. 1. After a short period of normal growth, the animals lost condition rapidly, developed typical scorbutic symptoms and deaths began to occur in about 4 weeks. It was evident that the diet was quite inadequate. Attempts were made to improve the diet by increasing the level of dried grass and raising the protein content by the introduction of dried yeast and dried skimmed milk in place of sugar-beet pulp (diets 4 and 5). With both these diets the results (Figs. 2 and 3) were somewhat similar to those with diet 3 (Fig. 1), but there was a slight and successive improvement in each case. A small portion of diet 5 was pelleted and gave good growth; but when the pellets were replaced by meal, growth immediately slowed down. Scorbutic symptoms on this diet, however, did not develop for a further 4–5 weeks.

It was clear that the diets were all seriously deficient in ascorbic acid and that much of the vitamin originally present in the grass meal must have been lost during storage. It seemed possible that the failure of these diets could be explained solely by the poor quality of the grass meal used. Confirmation of this was given by a chemical analysis made shortly after the feeding experiments were completed, by which time the grass meal was found to contain only 5 mg. of ascorbic acid per 100 g. (Table 2).

Nevertheless, two suggestive points emerged from these experiments. First, the cessation of growth when pelleted diet 5 was replaced by freshly prepared meal of identical composition demonstrated the importance of the physical state of the diet. Later experiments confirmed that a diet in pellet form is much more acceptable to guinea-pigs than a diet in meal form. The pelleting process is not, however, satisfactory for small batches and many of the experimental diets had to be used in meal form. Secondly, in all these experiments, the immediate growth of the animals before the onset of scurvy was slightly better than that of the controls over the same period. This suggested that the growth rate on the stock diet might be susceptible to improvement and that the high protein content of the experimental diets might be justified. A direct test of this point was made.

Growth of guinea-pigs on a high protein diet supplemented with fresh greens

A diet in cube form (diet 1), relatively rich in protein, which had given satisfactory results as a stock maintenance diet for rats and mice (Parkes, 1946) was used. The diet is completely deficient in ascorbic acid, and fresh greens were given each day. Hay was also given, as the rat diet has a very low fibre content. Evidence is conflicting as to the need of guinea-pigs for bulk in the diet. Kohler, Elvehjem & Hart (1938) succeeded in raising guinea-pigs on an all-liquid diet of mineralized milk, orange juice and grass juice, and they conclude that 'guinea-pigs do not need roughage in the diet, at least when milk is used as the basal ration'. Hogan & Ritchie (1934),

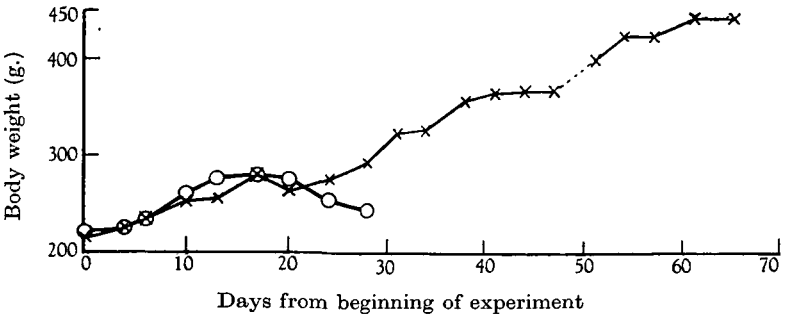

Fig. 1. Growth of guinea-pigs on diet 3 compared with that on stock diet. Twelve animals on each. ○ = Diet 3 and water. × = Bran/sugar-beet mash and greens. The dotted line indicates the death of an animal.

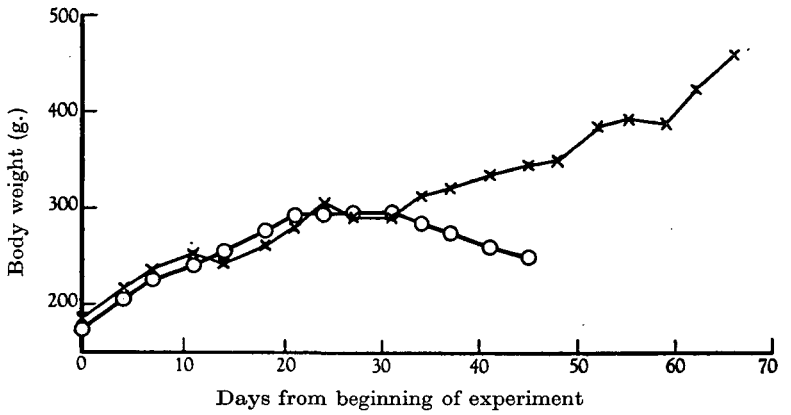

Fig. 2. Growth of guinea-pigs on diet 4 compared with that on stock diet. Twelve animals on each. ○ = Diet 4 and water. × = Bran/sugar-beet mash and greens.

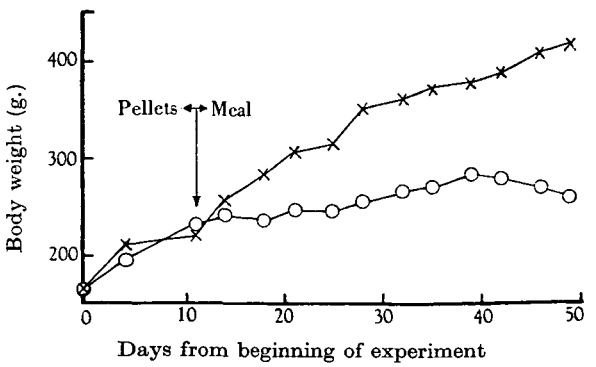

Fig. 3. Growth of guinea-pigs on diet 5 pellets (11 days) followed by diet 5 meal compared with that on stock diet. Six animals on each. ○ = Diet 5 pellets or meal and water. × = Bran/sugar-beet mash and greens.

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on the other hand, state that guinea-pigs need more bulk than rabbits and found that the former failed to thrive on a diet containing only 3 % of cellulose.

The results of feeding diet 1 with greens and hay to guinea-pigs are shown in Fig. 4. Growth rate (5·9 g. per day) was better than that (4·7 g. per day) of the controls receiving stock diet and fresh greens. The experiment was stopped after 8 weeks; it had shown that an improvement of the growth rate on stock diet could be effected.

The failure of even 30 % of dried grass to supply adequate vitamin C in the diet, the difficulty of obtaining grass meal of good quality, and the rapidity with which its ascorbic acid is lost on storage, made it apparent that other means of adding vitamin C to the diet must be sought and a series of experiments were planned to study the effect of fortifying the diet with synthetic ascorbic acid.

Growth of guinea-pigs on diets freshly fortified with ascorbic acid

Diet 5, which had given the most promising results in the early experiments, was fortified with ascorbic acid at a level calculated to give an average daily intake of 5 mg. per animal (diet 8). On this diet, made up in small batches, the animals throve and growth was equal to and more regular than that of the controls receiving stock diet (Fig. 5). The result showed that the elimination of fresh greenstuff was a practical possibility provided that the diet contained sufficient vitamin C. The experiment was stopped after 20 weeks. Diet 8 was expensive and appropriate modifications were introduced. For diet 7, the dried yeast and dried skimmed milk were replaced by ground-nut meal and meat and bone meal; linseed cake was added to provide a source of the accessory food factors for the guinea-pig described by Woolley (1942). One ton of diet 7 was prepared of which half was pelleted and the other half left as meal. Ascorbic acid, in amounts calculated to give an average daily intake of 10 mg. per animal, was added to some of the meal (diet 10) and, for comparison, diet 5 was fortified with ascorbic acid to provide an average daily intake of 10 mg. per animal (diet 11).

