

LXXXVIII. ASCORBIC ACID IN THE EYE-LENS AND AQUEOUS HUMOUR OF THE OX.

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IN an earlier communication [Birch and Dann, 1933] on the estimation and distribution of ascorbic acid and glutathione in animal tissues we drew attention to the distribution of ascorbic acid in organs other than the suprarenal cortex, as detected by the indophenol reagent. The presence in a number of tissues of reducing substances other than glutathione had become evident from the work of Mason [1930] and of Bierich and Rosenbohm [1933], but no evidence of the identity of such substances had been obtained. By applying the indophenol titration for ascorbic acid to tissue extracts we were able to demonstrate the presence of the acid in a number of organs and to give figures for the amounts present. At the same time it was pointed out that the indophenol titration may not be specific for ascorbic acid in tissue extracts, so that other tests are necessary to confirm the results obtained by the titration method.

The silver nitrate staining test was applied to a number of the tissues which had been found by means of the indophenol titration to contain ascorbic acid, with results which were not always in harmony with those obtained by the latter method. Some tissues found by the titration method to contain ascorbic acid gave little or no staining with silver nitrate, and led us to the conclusion already formulated by Harris and Ray [1933, 1] that a positive result with this test indicates the presence of ascorbic acid in a tissue but a negative result does not necessarily mean that ascorbic acid is absent.

In order to examine the reliability of results obtained by the titration method it was decided to carry out biological assays of the ascorbic acid found by the titration method to occur in unexpected sites. The tissues chosen for examination were the eye-lens and aqueous humour of the ox.

EXPERIMENTAL.

For the first attempt to assay biologically the ascorbic acid in the lens and the aqueous humour of the ox's eye the recovery test of Harris *et al.* [1932] was employed. Young guinea-pigs fed on a full diet of natural foodstuffs on attaining the weight of 300 g. were placed on a vitamin C-free diet consisting of bran 80 parts, ground oats 720, egg-yolk 40, salts 84, cod-liver oil 1 %, and were weighed daily thereafter. As soon as any animal showed a loss in weight on two successive days, it was given its first dose of the material under test and then dosed daily. The weight curves of the animals are shown in Fig. 1.

Since no simple method of extracting the ascorbic acid from the lens could be devised, the method of preparing the lens for dosing was to remove the lenses

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from a number of ox eyes which had been kept in cold storage after the slaughtering and to estimate by the indophenol micro-titration the amount of ascorbic acid in a sample of the lenses. Weighed amounts of the lenses calculated to contain (from the titration results) either 1 mg. or 3 mg. of ascorbic acid were then mashed up in a little water until the suspension could be taken up in a pipette and given to the guinea-pig. For doses of the aqueous humour, the humour was extracted from a number of eyes and pooled; an ascorbic acid

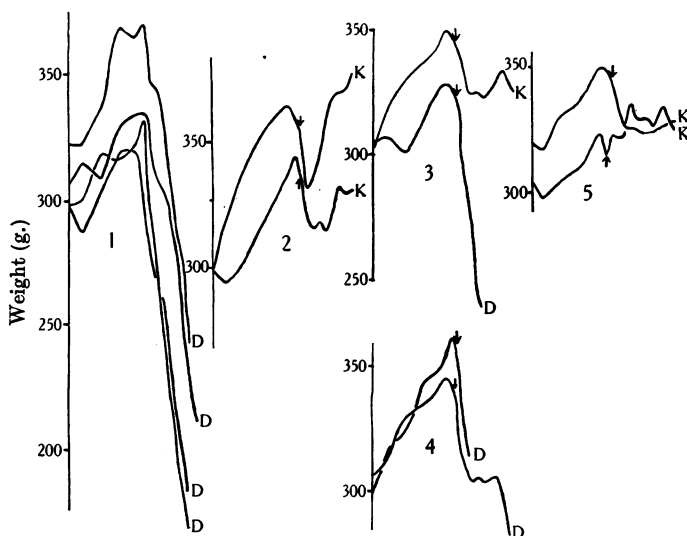


Fig. 1. Weight curves of guinea-pigs used in recovery test. The daily doses of the different groups were as follows: (1) No addition to basal diet; (2) 3.0 ml. of orange juice; (3) Eye-lens calculated from indophenol titration to contain 1 mg. ascorbic acid; (4) Eye-lens calculated to contain 3 mg. ascorbic acid; (5) Aqueous humour calculated to contain 2 mg. of ascorbic acid. Dosing commenced as indicated by the arrows. D signifies died; K signifies killed.

determination was then made on a portion of the mixed humour. The amount of humour calculated to contain 2 mg. ascorbic acid was then given to the guinea-pig from a pipette. The ascorbic acid estimation and preparation of doses were carried out each day, and a fresh supply of eyes was obtained as often as possible (generally every day).

