FEEDING AND BREEDING OF LABORATORY ANIMALS

XII. A NOTE ON THE BREEDING OF GUINEA-PIGS WITHOUT FRESH GREEN FOOD

By H. M. BRUCE

From the National Institute for Medical Research, London, N.W. 7

(With 1 Figure in the Text)

The high requirement of vitamin C by the guinea-pig is difficult to satisfy in a pelleted diet. Incorporation of a rich natural source of the vitamin such as Lucerne meal, or of synthetic ascorbic acid fails because there is a gradual loss of activity with storage, in addition to the loss during the routine pelleting process (Bruce & Parkes, 1947*a*). Thus, at present, the feeding of fresh green food remains the most economical way to ensure an adequate supply of vitamin C for a guinea-pig breeding colony.

In the search for a complete food for guinea-pigs a pelleted diet (diet 18) was evolved which when supplemented with fresh green food, hay and water, proved entirely satisfactory for growth, maintenance and reproduction. It has the following composition: bran, 15%; barley meal, 20%; ground nut cake, 15%; linseed cake, 10%; dried meat and bone meal, 8%; dried grass meal, 30%; calcium carbonate, 1%; sodium chloride, 1%. A full description of the experiments which led to the choice of the diet is given in Part III of this series (Bruce & Parkes, 1947*a*). A small breeding colony was maintained on the diet for over 2 years, during which time six generations of young were reared. A description of this colony is given in Parts V and VIII (Bruce & Parkes, 1947*b*; Bruce & Parkes, 1948).

As a result of this experience, and in the absence of evidence to the contrary, diet 18 was assumed to supply all the nutritional needs of the guinea-pig, with the exception of vitamin C and roughage. A direct test, the reproductive capacities of successive generations of animals receiving the diet supplemented only with synthetic ascorbic acid, has now been made, and the results are reported here.

MATERIAL AND METHODS

Housing and general care

This has been described in the earlier papers (Parts III, V and VIII).

Ascorbic acid

The solution was prepared immediately before use in freshly boiled glass-distilled water. It was fed by pipette twice a week at dose levels chosen to supply more than sufficient for the daily requirement. Zilva (1936) maintained animals on

https://doi.org/10.1017/S0022172400015102 Published online by Cambridge University Press

a scorbutic diet supplemented with 5 mg. ascorbic acid per day for several years; growth was good. This amount is higher than is generally considered necessary (Mannering, 1949). The vitamin C requirement of the guinea-pig for pregnancy has not been worked out. Previous experience with diet 18 and attempts to supply vitamin C by feeding dehydrated cabbage, showed that a daily intake of about 12 mg. ascorbic acid per pig was insufficient (Bruce & Parkes, 1947*a*). When the consumption of dried cabbage supplied about 20 mg. ascorbic acid per day, normal litters were produced. For these reasons, 5 mg. ascorbic acid per day during the period of growth and 20 mg. per day during the period of reproduction were chosen for the experiments described below.

The animals

For the start of the experiment two unselected pregnant females which had received diet 18, hay and fresh green food were paired with normal males. At the birth of the litters fresh green supplement was stopped and ascorbic acid doses were started. The males were removed 24 hr. after the birth of the litters. These young were regarded as the F_1 generation, i.e. the first generation to receive no green food throughout life. At least two pairs from each successive generation were kept for breeding; the older pairs were discarded when the next generation of young had mated. The sexes were housed together from weaning so that mating could take place at the earliest opportunity. Young female guinea-pigs may mature as early as 5 weeks of age, but young males are not generally mature until about 2–3 months old (Sewell Wright, 1922). Mating was always kept strictly within generations; some pairs were brother-sister, but not all.

RESULTS

Growth

The growth of four successive generations of males is given in Fig. 1. In the first generation growth was poor. The sample of ascorbic acid had been stored for some time with consequent loss of activity and the doses given proved insufficient. The use of a fresh sample remedied this, and growth in later generations was normal. For comparison, the average growth of males from the original breeding colony receiving fresh green food is also given. The substitution of ascorbic acid for fresh greens has had no effect upon rate of growth.