A direct comparison was made of the growth of guinea-pigs on diet 7 meal, diet 7 pellets, diet 10 meal, diet 11 meal, and on diet 7 pellets supplemented with fresh greens but without water. The results are shown in Figs. 6 and 7. There was only one group of control animals, and for ease of comparison their weight increase has been plotted separately against that of each of the experimental groups. The two groups of animals on diet 7 alone, either pelleted or as a meal, developed scurvy rapidly. The responses of the two groups were almost identical and only that of the group receiving meal is shown in the figure (Fig. 6). When diet 7 was supplemented with fresh green food, but without water (Fig. 6), growth was less good throughout than in the controls. One animal died after 79 days. It was evident that fresh green food was unable to meet the animal's requirement for water with a dry diet of this type. These results are similar to those obtained with rabbits under similar conditions (Bruce & Parkes, 1946). By contrast, both groups of animals receiving the fortified diets showed very good growth, better than that seen with the stock diet, in spite of the fact that both diets were in meal form (Figs. 6, 7).

Large-scale preparation of diets fortified with ascorbic acid

Diet 10 was prepared in bulk (1 ton) and half of it was pelleted, the remainder being kept in meal form. Chemical determinations of the ascorbic acid content of the two diets showed that over 70 % of the added ascorbic acid had been lost on pelleting (Table 2). The pellets were found to contain only 8 mg. of ascorbic acid per 100 g. as compared with 31 mg. per 100 g. for the unpelleted mixture.

Feeding tests were carried out on the fortified pelleted diet at intervals. Freshly prepared diet 10 either as pellets or as a meal supported good growth for about 14 weeks (Fig. 8), at the end of which time the animals had reached a weight of about 700 g.; response to the pelleted diet was again slightly better than the response to the meal although the latter had a higher ascorbic acid content. Increase in weight above 700 g. was exceedingly slow, but no symptoms of deficiency other than the retarding of growth were seen.

In a later test carried out after two months of storage, the pelleted diet was only able to support growth for from 7 to 8 weeks before some of the animals began to lose weight (Fig. 9) and had to be given supplements. Much individual variation was found among this group. It was evident that in spite of the continued slow but steady increase in weight of the older animals (Fig. 8) which had now received diet 10 for 12 weeks, the diet was becoming deficient in ascorbic acid loss of which had taken place during storage. Chemical analysis of the diet made after 4½ months confirmed the findings of the biological test (Table 2).

A final experiment started when the diet had been stored for 5 months showed (Fig. 10) that by this time nearly all the ascorbic acid had been lost. Growth was maintained for less than 3 weeks before a rapid decline set in and the animals had to be given a supplementary source of vitamin C. Dehydrated cabbage was eaten readily, in spite of the

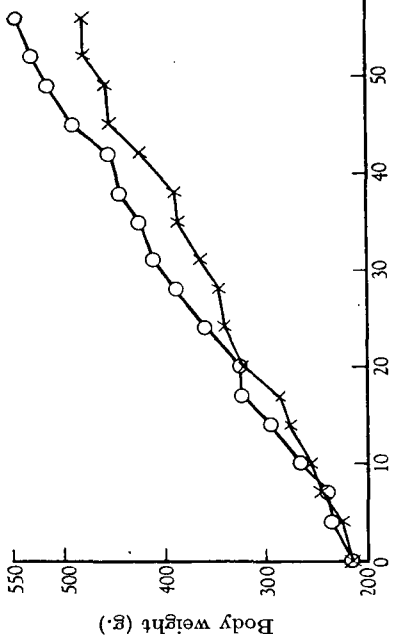

Fig. 4. Growth of guinea-pigs on diet 1 cubes supplemented with fresh greens compared with that on stock diet. Six animals on each. ○ = Diet 1 cubes, fresh greens and water. × = Bran/sugar-beet mash and greens.

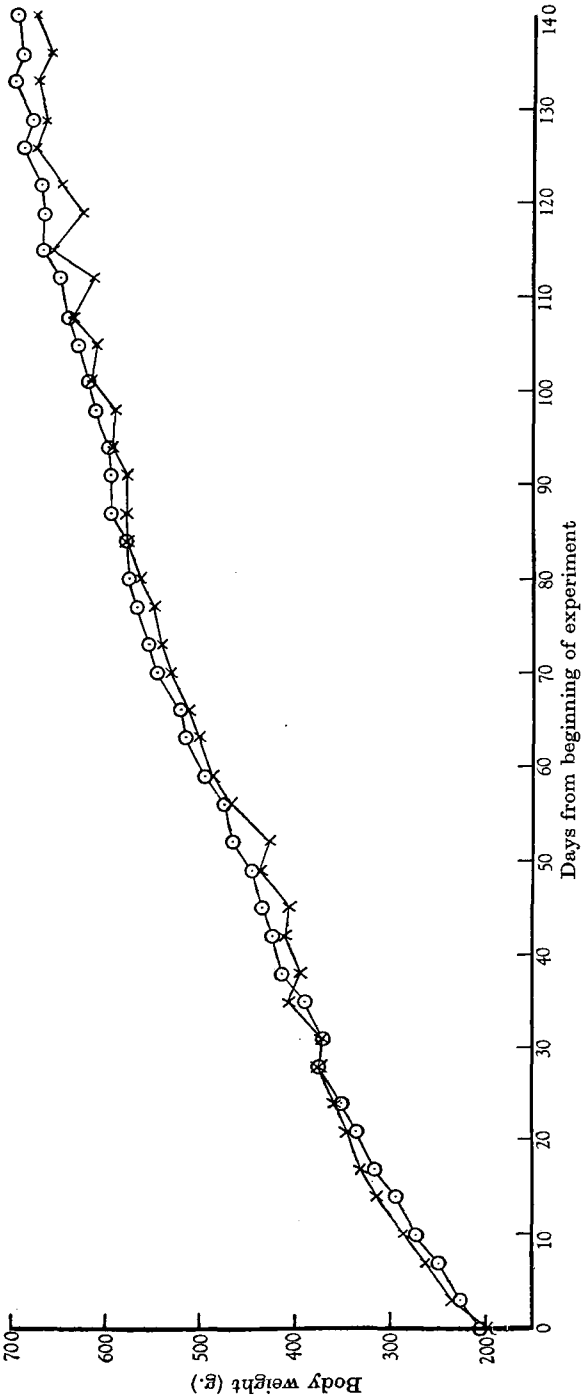

Fig. 5. Growth of guinea-pigs on diet 8 compared with that on stock diet. Six animals on each. ⊙ = Diet 8 and water. × = Bran/sugar-beet mash and greens.

