

(b) the bodies of the undernourished animals contained more fat, but less protein and calcium than those of the corresponding starved animals; (c) the concentration of haemoglobin in the blood of the undernourished young rats was lower than in the blood of the corresponding starved animals; (d) the livers of the adult undernourished animals contained much less fat, but more protein and glycogen than the livers of the adult starved animals.

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Vitamin C economy of rabbits

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The investigation to be described originated in the course of some studies on the relation between vitamin C and adrenal function, when it was found that the administration of ACTH or cortisone caused a massive enlargement of the liver in guinea-pigs and rabbits, but not in various other species examined, including rats, mice or chicks (Harris, Bland, Hughes & Constable, 1953). In searching for an explanation of this finding, the possibility had to be considered whether the special response of rabbits and guinea-pigs, as distinct from that of the other species, might conceivably be attributed to some common peculiarity in their vitamin C metabolism. This explanation seemed unlikely, for rabbits, in contrast with guinea-pigs, are not generally supposed to need vitamin C. Nevertheless, a study of the literature (see below) revealed the fact that past evidence about the vitamin C economy of rabbits was surprisingly conflicting. In these circumstances it was deemed desirable to make a direct test, in order to settle conclusively whether or not rabbits have in fact any need for vitamin C in their diet.

Species differences in vitamin C requirements. The opinion most generally accepted at present is that guinea-pigs and primates are the only animals unable to synthesize vitamin C in the body and therefore needing it in their food. The view has, however, been the subject of considerable controversy. At one time it was authoritatively maintained (Medical Research Council, 1932, p. 194) that the difference between the two classes of animals, those susceptible to scurvy and those apparently not susceptible, was 'one of degree only'. 'It is evident that the rat requires vitamin C, but only in very small amounts compared with such animals as the guinea-pig and monkey.' Again, it had been assumed (Holst & Frölich, 1907, 1912; Mellanby, 1929) that vitamin C was needed by dogs, but it was later shown that dogs could be maintained indefinitely in normal health on a vitamin C-free diet (Harris, 1931). Similarly, it was claimed that 'well-developed scurvy has been occasionally observed in pigs' (Medical Research Council, 1932, p. 195; see also Holst & Frölich, 1912; Zilva, Golding & Drummond, 1924; Plimmer, 1920), but recent investigations seem against such a conclusion (Braude, Kon & Porter, 1950).

Rabbits. The literature about rabbits has been especially conflicting. Findlay (1921), Lopez-Lomba & Randoin (1923), Mellanby & Killick (1926) and Mellanby (1930), among others, concur in the conclusion that the young rabbit needs vitamin C. For example, Findlay (1921) describes typical haemorrhages and lesions in the joints seen in young stillborn rabbits whose mothers had been reared on a scorbutogenic diet. Adult rabbits, according to Findlay, when deprived of vitamin C, gradually lost weight and eventually died; the addition of vitamin C to their diet in the form of swede juice was followed by an increase in their weight and an improvement in general condition.

Again, Mellanby & Killick (1926) refer to the signs of scurvy in rabbits, seen clinically, at post-mortem examination and histologically. Mellanby (1930) similarly describes the occurrence of scurvy in rabbits fed on bran, oats and small amounts of grass.

In conflict with all the foregoing conclusions, however, are the tacit assumption of Hogan & Hamilton (1942) that rabbits have no need of vitamin C and the finding of Bruce & Parkes (1946) that rabbits can thrive on a diet containing no more than 30% of poor-quality grass meal and therefore that 'the exogenous requirements of the rabbit for this vitamin must be very small'.

Browning (1931, p. 334) includes Holst & Frölich (1907, 1912) among those who found that young rabbits require vitamin C, but we have been unable to trace any allusion to this in their papers. She states also that the resistance to scurvy of adult rabbits, as contrasted with young rabbits, which need the vitamin, 'is attributed by Portier & Randoin (1920) and Portier & Lopez-Lomba (1921) to coprophagy and the production of vitamins in the intestinal canal': however, the original French texts do not appear to contain any allusion to scurvy or to vitamin C.

