XXXII. ACCESSORY FACTORS IN THE NUTRITION OF THE RAT.

BY ARTHUR HARDEN AND SYLVESTER SOLOMON ZILVA.

From the Biochemical Department, Lister Institute.

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THE researches of McCollum and Davis have revealed the fact that rats cannot grow or exist on an artificial diet unless two principles as yet chemically unknown are present. These were designated by McCollum and Kennedy [1916] as fat-soluble A and water-soluble B. The former is associated in nature mostly with fats of animal origin; the latter is soluble in water. McCollum and Kennedy have further found that the water-soluble B if not identical with the antineuritic factor is at least closely associated with it, and this has been confirmed by Drummond [1917]. More recently McCollum and Pitz [1917] and Pitz [1918] have expressed the view, based on observations made on guinea-pigs and rats, that scurvy is not a disease produced by the deficiency of an accessory factor and that the scorbutic condition produced in guinea-pigs by dietetic means is due to other unfavourable points in the diet. In a previous communication [1918, 2] we have already discussed their experiments on the guinea-pigs and have shown that their conclusions were not warranted by the experimental evidence brought forward in their papers. We hold the view that scurvy is a disease brought about by the dietetic deficiency of a principle distinctly different in its physical and other properties from the antineuritic and the fat-soluble A factors. It is evident from the extensive researches of Osborne and Mendel, and McCollum and his collaborators that by omitting the addition of antiscorbutics to their purified diet, no scorbutic lesions can be produced in rats, such as are found in human subjects, monkeys and guinea-pigs, when they subsist on diets deficient in antiscorbutics. Guinea-pigs when put on oats, bran and autoclaved milk show scorbutic symptoms 14–21 days after the commencement of the experiment. Our own observations on monkeys [1919] show that these animals manifest scorbutic lesions after 2½–3 months' subsistence on a diet free from

antiscorbutics. As regards human subjects there appear to be a few apparently reliable clinical records of cases in which, owing to adverse circumstances, the subjects have been entirely deprived of antiscorbutics for a considerable time. Judging from these, 6-8 months seems to be the time necessary for the subject to exist on a deficient diet before the disease is well declared. In perusing the literature one finds, however, that rats have been kept by various experimenters for a considerable length of time on scorbutic diets without showing any signs of scurvy. The experiments we are about to describe show nevertheless that rats existing on a scorbutic diet, although capable of gaining in weight and reproducing themselves, without any apparent manifestation of pathological symptoms for months, do not thrive so well as animals which have their diets supplemented with an antiscorbutic. This suggests that although rats are not very susceptible to scurvy they cannot absolutely dispense with antiscorbutics without restriction of their normal development.

EXPERIMENTAL.

Our investigations were carried out on several groups of rats receiving a purified basal diet of starch, caseinogen and inorganic constituents and the various accessory factors. The basal diet was made up as follows:

Starch	75 per cent.
Caseinogen	20 ,,
Salts	5 ,,

The salt mixture was the same as that employed by McCollum and his collaborators in their researches and had the following composition:

NaCl	...	...	1.73 g.	CaH ₄ (PO ₄) ₂ ·2H ₂ O	5.40
MgSO ₄ (anhydr.)	...	...	2.66	Calcium lactate	13.0
NaH ₂ (PO ₄)H ₂ O	...	...	3.47	Ferric citrate	1.18
K ₂ HPO ₄	...	...	9.54		

The fat-soluble A factor was supplied in the form of butter-fat purified by centrifuging the melted fat and decanting the supernatant layer. The antineuritic factor was introduced by adding autolysed yeast. The antiscorbutic was added in the form of lemon juice from which the citric and other organic acids had been removed by the method we have already described in a previous communication [1918, 1].

For the sake of convenience we shall designate in this paper the diets of the various groups by the accessory factors they contain, using the letters

A, B, and C for the fat-soluble A, antineuritic, and antiscorbutic factors respectively. The rats were kept separately in cages; couples belonging to the same group were brought together only occasionally for mating purposes. The food was supplied to them fresh in balls every day and the remains of the previous day were weighed daily. The drinking water was changed twice daily.

Fig. 1. Weight curves of 3 rats on an AC diet.

Fig. 1 represents the weight curve of 3 rats kept on an AC diet. The daily ration of each of these rats was made up as follows:

Basal mixture	...	...	...	...	10 g.
Antiscorbutic equiv. to lemon juice	...	...	...	...	5 cc.
Centrifuged butter fat	...	...	...	...	1.8 g.
Olive oil	...	...	...	...	1.6 g.

The above ingredients were incorporated in a single ball, the olive oil being added in order to obtain a convenient consistency in the paste. From $\frac{1}{4}$ – $\frac{3}{4}$ of the daily ration was consumed by the animals.

