CXXV. THE EFFECT OF INCOMPLETE DIETS ON THE CONCENTRATION OF ASCORBIC ACID IN THE ORGANS OF THE RAT.

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It has been known for some time from biological tests that the liver of animals contains vitamin C in very much higher concentrations than many of their other tissues. Recent observations have brought to light that the small intestine also is rich in the vitamin. Harde and Wolff [1934] were the first to demonstrate that, of the entire intestinal tract, the small intestine of the mouse kept on a scorbutic diet showed by the indophenol titration method a more or less constant ascorbic acid content about equal to that of the liver—an observation which led them to suggest that the vitamin was most probably synthesised by the former. A few months later Hopkins [1934] made a similar observation with regard to the rat and further extended the investigation in order to determine its connection with the vitamin content of the liver and the relation of the concentrations in these organs to the composition of the diet consumed by the rat. Soon after the publication of the above communication the writer [Zilva, 1935] produced evidence which militated against the view that the concentration of ascorbic acid in the small intestine of the mouse or rat was due to the synthesis of the vitamin in that organ. He was able to show that when guinea-pigs—animals which do not synthesise ascorbic acid—were kept on a good mixed diet containing cabbage *ad lib.* extracts of the walls of the small intestine possessed a reducing capacity for indophenol of an order similar to that of the mouse and the rat. When, however, the vitamin was withheld from the diet the reducing capacity fell almost to zero. Furthermore, when ascorbic acid was injected into the blood stream of vitamin C-depleted animals the reducing capacity for indophenol rose to about the level found in the small intestine of guinea-pigs subsisting on a diet rich in vitamin C. The results indicated that it was more probable that the mouse and the rat, like the guinea-pig, tend to concentrate and store rather than to synthesise ascorbic acid in the small intestine. From the writer's experiments with guinea-pigs it could be concluded in addition that the indophenol-reducing substance in the walls of the small intestine of the mouse and rat was mainly ascorbic acid. Similar results were also obtained independently by Jacobsen [1935].

In a recent publication Hopkins [1935]² with the assistance of B. R. Slater gives in greater detail the results and conclusions previously outlined in brief in his preliminary note [Hopkins, 1934]. He found that when rats existed on a

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² In discussing the origin of ascorbic acid in connection with animals which synthesise the vitamin Hopkins says in this communication "That it (ascorbic acid) is normally present in the latter (the small intestine) is now well known [Hopkins, 1934; Harde and Wolff, 1934; Zilva, 1935; *et al.*]". For the sake of precision the writer would like to point out that in his publication here referred to he demonstrated the presence of ascorbic acid in the intestine of the guinea-pig and not of the rat or mouse, the object being, as mentioned above, to stress the improbability of synthesis in the small intestine implied by Harde and Wolff and by Hopkins in their communications.

normal mixed diet the average concentrations of ascorbic acid in the liver and in the small intestine were the same. When however a carbohydrate diet was consumed by these animals the concentration in the liver rose immediately, becoming higher than in the intestine. On the other hand on diets lacking carbohydrates such as those consisting of protein and fat or fat alone and after 48 hours' starvation the concentration in the liver rapidly fell while that in the intestine rose. He therefore considers that the liver and the intestine represent sites of formation rather than of storage of the vitamin. When carbohydrate in the liver is inadequately available as a primary source for synthesis, the intestine can then utilise protein for this purpose. A mutual adjustment is thus possible in the productive activities of these organs.

This work appeared to call for repetition. From the data provided in this communication it will be seen that some of the major results could not be reproduced, whilst others when considered in their entirety are subject to an interpretation different from that given to them by Hopkins.

Technique.

Although the technique used by Hopkins was in principle the same as is usually applied to work of this nature, all the conditions were adjusted as nearly as possible to those observed by him in his work.

Stock. Rats raised in two different places, namely the Lister Institute and by a breeder in Hampshire were employed. The origin of these experimental animals was found, however, not to influence the results. Each experimental animal was kept in a separate cage.

Diets. Rats on the normal diet were offered a free choice of brown bread, milk, oats and bran. The carbohydrate diet contained three parts of starch and one part of cane sugar; the protein and fat diet two parts of caseinogen and one part of lard. The last two diets were made into a paste, the first with about one-quarter part by weight of water, the second with about an equal part of water. The fat diet consisted of lard only. To each of the diets about 5% of a balanced salt mixture was added. Clean water changed daily was offered *ad lib.* in all cases during the experiment, including the 48-hour fasting period. The amount of food consumed was recorded daily.

Preparation and extraction of tissues. All the animals were killed by stunning and bleeding. The livers and the intestines were taken out immediately, and after removal of all adhering tissues were worked up for analysis with the utmost speed. The intestines extending from the pyloric to the caecal end were emptied of their contents by a flow of tepid water, squeezed and well dried on filter-paper. The organs were then thoroughly ground with sand in three times their weight of dilute trichloroacetic acid, so as to yield a final extract containing 2.5% trichloroacetic acid.