Because of these puzzling discrepancies and of our findings, referred to above, about the unexpected similarity between rabbits and guinea-pigs in another respect, it was clear that further investigation was needed.

EXPERIMENTAL

Diets

In all the tests to be recorded the same basal, scorbutogenic diet was used; it consisted (in parts by weight) of: Sussex-ground oats 56, bran 13, white-fish meal 21, dried yeast powder 8, salt mixture 2. The supplements given weekly to all animals were: vitamin E (α -tocopheryl acetate), 5% (w/w) in arachis oil, two drops; vitamin K (2-methyl-1:4-naphthoquinone), 5% (w/w) in arachis oil, two drops; halibut-liver oil, two drops. During the final 15 weeks of the 25-week experimental period, in Exp. 3 (see below), each rabbit was offered in addition one slice, daily, of bread that had been dried overnight at 70° (National loaf, weight when dried approx. 30 g). The bread was included because in earlier experiments we had gained the impression that the inclusion of some more solid food was preferred by the animals.

Design of investigation

Young weanling rabbits ('Old English' Porton strain, Allington Farm, Ministry of Supply, Porton, Wilts), aged about 6 weeks and weighing about 1 kg, were used throughout. Three separate experiments were done, the first two being by way of preliminary trial.

Exp. 1. Four males were kept on the experimental diet for 54 days, two of them receiving supplements of 50 mg ascorbic acid daily throughout and the other two no ascorbic acid. Ascorbic acid was determined in the adrenal glands, liver and blood.

Exp. 2. Six young males were kept on the diet for 55 days, three of them having 50 mg ascorbic acid daily, the remaining three none. Ascorbic acid was determined in the adrenal glands.

Exp. 3. This was a larger-scale test, in which eighteen rabbits were used. For a preliminary period of 8 days all the animals were kept on the basal diet supplemented with 50 g cabbage daily. They were then divided into four sets:

Set 1, 'initial controls': four animals, two males and two females, were killed at the beginning of the experiment.

The remaining three sets were kept on experiment for a further 25 weeks:

Set 2, 'negative group': five animals, two males and three females, had the basal diet without any ascorbic acid added.

Set 3, 'positive group': five animals, two males and three females, received the basal diet with 50 mg ascorbic acid daily.

Set 4, 'cabbage group': four animals, two males and two females, had the basal diet with 50 g cabbage daily.

In this experiment ascorbic acid was determined in the liver, spleen, adrenal glands, kidneys, blood and urine.

Experimental procedures and methods

All the animals were weighed twice weekly for comparison of their growth curves, and their condition was judged by frequent inspection. At the conclusion of the experimental period they were killed by a blow on the back of the head. Routine

post-mortem examinations were made, and certain organs were removed for analysis. Ascorbic acid in the liver and kidneys was estimated by indophenol titration (Harris & Olliver, 1942), in the adrenal glands and spleen photometrically (Mindlin & Butler, 1937) and in blood by means of the dinitrophenylhydrazine test (Roe & Kuether, 1943).

For determining ascorbic acid in the urine, an unpublished method (Constable, Harris & Howard, 1956), based on the homocysteine reaction described by Hughes (1956), was used. The urine was collected in metabolism cages, in glycine-HCl buffer, pH 2.2. Pooled, 24 h specimens were analysed at random intervals during the final 15 weeks of the experiment, an average value for set 2 being based on fourteen such separate determinations, for set 3 on thirteen separate determinations and for set 4 on nine separate determinations.