From the weight curves in Fig. 1 it can be seen that the test was not successful. Three of the four animals receiving a supplement of eye-lens died before the first of the negative control animals, the average time between commencement of the experimental period and death being $4\frac{1}{2}$ days for the animals receiving eye-lens and $6\frac{1}{2}$ days for the negative controls. Thus no evidence of the presence of ascorbic acid in the lens is afforded by this test, which however suggests not so much the absence of ascorbic acid from the lens as the presence of some other substance exerting a positive toxic effect. The animals receiving aqueous humour lived through the experimental period of 9 days but did not show an increase of weight, as did the positive controls receiving 3 ml. of orange juice daily. The curves indicate that the dose of aqueous humour contained about 1.2 mg. ascorbic acid, or about two-thirds of the amount found by the titration. If the aqueous humour exerts in slighter degree a similar toxic action to the lens, then

the amount of ascorbic acid present would be greater than is indicated by the curves.

On account of the failure of this test with the lens a second test was arranged with alterations considered likely to diminish the effect of the toxic action of the lens. Instead of the curative test, the tooth structure method of Höjer as modified by Key and Elphick [1931] was used, as it has the advantage that at the beginning of the dosing period the guinea-pigs have not been entirely depleted of their vitamin C and therefore are likely to be more resistant to the toxic action of the dosing material. For convenience of dosing a large batch of ox eyes was obtained, and the lenses were removed, dried in a vacuum-desiccator and powdered. The dried powdered lenses were well ground and mixed to give a uniform material, which by titration against the indophenol was found to contain 0.9 mg. ascorbic acid per g. The indophenol reagent was standardised against pure ascorbic acid. The dose given to each animal daily was 3 g. of the powdered eye-lens, containing according to the titration 2.7 mg. of ascorbic acid. Four animals were used, together with three negative controls, four animals receiving 3.5 ml. orange juice daily and three animals receiving 2 ml. orange juice daily. After 14 days all the animals were killed and their lower jaws dissected out for examination of the teeth. During the dosing period one animal receiving the eye-lens had gained a little in weight, one had remained steady and the other two had fallen considerably. The results of the histological examination of the teeth are set out in Table I.

Table I.

Guinea-pig no.	Daily dose	Degree of protection from scurvy*
1	3 g. dried eye-lens	2.6
2	"	2.2
3	"	2
4	"	3.3
5	3.5 ml. orange juice	2.3
6	"	3.5
7	"	3
8	"	3.8
9	2 ml. orange juice	1.5
10	"	1
11	"	1.8
12	None	0
13	"	0.3
14	"	1.2

* Each figure in this column is the mean of three, allotted by three independent observers.

From the figures given it appears that ascorbic acid is undoubtedly present in the lens of the ox eye. The doses of orange juice given to the positive control animals were chosen to contain 2.1 mg. and 1.2 mg. of ascorbic acid in 3.5 ml. and 2.0 ml. respectively. Since this work was undertaken, however, it has been demonstrated by Harris and Ray [1933, 2] that the ascorbic acid content of the juice of different oranges varies a good deal more than was supposed. Hence it is difficult to give a quantitative figure for ascorbic acid content of a test dose used in a biological test unless the positive controls have been dosed with pure ascorbic acid. On the assumption that the orange juice used in our experiments was near to the average in ascorbic acid content (*i.e.* 0.6 mg. per ml. of juice) then the results set out in Table I indicate that the eye-lens contains ascorbic acid to the extent of about two-thirds of the amount suggested by indophenol titration.

SUMMARY.

The eye-lens and aqueous humour of the ox have been tested biologically by the tooth protection method and the recovery method respectively, and the presence of ascorbic acid found by the indophenol titration method has been confirmed.

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