Titration. All the titrations were carried out by running the indicator into 5 ml. and occasionally less of the colourless extracts adjusted to p_H 2.5 from a macro-burette, until the addition of two drops left the solution coloured for 10 sec. Each extract was titrated with both dimethylaminobenzoindophenol and 2:6-dichlorophenolindophenol. These figures usually agreed very well. When there was a small difference the average of the two titrations was used in the calculation of the result, the average dry weight of the organs being taken as a basis. In a number of representative experiments the extracts were also titrated with both indophenols firstly by delivering the indicators from a micro-burette into the extracts and secondly by running the extracts from such a burette into a measured quantity of 2:6-dichlorophenolindophenol of known strength

(0.2 ml. *N*/200 or 0.05 ml. *N*/100). The control micro-determinations were carried out independently by Mr A. E. Kellie to whom I should like to express my thanks for assistance in this matter. The indicators were standardised daily against pure ascorbic acid.

Determination of dry matter contents. The adhering fatty and other tissues were carefully removed from the organs. They were then dried at 37° for about 24 hours, after which they were ground to a fine powder and dried to constant weight at 100°. All the intestines and some of the livers were extracted with ether in a Soxhlet apparatus during 1 working day previous to drying at 100°.

Discussion of results.

Before considering the general results reference must be made to the conditions under which the determinations of ascorbic acid were carried out. The writer and his colleagues have during the last 10 years been employing the indophenol technique with good results when this reduction method could be applied specifically in the assessment of the vitamin. The standardised indicator, phenolindophenol or dimethylaminobenzoinidophenol used by them was always delivered into about 5 ml. of test solution until the addition of a few drops left the solution coloured for a specified time. Later Tillmans, using the somewhat more highly coloured 2:6-dichlorophenolindophenol which, as well as the above indophenols, is decolorised by ascorbic acid and compounds of a similar or greater reducing potential, also obtained good results by titrating in the same manner. Still later Szent-Györgyi made the useful suggestion of titrating in acid solution in order to suppress the reducing activity of some accompanying substances [cf. Harris and Ray, 1933]. This was followed by the introduction by numerous workers of minor modifications the utility of which is not immediately obvious. One of these [Birch *et al.*, 1933] consisted of delivering the test solution into the indicator instead of the reverse way of titration. Hopkins utilised this variant of indophenol titration and found it an excellent method. In view of the lack of agreement between some of the results in the two investigations it was necessary to ascertain whether it could be traced to the slight difference in the titration procedure. The results obtained (Table I) by the various procedures show that such differences as were observed could not be traced to one particular mode of titration but were due to a general error inherent in the indophenol technique as applied to animal tissue extracts. It may therefore be concluded that the divergencies in the results are not due to the minor variation in the technique and that the inverted order of titration with 2:6-dichlorophenolindophenol, apart from the personal predilection, does not, when considered objectively, endow the results with any higher degree of accuracy.

In this investigation not only are data given of the concentrations of ascorbic acid in the intestine and liver of the rats kept on the various diets but also of the total contents of the vitamin in these organs together with their weight. These latter data were not provided or considered by Hopkins, but, as will be seen later, they form evidence of an essential character in the argument of the thesis.

For the sake of convenience the average results of all the experiments are given in Table VIII, p. 867.

The first experiment to be considered will be that of the rats kept on the stock diet, since the results obtained with them must serve as the standard for comparison. Considering that these rats had a free choice of a mixed diet it was difficult to record the quantity of food consumed by them per day but from various observations it may be estimated to be about 10–15 g. calculated on dry matter. In this and in all the other experiments, the results are divided into

Table I.

