CIX. VITAMIN C AND THE RAT'S DIET.

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THE rôle of the antiscorbutic vitamin in the rat's diet is a question upon which there appear to be different views. On the whole nutrition workers in America omit orange and lemon juice from synthetic diets, while in England it is usual to add small quantities. Simmonds [1924] states that "the rat is apparently immune to scurvy and does not require this vitamin in its diet," and Mendel [1923], in discussing the essential factors of a diet, says that the rat does not seem to suffer from a lack of antiscorbutic foods. Parsons [1920] found that the livers of rats fed for long periods on a typical scorbutic diet were rich in the antiscorbutic substance and suggests that the rat needs this factor for its normal metabolism, but is capable of synthesising it. Many American workers do not even mention vitamin C in discussing the composition of their rats' diets, but tacitly assume that this food factor is not needed by the animal. For instance, Anderson and Mendel [1928] supplement their experimental diets by daily doses of yeast and cod-liver oil to provide "an adequate intake of the indispensable vitamins." Sure [1924, 1925] uses cod-liver oil and Harris uses yeast-vitamin powder, but no orange or lemon juice either with his skimmed milk or pure synthetic diets, and Elmslie and Steenbock [1929] give yeast as a source of the vitamin B complex and cod-liver oil to supply vitamins A and D, but no vitamin C. Kennedy and Palmer [1922] also use no vitamin C in their basal diet and express the view that very few workers regard vitamin C as a necessary constituent in the diet of growing rats. Evans and Bishop [1923] used no source of vitamin C in their synthetic diet, but in trying to cure sterility they gave orange juice to some rats and fresh green lettuce to others; they found the latter to be a most efficient cure, but apparently noted no effects due to addition of the orange juice. On the other hand, Harden and Zilva [1918] consider that rats grow better when the antiscorbutic factor is present in the diet, and their growth curves exhibit an appreciable improvement. Drummond [1919] agrees with the work of Harden and Zilva and states that "the rat requires the antiscorbutic factor in order to achieve a normal development." Kennedy [1926] says that although the rat appears to thrive without vitamin C he added concentrated orange juice in case it might be essential for reproduction. The experiments of Bacharach [1925]

showed that no disadvantage attended the omission of lemon juice from the rat's diet during the experimental period involved in testing for vitamin B, and other workers [Coward, Key, Morgan and Cambden, 1929; Drummond and Marrian, 1926; Reader, 1929; Willimot, 1926] now omit lemon juice from a vitamin B-free diet. Coward [1929, 1930] and Coward, Key and Morgan [1929] also omit vitamin C from their vitamin A-free diet.

The amount of orange or lemon juice added to synthetic diets varies a little, but as a rule 5–6 cc. per 90–100 g. dry solid is given by nutrition workers in England [Boas, 1926; Chick, 1926; Chick and Roscoe, 1926; Luce and MacLean, 1925; Reader and Drummond, 1926].

The diets used by Harden and Zilva [1918] contained much larger amounts of lemon juice, *i.e.* 40 instead of 5 cc. Since the growth of rats fed on synthetic diets is far below that obtained on a good mixed diet of kitchen scraps, it is worth while to try any factor which might produce an improvement. Therefore experiments were made in which rats were given large amounts of lemon juice in the synthetic diet to see if it would be an advantage to incorporate more than the usual 5 cc. of orange or lemon juice in the basal diet.

EXPERIMENTAL.

(a) *Animals and method.*

Piebald rats bred in the laboratory were used in this work. Twelve animals, 6 ♀ and 6 ♂ (except in the trial experiment with lemon juice, where owing to a shortage of rats only 5 ♀ and 4 ♂ were available) were used for each experiment and were kept in a large metal cage with a screen, so that they had no access to excreta. The pregnant does were removed to smaller wooden cages a few days before the birth of a litter. The rats were weighed daily for the first 4 weeks, after which the weighing was omitted on Saturdays and Sundays. After the birth of the first litter, when the rate of growth is slower, the males were weighed only once a week, though the mother and her young were weighed daily.

(b) *Diets.*

Two basal diets were used, one containing caseinogen and the other edestin, as it was thought advisable to use two different proteins in case one produced greater growth than the other, as a result of which the effect of vitamin C might be more marked. The composition of the basal diets was as follows:

Diet AF	Diet AF Ed
Caseinogen 20 g.	Edestin 20 g.
Potato starch 56 g.	Potato starch 56 g.
Mineral salts 8 g.	Mineral salts 8 g. [Hartwell, 1922].
Butter 16 g.	Butter 16 g.
Marmite 15 g.	Marmite 15 g.

The diet was prepared by the "cooked" method described in a previous paper [Hartwell, 1925]. Lemon juice strained through muslin and the green leaves of cabbage finely minced were used as sources of vitamin C, and both were well beaten into the diet when it had cooled. Food and distilled water were given *ad lib.*, but special feeding dishes were used in order to prevent scattering of the food and thus to obtain an approximate intake per cage.

(c) *Experiments.*

1. *Diet AF.* This experiment was made in duplicate in order to see what differences in growth might occur with the same diet.

