84. THE ACTION OF *l*-ASCORBIC ACID ON THE *IN VITRO* RESPIRATION OF LIVER TISSUE FROM GUINEA-PIGS ON A RESTRICTED DIET. I

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In order to explore the possible connexion between *l*-ascorbic acid and tissue respiration, Harrison [1933] studied manometrically the respiration of tissues—mainly liver tissue—from normal and scorbutic guinea-pigs in the presence and in the absence of small quantities of *l*-ascorbic acid. He found that the addition of the vitamin to liver tissues of scorbutic animals increased the rate of O₂ uptake.

Quastel & Wheatley [1934], in investigating the effect of *l*-ascorbic acid on the oxidation of fats in the liver, found that slices from guinea-pigs fed on a scorbutic diet for 15 days or more usually showed an increased respiration in the presence of *l*-ascorbic acid which was significantly higher than that obtained with liver tissue from normal animals. The scorbutic animals employed by these workers after having reached a maximum weight eventually lost about 10–15% of the maximum value before being utilized for the experiment. Quastel & Wheatley thus obtained results somewhat similar to those of Harrison.

Phillips *et al.* [1934] were unable to confirm Harrison's results but it must be added that their technique differed widely from that employed by Harrison. For instance they utilized minced tissue in a phosphate-Ringer solution in air with 0.12 mg. of *l*-ascorbic acid per 200 mg. of tissue, whilst Harrison employed liver slices in a phosphate buffer solution in O₂ with 0.25 mg. of *l*-ascorbic acid per 200 mg. of sliced tissue.

Stotz *et al.* [1937] observed only irregular increases in the respiration on addition of *l*-ascorbic acid to liver slices from scorbutic and normal animals and they found no significant difference in behaviour between the tissue from normal and vitamin C-deficient guinea-pigs. They too used a medium different from that employed by Harrison.

It occurred to the present writers that the increased tissue respiration observed by Harrison might have been due not to the specific deficiency of vitamin C but to another dietetic restriction. Soon after the appearance of his communication we attempted to test this hypothesis experimentally, but in spite of various efforts, we were unable to reproduce Harrison's results. Nevertheless, Harrison's figures were so convincing that it became apparent that the discrepancy between his and our results must have been due to an undetected difference in technique. This was particularly probable, because Harrison used a differential correction for the oxidation of *l*-ascorbic acid in the medium which made the increases in his Q_{O₂} values appear to be lower than they really were. His correction was effected by introducing into the control flask of the Barcroft differential manometer a solution of ascorbic acid similar to that present in the

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flask containing the tissue. The aerobic oxidation of the added *l*-ascorbic acid in the control therefore proceeded at a higher rate than in the other flask owing to the absence of the protective action of the liver tissue. After a number of intermittent attempts, stretching over several years, we were eventually able to repeat Harrison's results with perfect regularity, but, as anticipated, we found that on addition of *l*-ascorbic acid an increase in the surviving respiration of the liver tissue could also be obtained when the animals had previously received a quantitatively restricted diet with an ample supply of vitamin C. The phenomenon is therefore not specific for scurvy in guinea-pigs.

Technique

Healthy growing guinea-pigs weighing 250–350 g. were used. The control or normally fed animals were offered a mixture of one part of oats and two parts of bran by volume and cabbage *ad lib*. Another group of animals received a diet restricted quantitatively so as to bring about some inanition, the quantity of the basal diet of oats and bran being varied daily so that they gradually lost weight and reached a steady value of *c.* 80% of their original weight after 15–20 days. The liver tissue was examined for its respiratory capacity in the presence and in the absence of ascorbic acid after the animals had subsisted on the restricted diet for 21–28 days. During this period, with the exception of the last 4–6 days when a daily dose of 10 mg. of ascorbic acid was given, the guinea-pigs received 15 g. of cabbage daily. These dietetically restricted animals therefore consumed daily a quantity of vitamin C which, although not high enough to saturate them fully, was many times higher than the dose required to protect them from macroscopic and microscopic lesions of scurvy and any difference observed in the respiration of their liver tissue could therefore not have been ascribed to scurvy. The third group of guinea-pigs received *ad lib.* a scorbutic diet made up of

Bran	...	...	6 parts, by volume
Barley meal	...	2	"
Middlings	...	3	"
Fishmeal	...	1	"
Crushed oats	...	4	"

60 ml. of autoclaved milk made up from a dried powder were offered in addition to these animals.

Respiration was measured in a Warburg-Barcroft apparatus at 37° [cf. Dixon, 1934]. After stunning and bleeding the animals, the livers were excised and weighed. Tissue from corresponding lobes of the liver was cut with a razor blade and only thin slices (*c.* 0.3 mm.) were used. The cut slices were suspended in a shallow layer of buffer in a Petri dish and uneven or ragged ones were rejected. The suitable slices were then trimmed and cut into uniform pieces roughly 0.5 cm. across and an equal number, taken at random, was placed in each flask. This precaution was necessary since slices from different portions of the liver possess significantly different rates of respiration. In this way approximate uniformity of the mass of tissue in each flask was also ensured. As in Harrison's experiments the slices were suspended in *M*/15 Na-K phosphate buffer *pH* 7.4, the total volume of medium being kept at 2.5 ml. Manometer readings were taken every 10 min. during the 1st hr. and again at the end of the 2nd hr. After each experiment the *pH* of the medium was checked by capillator. The tissue was then removed for determination of dry weight and the contents of those flasks to which ascorbic acid had been added were rinsed out, acidified

and titrated with standardized 2:6-dichlorophenolindophenol. The O_2 uptake for each flask was calculated as $\mu l.$ at N.T.P. per mg. of dried tissue. In each experiment 0.25 mg. of ascorbic acid was added to 4 of the 8 flasks containing the tissue and the mean of each 4 readings is recorded in the results.