Liver					Intestine					
Macro direct Dim. mg./g.	Macro direct Dichlor. mg./g.	Micro direct Dim. mg./g.	Micro direct Dichlor. mg./g.	Micro inverted Dichlor. mg./g.	Macro direct Dim. mg./g.	Macro direct Dichlor. mg./g.	Micro direct Dim. mg./g.	Micro direct Dichlor. mg./g.	Micro inverted Dichlor. mg./g.	
48 hours without food.										
0.29	0.30	0.30	0.29	0.31	0.16	0.16	0.18	0.17	0.20	
0.21	0.20	0.22	0.20	0.21	0.18	0.17	0.20	0.19	0.20	
0.23	0.22	0.22	0.22	0.23	0.27	0.27	0.28	0.26	0.30	
0.23	0.21	0.26	0.24	0.23	0.23	0.23	0.27	0.24	0.28	
0.20	0.21	0.22	0.20	0.20	0.21	0.20	0.23	0.23	0.24	
0.31	0.33	0.33	0.29	0.33	0.31	0.30	0.28	0.25	0.28	
0.30	0.29	0.29	0.28	0.30	0.27	0.27	0.28	0.28	0.29	
0.15	0.17	0.17	0.18	0.16	0.32	0.40	0.34	0.35	0.34	
0.18	0.18	0.16	0.16	0.16	0.30	0.31	0.30	0.29	0.31	
0.28	0.30	0.30	0.31	0.34	0.31	0.30	0.28	0.28	0.24	
Av.	0.24	0.24	0.25	0.24	0.25	0.26	0.26	0.26	0.25	0.27
3 days on carbohydrate diet after 2 days' fast.										
0.20	0.20	0.17	0.17	0.23	0.10	0.10	0.10	0.10	0.14	
0.29	0.29	0.26	0.27	0.33	0.14	0.16	0.18	0.17	0.17	
0.35	0.35	0.30	0.30	0.34	—	0.16	0.16	0.15	0.18	
0.34	0.34	0.30	0.30	0.31	0.16	0.16	0.15	0.14	0.16	
0.24	0.24	0.24	0.23	0.25	0.10	0.10	0.12	0.10	0.11	
0.36	0.36	0.35	0.34	0.37	0.10	0.12	0.13	0.13	0.14	
0.22	0.22	0.21	0.21	0.22	0.15	0.15	0.18	0.17	0.18	
0.29	0.29	0.29	0.28	0.28	0.18	0.18	0.19	0.19	0.20	
Av.	0.29	0.29	0.27	0.26	0.29	0.13	0.14	0.15	0.14	0.16
3 days on protein-fat diet after 2 days' fast.										
0.31	0.32	0.36	0.35	0.36	0.35	0.35	0.31	0.31	0.32	
0.16	0.17	0.14	0.15	0.18	0.24	0.23	0.24	0.21	0.25	
0.21	0.19	0.20	0.18	0.23	0.21	0.19	0.20	0.20	0.23	
Av.	0.23	0.23	0.23	0.26	0.27	0.26	0.25	0.24	0.27	
Dim. = dimethylaminobenzoindophenol.					Dichlor. = 2:6-dichlorophenolindophenol.					

Dim. = dimethylaminobenzoinodphenol. Dichlor. = 2:6-dichlorophenolindophenol.

two sections—one consisting of those obtained with animals weighing between 150 and 200 g., such as are most of those used by Hopkins, and the other of those of heavier rats. It is seen (Table II) that as the animals grew the total amount of ascorbic acid in both organs increased but the concentrations remained more or less the same. In comparing the respective results of the two communications it was found that whilst the average concentration observed in the livers was essentially the same in both cases, that of the intestines as recorded by Hopkins (0.26 mg./g.) is rather higher than the figure found in the present investigation (0.19 mg./g.).

The results next to be considered are those obtained with the animals receiving no food for 48 hours (Table III). Their livers showed the same concentration as that found in the case of the rats on the stock diet. On the other hand the intestines of the fasted animals belonging to the 150–200 g. group showed a rise of possible significance in the vitamin concentration as compared with the same group on the stock diet. This difference is similar to but of a lower order than that found by Hopkins (0.26 mg./g. for stock and 0.40 mg./g. for fasted rats). The latter suggested that the increase in the concentration in the gut was due to synthesis of the vitamin in the organ owing to “a specific effect of failure in carbohydrate supply”. The present writer's figures offer a different interpretation from the above. A fast of 48 hours' duration, as would naturally

Table II.

Stoak diet.							
Wt. of rat g.	Sex	Wet wt. of liver g.	Ascorbic acid liver		Wet wt. of intestine g.	Ascorbic acid intestine	
			mg./g.	Total content mg.		mg./g.	Total content mg.
150	♀	5.5	0.25	1.4	6.0	0.21	1.3
152	♀	5.5	0.22	1.2	6.0	0.20	1.2
152	♀	5.5	0.24	1.3	6.0	0.18	1.1
157	♀	6.0	0.23	1.4	6.0	0.16	1.0
172	♂	5.0	0.24	1.2	7.0	0.19	1.3
175	♂	5.5	0.22	1.2	7.0	0.17	1.2
195	♂	7.0	0.19	1.3	7.5	0.20	1.5
160	♂	6.0	0.27	1.6	6.0	0.18	1.1
155	♀	5.0	0.26	1.3	5.0	0.26	1.3
160	♂	5.5	0.27	1.5	6.0	0.20	1.2
135	♂	4.0	0.23	0.9	6.0	0.20	1.2
155	♂	5.0	0.36	1.8	6.0	0.25	1.5
140	♂	5.5	0.31	1.7	7.0	0.17	1.2
197	♂	6.5	0.26	1.7	7.5	0.17	1.3
180	♂	6.0	0.30	1.8	8.0	0.19	1.5
170	♂	5.0	0.26	1.3	8.0	0.14	1.1
Av.	163	5.5	0.26	1.4	6.6	0.19	1.3
	200	7.0	0.21	1.5	9.0	0.19	1.7
	210	5.5	0.27	1.6	5.5	0.32	1.8
	*255	11.0	0.22	2.4	11.0	0.25	2.8
	265	10.0	0.24	2.4	5.5	0.20	1.1
	295	11.0	0.31	3.4	11.0	0.16	1.8
	335	13.0	0.20	2.6	10.0	0.19	1.9
	410	13.0	0.28	3.6	9.0	0.19	1.7
	230	8.0	0.34	2.7	6.5	0.29	1.9
	270	10.0	0.32	3.2	9.0	0.21	1.9
Av.	274	9.8	0.27	2.6	8.5	0.22	1.8

