THE DEPLETION OF VITAMIN C IN THE LIVER OF THE GUINEA PIG ON A SCORBUTIC RATION.*

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The dissimilarity in the antiscorbutic requirement of the rat and the guinea pig has been frequently pointed out. This dissimilarity led to an investigation by one of us (H.T.P.) of the antiscorbutic content of the liver of the rat. The results showed a high content of antiscorbutic vitamin in the liver of the rat, which was apparently uninfluenced by the antiscorbutic content of the diet over several months' time (1). A later experiment (2) carried out with greater detail showed no perceptible decrease in the antiscorbutic content of the rat's liver, even though the rats had been restricted for two generations to a purified ration containing, at most, only traces of antiscorbutic vitamin. These early results were also confirmed by Lepkovsky and Nelson (3) who fed a somewhat different ration over the period of two generations in the attempt to deplete the rats of vitamin C, and used smaller doses of the rat liver.

It was suggested in the first report cited (1), that:

"It is conceivable that the liver cells are so constituted that a considerable supply of the antiscorbutic factor may be contained in them while at the same time the content of other tissues is below the level at which proper functioning is possible. It is proposed to test this point by determining also the antiscorbutic content of livers of guinea pigs suffering from scurvy as contrasted with the livers of normal guinea pigs."

It was for the purpose of carrying out this phase of the problem that the present investigation was undertaken.

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EXPERIMENTAL.

Rations.

Two scorbutic rations were used in the feeding of the guinea pigs. Ration I was the soy bean scorbutic ration used in the first investigation cited (1).

Ration I.

	per cent
Soy beans or soy bean flour	84
NaCl	3
Ca lactate	3
Dried yeast	3
Filter paper	2
Butter fat	5

For this ration, both soy bean flour (Hepco) and soy beans cooked in the pressure cooker for 1 hour at 15 pounds pressure were used. To prepare this ration, the filter paper is torn into small pieces and beaten in a quart of distilled water, until a fine pulp is obtained. This is poured on the mixed dry ingredients and rubbed until uniform. When dry it is ground or crushed and mixed thoroughly with melted butter fat. Baker's compressed yeast was used.

Ration II was that used by Steenbock and coworkers (4).

Ration II.

	per cent
Rolled oats	69
Alfalfa meal (autoclaved 30 minutes at 15 lbs. pressure)	25
Casein	5
Common salt	1

The casein was purified following the technique outlined by Steenbock and associates (5) with the following slight modifications: Tap water was used during the whole period; and the water was changed only once each day for 7 days.

This second ration was included in the investigation for purposes of comparison, to make sure that the experimental conditions of this and other investigations were comparable. No significant differences were noted in the severity of the scurvy symptoms induced by the two diets, or the time at which these appeared. There were, however, some differences noted in the readiness with which the animals adapted themselves to the two diets, and the regularity of growth upon them.

Animals.

The determination of the concentration of the antiscorbutic vitamin in the livers of guinea pigs, fed rations varying in their content of this vitamin, was made by feeding varying doses of the livers to a group of guinea pigs (Group A) which showed the early symptoms of scurvy, including unmistakable swelling of wrists, after a preliminary period on a scorbutic diet. Guinea pigs, Nos. 102 and 106, were fed Ration I and Nos. 110, 111, 118, 121, 122, and 133 were fed Ration II. The point at which doses of liver were begun is indicated on Charts I and II.

TABLE I.

Serial No. of guinea pig.	No. of days on scorbutic diet.	Maximum weight of guinea pig.	Weight of guinea pig when killed.	Weight of liver.
		gm.	*gm.*	*gm.*
112	20	185	126	5
117	20	205	164	9.6
129	20	261	215	15.03
108	21	185	127	8
119	21	179	130	7.6
118	22	200	127	6.5
109	22	235	150	9.2
123	22	375	318	18.09
113	23	173	128	7.8
115	23	262	198	12.45
107	24	270	196	12
125	24	414	332	19.2
124	26	393	245	13.13
128	28	357	195	8
130	28	383	255	12

A group of guinea pigs (Group B) was fed Ration II for from 20 to 28 days. In no case was a guinea pig used until marked symptoms of scurvy had developed. Table I gives the weights of the guinea pigs and of the livers used. It was thought to be of interest to record these data since there is no close agreement in the literature as to the relative change in the weight of the liver during the period of depletion of vitamin C. The literature on the subject is reviewed, and new experimental data presented by Bessesen (6).