It is seen from the weight curves that none of the animals has gained appreciably in weight during any period of the experiment. This becomes more marked as the rats were at an age when development under normal conditions progresses rapidly. After about three weeks the animals commenced losing in weight and died five weeks after the commencement of the experiment. Rat No. 8 was killed by the buck after $4\frac{1}{2}$ weeks when left with it in a cage for mating purposes. Rats Nos. 7 and 9 showed decided neuritic lesions before death. Rat No. 7 first showed marked paralysis in the hind limbs four days before death. This condition became gradually worse. Rat No. 9 showed even more striking symptoms. The following was its condition three

days before death¹. Rotated as ingoing corkscrew. Position of left ear approximated left shoulder. Left eye to the ground. Tone increased on left side. Body was bent anticlockwise, i.e. concavity to the left. No circus movements observed. Animal was able to use fore and hind legs for grasping objects apparently without incoordination. Could not walk in a normal manner. It stood on a wide base. Difficulty appeared to be due to the increase in tone of muscles of limb. A day later the animal showed circus movements and died on the subsequent day. Portions of the sciatic nerves of rats No. 7 and No. 9, teased and treated with osmic acid, were examined histologically by Miss F. M. Tozer. In Miss Tozer's opinion the greater part of the nerve of rat No. 9 was sound. There were however a fair number of curiously eroded abnormal looking fibres concerning which in the absence of further evidence it is difficult to give a definite opinion. Their appearance is possibly suggestive of a very early stage of degeneration. The teased preparation of the nerve of rat No. 7 showed no degenerating fibres.

Fig. 2. Weight curves of 3 rats on a purified B diet.

Fig. 2 represents the weight curves of three rats fed on a B diet. The daily ration of these animals was as follows:

Basal mixture...	...	12 g.	Water	...	...	2½ cc.
Autolysed yeast	...	5 cc.	Olive oil	...	...	2½ cc.

From $\frac{1}{2}$ – $\frac{3}{4}$ of this ration was consumed by the animals daily. All these animals succumbed to the fat-soluble A factor deficiency within a period varying from 2 to 3½ months. During the first month they all gained in weight. Their weight then became more or less stationary, but the animals declined a little before death. Two of these rats, namely 24 and 26, showed xerophthalmia in acute form.

¹ We are indebted to the late Dr F. E. Batten for this description of the condition of the animals.

That the antiscorbutic factor does not fulfil the same physiological function as the fat-soluble A factor is seen from Fig. 3 which represents the weight curves of three rats fed on a BC diet. The daily ration of this group was made up as follows:

Basal mixture	...	...	...	12 g.
Antiscorbutic equiv. to lemon juice				5 cc.
Autolysed yeast juice	...	...		5 cc.
Olive oil	...	...	...	0.75 cc.

Fig. 3. Weight curves of 3 rats on a pure BC diet.

About three-quarters of the daily ration was consumed by the rats. The weight curves of this set do not differ much from those of the previous group. Rat 29 died two months and rats 27 and 28, $3\frac{1}{2}$ months after the commencement of the experiment. They all developed xerophthalmia. It is evident from this experiment that the addition of an antiscorbutic to a diet lacking the fat-soluble A does not rectify the deficiency.

In the last two experiments the caseinogen employed was previously extracted with ether and alcohol. Experiments of the same character as these two were made in which the caseinogen had not been previously purified. The results obtained are of sufficient interest to be recorded. Fig. 4 shows the weight curves of a group of rats fed on B diet with unpurified caseinogen and Fig. 5 represents the same of a group of rats fed on a BC diet with unpurified caseinogen.

The general characters of these curves are the same as those of the animals fed on similar diets with purified caseinogen. The main difference lies

in the prolonged period of time during which the animals lived on the experimental diet. After about two months, growth was entirely suspended and a decline in weight was observed towards the end of the experiments. Many of these animals displayed xerophthalmia. During the progress of the experiment the buck of each set was on several occasions mated with the does, but no litters were produced. This prolonged sustenance on a diet greatly deficient in the fat-soluble A factor afforded us the opportunity of observing certain marked histological changes in the *post-mortem* tissues of these animals which are of decided interest. We shall describe and discuss these results in another communication.

Fig. 4. Weight curves of 3 rats on a crude B diet.

Fig. 5. Weight curves of 3 rats on a crude BC diet.

Two groups of rats were also fed on AB and ABC diets. These were made up as follows:

	AB	ABC
Basal mixture	10 g.	10.6 g.
Purified butter	1.85 g.	1.7 g.
Autolysed yeast	5 cc.	5 cc.
Olive oil	2.5 cc.	0.25 cc.
Antiscorbutic equiv. to lemon juice	—	5 cc.

Fig. 6. Weight curves of 2 ♂ rats on ABC and AB diets respectively.

Fig. 7. Weight curves of 2 ♀ rats on ABC and AB diets respectively.

.... indicates that no weighings were made owing to the birth of a litter.

These two groups grew satisfactorily and reproduced themselves. The ABC group however flourished decidedly better than the AB group. This can be seen from Figs. 6 and 7 which represent the weight curves of animals

belonging to those two groups. Fig. 6 represents the weights of the two respective bucks and Fig. 7 of the two does on the two diets. In both cases the difference in growth is decidedly marked. This observation does not appear to be accidental as similar observations have been made in this laboratory on other animals kept on similar diets and so far no deviation from the above behaviour has been recorded.

If animals kept on the AB diet do not develop to their fullest capacity without having the antiscorbutic added in their diet, it would appear that rats are susceptible to an antiscorbutic deficiency although they do not develop definite lesions. Further investigations will definitely decide this point.

SUMMARY.

1. An antiscorbutic does not fulfil the physiological function of the fat-soluble A when it replaces it in the diet of the rat.

2. The authors confirm the observation of McCollum and his colleagues and of Drummond that by depriving rats of the antineuritic factor a dietetic deficiency is brought about as a result of which neuritic lesions are produced in the animals and a fatal termination ensues if the diet is not rectified in time.

3. Rats subsisting on a diet containing the antiscorbutic as well as the water-soluble and fat-soluble A factors grow better than rats from the diet of which the antiscorbutic factor is absent.

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