XXIII. NOTE ON THE ETIOLOGY OF SCURVY IN GUINEA-PIGS.

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As a result of experiments carried out on rats and guinea-pigs McCollum and Pitz [1917] came to the conclusion that scurvy in the guinea-pig is not a disease brought about by the deficiency of an accessory factor in the diet but that the scorbutic condition is caused by a diet chemically and physically unfavourable. It is particularly the physical condition of the scorbutic diet of oats which is supposed by them to have a detrimental effect on the digestive tract of the guinea-pig, causing constipation and thus facilitating the absorption of toxins produced by the bacterial flora of the intestine. These workers were unable to produce scurvy in rats by feeding them on diets of which oats formed the basal constituent, they were however able to induce scurvy after a certain period only in some of their guinea-pigs which were fed on rolled oats and fresh whole milk. Adopting that technique McCollum and Pitz tried a variety of substances which were intended either to obviate constipation in their experimental guinea-pigs or to attenuate the bacterial development in the alimentary tract of the animals. As some of the substances tested produced a beneficial result the authors considered their hypothesis to be substantiated by their observations. Such conclusions however are not in our opinion warranted by the experimental evidence given in their paper. Fresh whole milk was given to the animals *ad lib*. Milk if not subjected to any heating is antiscorbutic and it has been shown recently by Chick, Hume and Skelton [1918] that if large enough quantities of fresh milk are taken by guinea-pigs scurvy does not supervene. We agree with the suggestion of the last mentioned investigators that had the quantities of milk consumed by the animals in McCollum and Pitz's experiments been measured, the beneficial effect observed in their animals treated with the various substances could in all probability have been traced to the antiscorbutic potency of the augmented quantities of fresh milk consumed.

McCollum and Pitz, in the same communication, ascribe the antiscorbutic activity of orange juice to its content of sodium and potassium citrates which

possess laxative properties and to the retardation of the development of bacteria by the action of citric acid contained in the juice. These explanations appear hardly feasible in view of the observations made by us in connection with the antiscorbutic content of lemon juice. In the preceding paper [Harden and Zilva, 1918] we have shown that by removing the free citric acid from lemon juice a residue is obtained whose inorganic content is almost insignificant, which nevertheless contains almost the entire antiscorbutic content of the original juice. It is therefore evident that the activity of the extract cannot be ascribed either to its content of citric acid or of sodium and potassium citrates as the inorganic residue of the preventive dose of 1.5 c.c. only corresponds to about 2-3 mg. of potassium or sodium citrate.

Pitz [1918] in a recent communication describes experiments which in his opinion offer further evidence in support of the hypothesis that scurvy in the guinea-pig is not due to the absence or deficiency of a certain factor in the diet but to the absorption of toxic products of bacterial activity brought about by the long retention of the faeces in the caecum caused by the unfavourable physical consistency of the diet. In his experiments he adopted the procedure of feeding the guinea-pigs with different sugars in order to induce an acidophilic bacterial flora instead of the usual putrefactive one and thus to obviate the possibility of the animals absorbing the toxic products of putrefaction which in his opinion are responsible for the production of scurvy in guinea-pigs. He claims to have achieved quite a decided success by adding lactose to the animals' diet; with other carbohydrates a marked beneficial effect was also observed. In this investigation Pitz again employed the same technique as was used by him previously in collaboration with McCollum, giving the guinea-pigs rolled oats and fresh whole milk *ad lib.* as a basal diet. We are therefore again unable to interpret Pitz's results in the same way as he does. Moreover we had records of some experiments at the time of the appearance of Pitz's paper which did not bear out that author's observations. In connection with another investigation we had the opportunity of feeding guinea-pigs on cane sugar, fructose, and a syrup obtained after the fructose has been crystallised out from the products of the hydrolysis of inulin for which we are indebted to Prof. J. C. Irvine. These sugars, in the quantities we used, did not show the slightest antiscorbutic potency. Since the appearance of Pitz's publication we have also tested lactose, using high quantities and employing our technique, and as was expected no antiscorbutic activity was found to be possessed by the sample of the lactose used by us. We therefore consider it of interest to publish the record of our experiments.