The other group of guinea pigs (Group C) was fed a ration high in antiscorbutic vitamin. In order to have as close a check

with Group B as possible the basal diet of Group C was identical in its percentage composition with Ration II. However, no pains were taken to autoclave the alfalfa meal or purify the casein. The presence of abundant antiscorbutic vitamin in the diet was assured by offering fresh cabbage at all times.

Preparation of Liver.

The preparation of the guinea pig livers followed closely the method previously described (2) for preparation of the rat's liver. The guinea pig, which was to furnish the liver, was chloroformed and the liver removed a short time after death ensued. One exception should be noted in the case of a pig which died with pronounced scurvy symptoms. In this case, its liver was fed within a few hours after the death of the animal. Since the guinea pig differs from the rat, in that it has a gall bladder, this was removed before the amounts to be fed were weighed out.

In feeding the livers of normal guinea pigs, the size of the livers (varying from 21 to 31.8 gm.) was such that an efficient utilization of material could best be accomplished by feeding twice the prescribed amount on alternate days. This method was utilized also in the case of Guinea Pig 133, fed a 7.5 gm. "daily" dose of scorbutic guinea pig liver. In the other instances, however, the liver of one guinea pig on the scurvy ration was sufficient to furnish the daily doses of the three guinea pigs fed.

RESULTS.

The results of the experiment are shown in Charts I and II. It will be seen that in the case of the guinea pigs given the doses of the livers of normal guinea pigs, the loss in weight was checked in from 1 to 4 days after the addition of liver was started, and the gain in weight continued for from 14 to 16 days until the autopsy was held. A corresponding increase in the activity and well-being of the pigs was also noted. The autopsy showed that scurvy symptoms had almost disappeared in every case.

The scorbutic guinea pigs, given the doses of livers of scorbutic pigs, continued to lose weight steadily until death, which ensued from 5 to 9 days after addition of liver was started. Autopsy of these pigs showed pronounced scurvy symptoms.

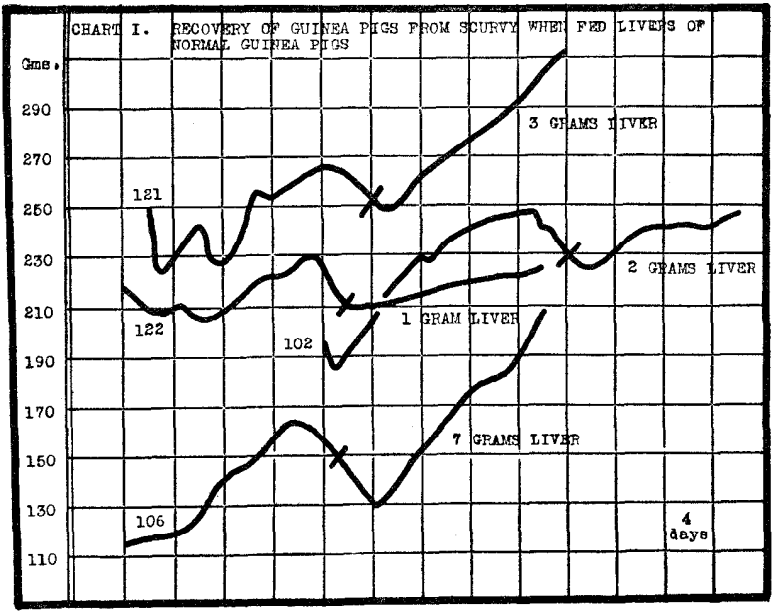

CHART I.