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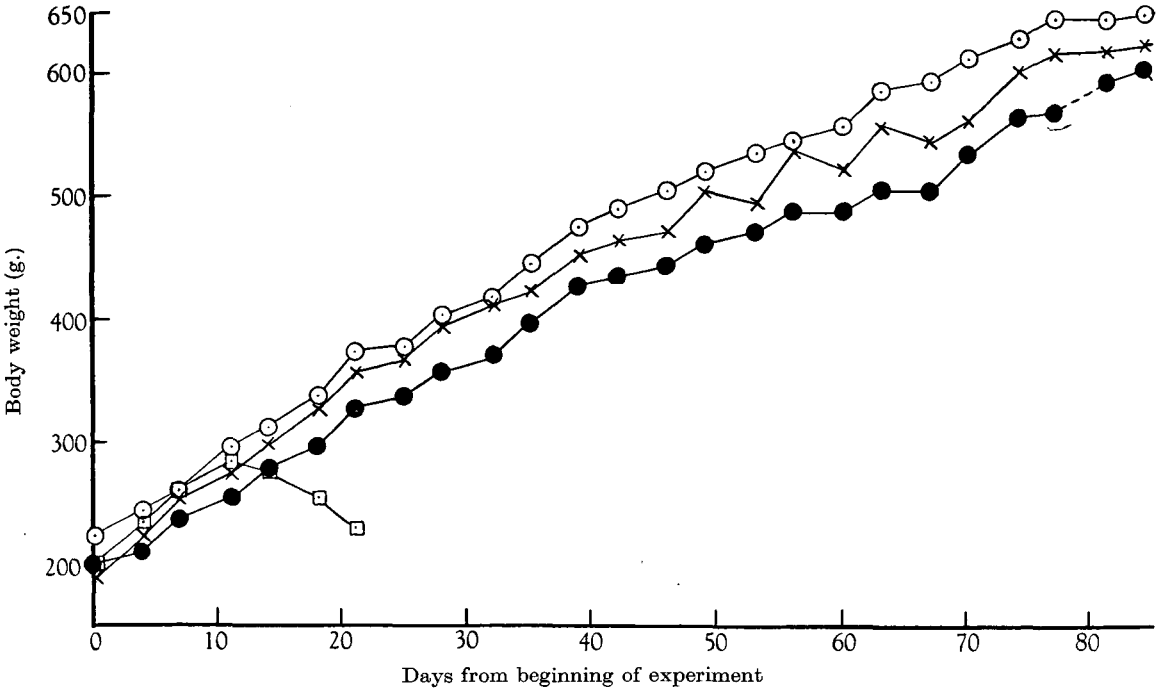

Fig. 6. Growth of guinea-pigs on diet 7 meal, diet 7 pellets with greens but no water, and diet 10, compared with that on stock diet. Six animals on each. N.B. Same control group as in Fig. 7. ⊡ = Diet 7 meal. ● = Diet 7 pellets with greens but no water. ⊙ = Diet 10. × = Bran/sugar-beet mash and greens. The dotted line indicates death of one member of the group.

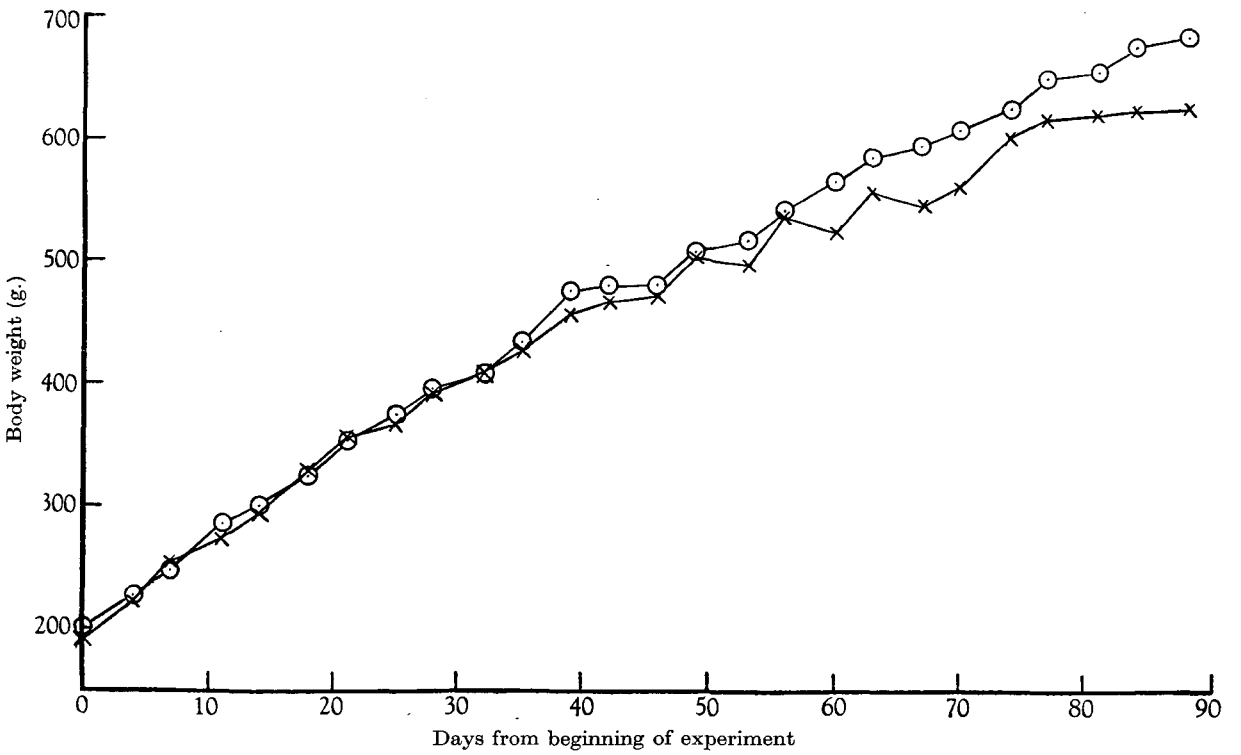

Fig. 7. Growth of guinea-pigs on diet 11 compared with that on stock diet. Six animals on each. N.B. Same control group as in Fig. 6. ⊙ = Diet 11 and water. × = Bran/sugar-beet mash and greens.

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failure of appetite usually associated with vitamin C deficiency, and growth restarted at once.

Most striking of the results of these three experiments was the great tolerance exhibited by the adult animals to the shortage of ascorbic acid which developed gradually due to storage of the diet. Dann & Cowgill (1935) state that the vitamin C requirement of guinea-pigs is directly proportional to body weight, and is almost exactly 1 ml. lemon juice per 100 g. (equivalent to about 0·5 mg. ascorbic acid per 100 g.). Accordingly, animals weighing about 700 g. should require 3·5 mg. ascorbic acid daily. Diet 10 was undoubtedly not able to supply anything like this amount after 16–17 weeks of storage. The younger animals (Test 2) began to lose weight at from 500 to 600 g., showing that diet 10 was unable to supply the 2·5–3·0 mg. daily required to maintain that body weight; yet the adults receiving precisely the same diet—these experiments were carried out concurrently—were able to maintain a weight of 700 g.

Even more striking was the further comparison made with the third test. The young guinea-pigs which received diet 10 after it had been stored for 21 weeks began to lose weight as rapidly as if the diet contained no ascorbic acid, and this was nearly 4 weeks before the adults started to lose weight.

It was evident from these results that for bulk preparation of diets, synthetic ascorbic acid was unsuitable. There remained the possibility that the pre-mixing of the ascorbic acid with the minerals in diet 10, by providing an alkaline medium within the diet, accentuated the effect of the unavoidable heat and moisture, and caused a greater destruction than would have resulted from the pelleting process alone. To check this, a batch of diet was prepared from which the calcium carbonate was omitted and in which the pre-mix of the ascorbic acid was made with grass or barley meal (diet 19). Half was pelleted and the other half was kept as meal. Chemical analyses showed that the destruction of the ascorbic acid was of the same order as in diet 10, and the idea of fortification of the pellets with synthetic ascorbic acid was abandoned, and a better source of natural vitamin C was sought.

The possibility of using a fruit juice, blackcurrant, rose-hip, or concentrated orange juice, was ruled out on grounds of expense and inconvenience. Two other possible sources were suggested: (i) dried lucerne meal, which may have a high ascorbic acid content, and (ii) dehydrated cabbage.

A detailed study of the ascorbic acid content of lucerne, and its behaviour on drying and on storage has been made by Marston, Quinlan-Watson & Dewey (private communication). According to these authors a good quality sample of lucerne meal, dried under optimum conditions, should contain between 0·65 and 1·0% of ascorbic acid, which might be preserved for considerable periods. The only sample available for the present experiments had a comparatively low content of ascorbic acid, about 60 mg. per 100 g. (Table 2), and it was necessary to incorporate this material in the diets at the same level as the dried grass.