Reproduction

All the animals given the opportunity to mate were fertile. Sexual maturity occurred at the normal time, and first litters were born when the females were about 20 weeks old. As the object of this work was to gain further information about the adequacy of diet 18, successive generations were bred from first litters, although these are usually smaller than later litters (Sewell Wright, 1922; Haines, 1929; Bruce & Parkes, 1948) and not the most vigorous. Reproduction in three generations reared with synthetic ascorbic acid and without fresh green food is given in Tables 1 and 2. Birth weight, litter size and growth rate are normal; there

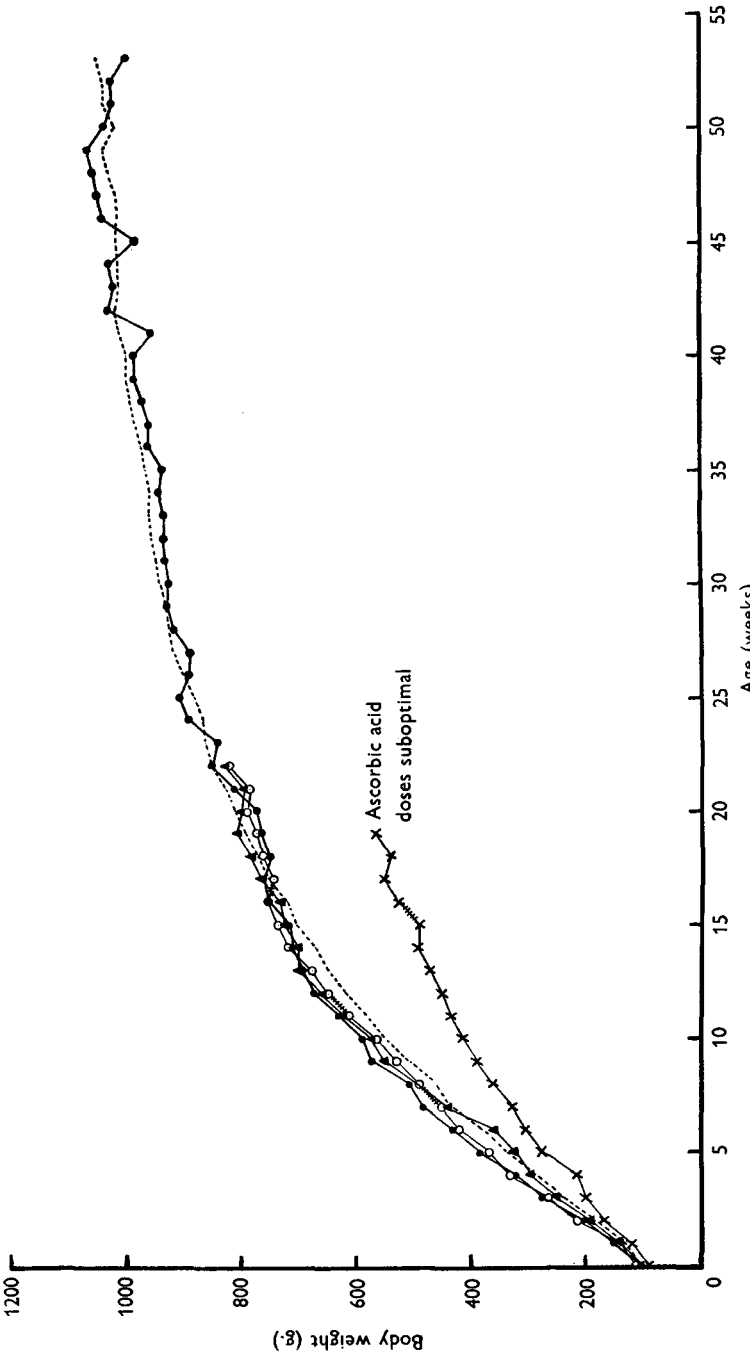

Fig. 1. Growth of young guinea-pigs without fresh green food. Four successive generations of young males reared on diet 18 and hay supplemented with ascorbic acid. ×, F_1, 3 males; ●, F_2, 2 males; ○, F_3, 7 males; ▲, F_4, 3 males; - - -, normal colony males receiving green food.; ####, one animal discarded.

https://doi.org/10.1017/S0022172400015102 Published online by Cambridge University Press

was even an increase in average litter size by the third generation, and the substitution of ascorbic acid for fresh greens did not affect reproduction.