Table 1. *The respiration of guinea-pig liver slices in the presence and absence of ascorbic acid*

Sex of animal	No. of days on diet	Mean Q_{O_2}								
		Weight g.			1st hr.			2nd hr.		
		Initial	Max.	Final	No addition	Ascorbate added	% increase	No addition	Ascorbate added	% increase
Normal diet										
F.	—	—	—	295	3.79	3.54	-6.6	2.92	3.54	21.2
M.	—	—	—	340	2.80	3.19	13.9	2.46	2.87	16.7
F.	—	—	—	350	4.40	4.78	8.6	—	—	—
M.	—	—	—	305	3.99	4.04	1.3	3.54	3.67	3.7
F.	—	—	—	320	2.92	3.57	22.3	2.88	3.46	20.1
F.	—	—	—	340	3.90	3.98	2.1	3.33	3.37	1.2
F.	—	—	—	360	3.46	4.54	31.2	2.98	4.11	37.9
F.	—	—	—	390	4.93	5.47	11.0	4.15	4.84	16.6
M.	—	—	—	350	4.07	4.36	7.1	3.17	3.49	10.1
M.	—	—	—	350	4.38	4.47	2.1	—	—	—
F.	—	—	—	260	6.44	6.33	-1.7	5.53	5.40	-2.3
F.	—	—	—	265	3.99	4.09	2.5	3.32	3.60	8.4
Mean	—	—	—	327	4.09	4.36	7.8	3.43	3.84	13.4
Scurbutic diet										
F.	26	260	320	270	3.55	5.12	44.2	—	—	—
M.	25	270	327	255	3.68	3.96	7.6	—	—	—
F.	26	275	305	210	3.49	3.92	12.3	—	—	—
M.	21	290	350	345	3.37	4.54	34.7	2.86	4.07	42.3
M.	21	290	320	260	3.86	5.17	33.9	3.24	4.54	40.1
M.	21	310	365	365	4.33	6.26	44.6	3.92	5.52	40.8
F.	21	290	350	265	4.17	5.84	40.0	3.54	5.01	41.5
M.	22	275	345	285	2.52	3.89	54.4	2.29	3.44	50.2
M.	21	270	355	315	2.83	4.05	43.2	2.42	3.44	42.1
F.	21	265	315	270	4.58	5.27	15.1	3.55	4.44	25.1
M.	21	280	360	345	3.77	5.34	41.7	2.97	4.45	49.8
M.	21	285	380	370	3.24	4.53	39.8	2.73	3.83	40.2
M.	21	275	335	335	4.24	5.99	41.3	3.42	4.94	44.4
M.	26	285	370	275	2.58	3.80	47.1	2.23	3.64	63.2
M.	27	300	405	287	2.61	4.20	60.9	2.22	3.64	64.5
Mean	—	281	347	297	3.52	4.79	37.4	2.95	4.25	45.3
Restricted diet										
		Initial wt.	Final wt.	% loss in wt.						
F.	—	275	200	27	3.91	5.46	39.6	3.11	4.65	49.5
M.	—	275	212	23	3.28	4.06	23.8	2.68	3.64	35.8
F.	—	300	220	27	5.77	6.62	14.7	4.92	5.66	15.3
M.	—	300	225	25	3.21	9.15	185.1	2.88	8.67	201.0
M.	—	300	222	26	3.63	4.95	26.5	2.91	3.66	25.8
F.	—	300	225	25	4.18	5.05	20.8	3.50	4.08	16.6
F.	—	295	230	22	3.24	5.24	61.7	2.57	4.20	63.3
M.	—	300	227	24	2.90	4.58	57.9	2.54	4.01	57.8
F.	—	285	215	25	4.45	5.78	29.9	3.75	5.66	51.0
F.	—	295	230	20	4.73	6.70	41.6	3.76	5.38	43.1
F.	—	280	230	18	4.45	4.68	5.2	3.73	3.93	5.4
F.	—	290	225	22	4.39	5.12	16.8	3.67	4.58	24.8
Mean	—	291	222	24	4.01	5.62	43.6	3.33	4.84	49.1

Results

The results are given in Table 1, from which it will be seen that the increase in respiration on the addition of ascorbic acid to liver slices of guinea-pigs on a normal diet was markedly less than occurred with those animals on a restricted diet or on a scorbutic diet: the rise in Q_{O_2} in these two groups were of the same order. These results will be discussed in conjunction with those reported in the following paper [Snow & Zilva, 1941]. It may perhaps be appropriate to stress here one point, namely that the *total* respiration of the liver tissue from the animals on the restricted diet after the addition of ascorbic acid was on the whole higher than that of the liver tissue from the animals on a normal diet. The bearing of this observation on the general problem will be considered later.

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