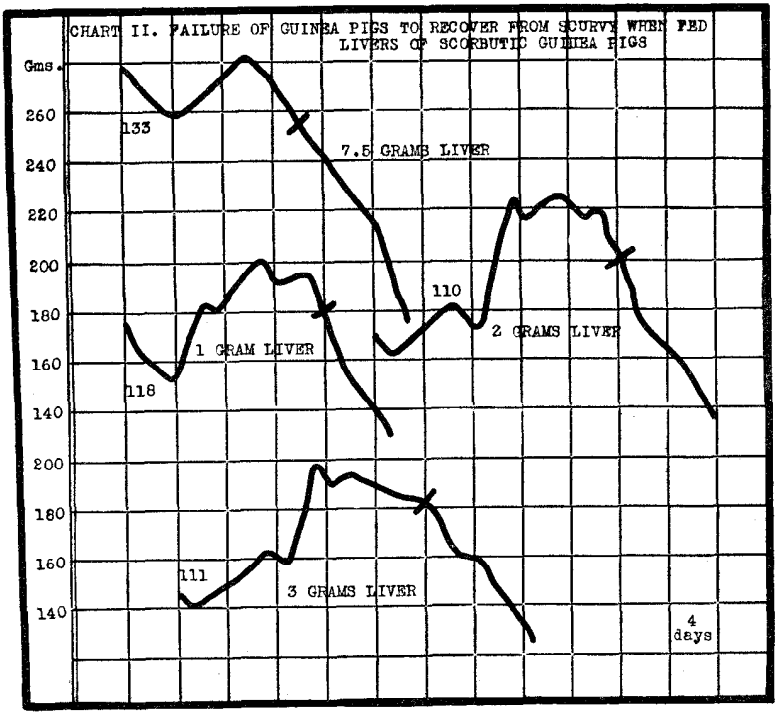

CHART I.

DISCUSSION.

From the results presented it will be seen that in the liver of the normal guinea pig, vitamin C is found in abundance; and that in the liver of the guinea pig fed a diet lacking in this factor, vitamin C becomes strikingly depleted. That the concentration of vitamin C in the normal guinea pig liver is comparable with that in the rat liver is indicated by the close similarity of the growth curves in the present study and in that of Lepkovsky and Nelson (3) wherever comparable doses of liver were used.

The depletion of vitamin C in the guinea pig's liver, when this factor is lacking in that animal's food is another example of such vitamin depletion in the liver already demonstrated in the rat in regard to vitamin A by Steenbock, Sell, and Nelson (7), and in regard to vitamin B by Osborne and Mendel (8).

The present investigation, in addition to these other two instances of depletion, renders all the more surprising the persistence of vitamin C in the liver of the rat even after long intervals on a diet containing at most only traces of this factor. It makes very improbable the hypothesis quoted at the beginning of this paper; *i.e.*, that the persistence of this factor in the rat's liver under such conditions might be accounted for by assuming that a peculiar relationship exists in the liver in regard to vitamin C whereby this factor might persist in this organ after its depletion in the rest of the body. It, therefore, again emphasizes the reasonableness of the hypothesis emphasized in the first report (1) of this series; *i.e.*, that the rat produces vitamin C in metabolism from some source not available to the guinea pig. Whether this is a true synthesis, or a conversion of some closely related compound is being investigated.

BIBLIOGRAPHY.

1. Parsons, H. T., *J. Biol. Chem.*, 1920, xliv, 587.
2. Parsons, H. T., and Hutton, M. K., *J. Biol. Chem.*, 1924, lix, 97.
3. Lepkovsky, S., and Nelson, M. T., *J. Biol. Chem.*, 1924, lix, 91.
4. Hart, E. B., Steenbock, H., and Ellis, N. R., *J. Biol. Chem.*, 1920, xlii, 383.
5. Steenbock, H., Sell, M. T., and Nelson, E. M., *J. Biol. Chem.*, 1923, lv, 399.
6. Bessesen, D. H., *Am. J. Physiol.*, 1922–23, lxiii, 245.
7. Steenbock, H., Sell, M. T., and Nelson, E. M., *J. Biol. Chem.*, 1923, lvi, 327.
8. Osborne, T. B., and Mendel, L. B., *J. Biol. Chem.*, 1923, lviii, 363.