* Pregnant.

be expected, produced a loss in the dry matter including a large part of the ether-extractable fraction (Table IV) accompanied by a corresponding fall in the wet weight of the intestine, and, as the total vitamin content of this organ remained unaltered, the concentration per g. of tissue in the lighter group of animals (such as used by Hopkins), therefore, rose in consequence. It is essential in interpreting results such as these that the weight of the organ and its total content of ascorbic acid and not the concentration alone be taken into account—a consideration omitted by Hopkins from his argument. It may be further noted in this connection that there was a similar diminution in the weights of the livers but in this case it was accompanied by a corresponding diminution in the total vitamin content of these organs. This observation however has no direct connection with the present argument. Further work will be required to ascertain whether the decrease is significant.

The experiment which has the strongest bearing on the thesis under discussion is that carried out on rats receiving a carbohydrate diet. The results of the present investigation are given in Table V. The first point that emerges from them is that the incomplete diet had a detrimental effect on the animals. This is reflected in their appreciable loss of weight. Furthermore when the figures for the dry matter content and for the ether-extractable fraction of the intestine are examined (Table IV) a condition similar to but not quite so marked as that found in the case of the fasted rats is observed, namely a loss in weight in each of the fractions as compared with the corresponding figures recorded in the analysis of

Table III.

48 hours without food.

Wt. of rat		Sex	Wet wt. of liver g.	Ascorbic acid liver		Wet wt. of intestine g.	Ascorbic acid intestine	
Initial g.	Final g.			mg./g.	Total content mg.		mg./g.	Total content mg.
140	110	O ₃ O ₃ O ₃	4.0	0.15	0.6	4.0	0.32	1.3
210	195		6.0	0.28	1.7	6.0	0.31	1.9
185	160		5.0	0.18	0.9	5.0	0.30	1.5
130	115		3.2	0.41	1.3	4.5	0.27	1.2
135	115		3.5	0.34	1.2	4.5	0.29	1.3
175	145		4.3	0.26	1.1	6.0	0.21	1.3
180	160		4.5	0.36	1.6	5.3	0.26	1.4
185	162		5.4	0.31	1.7	5.6	0.21	1.2
195	170		5.0	0.28	1.4	4.5	0.36	1.6
145	120		4.0	0.18	0.7	6.0	0.22	1.3
210	185	O ₃ O ₃ O ₃	5.5	0.24	1.3	5.5	0.25	1.4
215	192		5.5	0.33	1.8	6.0	0.28	1.7
150	132		4.5	0.13	0.6	7.0	0.19	1.3
195	172		5.0	0.28	1.4	6.0	0.25	1.5
205	185		5.0	0.22	1.1	7.5	0.19	1.4
Av.	177	155	4.7	0.26	1.2	5.6	0.26	1.4
280	250	O ₃ O ₃ O ₃	7.0	0.29	2.0	7.0	0.16	1.1
300	270		8.0	0.21	1.7	8.5	0.18	1.5
285	252		6.0	0.23	1.4	7.0	0.27	1.9
410	380		9.0	0.23	2.1	6.0	0.23	1.4
375	347		8.0	0.20	1.6	7.0	0.21	1.5
295	280		7.0	0.31	2.2	6.0	0.31	1.9
280	240		7.0	0.27	1.9	8.0	0.29	2.3
310	260		8.0	0.30	2.4	6.0	0.27	1.6
280	252		6.5	0.25	1.6	7.0	0.17	1.2
360	332		8.6	0.28	2.4	8.5	0.32	2.7
250	225	O ₃ O ₃ O ₃	6.0	0.25	1.5	6.7	0.16	1.1
Av.	311	281	7.4	0.26	1.9	7.1	0.23	1.7

the intestines of the rats on the stock diet. 3 days on the incomplete diet therefore did not bring the condition of the animals up to the level observed in the stock rats. In the light group of the previously fasted animals there was some rise in the total ascorbic acid content of the liver. This increase was, however, not observed in the case of the heavier group and of the group which subsisted for 6 days on the diet without a previous fast. The vitamin concentrations of these 3 groups were 0.30, 0.31 and 0.26 mg./g. respectively as compared with 0.26 and 0.27 mg./g. the figures for the corresponding groups in the stock diet animals. It seems rather doubtful whether this rise of 15% in the concentration of the reducing substance extracted from the livers of the previously fasted animals is fully significant, especially as no increase in concentration, such as was found by Hopkins, could be recorded in the animals which had no previous fast. His figures were 0.35 mg./g. for the animals after 2 days' fast and 3 days on the carbohydrate diet and 0.32 mg./g. for those on 5-7 days of the diet without previous fast as compared with 0.26 mg./g. for the rats on the stock diet, thus registering an increase of 34.4 and 23% respectively, figures much higher than those above. Unlike his results there was also a greater variation in the liver vitamin concentrations of the individual animals in the present investigation. From Table V it is further seen that a significant fall is recorded in the average vitamin content and in the average vitamin concentration in the intestines of all the groups on the carbohydrate diet as compared with the stock diet group, in spite of the loss in the wet weight. This observation is also at variance with that

Table IV.