Growth of guinea-pigs on diets containing lucerne meal

Two diets were prepared containing lucerne meal in place of dried grass meal: diet 13, otherwise similar to diet 5 and prepared in meal form at the Institute, and diet 14, otherwise similar to diet 7 and prepared in bulk in both meal and pellet form. Chemical determinations of the ascorbic acid content of diet 14 carried out when it was freshly made showed a close agreement between the calculated and analytical values for the meal and also showed that the natural ascorbic acid of the lucerne meal was as easily destroyed by the pelleting process as the synthetic ascorbic acid. Diet 14 meal contained 22 mg. ascorbic acid per 100 g.; diet 14 pellets contained only 10 mg. per 100 g. (Table 2).

Growth on diet 13 was quite good, 5 g. per day, for about 8 weeks (Fig. 11), by which time the average weight was about 500 g.; after this growth fell to just over 1 g. per day, at which it was maintained until the end of the experiment. These animals became exceptionally thirsty and the break in the curve between the 16th and 17th week can be explained by a temporary shortage of water. The 500 ml. hitherto sufficient for three of these animals became inadequate; when the daily allowance was doubled, growth was resumed. After 196 days the experiment was stopped.

Results with diet 14 were disappointing (Fig. 12). The animals which received the meal showed only poor growth from the start. Two deaths occurred, one after 35 days, the other after 123 days. In neither case was there an appreciable fall in weight before death or macroscopic signs of scurvy. The animal which died at 123 days weighed only 350 g. None of the animals had reached 600 g. by this time and the experiment was stopped. Growth on the pelleted diet was promising for the first 3 weeks; after this it ceased suddenly and the animals began to lose weight; one animal died on the 39th day with severe scorbutic lesions of both knees. At this point it was evident that the animals were seriously short of vitamin C, and supplementary feeding with dehydrated cabbage was started. Tests at this time showed that the animals were only eating about 20 g. of diet daily (Table 3) an amount considerably below normal for such animals. Ascorbic acid was therefore given in addition to dehydrated cabbage. Recovery of appetite was rapid and after 14 days ascorbic acid dosing was discontinued. By this time the animals were eating well over 1 g. dehydrated

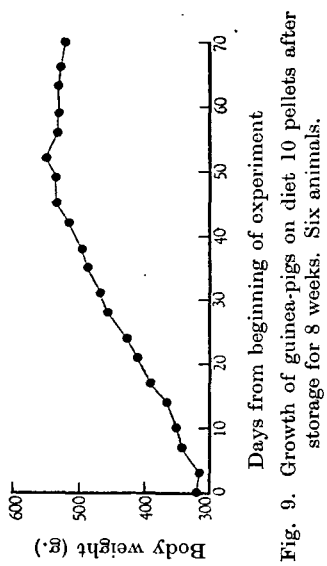

Fig. 9. Growth of guinea-pigs on diet 10 pellets after storage for 8 weeks. Six animals.

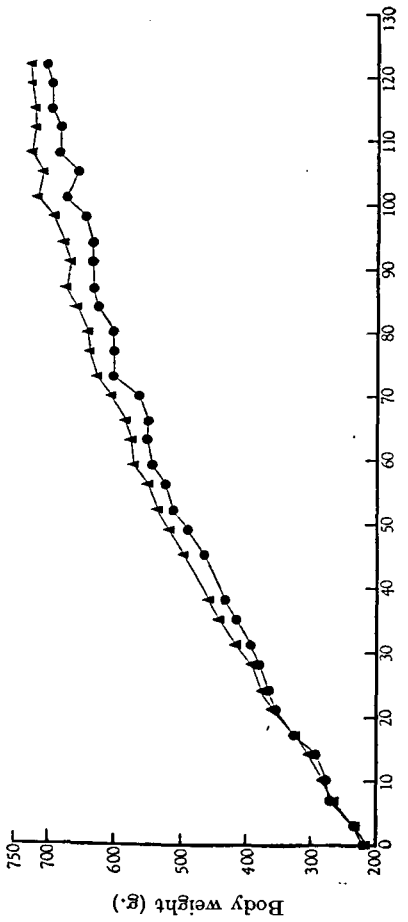

Fig. 8. Growth of guinea-pigs on diet 10 pellets compared with that on diet 10 meal freshly made. Six animals on each. (Early part of Fig. 15.) ▲ = Diet 10 pellets, water and hay. ● = Diet 10 meal, water and hay.

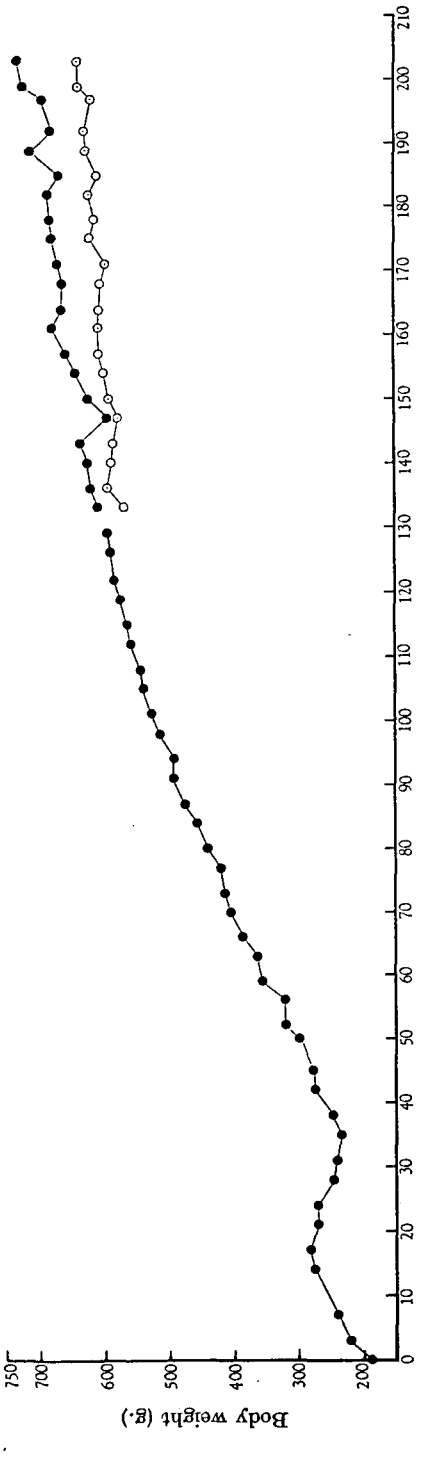

Fig. 10. Growth of guinea-pigs on diet 10 pellets after storage for 5 months. Six animals. Arrow *A* indicates addition of dehydrated cabbage. Arrow *B* indicates replacement of dehydrated cabbage by ascorbic acid for half the animals.