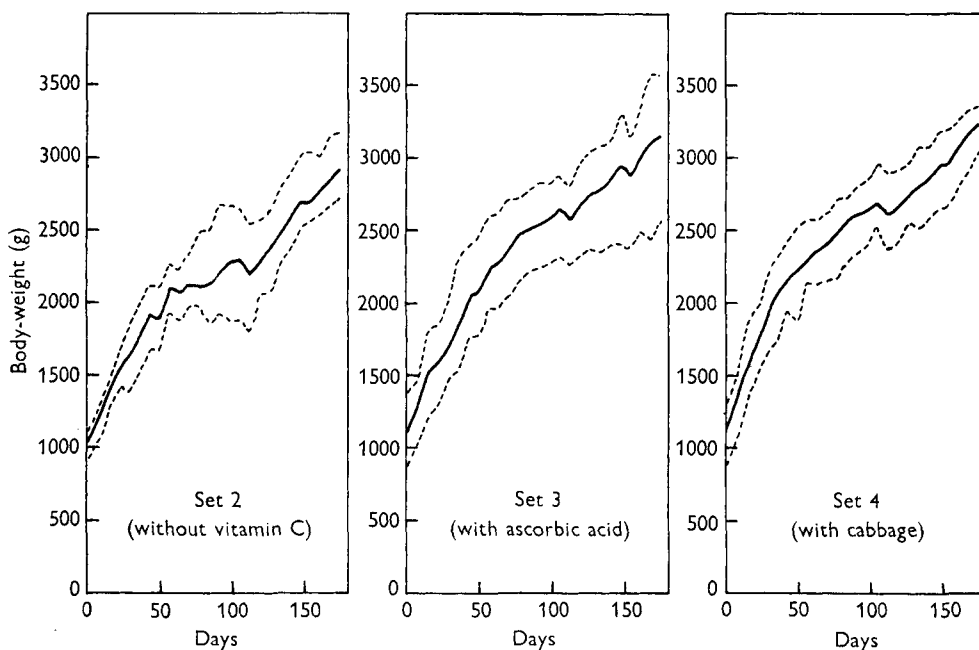


Fig. 1. Exp. 3. Body-weight curves of rabbits in sets 2-4. —, mean; ----, range.

RESULTS

Growth rates. In all three experiments, the rabbits gained weight at about the same rate, whether they were kept on the vitamin C-free diet or on the diets supplemented with ascorbic acid or with cabbage. Fig. 1 shows the mean body-weights, and the spread of the individual weight curves, throughout the course of Exp. 3. It is apparent that the range of weight gains was about the same for all three groups. There was no statistically significant difference between the weight gains of the three groups, when compared, for example, after periods of 50, 100 or 150 days.

Essentially the same finding emerged also from Exps. 1 and 2, in which, however, smaller numbers of animals were involved.

Table 1. *Exp. 3. Mean values, with range, for vitamin C content of organs, blood and urine of rabbits on diets with and without vitamin C*

Diet	Duration of experiment (days)	No. of animals	Body-weight (g)	Adrenal glands			Spleen			Kidneys		
				Weight (mg)	mg/100 g	Ascorbic acid Total (mg)	Weight (g)	mg/100 g	Ascorbic acid Total (mg)	Weight (g)	mg/100 g	Ascorbic acid Total (mg)
Normal, mixed	0	4	1083 (885-1232)	88 (31-96)	263 (239-288)	0.23 (0.193-0.276)	0.82 (0.493-1.281)	43.4 (33.0-49.5)	0.339 (0.220-0.425)	10.1 (9.40-11.0)	4.98 (3.07-6.66)	0.513 (0.288-0.700)
Basal, no vitamin C	175	4	2918 (2765-3165)	545 (252-930)	236 (111-394)	1.06 (0.935-1.20)	1.34 (0.798-1.74)	43.6 (36.4-47.0)	0.662 (0.290-0.823)	18.4 (15.4-20.8)	8.91 (6.35-10.7)	1.62 (1.20-2.04)
Basal, with synthetic ascorbic acid 50 mg/day	175	5	3148 (2565-3565)	484 (319-629)	246 (150-345)	1.23 (0.73-1.94)	1.27 (0.716-1.91)	49.0 (32.6-65.1)	0.610 (0.317-0.947)	18.9 (15.1-23.2)	9.60 (7.40-13.0)	1.78 (1.26-2.27)
Basal, with cabbage 50 g/day	175	4	3272 (2995-3480)	495 (344-904)	363 (217-532)	1.58 (1.15-1.95)	1.30 (0.90-1.95)	48.3 (46.4-50.6)	0.670 (0.455-0.957)	21.1 (17.6-25.5)	10.8 (9.30-12.0)	2.25 (1.89-2.55)