Table 1. *Average reproduction of first and second generation guinea-pigs on diet* 18 *with ascorbic acid but no fresh greens*

	Ascorbic acid	Average previously found for a normal colony receiving green food
Total number of litters	8	—
Total number of young born	21	—
Average litter size at birth	2·6	3·0
Average birth weight of living young (g.)	104	104
Average age of young at 200 g. (days)	15·2	14·9

Table 2. *Average reproduction of third generation guinea-pigs on diet* 18 *with ascorbic acid but no fresh green food*

	Ascorbic acid	Average previously found for a normal colony receiving green food
Total number of litters	5	—
Total number of young born	21	—
Average litter size at birth	4·2	4·0
Average birth weight of living young (g.)	97·0	96·0
Average age of young at 200 g. (days)	19·9	17·8

There was, however, an unexpected fall in the weaning rate of the fourth generation of young. The cause of death was not apparent. There were no scorbutic symptoms and there was no evidence of infection. It seemed likely that, as parental stores became depleted in the course of several generations, a dietary deficiency affecting the vigour of the young, and which was not a deficiency of vitamin C, was becoming manifest. Further, in one litter, four out of five young died suddenly between 5 and 7 weeks of age. The symptoms of the disease, staring coat, distended abdomen and muscular weakness, developed rapidly and death took place within a very few days of their appearance. There was little or no loss of weight before death. In one animal paralysis of the hindlegs developed 24 hr. before death, and this suggested a deficiency of vitamin E. The surviving animal in the litter, a female, was therefore given small doses of alpha-tocopherol acetate in olive oil, by mouth. In due course she successfully completed two pregnancies and reared the litters, both of which contained two young. She received 50 mg. of alpha-tocopherol during each gestation period.

Acute vitamin E deficiency in the guinea-pig results in degeneration of skeletal muscle, paralysis and death. In less severe deficiency, disturbances of reproduction similar to those occurring in rats have been found (Pappenheimer & Goettsch, 1941; Pappenheimer, 1944). There are very few references in the literature to this effect on reproduction in the guinea-pig, and it is therefore of interest to describe

the condition as it was observed in the animals used for this work. The development of vitamin E deficiency in the third-generation female whose progeny showed the characteristic paralysis which identified the cause of the trouble, was gradual and insidious. The first litter, born when the female was 20 weeks old, contained four young of which two were weak and died within a few days of birth. Such an event was not unusual in the normal colony, only 65 % of litters containing four young (78 out of 120) being weaned without loss, and hence it was accepted without suspicion of the underlying cause. The second litter contained five young; all were weaned, but four developed vitamin E deficiency and died at 4–5 weeks of age. The third litter, of three young, was premature, parturition taking place after only 56 days gestation. Both the second and the third pregnancies resulted from a mating at post-partum oestrus. The female had thus been in a continuous state of pregnancy for nearly 8 months and would have had a maximal requirement for all essential nutrients.

From this time, as a precaution, alpha-tocopherol was given to young whose growth was slower than normal. Two litters (F_4, six young; F_5, four young) were immediately affected. Both had stillborn young. Of the seven living young, five received alpha-tocopherol, and all showed an improvement in growth rate after the doses were started, which was maintained when the doses were stopped. It was clear that some of the young of these generations were suffering from slight vitamin E deficiency. It should be emphasized that fresh green food supplies vitamin E as well as vitamin C, so that in the normal colony this does not happen.

Table 3. *Proportion of young weaned in successive generations of guinea-pigs receiving diet* 18 *and synthetic ascorbic acid*

Generation of parents	No. of litters allowed	Total no. of young		Proportion of young weaned	
		Born	Weaned	As % of all births	As % of young born alive
F_1	2	6	6	100	100
F_2	6	15	12	80	92
F_3	4	15	7	47	58
Alpha-tocopherol doses given where necessary to the following:					
F_3	1	6	4	67	100
F_4	6	14*	13*	93	93
F_5	1	2	2	100	100
Previously recorded for normal colony receiving green food	225	1240	1049	85	94

* One young died at 6 days old. It had not received alpha-tocopherol.

The gradual appearance of vitamin E deficiency in some of the animals was most apparent as an increase in mortality before weaning in the third and fourth generations (Table 3). There was much individual variation. The young not manifesting the deficiency were vigorous and grew well. After the addition of alpha-

https://doi.org/10.1017/S0022172400015102 Published online by Cambridge University Press

tocopherol to the diet of those whose slow rate of growth indicated depleted vitamin E reserves, the mortality before weaning returned to normal, thirteen out of fourteen young of the fifth generation, and two young of the sixth generation having been reared so far.