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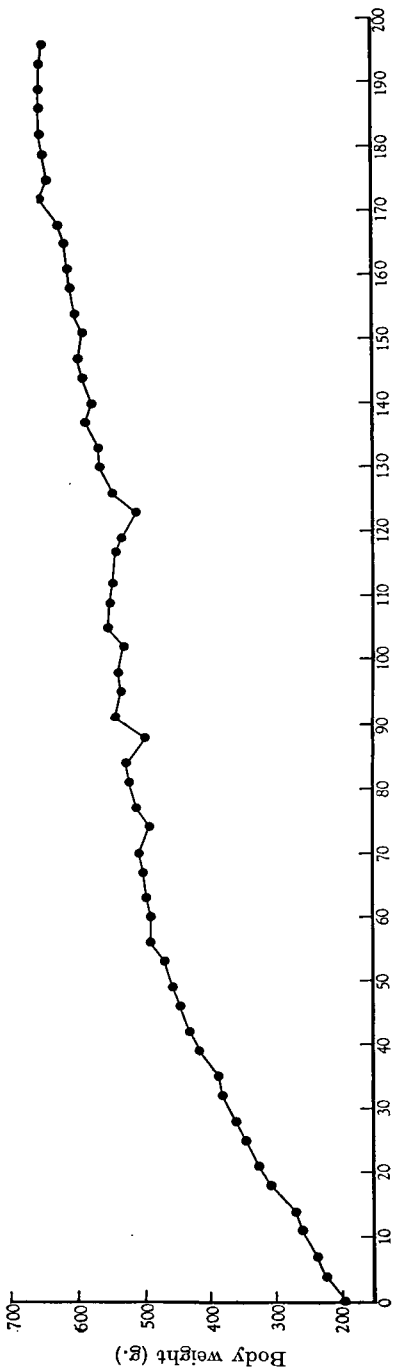

Fig. 11. Growth of guinea-pigs on diet 13 meal. Six animals.

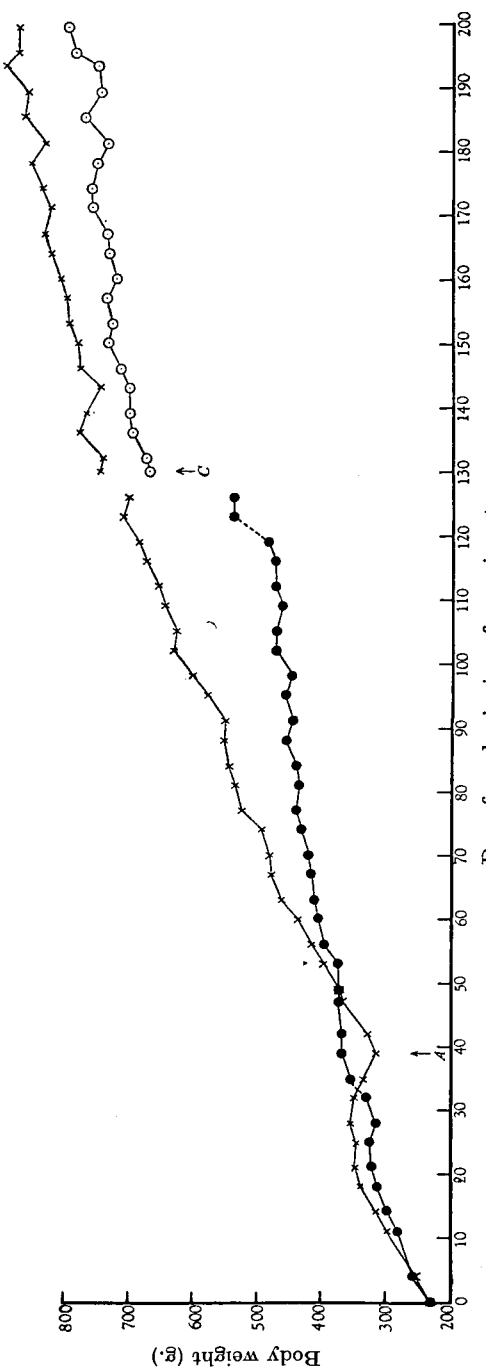

Fig. 12. Growth of guinea-pigs on diet 14 meal and pellets. Five animals on each. ● = Diet 14 meal. × = Diet 14 pellets. Supplementary feeding with dehydrated cabbage and dosing with ascorbic acid was started at *A*. At *B* the ascorbic acid dosing was stopped. Arrow *C* indicates replacement of dehydrated cabbage by ascorbic acid for half the animals. The dotted line indicates the death of an animal.

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cabbage daily, equivalent to 2·5–3·0 mg. ascorbic acid. Growth was resumed as soon as the supplements were started. The results will be discussed in a later section.

In view of the fact that there seemed to be a considerable amount of ascorbic acid in diet 14 even after pelleting, the development of scurvy was entirely unexpected. A second sample of the diet analysed as soon as the scorbutic symptoms appeared confirmed the original value found by chemical analysis, namely 10 mg. ascorbic acid per 100 g. A daily food intake of 20 g. of diet should have provided about 2 mg. ascorbic acid, enough to give complete protection from scurvy for at least 90 days (Zilva, 1941). This discrepancy between biological response and chemical analysis has not yet been explained. The behaviour of the animals receiving the pelleted diet suggests that the poor response to diet 14 meal was also due, at least in part, to a shortage of ascorbic acid. This group of animals may have been particularly sensitive or there may be some constituent in the diet which prevented the guinea-pigs from obtaining the ascorbic acid present. It has not been possible to repeat the experiment. Should the discrepancy be confirmed it will be an exception to the extensive agreement so far reported between the two methods of assay (Harris & Olliver, 1942).

A further reduction in the ascorbic acid content of diet 14 took place on storage even when the samples were kept in nitrogen (Table 2). This confirmed the suspicions aroused by the low ascorbic acid content of the sample when it was obtained, that the original dehydration process had failed to destroy the oxidases. Hence the loss on storage of the diet was likely to be considerable since there would be destruction due both to the presence of enzymes in the lucerne meal constituent and to the presence of the moisture (6–7 %) in the diet. Marston, Quinlan-Watson & Dewey reported a fairly rapid destruction of ascorbic acid when the sample contained 11 % moisture, but a slow destruction in samples containing up to 4 % of water packed in an inert atmosphere.

In view of the failure of synthetic ascorbic acid and of the natural product in dried grass and dried lucerne meal to withstand pelleting and storage under practical conditions, it seemed unlikely that the ascorbic acid of dehydrated cabbage would behave differently, even though the product at the start was of first quality and had been prepared under optimum conditions to ensure destruction of the enzymes. No attempt was therefore made to incorporate dehydrated cabbage into a diet, and feeding tests were limited to its use as a supplement to avoid the seasonal variations in quality and quantity which are inevitable with fresh greenstuff.

Table 3. *Consumption of pelleted diets by guinea-pigs*

Diet	No. of guinea-pigs	Approx. age at start of period (months)	Length of period recorded (days)	Approx. weight change during period (g.)	Average daily consumption (g.)	Physiological state
18 + supplement	18	1	10	240–290	29	Normal
14 + supplement	2	2½	11	310–337	20	Recovering from vitamin C deficiency
	—	—	10	337–400	29	
	2	2½	11	375–405	20	Records started when the supplement was given
	—	—	10	405–470	29	
10 + supplement	2	9	41	810–930	34	Normal
	2	9	41	860–990	42	Normal
7 + supplement	8	11–13	25	850–890	36	Normal
	—	—	38	890–940	38	
7 alone	2	9	35	910–840	35	Developing vitamin C deficiency
7 + ascorbic acid	—	—	10	840–856	37	Recovering from vitamin C deficiency
	—	—	10	856–890	46	
	—	—	8	890–915	48	
7 alone	2	11–13	28	780–700	Diet always fouled. No record	Developing vitamin C deficiency
	—	—	4	700–645	5	Severe vitamin C deficiency
	—	—	6	645–death	None	

Growth of guinea-pigs on diet 7 supplemented with dehydrated cabbage

A direct comparison was made between dehydrated cabbage and fresh greens as supplements to

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diet 7 with twelve animals (six males and six females) in each group. Growth up to the time the animals were ready for breeding is shown in Fig. 13. One female receiving fresh greens failed to grow beyond 230 g. and died after 33 days. This animal has been discarded from the group. The growth rate of the animals receiving dehydrated cabbage, 5·3 g. per day, was, throughout, slightly less good than that of the group receiving fresh greens, 6·9 g. per day, but was sufficiently encouraging to justify further consideration. Standard dehydrated cabbage is not at present available and, at best, might cost several hundred pounds a ton, but it was suggested to us that sub-standard material, withheld from human consumption, might be suitable for animal feeding, and when available would be much less expensive.