Diet	Duration of experiment (days)	No. of animals	Body-weight (g)	Liver			Blood			Urine		
				Weight (g)	mg/100 g	Ascorbic acid Total (mg)	Volume* (ml.)	mg/100 ml.	Ascorbic acid Total (mg)	Blood plus certain organs† Ascorbic acid Total (mg)	mg/day	Ascorbic acid Total calculated for 175 days (mg)
Normal, mixed	0	4	1083 (885-1232)	43.1 (35.5-54.1)	18.9 (12.5-25.7)	8.43 (4.45-13.8)	75.7 (62-86)	—	1.141 (0.94-1.29)	10.7 (6.62-16.4)	—	—
Basal, no vitamin C	175	4	2918 (2765-3165)	99.1 (88.6-116)	15.6 (11.9-19.6)	15.8 (10.4-21.9)	263 (188-221)	0.91 (0.68-1.16)	1.86 (1.30-2.57)	20.9 (14.6-28.6)	0.39 (0.11-0.56)	68.2 —
Basal, with synthetic ascorbic acid 50 mg/day	175	5	3148 (2565-3565)	140 (101-164)	10.2 (11.0-26.4)	25.7 (18.1-30.0)	210 (178-249)	1.44 (1.09-1.82)	3.16 (2.35-4.44)	32.4 (23.2-43.0)	0.75 (0.27-1.74)	131 —
Basal, with cabbage 50 g/day	175	4	3272 (2995-3480)	131 (110-165)	24.2 (23.8-24.4)	31.7 (26.8-39.4)	228 (209-242)	1.49 (1.14-1.79)	3.44 (2.39-4.35)	39.7 (36.5-47.3)	1.98 (0.51-3.00)	347 —

* Calculated from data of Courtice (1943-4).
 † Adrenal glands, spleen, kidneys, liver and blood: combined total.
 ‡ Calculated on the assumption that the ascorbic-acid concentration in the blood was 1.5 mg/100 ml.

Note. The values for the mean total ascorbic acid in different organs are calculated as the means of the separate individual values. They therefore, do not necessarily agree exactly with the products of the mean weight of the organ and the mean concentration of ascorbic acid.

Intercurrent deaths. In Exp. 1, one animal on the vitamin C-free diet died after 19 days; in Exp. 2 one animal from each group (after 11 days in the group not given ascorbic acid and after 49 days in the group given ascorbic acid); and in Exp. 3 one animal in set 2 after 21 days. No signs suggestive of scurvy could be found *post mortem*, and death was attributed to incidental respiratory infection.

Absence of scorbutic signs. In Exps. 1 and 2, the rabbits were kept on a vitamin C-free diet for 8 weeks and in Exp. 3 for 25 weeks. No signs of scurvy were observed during the life of the animals or on careful post-mortem examination at the conclusion of the trials.

Table 2. *Exp. 3. Mean values for, and increase in, the total vitamin C content of organs and blood of rabbits kept for 25 weeks on a vitamin C-free diet (sets 1 and 2 compared)*

Organ or blood	At beginning of experiment (mg)	At end of experiment (mg)	Increase with its standard error (%)
Adrenal glands	0.230	1.06	360 ± 31.96***
Spleen	0.339	0.602	78 ± 39.6†
Kidneys	0.508	1.62	219 ± 47.45**
Liver	8.43	15.8	86 ± 40.23†
Blood	1.14‡	1.86	63 ± 24.29*

*** denotes $P < 0.001$. ** denotes $P < 0.01$. * denotes $P = 0.05-0.01$.

† denotes $P = 0.10-0.05$.

‡ Calculated on the assumption that the ascorbic-acid concentration in the blood was 1.5 mg/100 ml.

Vitamin C in organs of animals on deficient diet. It will be seen (Table 1) that the concentrations of vitamin C in the various organs examined in Exp. 3 were not substantially different in those rabbits that had received no vitamin C for 25 weeks from those in the control rabbits that were killed at the beginning of the test. On the other hand, since the animals, and their organs, were considerably larger at the end of the experiment than at its beginning the actual total quantities of vitamin C present in the various organs were much greater at the end than at the beginning (Tables 1 and 2). This fact, and the finding that vitamin C continued to be excreted at a considerable rate in the urine of rabbits on the vitamin C-free diet (Table 1), can only be interpreted in one way, by assuming that the rabbit can synthesize its own vitamin C.