The substitution of synthetic ascorbic acid for fresh green food therefore caused no deterioration in the nutritive value of the diet, and it can be concluded that diet 18 contains all the essential food factors required by the guinea-pig (Mannering, 1949) with the exception of vitamin C and roughage. The experiments described above indicate that the vitamin E content of the pellets alone is inadequate for guinea-pigs, but as green food is always given with the pellets, both vitamin C and vitamin E requirements are fully met under normal conditions. Hay, also, is always given to augment the roughage.

DISCUSSION

Is synthetic ascorbic acid always as effective as vitamin C in a natural form? On this question the evidence is conflicting as regards both clinical and experimental scurvy.

Elmby & Warburg (1937) described three patients unable to absorb ascorbic acid or to retain it after injection, who were cured with lemon juice. McGovern, Gannon & Wright (1939) found that synthetic ascorbic acid was ineffective in the cure of scurvy among several members of one family, unless very high doses were given. Here, too, the condition was ultimately kept in check with fruit juices. Reports of similar effects have also appeared in the Fiat Review of German Science (1939–46). Stutz & Weisspfennig (1939) found that a dose of 50 mg. ascorbic acid per day, although adequate at home, was insufficient to prevent signs of scurvy among a crew during a voyage to the West Indies, and that lemon juice was more effective. Pezold (1941) was able to induce a state of saturation with smaller amounts of vitamin C as rose hips than as ascorbic acid. On the other hand, the utilization of vitamin C by normal people is apparently the same whether the source is synthetic ascorbic acid, orange juice (Hawley, Stephens & Anderson, 1936), frozen raspberries (Todhunter & Fatzer, 1940) or raw cabbage and canned tomato juice (Clayton & Borden, 1943).

Jacobsen (1935) found a lower concentration of vitamin C in the adrenals, small intestines and livers of guinea-pigs dosed with ascorbic acid than in those receiving fresh cabbage. Hawley, Daggs & Stephens (1937) also report a better retention of vitamin C by guinea-pigs with cabbage, alfalfa and orange juice than with crystalline vitamin C.

By contrast, as reported in the Fiat Review (1939–46), Scheunert & Reschke (1941) determined the smallest amount of vitamin C necessary to keep guinea-pigs free from scurvy for 35 days and found no difference between synthetic ascorbic acid, lemon juice, apples or white cabbage. They explain the differences found by other workers as due to substances such as the B vitamins and minerals which are present in the natural source of vitamin C but absent when synthetic ascorbic acid is given.

In 1936, Armentano and his co-workers described a substance, not vitamin C, present in Hungarian red pepper and in lemon juice, which could increase capillary strength in man. Subsequent work led to the isolation of a crystalline material. The active principle was called 'vitamin P'. Since then a great deal of work, much of it unconvincing, has been done on the subject. There seems little doubt that an increased capillary fragility resulting from a dietary deficiency can be induced in guinea-pigs even when they are receiving adequate amounts of vitamin C. Todhunter, Robbins, Ivey and Brewer (1940) found that vitamin C as ascorbic acid or lemon juice was equally well utilized by guinea-pigs when judged by weight gains, blood plasma levels or storage of vitamin C in the adrenals, but that the animals receiving lemon juice had fewer haemorrhages when scored for scurvy. These authors conclude, 'the data indicate the possibility that lemon juice contains an additional factor which is concerned in the prevention of haemorrhages characteristic of scurvy'. Later, Bourne (1943) and Hughes & Parkes (1946) were able to confirm this. There is no evidence, as yet, to show whether, as stated by Scarborough & Bacharach (1949) in a recent and extensive review on vitamin P, 'an unduly low capillary resistance confers any disadvantage on the organism'. No tests of capillary resistance were made on the animals receiving diet 18 with ascorbic acid to supply vitamin C.

In the guinea-pig a deficiency of either vitamin C (Warkany, 1945) or vitamin E (Pappenheimer & Goettsch, 1941) will cause disturbances of reproduction. Either deficiency may result in haemorrhages and an increase in the number of abortions, depending on the severity of the deficiency. These are specific effects on reproduction, and it cannot be inferred that scorbutic haemorrhages are connected with a shortage of vitamin E. Crampton & Bell (1947), using reproduction as a test of dietary adequacy, found more reproductive failures (absorptions and haemorrhages) when synthetic ascorbic acid was given than with fresh orange juice, decitrated lemon juice or synthetic orange juice. When fresh lawn clippings or cabbage was the source of roughage, however, no reproductive failures occurred and the diet appeared to be completely fortified. This suggests that the amounts of vitamin C, vitamin E or both may have been insufficient, since both would be supplied by grass and cabbage. The interpretation of the experimental results may thus have been complicated by unsuspected deficiencies in their basal diet.