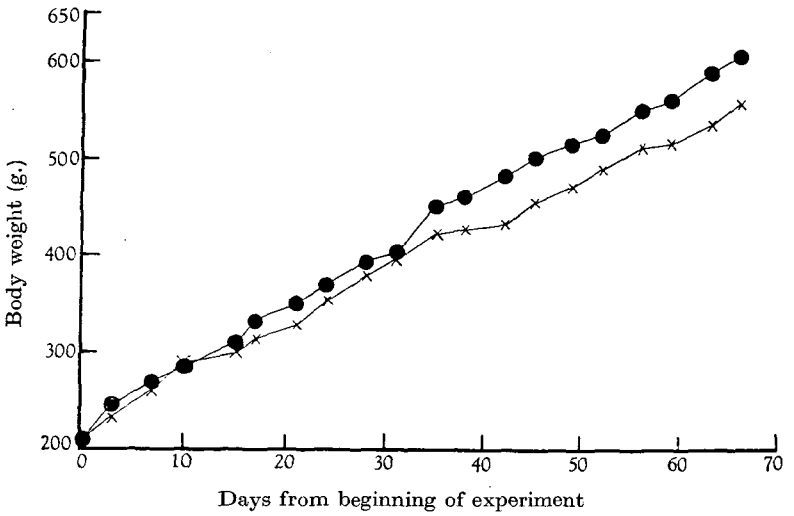

Fig. 13. Growth of guinea-pigs on diet 7 pellets supplemented with fresh greens, and on diet 7 pellets supplemented with dehydrated cabbage. Six male and six female animals on each. ● = Diet 7 and fresh greens. × = Diet 7 and dehydrated cabbage.

We have had the opportunity of testing such material. One sample had an abnormally high sulphur dioxide content, and the other had been scorched during dehydration. The ascorbic acid content of the two samples are given in Table 2. If the cost of this material were about £100 per ton, a daily ration per guinea-pig of 5 g. would cost about $\frac{1}{10}d.$, considerably less than the cost, at current prices, of the ration of fresh green food.

The results of feeding the sub-standard material are shown in Fig. 14. There was little difference over the period studied (14 weeks) between the growth of the animals on standard cabbage and that of those on the high sulphur dioxide material. The latter, however, seemed slightly unpalatable and the maximum daily intake was about 1·7 g., equivalent to about 4 mg. of ascorbic acid. This amount was more than enough for growth at this age, but it is doubtful whether as the body weight increased the animals would have continued to eat sufficient to meet their needs. The scorched sample proved definitely unpalatable; the maximum intake was 1 g. daily, equivalent to less than 2 mg. of ascorbic acid, and growth was unsatisfactory. No such difficulty was encountered with the standard material; by the end of the experiment the animals were eating readily up to 5 g. daily, equivalent to about 12 mg. of ascorbic acid.

Reproduction of guinea-pigs receiving dehydrated cabbage as a supplement of diet 7

Six females and six males which had received standard dehydrated cabbage from weaning were mated. The animals were housed in pairs and it was not possible to obtain individual records for the consumption of dried cabbage. The values referred to are average intakes, assuming that both animals of a pair were eating the dehydrated cabbage in equal amounts. All six females became pregnant. Three, having only a restricted intake of dehydrated cabbage showed failures typical of vitamin C deficiency. One female had four normal embryos, but died from intrauterine haemorrhage during gestation. For the 12 days before death this animal had been eating sufficient dehydrated cabbage to supply 11–12 mg. of ascorbic acid each day, but during the early part of gestation intake had been much lower than this. Two other females aborted, both of these ultimately produced normal young when their intake of dried cabbage increased. The remaining

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three animals produced normal young. In all, five litters were born with a total of sixteen young of which two were born dead, three died before weaning, and eleven were reared. The average weight of live young at birth was 84 g. with a range of 60–130 g. The young reached a weight of 200 g. in an average of 16 days, with a range of from 8 to 20 days. They continued to eat dehydrated cabbage readily after weaning, and as early as 4 weeks old when about 300 g. in weight they were eating 4–5 g. daily. The average weight increase of these young guinea-pigs was 6–7 g. per day from birth until 5 weeks of age, when the record was discontinued. In spite of this good growth, the young were not in good condition. Their coats were rough rather than smooth and glossy, and the animals were somewhat listless.

It is unsatisfactory to draw conclusions from such small numbers, but these results suggest that while normal reproduction is possible in guinea-pigs fed on dehydrated cabbage, individual variations in appetite might cause serious difficulties in large-scale work. Breeding animals in particular might fail to eat sufficient dehydrated cabbage to supply their excessive needs for vitamin C in pregnancy.

Reproduction on diet 7 supplemented with fresh greenstuff will be reported in a later paper.

Supplements necessary with diet 7

In the course of the work recorded above, one unexplained death occurred after 102 days of an animal maintained on freshly made diet 10 meal, and one after 79 days of an animal maintained on diet 7 supplemented with fresh greens. This raised the question of whether diet 7 might have deficiencies for guinea-pigs other than that of vitamin C, especially since a multiplicity of factors allegedly required by guinea-pigs have been described in the literature. Moreover, the animals used in some of the earlier feeding tests were given diet 10 pellets at the end of the original experiment, and some rather odd results were obtained. This diet was short of vitamin C at the time, and a high mortality occurred among the animals, mostly after from 7 to 9 weeks. Not all animals, however, lost weight before death or showed scorbutic lesions on post-mortem examination, neither were any signs of infection detected. Moreover, no deaths had been recorded over the same period among animals receiving the stock diet and fresh greens.

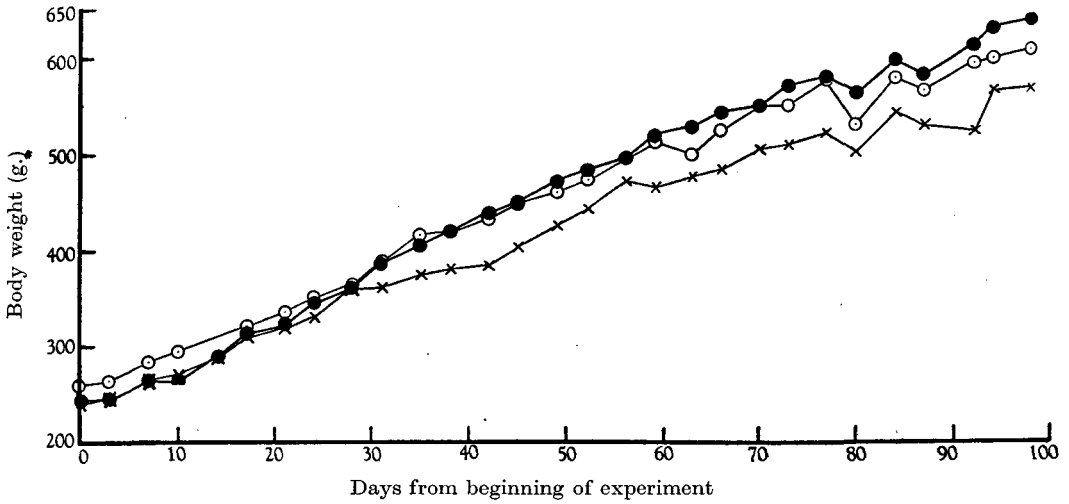

Fig. 14. Growth of guinea-pigs on diet 7 pellets supplemented with standard, high SO_2 or scorched dehydrated cabbage. Six animals on each. ● = Diet 7 and standard dehydrated cabbage. ⊙ = Diet 7 and high SO_2 dehydrated cabbage. × = Diet 7 and scorched dehydrated cabbage.