Wt. of rat		Sex	Wet wt. of liver g.	Total dry wt. g.	Wt. of etheral extract g.	% Total dry wt. Wet wt.	Wet wt. of in- testine g.	Total dry wt. g.	Wt. of etheral extract g.	% Total dry wt. Wet wt.
Initial g.	Final g.									
Stock diet.										
	150	O O O O O O O O O	6.50	1.77	—	27.21	6.31	1.21	0.30	19.18
	135		7.34	1.88	—	25.66	6.71	1.17	0.23	17.43
	150		6.09	1.70	0.16	27.93	6.86	1.06	0.21	15.44
	155		5.42	1.49	0.13	27.48	5.00	0.97	0.25	19.38
	250		8.76	2.63	—	29.98	6.41	1.44	0.54	22.50
	250		8.81	2.62	—	29.70	7.46	1.22	0.26	16.39
	290		12.18	3.16	—	25.98	9.87	1.77	0.28	17.98
	145		11.70	3.33	—	28.48	6.25	1.14	0.26	18.22
	152		—	—	—	—	4.98	1.02	0.28	20.54
	150	—	—	—	—	7.10	0.94	0.24	13.21	
Av.	183		7.42	2.06	0.15	27.80	6.69	1.19	0.28	18.03
48 hours without food.										
177	137	O O O O O O O O O O O O O O O	3.87	1.07	0.09	27.65	5.01	0.60	0.09	12.07
175	137		3.97	1.11	0.11	28.08	4.29	0.67	0.12	15.63
180	150		4.56	1.28	—	28.14	5.23	0.77	0.10	14.82
177	148		4.02	1.10	—	27.43	4.62	0.69	0.12	14.84
185	152		4.48	1.27	—	28.33	5.75	0.76	0.11	13.30
180	145		4.10	1.20	—	29.33	4.75	0.68	0.10	14.32
270	245		5.90	1.88	—	31.81	4.80	0.87	0.19	18.17
305	285		7.55	2.36	—	31.28	5.98	1.10	0.21	18.37
*300	270		7.28	2.35	—	32.31	5.82	0.81	0.10	13.85
270	220		6.31	1.99	—	31.46	6.92	1.02	0.26	14.73
280	250		6.83	2.18	—	31.85	7.48	1.14	0.20	15.26
170	130		—	—	—	—	5.95	0.75	0.09	12.52
160	145		—	—	—	—	5.14	0.62	0.15	12.13
*175	125		—	—	—	—	4.60	0.58	0.07	12.49
Av.	215		181	5.35	1.62	0.10	29.79	5.45	0.79	0.14
3 days on carbohydrate diet after 2 days' fast.										
200	195	O O O O O O O O O	6.70	1.83	0.22	27.39	6.18	0.88	0.19	14.29
200	200		6.16	1.56	0.19	25.31	6.25	0.98	0.13	15.65
215	217		7.14	1.87	0.23	26.18	6.91	1.03	0.19	14.94
170	152		6.90	2.06	0.37	29.83	5.14	0.66	0.10	12.80
165	135		6.10	1.68	0.18	27.60	5.93	0.94	0.17	15.81
180	155		7.12	1.97	0.18	27.67	5.76	0.92	0.15	16.05
180	177		6.88	1.95	—	28.31	5.58	0.90	0.16	16.21
180	190		7.73	2.23	—	28.85	6.03	1.01	0.19	16.78
180	177		5.56	1.56	—	28.11	5.36	0.80	0.12	14.91
Av.	186	178	6.70	1.86	0.23	27.69	5.90	0.90	0.16	15.27
3 days on protein-fat diet after 2 days' fast.										
150	127	O O O O O O	5.67	1.71	0.24	30.11	6.26	1.00	0.17	15.99
140	130		4.36	1.32	0.12	30.13	6.19	0.74	0.08	11.93
150	135		5.67	1.75	0.22	30.81	6.82	0.87	0.14	12.81
172	152		6.03	1.80	—	29.77	7.12	0.99	0.15	13.96
180	157		5.13	1.55	—	30.14	5.46	0.81	0.08	14.77
160	145		6.08	1.71	—	28.17	6.38	1.03	0.19	16.13
Av.	159	141	5.49	1.64	0.19	29.86	6.37	0.91	0.13	14.26
3 days on fat diet after 2 days' fast.										
160	115	O O O O	3.72	1.19	0.28	31.88	4.64	0.73	0.14	15.83
190	160		7.37	2.04	0.43	27.72	9.90	1.61	0.34	16.27
175	140		5.81	1.63	0.45	28.07	7.58	1.35	0.37	17.84
180	145		5.60	1.84	0.69	32.90	6.38	1.09	0.28	17.12
Av.	176	140	5.63	1.68	0.46	30.14	7.13	1.20	0.28	16.77

* Pregnant.