Effect of dietary vitamin C on vitamin C content of organs. In the groups that received supplements of vitamin C during the course of the test, either as synthetic ascorbic acid or in cabbage, the mean concentrations of ascorbic acid (and the total quantities present) in the various organs were somewhat higher than in the group having no vitamin C in their diet (Table 1), the effect being most significant, statistically, for the liver and the blood (Table 3).

Comparison with vitamin C values in other species. On comparing the values given in Table 4, it will be noted that the concentrations of ascorbic acid in the organs of the rabbit are somewhat similar to those that we have found in the rat and chick, species that are able to synthesize their own vitamin C, but appreciably higher than the concentrations that we have commonly observed in guinea-pigs, receiving supplements of cabbage, 15 g/day.

Table 3. *Exp. 3. Mean values with their standard errors for percentage increase (+) or decrease (—) in weight of organs and vitamin C content of organs and blood in groups of rabbits that received vitamin C for 175 days compared with the group kept for the same time on the vitamin C-free diet (sets 3 and 4 compared with set 2)*

Diet	Adrenal glands			Spleen			Kidneys			Liver			Blood			
	Weight	Concentration	Total content	Weight	Concentration	Total content	Weight†	Concentration	Total content	Weight†	Concentration	Total content	Concentration	Total content	Concentration	Total content
Basal, with synthetic ascorbic acid 50 mg/day	-11.4 (±25.27)	+4.2 (±28.85)	+15.5 (±30.77)	-5.6 (±23.78)	+12.5 (±16.12)	+1.4 (±27.92)	+41.5 (±14.97)*	+23.2 (±26.80)	+62.4 (±27.56)*	+31.9 (±13.61)*	+58.4 (±20.85)*	+70.0 (±25.45)*	+53.7 (±20.54)*	+84.7 (±26.92)*		
Basal, with cabbage 50 g/day	-9.1 (±36.0)	+53.7 (±38.34)	+49.4 (±19.46)*	+3.6 (±25.38)	+10.8 (±5.98)	+11.3 (±28.90)	+38.9 (±16.04)*	+20.7 (±12.72)	+38.9 (±16.04)*	+55.0 (±13.65)**	+100.8 (±24.50)**					

** denotes $P < 0.01$. * denotes $P = 0.05-0.01$.

† The two corresponding values for liver weights expressed in terms of weight of liver per unit of body-weight are as follows: 31.1 (±11.35)* and 18.4 (±11.31).

Effect of vitamin C on size of liver. An unexpected finding was that the mean weight of the liver in those rabbits that had received no vitamin C for 175 days was substantially less than in the rabbits that had received supplements of synthetic ascorbic acid or cabbage (Table 1). The difference, however, was of doubtful significance statistically (Table 3).

Table 4. *Vitamin C content of organs and blood of different species*

Species	Vitamin C intake (mg/day)	Typical values for ascorbic-acid concentration (mg/100 g)		
		Adrenals	Liver	Blood
Guinea-pig	15*	130	14	0.8
Rat	0	440	40	1.0
Chick	0	140	33	1.2
Rabbit	0	236	16	0.9
	50	246	19	1.4
	50†	363	24	1.5

* Approximate intake, derived from consumption of 15 g cabbage daily.

† Approximate intake, derived from consumption of 50 g cabbage daily.

DISCUSSION

Our principal findings can be summarized in the statement that rabbits kept on a vitamin C-free diet, for periods of up to 25 weeks, remained as healthy, and gained weight at approximately the same rate, as those receiving vitamin C as synthetic ascorbic acid or in cabbage. On post-mortem examination at the end of the experiment, there were no signs of scurvy or other obvious abnormalities. Moreover, the total amounts of vitamin C found in the various organs were considerably greater than those in the control animals killed at the beginning of the experiment, and during the test vitamin C was being continuously excreted in the rabbits' urine. As we have said, there seems no other way of interpreting these results than by concluding that the rabbit can synthesize the vitamin in its body. In this connexion it also seems significant that the concentrations of vitamin C in the various organs were somewhat similar to those found in rats and chicks, species known to be able to synthesize their own vitamin C, and appreciably higher than those present in the organs of guinea-pigs receiving cabbage in fairly liberal quantities.