The first attempts to prevent deaths among the remaining animals failed to give very conclusive results. Growth, in two animals, one of which had shown a typical fall in body weight, the other a fluctuating weight between 650–700 g. for about 14 weeks, was resumed slowly when a daily supplement of fresh greens was given, but only after a period of 6–7 weeks. In two more animals, cod-liver oil, given to supply additional vitamin A, failed to arrest the decline in weight. After 14 days the cod-liver oil was replaced by ascorbic acid, 10 mg. per day, and the decline in weight stopped immediately; growth, however, was not resumed. After 40 days, the ascorbic acid was in turn replaced by dehydrated cabbage. One animal died within 4 days, having refused to eat the dried cabbage; the other

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immediately started to grow again. Of three animals which were given supplements of dehydrated cabbage without previous dosing with ascorbic acid, one died after 30 days, although the dried cabbage had apparently been eaten well; the other two showed a fluctuating and very gradual increase in weight. Thus none of the three supplements tried had been indubitably successful; even the response to fresh greens was reluctant and not unbroken. Dehydrated cabbage had been successful only in three out of five cases. Ascorbic acid had been able on storage. The muscular dystrophy associated with the feeding of cod-liver oil to herbivora develops only with synthetic diets. Stock diets, composed of natural foods, were able to cure as well as to prevent the lesions developing in guinea-pigs which were receiving 0·5 ml. cod-liver oil daily in the experiments of Madsen, McCay & Maynard (1935). There seemed little danger therefore in using cod-liver oil as a source of vitamin A which would not also supply other possible factors required by the guinea-pig.

Table 4. *Response of individual animals to supplements*

Animal no.	No. of days to reach max. wt.	Total loss of wt. before dose (g.)	Total period of loss in wt. (days)	Supplement	No. of days dosed	Result
134	52	60	14	Fresh greens	155	Growth started again after 5 days; total response 310 g. in 150 days
137	98	80	24	Ascorbic acid	4	No change
		110	28	↓ Dried cabbage	94	Growth started again after 7 days; total response 350 g. in 87 days
133	45	80	18	Cod-liver oil	17	Continued loss, 90 g. in 18 days
		170	36	↓ Ascorbic acid	48	Loss of weight immediately arrested; growth started after 3 days; total response 180 g. in 48 days
				↓ Dried cabbage	87	Immediate loss of 70 g. in 4 days, followed by growth; total response 250 g. in 87 days
138	63	110	14	Diet 13	10	Continued loss in weight; refused to eat
		210	24	↓ Ascorbic acid	11	Continued loss in weight; died after 12 days of ascorbic acid dosing
136	101	210	14	Ascorbic acid	2	Died after 2 days
135	108	90	14	Ascorbic acid 10 mg.	57	Weight fluctuated about a mean of 640 g. for 57 days
				↓ 20 mg.	> 220	Weight fluctuated about a mean of 640 g. for a further 46 days; growth then restarted slowly and is still being maintained after more than 12 months.

to arrest the decline in weight but not to initiate growth for the period studied. In view of the slow response to fresh greens it seemed possible that the ascorbic acid doses had not been continued long enough, but it seemed that these failures might not be due to uncomplicated vitamin C deficiency, especially in view of the fact that recovery from scurvy is usually rapid when vitamin C is supplied.

Further attempts to settle this problem were made by comparing the effectiveness of fresh greens, dehydrated cabbage and ascorbic acid as supplements to diet 7. In addition, a few more animals were given cod-liver oil, to cover the possibility that a deficiency of vitamin A might also have developed in the diet

The great variation in the development of scurvy in animals receiving diet 10 after 2 months of storage, to which reference has already been made, made it impossible to treat these animals as a single group. Individual treatments and responses have been tabulated (Table 4), and it can be seen that in animals nos. 136 and 138, the deficiency once started developed with great rapidity and was too severe to respond to treatment by the time the supplements were given. Dehydrated cabbage was as effective as a curative agent as fresh greens (nos. 134, 137); one animal (no. 133) gave an immediate growth response to ascorbic acid which was maintained, but at a slightly slower rate when

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dehydrated cabbage was substituted for ascorbic acid, and finally, one animal (no. 135) made a very slow recovery on ascorbic acid alone. This animal now weighs over 1 kg. and has received ascorbic acid alone as a supplement to diet 7 for over 12 months.

Further evidence that diet 7 satisfies the nutritional needs of guinea-pigs except for ascorbic acid

To clarify the position, direct comparison of the supplements was made in a series of experiments. Most of the work was carried out on animals which were adult, or nearly adult, and had been used in earlier experiments. It was felt that these conditions offered the best chance of detecting the existence of a slowly developing deficiency, which might be masked in younger animals on test for shorter periods.

Direct comparison on the supplements: (1) The twelve animals which had received diet 10 when it was freshly prepared, either pelleted or as a meal (Fig. 8), were regrouped after 122 days into three lots, one pair of each lot having originally received meal and the other pellets.

Group 1 received fresh greens.

Group 2 received cod-liver oil.

Group 3 received no supplement.

All continued to receive the diet in the form in which they had originally received it, i.e. meal or pellets. In Group 1, the growth rate was at once increased, but no difference was observed in the growth of the group receiving cod-liver oil. These animals, and the control group receiving the unsupplemented diet, continued to show much the same slow increase as before (Fig. 15). It was evident that the retardation of the growth rate which all three animals had shown once they reached about 700 g. could not be due to a shortage of vitamin A. After 7 weeks, cod-liver oil doses were stopped and 3 weeks later dehydrated cabbage, 5 g. per animal per day, was given in its place. These adult animals ate dehydrated cabbage readily; growth restarted at once and continued at about the same rate as that in the group receiving fresh greens. Dehydration of the cabbage had, therefore, not destroyed whatever factor was necessary for the resumption of growth.

The controls, Group 3, ceased to increase in weight about 6 weeks after the supplementary feeding had been started for the other two groups. By this time they had been continuously, for 6 months, on diet 10, which had been made in bulk and of which the vitamin C content had been steadily diminishing. The two controls receiving the pelleted diet began to lose weight rapidly and died after 203 and 207 days respectively. Both had scorbutic lesions of the knees and elbows. The remaining two receiving the diet in meal form showed no signs of deficiency. They remained in good condition and continued to show a steady but slow increase in weight as long as the supply of diet lasted (294 days). The difference in the behaviour of the two pairs of controls was no doubt due to the fact that the ascorbic acid content of the meal was initially much higher than that of the pellets. The two surviving controls were finally given diet 7 pellets with a supplement of ascorbic acid, 10 mg. per animal per day. Eight days after the ascorbic acid dosing was started the growth rate increased. It was continued at the rate of about 3 g. per day for a further 5 weeks when the experiment was stopped. By this time the animals, which had received only ascorbic acid, had attained a weight of 940 g., very nearly equal to that of the dried cabbage group (970 g.).

This experiment therefore gave no evidence of the existence of a deficiency in diet 7 other than of vitamin C.

(2) In a second experiment, three groups were made up from 12 adult control animals from earlier experiments. All had received stock diet and fresh greens, six for 249 days and six for 242 days; they weighed about 800 g. All three groups were changed to diet 7, batch 2, which, at that time, had been stored for 3 months. Supplements were: group 1, fresh greens; group 2, dehydrated cabbage; group 3, no supplement. The immediate response in all three groups was a resumption of growth (Fig. 16) which was probably due to the increased protein content of diet 7 as compared with that of the stock diet. The control group 3 ceased to increase in weight after about a month. They began to lose condition and a rapid decline in weight set in. The older pair died after 59 and 63 days of receiving the vitamin C deficient diet, both having characteristic scorbutic lesions of the joints. After 59 days the younger pair, which had not lost weight so rapidly as the others, were given ascorbic acid, 20 mg. per day. Growth was resumed as soon as dosing started (Fig. 16) and appetite, which had fallen off with the development of the deficiency, recovered rapidly (Table 3). Growth was still being maintained after 103 days, when all the animals weighed about 1 kg. and the experiment was stopped. No difference was found between fresh greens and dehydrated cabbage among these animals.