Table V.

Wt. of rat		Sex	Food intake g.	Wet wt. of liver g.	Ascorbic acid liver		Wet wt. of intestine g.	Ascorbic acid intestine	
Initial g.	Final g.				mg./g.	Total content mg.		mg./g.	Total content mg.
3 days on carbohydrate diet after 2 days' fast.									
180	135	♂	5	3.8	0.29	1.1	5.5	0.15	0.8
160	137	♂	8	5.0	0.28	1.4	5.0	0.14	0.7
160	140	♂	9	6.5	0.28	1.8	5.0	0.18	0.9
165	140	♂	10	5.0	0.26	1.3	5.5	0.15	0.8
175	150	♂	11	5.5	0.26	1.4	5.5	0.11	0.6
160	150	♂	12	4.5	0.33	1.5	6.0	0.13	0.8
170	152	♂	13	5.5	0.37	2.0	6.0	0.15	0.9
*177	155	♀	12	5.0	0.38	1.9	5.5	0.17	0.9
190	155	♀	11	6.0	0.28	1.7	6.2	0.15	0.9
190	165	♀	10	6.2	0.23	1.4	7.5	0.10	0.8
183	165	♀	11	5.8	0.24	1.4	5.5	0.15	0.8
180	170	♀	11	5.0	0.28	1.4	5.5	0.15	0.8
190	170	♀	10	6.0	0.32	1.9	6.0	0.21	1.3
195	172	♀	14	5.0	0.38	1.9	6.0	0.17	1.0
190	175	♀	7	6.5	0.34	2.2	6.0	0.18	1.1
215	197	♀	14	7.0	0.29	2.0	6.5	0.17	1.1
200	190	♀	14	10.0	0.28	2.8	5.7	0.16	0.9
190	180	♀	13	7.3	0.38	2.8	5.0	0.18	0.9
185	172	♀	11	7.5	0.36	2.7	6.0	0.13	0.8
195	185	♀	13	7.5	0.20	1.5	6.0	0.10	0.6
180	165	♀	9	8.0	0.29	2.3	5.7	0.16	0.9
175	145	♀	7	5.5	0.35	1.9	5.0	0.16	0.8
190	162	♀	8	6.5	0.34	2.2	5.7	0.16	0.9
195	170	♀	8	7.0	0.24	1.7	5.0	0.10	0.5
190	170	♀	7	7.0	0.36	2.5	5.0	0.12	0.6
175	142	♀	6	6.0	0.22	1.3	6.0	0.15	0.9
170	150	♀	6	6.5	0.29	1.9	5.5	0.18	1.0
Av.	186	161	10	6.2	0.30	1.9	5.7	0.15	0.9
215	200	♀	15	8.5	0.32	2.7	5.5	0.15	0.8
220	200	♀	14	8.0	0.22	1.8	7.0	0.11	0.8
230	205	♀	10	7.5	0.33	2.4	6.0	0.15	0.9
215	205	♀	13	7.0	0.33	2.3	5.5	0.16	0.9
230	220	♀	14	5.5	0.40	2.2	6.5	0.22	1.4
210	220	♀	12	6.5	0.24	1.5	5.5	0.13	0.7
225	225	♀	16	8.0	0.36	2.9	7.0	0.17	1.2
230	237	♀	13	8.5	0.25	2.1	7.0	0.11	0.8
Av.	222	214	13	7.4	0.31	2.2	6.3	0.15	0.9
6 days on carbohydrate diet.									
190	185	♀	10	6.0	0.27	1.6	6.0	0.14	0.8
185	190	♀	13	6.0	0.37	2.2	6.0	0.20	1.2
170	152	♀	11	5.0	0.26	1.3	6.0	0.18	1.1
167	140	♀	9	5.0	0.24	1.2	5.0	0.14	0.7
170	150	♀	13	4.0	0.30	1.2	5.5	0.16	0.9
162	142	♀	10	4.0	0.25	1.0	5.0	0.20	1.0
160	150	♀	8	5.5	0.22	1.2	5.0	0.14	0.7
170	150	♀	8	5.0	0.18	0.9	6.5	0.09	0.6
150	137	♀	7	4.5	0.18	0.8	5.0	0.12	0.6
260	247	♀	10	7.5	0.33	2.5	6.5	0.18	1.2
270	265	♀	13	9.0	0.27	2.4	7.0	0.19	1.3
Av.	187	163	10	5.6	0.26	1.5	5.8	0.16	0.9

* Pregnant.