EXPERIMENTAL.

In testing the antiscorbutic potency of a substance the following technique is employed in our laboratory. Guinea-pigs weighing from 250 g. to 450 g. are put on a basal diet of crushed oats and bran (one volume of oats to two volumes of bran) and a daily ration of 50 c.c. of milk autoclaved for one hour at 120°. On this diet the animal commences to drop in weight after about fourteen days and usually dies of scurvy within thirty days. In our experience no animal has ever yet failed to die of scurvy on such a diet. The substance to be tested is administered in daily rations *per os* or in some cases it is dissolved in milk which is quantitatively consumed by the animal. Fig. 1 represents the weight curves of guinea-pigs which received the scorbutic diet described above and

Fig. 1

○-.-○-.-○-.-○ Schering's Fructose.

○—○—○—○ uncrystallisable residue from the preparation of fructose from inulin.

1 g. daily of Schering's fructose and of the uncrystallisable residues from the preparation of fructose from inulin respectively. Both these sugars were dissolved in the milk and were fully consumed by the animals until the last few days when, owing to their grave condition, the guinea-pigs were unable to consume much food. These animals died twenty-eight and thirty-four days after the commencement of the experiment and the post mortem examination disclosed a very acute condition of scurvy.

We have carried out no special experiments with cane sugar, but in the course of our work on scurvy we have on numerous occasions used generous doses of cane sugar for sweetening purposes without noticing the slightest beneficial effect, and we are convinced that it is scorbutically inactive.

Lactose was tested on three guinea-pigs receiving a basal diet of oats and bran and 50 c.c. of autoclaved milk. As in Pitz's experiments high doses were given. Two guinea-pigs received 6 g. of lactose and one 3 g. per diem in their milk. As will be seen from Table I about four-fifths of this was consumed daily. All the pigs died of scurvy within thirty days so that the onset was not even delayed. Fig. 2 shows the weight curves of the animals in question, which are typical weight curves of guinea-pigs kept on a scorbutic diet of oats, bran and autoclaved milk. A few days before their death the animals lost their inclination for food. In order to insure high consumption of lactose even at that stage the oats and bran were also mixed with lactose as well as the autoclaved milk.

Fig. 2

It is evident from the above experiments that the sugars tested afford no protection whatsoever against scurvy, and the data tend further to strengthen our belief that any amelioration observed by McCollum and Pitz in their experimental animals was due to the enhanced consumption of the raw milk and not to the antiscorbutic potency of the substances tested. As long as these observers continue to employ their present technique we consider that their results are open to criticism.

TABLE I.

Days	Pig No. 269 (3 g. lactose)		Pig No. 270 (6 g. lactose)		Pig No. 271 (6 g. lactose)	
	Milk	Oats and bran	Milk	Oats and bran	Milk	Oats and bran
1	40 c.c.	19 g.	38 c.c.	24 g.	35 c.c.	10 g.
2	40	14	40	31	40	25
3	40	31	45	29	45	24
4	35	16	35	21	40	21
5	50	18	27	25	35	19
6	45	18	40	26	35	21
7	40	17	40	27	45	19
8	40	16	45	26	45	18
9	44	16	45	23	50	21
10	40	14	35	21	40	26
11	40	9	10	26	45	24
12	10	14	25	28	45	22
13	30	16	40	25	40	21
14	45	14	35	23	45	19
15	40	11	35	24	30	19
16	30	16	40	18	45	21
17	50	14	45	18	40	16
18	45	6	30	8	40	11
19	30	6	30	10	40	11
20	45	4	30	6	30	6
21	35	6	25	12	30	1
22	30	3	25	6	25	0
23	35	7	15	7	35	0
24	25	5	20	5	28	5
25	20	5	15	5	40	5
26	10	5	10	2	10	3
27	12	1	—	—	6	2
28	0	1	—	—	10	3
29	10	1	—	—	0	2
30	—	—	—	—	0	0

REFERENCES.

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