In these two experiments with adult animals, ascorbic acid alone had proved as effective a supplement to diet 7 as either fresh greens or dehydrated cabbage. A final attempt to demonstrate the existence of a possible deficiency in diet 7, other than vitamin C, was made with younger, rapidly growing animals by substituting ascorbic acid for dehydrated cabbage. It was thought that if the dehydrated cabbage had been supplying any

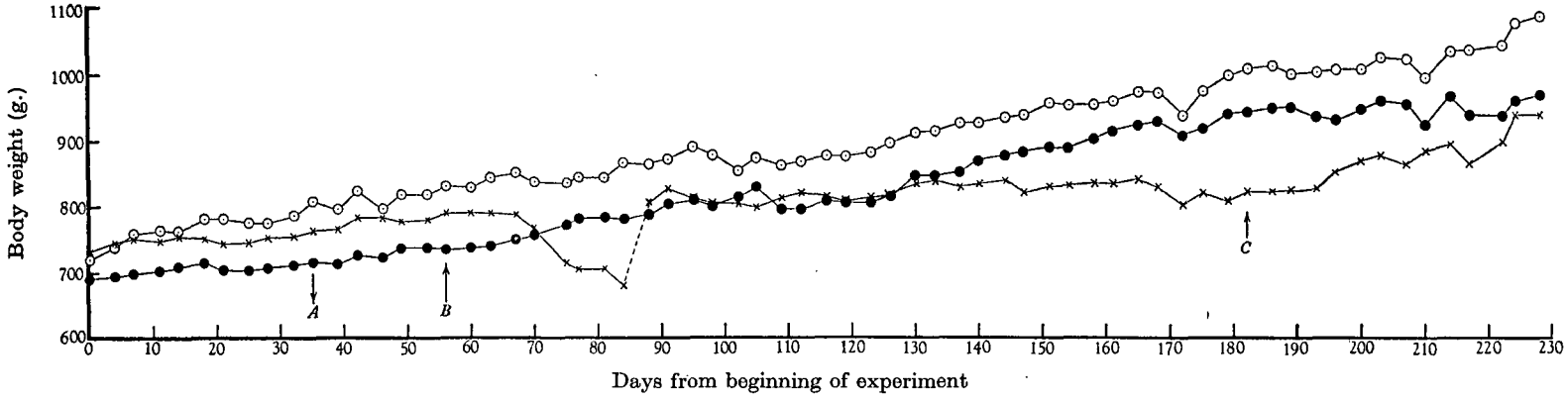

Fig. 15. The response of adult guinea-pigs which had received diet 10 for 5 months to supplements of cod-liver oil, dehydrated cabbage, ascorbic acid or fresh greens. Twelve animals in all. (Continuation of Fig. 8.) × = Diet 10 alone. Arrow *C* indicates addition of ascorbic acid. ● = Diet 10 and cod-liver oil for 35 days (end of dosing indicated by arrow *A*), followed by dehydrated cabbage (arrow *B*). ⊙ = Diet 10 and ascorbic acid or fresh greens. Dotted line indicates death of one member of the group.

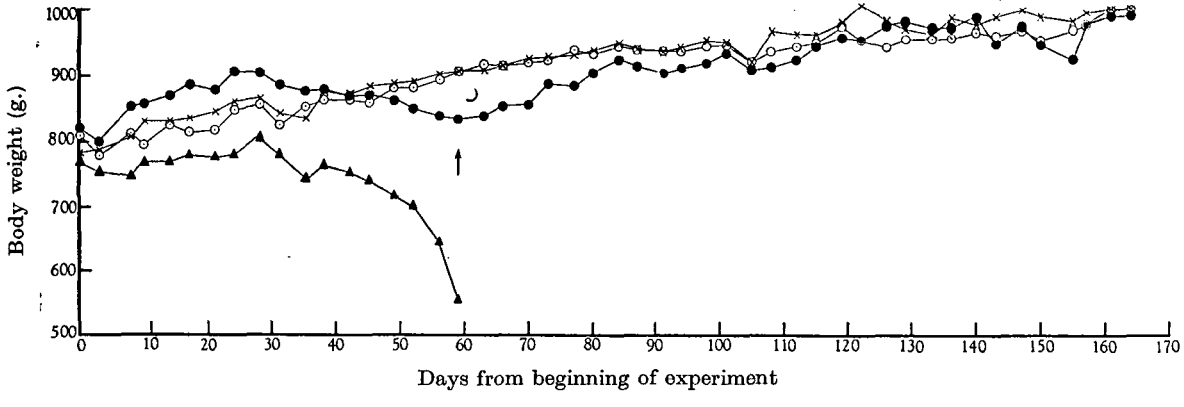

Fig. 16. Comparison of fresh greens, dehydrated cabbage and ascorbic acid as supplements to diet 7 for adult guinea-pigs. Four animals on each. ● = Diet 7 alone (younger pair of guinea-pigs). Arrow indicates addition of ascorbic acid. ▲ = Diet 7 alone (older pair of guinea-pigs). × = Diet 7 and fresh greens. ⊙ = Diet 7 and dehydrated cabbage.

special food factor, the ascorbic acid might fail to maintain growth.

Substitution of dehydrated cabbage by ascorbic acid. The two groups, which as young animals had developed rapid and severe scurvy (Figs. 9 and 12) on diet 14 and diet 10 after 6 months' storage, had both shown immediate and sustained recovery with dehydrated cabbage for over 90 days. Half the animals in each of these groups were then given 10 mg. of ascorbic acid per day instead of dehydrated cabbage. The experiment was stopped after 70–80 days of dosing with ascorbic acid. In neither case could a significant difference be detected between the animals which had received dehydrated cabbage throughout and those which had been changed to ascorbic acid. The groups are very small, 2 or 3 animals only, and these results therefore serve only as confirmatory evidence that there is no difference in effectiveness between dehydrated cabbage and ascorbic acid as supplements to diet 7.

SUMMARY

1. A dry pelleted diet is described which, supplemented with fresh greenstuff, dried cabbage, or ascorbic acid, is highly satisfactory for the growth and maintenance of guinea-pigs.

2. No evidence could be found that the pelleted diet alone was deficient in any other factor than vitamin C required by guinea-pigs, fresh greens and dehydrated cabbage being effective because of their high content of this vitamin.

3. Vitamin C added to the diet as crystalline ascorbic acid or as present in dried lucerne meal is mostly destroyed by the pelleting process and is further reduced during storage.

4. The aim of evolving a dry pelleted stable diet completely adequate for guinea-pigs has not yet been realized.

It is a pleasure to acknowledge our indebtedness to Miss M. Olliver, Messrs Chivers & Sons Ltd., who carried out the ascorbic acid determinations and gave us much valuable advice.

Our thanks are also due to Roche Products, who gave us the ascorbic acid, to the Director of Messrs Chivers & Sons Ltd. for the generous gift of 1 cwt. of standard dehydrated cabbage, to the Dehydration Division of the Ministry of Food for supplying two samples of sub-standard dehydrated cabbage, and to Mr D. G. Short, senior technician in charge of the animals at the Institute, who prepared the small-scale diets.

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(*MS. received for publication* 11. II. 46.—Ed.)