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made by him since he found that the intestinal concentration of the vitamin was unaffected by the carbohydrate diet. A point of particular interest recorded here is that the total vitamin content of the intestines of the heavier animals instead of being proportionately greater than that of the corresponding lighter animals, as was the case in both the preceding experiments and in the livers of the animals of this experiment, was actually only equal to it. Thus in effect the fall in the case of the larger animals was even much more marked than in the smaller animals. It is therefore seen from the above data that the crucial evidence of Hopkins's hypothesis cannot be easily reproduced, a fact which, even without questioning the identity of the substance responsible for the increased reduction, dis-

Table VI.

Wt. of rat		Sex	Food intake g.	Wet wt. of liver g.	Ascorbic acid liver		Wet wt. of in- testine g.	Ascorbic acid intestine		
Initial g.	Final g.				mg./g.	Total content mg.		mg./g.	Total content mg.	
3 days on protein-fat diet after 2 days' fast.										
	177	155	♀	8	5.0	0.16	0.8	7.0	0.23	1.6
	177	160	♀	7	5.3	0.17	0.9	8.0	0.18	1.4
	180	157	♀	8	5.5	0.35	1.9	7.0	0.31	2.2
	*180	170	♀	9	8.5	0.20	1.7	7.4	0.20	1.5
	180	150	♀	8	5.7	0.18	1.0	6.5	0.18	1.2
	*182	155	♀	7	6.5	0.26	1.7	7.0	0.27	1.9
	185	160	♀	8	6.5	0.26	1.7	7.0	0.24	1.7
	200	180	♀	7	8.0	0.25	2.0	8.0	0.25	2.0
	220	200	♀	9	6.5	0.29	1.9	6.0	0.30	1.8
	200	200	♀	8	8.7	0.17	1.5	8.0	0.26	2.1
	180	162	♀	7	7.0	0.19	1.3	7.0	0.29	2.0
	175	155	♀	5	6.0	0.23	1.4	5.0	0.26	1.3
	220	192	♀	9	6.0	0.20	1.2	10.0	0.22	2.2
	220	192	♀	7	6.5	0.20	1.3	9.0	0.24	2.2
Av.	191	170		8	6.6	0.22	1.5	7.4	0.25	1.8
	260	225	♀	9	9.0	0.27	2.4	9.0	0.21	1.9
	280	262	♀	10	10.0	0.24	2.4	11.0	0.17	1.9
	240	230	♀	8	7.5	0.21	1.6	8.0	0.15	1.2
	290	270	♀	9	10.0	0.20	2.0	10.0	0.16	1.6
	285	270	♀	9	8.5	0.21	1.8	9.0	0.17	1.5
Av.	271	251		9	9.0	0.23	2.0	9.4	0.17	1.6
5 days on protein-fat diet.										
	140	127	♀	9	5.2	0.19	1.0	6.0	0.20	1.2
	142	135	♀	5	5.0	0.18	0.9	6.0	0.22	1.3
	142	130	♀	5	5.5	0.22	1.2	6.0	0.23	1.4
	150	115	♀	4	8.5	0.21	1.8	9.5	0.23	2.2
	160	150	♀	6	5.0	0.18	0.9	6.2	0.15	0.9
	210	197	♀	7	8.0	0.25	2.0	6.7	0.28	1.9
	215	175	♀	3	6.0	0.18	1.1	6.0	0.15	0.9
	215	180	♀	5	7.0	0.27	1.9	7.0	0.20	1.4
Av.	172	151		6	6.3	0.21	1.3	6.7	0.21	1.4
12 days on protein-fat diet.										
	170	137	♀	5	5.5	0.24	1.3	5.5	0.20	1.1
	167	150	♀	6	6.5	0.21	1.4	6.0	0.17	1.0
	170	155	♀	6	6.5	0.19	1.2	6.5	0.23	1.5
	175	170	♀	8	7.5	0.27	2.0	8.0	0.25	2.0
	170	175	♀	6	6.0	0.20	1.2	8.0	0.20	1.6
	205	202	♀	6	7.0	0.19	1.3	7.0	0.21	1.5
	230	240	♀	7	9.5	0.20	1.9	9.0	0.19	1.7
Av.	184	176		6	6.9	0.21	1.5	7.1	0.21	1.5

* Pregnant.

courages the ready acceptance of his theory that the liver of the rat is the seat of formation of ascorbic acid from carbohydrates as the ultimate source.

The experiments with the protein-fat diets also do not offer evidence in agreement with the general hypothesis. The animals on this diet thrive even worse than those on the carbohydrate diet. They consumed less food and lost considerably in weight during the experiment. The majority of the rats developed diarrhoea or soft stools soon after being placed on the diet and their intestines at the *post mortem* examination were somewhat oedematous in appearance. These organs, especially in the light group previously fasted, disclosed a rise in the wet weight (Table VI), no doubt due to the oedematous condition, and signs of loss in dry matter *etc.* reminiscent of starvation (Table IV). In fact the condition of the experimental animals was such that it would have been unsafe to assume synthesis had any increase in the concentration of the vitamin in the intestine been observed. However with the possible exception of the lighter group of the previously fasted animals, no such rise in the concentration of the ascorbic acid was observed. It should be noted that the total content in the intestines of the heavy group here was not greater than that in the lighter group, as was the case also in the similar group on the carbohydrate diet.