No difference in the reproduction of guinea-pigs on diet 18 could be found between vitamin C supplied as ascorbic acid in the experiments described in this paper and vitamin C supplied as fresh cabbage to the normal guinea-pig colony.

SUMMARY

1. Five generations of guinea-pigs bred successfully on diet 18 supplemented with crystalline ascorbic acid but without fresh green food.

2. No difference in reproductive performance was found between these animals and those of the normal breeding colony receiving unlimited supplies of fresh cabbage.

3. Symptoms of vitamin E deficiency appeared among some young of the fourth

and fifth generations, showing that diet 18 alone contained barely enough of this factor for continuous reproduction through many generations.

4. Diet 18, supplemented by hay and fresh green food to supply vitamin C and additional vitamin E, provides all the essential factors required by the guinea-pig.

I should like to thank Dr A. S. Parkes, F.R.S., for his continued interest in the work.

REFERENCES

ARMENTANO, L. (1936). See quotations by Scarborough & Bacharach, 1949.
BOURNE, G. (1943). *Nature, Lond.*, **152**, 659.
BRUCE, H. M. & PARKES, A. S. (1947*a*). *J. Hyg., Camb.*, **45**, 70.
BRUCE, H. M. & PARKES, A. S. (1947*b*). *J. Hyg., Camb.*, **45**, 327.
BRUCE, H. M. & PARKES, A. S. (1948). *J. Hyg., Camb.*, **46**, 434.
CLAYTON, M. M. & BORDEN, R. A. (1943). *J. Nutrit.* **25**, 349.
CRAMPTON, E. W. & BELL, J. M. (1947). *Sci. Agric.* **27**, 57.
ELMBY, A. & WARBURG, E. (1937). *Lancet*, **2**, 1363.
FIAT REVIEW OF GERMAN SCIENCE (1939–46). *Pharmacology and Toxicology*, Part I, p. 109.
HAINES, G. (1929). *Proc. Soc. Anim. Prod.* p. 49.
HAWLEY, E. E., DAGGS, R. G. & STEPHENS, D. J. (1937). *J. Nutrit.* **14**, 1.
HAWLEY, E. E., STEPHENS, D. J. & ANDERSON, G. (1936). *J. Nutrit.* **11**, 135.
HUGHES, E. G. & PARKES, M. W. (1946). *Emil Christoph Barrell Jubilee Volume*, p. 216. Reinhardt, Basle.
JACOBSEN, E. (1935). *Skand. Arch. Physiol.* **72**, 259.
MANNERING, G. J. (1949). *Vitamins & Hormones*, **7**, 201.
MCGOVERN, T., GANNON, G. E. & WRIGHT, I. S. (1939). *Amer. J. med. Sci.* **197**, 310.
PAPPENHEIMER, A. M. (1944). *Amer. J. Path.* **20**, 239.
PAPPENHEIMER, A. M. & GOETTSCH, M. (1941). *Proc. Soc. exp. Biol., N.Y.*, **47**, 268.
PEZOLD, F. (1941). *Dtsch. med. Wschr.* p. 897.
SCARBOROUGH, H. & BACHARACH, A. L. (1949). *Vitamins & Hormones*, **7**, 1.
SCHEUNERT, A. & RESCHKE, J. (1941). *Vitamins & Hormones*, **1**, 195.
SEWELL WRIGHT (1922). *Bull. U.S. Dep. Agric.* no. 1090.
STUTZ, E. & WEISSPFENNIG, W. (1939). *Dtsch. Mil.-Arzt.* **4**, 212.
TODHUNTER, E. N. & FATZER, A. S. (1940). *J. Nutrit.* **19**, 121.
TODHUNTER, E. N., ROBBINS, R. C., IVEY, G. & BREWER, W. (1940). *J. Nutrit.* **19**, 113.
WARKANY, J. (1945). *Vitamins & Hormones*, **3**, 89.
ZILVA, S. S. (1936). *Biochem. J.* **30**, 1419.

(*MS. received for publication* 13. v. 50.)