2. *Diet AF + lemon juice.* The proportion of lemon juice added was 40 cc. to every 84 g. of dry diet (*i.e.* caseinogen, starch and mineral salts). A trial experiment was made using 5 ♀ and 4 ♂ to see if the animals could tolerate this amount of lemon juice, or if such a diet would be too acid and therefore cause intestinal disturbances. The young rats developed diarrhoea after 3–4 days on the diet, but after 10 days the faeces were quite normal. Temporary digestive disturbances often occur when weanling rats are put on synthetic diets, but usually the young animals appear to be able to adjust themselves to their new diet in a week or 10 days. In a second experiment 6 ♀ and 6 ♂ were used, and the experiment was continued for 15 weeks.

3. *Diet AF + cabbage.* 72 g. minced green cabbage leaves were added to the basal diet instead of 40 cc. of lemon juice. For the first 2 weeks minced lettuce was given, but this proved so expensive that green cabbage was used instead.

4. *Diet AF Ed.* This diet was similar to diet AF, but pure edestin¹ was used instead of caseinogen.

5. *Diet AF Ed + lemon juice.* 40 cc. lemon juice (strained) were added to 84 g. of the dry diet as in the experiment AF + lemon juice.

RESULTS AND DISCUSSION.

(1) *Growth.* In all experiments the rats grew well, though the rate of growth was not equal to that of the control animals fed on a mixed diet of kitchen scraps + bread and milk. The addition of lemon juice appeared to have no appreciable effect on the rate of growth. Fig. 1 shows the average growth curves of the males and of the females up to the time they became pregnant. The curves show that there is no greater difference in weight between the rats receiving lemon juice and those getting no vitamin C than there is between the two curves obtained at different times on diet AF alone. In the trial experiment with lemon juice the growth is just better than when no lemon juice was included in the diet. The growth curves when cabbage was used as a source of vitamin C show similar results. When edestin was given

¹ I am indebted to Messrs E. T. Pearson & Co., Ltd., for the pure edestin used in these experiments.

Fig. 1. Growth curves of male and female (shorter owing to pregnancy) rats. Each curve is the average for six rats, except diet AF + lemon juice, Exp. 1.

- 1. —•—•— Diet AF. Exp. 1.
- 2. - - - - - Diet AF. Exp. 2.
- 3. —△—△ Diet AF + lemon juice. Exp. 1.
- 4. - - - △ - - - Diet AF + lemon juice. Exp. 2.
- 5. —○—○ Diet AF + cabbage.
- 6. —□—□ Diet AF Ed.
- 7. —▲—▲ Diet AF Ed. + lemon juice.

instead of caseinogen the rate of growth appeared to be just better when lemon juice was added, but this improvement is very slight, the actual difference in weight being no greater than that obtained in the two separate experiments on diet AF.

(2) *General condition.* Although the animals grew well, their general condition was not up to standard. None of them was quite so firm to feel as the stock animals and practically every rat exhibited loss of fur, though the coat grew again without any alteration in the diet. The lemon juice caused no improvement in the caseinogen diet, but with edestin the fur did not come out quite so badly when lemon juice was added.

(3) *Reproduction.* On the whole reproduction was very poor and therefore the experiments were not continued after the first litters were born. Both with presence and absence of lemon juice the young were frequently born dead or died within a few days of birth, only a few were reared and the numbers per litter were low compared with stock animals.

From these experiments, therefore, it appears that the addition of lemon juice to the basal diet produces no improvement in breeding, but green cabbage leaves have a beneficial effect. Hence it seems that vitamin C itself is not the factor to which the improvement is due, and it is obvious that the raw cabbage contained some food factor of importance in breeding, which was not present in the lemon juice. It seems unlikely that the diets used were at all deficient in vitamin E, for Sure [1927] finds that 10 % of butter-fat in a synthetic ration assures fertility and successful lactation in the rat, and the diets described in this paper contained 16 % of butter. Mattill and Clayton [1926] consider it probable that milk-fat contains more vitamin E than has been estimated, though Evans and Bishop [1923] find that large quantities of this fat are required to cure sterility. However, the curative dose may be greater than the preventive ration. No information can be found as to the relative amounts of vitamin E in lemon juice and cabbage, therefore the reason for the improvement produced by cabbage leaves must await further research. It is interesting, however, to note that no improvement in growth was obtained by the addition of cabbage as compared with the lemon juice, affording further evidence that the dietary requirements vary with different periods of existence.

The experiments described here do not support the view of Harden and Zilva [1918] that the addition of the antiscorbutic vitamin to the rat's diet produces better growth. These workers obtained distinctly better growth when they added lemon juice with the citric acid removed, but as suggested by Palmer and Kennedy [1927] the lemon juice may have contained vitamin A or B, or both, and therefore the favourable result may not have been exclusively due to vitamin C. Since the experiments of Harden and Zilva much work has been done on growth with various diets and it is probable that there are several possible explanations of their results. For instance, their AB diet contained different amounts of the various constituents as compared with their ABC diet—thus in the AB diet there were 2.5 cc. olive oil, while the ABC diet had only 0.25 cc.

More recent work has shown that the actual proportion of constituents of a diet is an important factor and this in itself may account for the different rates of growth. It is also possible that the modified technique adopted now, *i.e.* keeping animals on screens away from their excreta, will require an alteration in the amounts of the different food factors required. In previous work [Hartwell, 1926] good growth was obtained with 2 g. marmite per 100 g. solid, but more recent work when rats were kept on screens has shown this figure to be too low. With their excreta accessible animals make up deficiencies in their diets from this source, but a more generous supply in their food may well produce better growth.

SUMMARY.

The addition of the antiscorbutic vitamin to a synthetic diet produced no improvement in the rate of growth or reproduction of the rat.

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