Nor could the rise in the intestinal concentration in rats existing on a diet of fat alone be confirmed (Table VII). These results, however, hardly deserve

Table VII.

3 days on fat diet after 2 days' fast.

Wt. of rat		Sex	Food intake g.	Wet wt. of liver g.	Ascorbic acid liver		Wet wt. of in- testine g.	Ascorbic acid intestine	
Initial g.	Final g.				mg./g.	Total content mg.		mg./g.	Total content mg.
160	142	♀	7	5.0	0.24	1.2	5.0	0.26	1.3
165	137	♀	6	4.5	0.27	1.2	7.0	0.20	1.4
165	137	♀	8	5.2	0.10	0.5	7.0	0.11	0.8
167	140	♀	6	4.5	0.27	1.2	7.0	0.20	1.4
167	142	♂	8	5.0	0.20	1.0	7.0	0.13	0.9
180	152	♂	8	5.0	0.22	1.1	7.0	0.20	1.4
Av.	167		7	4.9	0.22	1.0	6.7	0.18	1.2

serious comment. The animals in this experiment lost much more of their weight, and were in a very poor condition. At the *post mortem* examination lumps of undigested lard were found in the stomach. The livers showed definite fatty degeneration. The analysis of this organ of the rats on the diet indicated a great rise in the weight of the ether-extractable fraction (Table IV).

The results obtained in this communication do not therefore justify the hypothesis under consideration. Conceding even that on feeding a carbohydrate diet there really is a preferential rise in the indophenol-reducing capacity per unit of tissue in the liver and that a similar rise takes place in the intestine on protein or fat diets and during a fast, do such increases necessarily imply synthesis of ascorbic acid in these organs? In the first place it must be borne in mind that although, as previously shown [Zilva, 1935; Jacobsen, 1935], little doubt can exist that the capacity for reducing indophenol is in the main due to the presence of ascorbic acid in these organs, it does not necessarily follow that a rise in the reducing capacity especially of liver extracts or in extracts of oedematous tissues is due to a rise in the concentration of the vitamin. There are substances of physiological significance such as alloxantin which decolorise

Table VIII. *Average results.*

Wt. of rat		Food intake g.	Wet wt. of liver g.	Ascorbic acid liver		Wet wt. of in- testine g.	Ascorbic acid intestine	
Initial g.	Final g.			mg./g.	Total content mg.		mg./g.	Total content mg.
Stock diet (Table II).								
—	163	—	5.5	0.26	1.4	6.6	0.19	1.3
—	274	—	9.8	0.27	2.6	8.5	0.22	1.8
48 hours without food (Table III).								
177	155	—	4.7	0.26	1.2	5.6	0.26	1.4
311	281	—	7.4	0.26	1.9	7.1	0.23	1.7
3 days on carbohydrate diet after 2 days' fast (Table V).								
186	161	10	6.2	0.30	1.9	5.7	0.15	0.9
222	214	13	7.4	0.31	2.2	6.3	0.15	0.9
6 days on carbohydrate diet (Table V).								
187	163	10	5.6	0.26	1.5	5.8	0.16	0.9
3 days on protein-fat diet after 2 days' fast (Table VI).								
191	170	8	6.6	0.22	1.5	7.4	0.25	1.8
271	251	9	9.0	0.23	2.0	9.4	0.17	1.6
5 days on protein-fat diet (Table VI).								
172	151	6	6.3	0.21	1.3	6.7	0.21	1.4
12 days on protein-fat diet (Table VI).								
184	176	6	6.9	0.21	1.5	7.1	0.21	1.5
3 days on fat diet after 2 days' fast (Table VII).								
167	142	7	4.9	0.22	1.0	6.7	0.18	1.2

indophenol much more rapidly than sulphydryl compounds in acid solution and the presence of these as well as the latter in amounts which would increase the reduction to the extent noted by Hopkins could hardly be detected by studying the velocity of the reaction as was done by his colleague G. A. Millikan. Conceding further that the increase was really due to ascorbic acid, such an increase could just as well be assumed to be due to the transfer of the vitamin from the other parts of the body to the organ in question in order to fulfil a physiological need conditioned by the constitution of the diet. Such a view is in the opinion of the writer a less hazardous speculation than that which assumes the rise in the concentration of the vitamin in an organ to be due to local synthesis induced by the consumption of a diet predominating in a certain constituent capable of acting as an ultimate precursor of the vitamin. The fact that the diets were, as seen above, so drastic in character as to bring about marked disturbances in the metabolic functions of a hardy experimental animal like the rat calls particularly for forbearance in the choice of the latter interpretation. Taking these arguments into consideration in conjunction with the observed divergencies in the essential results of the two communications, it would appear that Hopkins's hypothesis, attractive as it may be, is so far not fully supported by experimental evidence.

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