Also in line with the foregoing interpretation is our finding that the provision of vitamin C in the rabbit's diet had the effect of further increasing the content of the vitamin in the organs, especially the blood and liver. The explanation, no doubt, is that the rabbit, besides being able to synthesize adequate amounts of vitamin C in the body, can also derive additional supplies from the diet when it contains any.

We are left with the somewhat puzzling fact that Findlay and other authors have published accounts of supposed signs of scurvy seen in rabbits. Perhaps the most likely explanation is that the ill effects they described were caused by deficiency of other dietary factors at that time not yet known, or less well recognized, possibly sometimes including vitamin K. In the experiments of Findlay (1921), for example, the basal diet consisted of bran, oats and water, and must therefore have been somewhat

deficient in a variety of nutrients; the beneficial action observed on administration of swede juice, although attributed at the time to its vitamin C content, might well have been due to other constituents. If this interpretation is correct, it is in line with the explanation, now generally accepted, of the early observation by Harden & Zilva (1918) and Drummond (1919) that young rats grew better on a vitamin C-free diet when it was supplemented with orange juice or other food material designed to convey vitamin C alone, but in fact supplying also additional vitamins, particularly those of the B group.

It seems likely also that, in several of the early accounts of scurvy in pigs, dogs and rabbits, mentioned above in the introduction, some of the bony abnormalities described were those not of scurvy but of rickets. In this connexion we may recall the earlier confusion about 'scurvy-rickets' in human infants, considered at one time to be a combination of scurvy and rickets, but later established, after the work of Barlow (1894), and others, to be simply infantile scurvy.

Turning to the more quantitative aspects of our metabolic experiments with rabbits, we may note that when the additional vitamin C was supplied in the diet, a relatively small increase only was found in the tissues and the urine. We can conclude therefore that the rabbit, in common with other species previously studied, has only limited powers of storage of vitamin C (in contrast for example with the fat-soluble vitamins), and that a considerable destruction of vitamin C must occur in the body.

In the tests on guinea-pigs, the amount of vitamin C found in the tissues was relatively low, compared with the other species. It may be pointed out, however, that the intake of vitamin C, although several times greater than that needed to prevent any known signs of scurvy, was nevertheless considerably less than would be consumed by the animals in their natural habitat, when their body stores would be correspondingly greater. Similarly, the supplements of vitamin C given to the rabbits should not be regarded as specially large, compared with what they could obtain in the field.

An incidental finding, in our experiments, was the suggestion of some possible increase in liver weight in those rabbits given supplements of vitamin C, either as ascorbic acid or as cabbage. This was surprising and needs further study to ascertain whether or not the difference noted was fortuitous.

SUMMARY

1. Young rabbits were kept for periods of up to 25 weeks on a vitamin C-free diet. Control groups received allowances of synthetic ascorbic acid (50 mg/day) or of cabbage (50 g/day).

2. The rabbits on the vitamin C-free diet gained weight at a similar rate to the controls and continued to excrete considerable amounts of vitamin C in their urine. At the close of the experiment, the total quantities of ascorbic acid present in various organs (adrenal glands, spleen, kidneys, liver) were considerably higher than in corresponding animals killed at the beginning of the experiment. There were no signs of scurvy.

3. With vitamin C in the diet, the amounts in the organs were greater still.

4. The findings are interpreted to mean that the rabbit can synthesize its own vitamin C and can also derive further supplies from any in the diet.
5. In line with this interpretation was the finding that the concentrations of ascorbic acid in the organs of the rabbit were of a similar order to those found in the rat and chick and appreciably higher than in those of guinea-pigs receiving reasonably liberal allowances of vitamin C.
6. We are thus unable to substantiate the descriptions in the earlier literature of the occurrence of scurvy in rabbits. Consequently the previous finding in this laboratory, of a resemblance between the rabbit and the guinea-pig in responding to ACTH or cortisone by a massive increase in the weights of their livers, does not appear attributable to a common need for vitamin C.

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