CCLXXII. VITAMIN C IN THE SUPRARENAL MEDULLA.

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(*Received November 2nd, 1933.*)

It is already known that the suprarenal gland is an extremely potent source of vitamin C, but hitherto the activity has been thought to be restricted to the cortex. In the present paper, however, it is shown that the medulla of the suprarenal is also intensely active, its potency falling but little short of that of the cortex. Our results indicate that ox suprarenal cortex has an activity of about 30 international units per gram and ox medulla of about 20 units, *i.e.* no less than two-thirds of the former. In other words the cortex has about thrice and the medulla about twice the potency of fresh orange juice or lemon juice (the international standard of vitamin C activity).

Szent-Györgyi [1928], struck by the peculiar silver-reducing power of the cortex, isolated from it the substance thought to be responsible—first called hexuronic acid, but later, after its identification with vitamin C, renamed ascorbic acid. The intense antiscorbutic activity of the suprarenal cortex was demonstrated in papers from this laboratory [Harris, Mills and Innes, 1932; Harris and Ray, 1932; 1933, 1] and the degree of activity was shown to be commensurate with its richness in hexuronic acid. A chemical method for estimating the latter, based on modifications in the use of Tillmans's oxidation-reduction indicator 2 : 6-dichlorophenolindophenol was worked out and applied to various animal and vegetable materials, and the surprising discovery was made that certain tissues, including the suprarenal medulla, not hitherto recognised as sources of vitamin C, also gave a high titration value [Harris and Ray, 1933, 1, 2; Birch, Harris and Ray, 1933; Birch and Dann, 1933; Harris, 1933, 1]. This result at once suggested that the absence of the silver reaction was in reality little guide as to the presence or absence of vitamin C, a conviction which was strengthened by our further observation that the medulla and cortex of different species, as well as a variety of other tissues (*e.g.* liver), might stain or not in the most erratic order with little apparent parallelism with their true antiscorbutic activities. Again liver extract and other tissue extracts although rich in vitamin C did not reduce silver. Similarly Gough and Zilva [1933] have recently noted that human suprarenals may fail to darken at all with silver (their vitamin C potency, however, not having been tested). The chemical test for vitamin C, although it is known to be reasonably specific, may give high results in certain exceptional cases, and it was therefore necessary to check the titration results on the suprarenal medulla against direct feeding tests. The biological determination gave results in complete agreement with the indophenol titration. We have recently repeated these feeding tests and exactly confirmed our earlier results.

Experimental.

The technique, using the histological tooth structure method, was the same as that described in earlier papers [*e.g.* Harris and Ray, 1932].

In the first test a group of three guinea-pigs received daily 1 g. of freshly excised ox suprarenal medulla and control groups received 1, 2 and 3 cc. of orange juice. The second test was similar except that the last group was omitted. The results are shown in Table I, and are analysed in Fig. 1.

Table I. *Antiscorbutic activity of ox suprarenal medulla.*

Supplement	Degree of protection: Individual animals	Mean
First determination		
Suprarenal medulla, 1 g.	2, 2, 3	2·3
Negative control	0, 0·5, 1	0·5
Orange juice, 1 cc.	1, 2, 1, 1·5	1·4
2 cc.	3, 2·5, 2·5, 2	2·5
3 cc.	3·5, 3·0, 1·5, 3·5	2·9
Second determination		
Suprarenal medulla, 1 g.	1·5, 1·5, 2	1·7
Negative control	0, 0, 0	0
Orange juice, 1 cc.	0·5, 1, 1·5	1
2 cc.	1, 2, 2·5	1·8

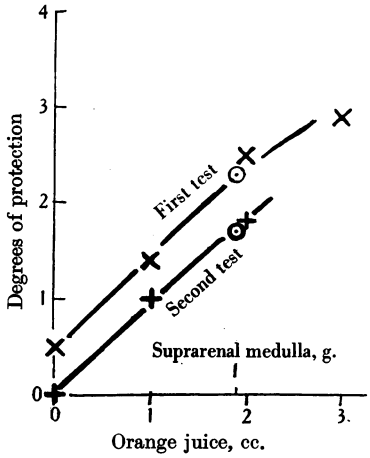

Fig. 1. Comparison of protective power of suprarenal medulla and orange juice.

× orange juice; ⊙ medulla.

Results. As is seen from Fig. 1, 1 g. of suprarenal cortex was found to have the same biological activity as 1·9 cc. of orange juice. This same figure was obtained in both series of tests. It agrees exactly also with that calculated from the chemical titration, according to which 1 cc. of orange juice was found to contain 0·6 mg. ascorbic acid, and 1 g. of medulla 1·1 mg. (see Table II).

Lack of correlation with silver stain. It is seen that although the ox medulla does not stain with silver in Szent-Györgyi's method, and the cortex does, nevertheless the medulla contains vitamin C in almost the same order of

Table II. *Vitamin C content of ox suprarenal medulla: agreement between biological and chemical results.*

	Amount of orange juice equivalent to 1 g. of medulla
By biological test	1·9 cc.
By chemical test	1·8 cc.

concentration as does the cortex. It is apparent at once, therefore, that the absence of a silver stain is no infallible indication that large amounts of vitamin C may not be present in a given tissue. Liver and certain other tissues appear in fact to contain a substance or system which protects silver nitrate from reduction by ascorbic acid. Thus we find that when ascorbic acid is mixed with the tissue extract it no longer reduces silver.

Physiological considerations.

1. The suprarenal not a reserve store of vitamin C. At first sight the fact that the suprarenal contains a concentration of vitamin C so many times greater than that present in the body as a whole might be thought to indicate that it serves as a reserve or storehouse, in the same way that the liver is known to put away reserves of vitamin A for the future use of the body. This interpretation indeed seems to have been already adopted in some quarters. The following considerations, however, show that this conception cannot be accepted. In the first place, the total amount of ascorbic acid in the adrenals of the guinea-pig is no more than about 0·5 mg.; *i.e.* not sufficient to provide the vitamin C needs of the body for more than about 24 hours. This should be contrasted with vitamin A, of which enough can easily be held in the liver to provide an animal with its needs for a whole lifetime. Secondly, we find that the amount in the suprarenal does not appear to be raised significantly above normal limits after extra allowances of vitamin C have been given. This finding again is in sharp contrast with all experience with vitamin A, where the reserves, and hence the time of depletion, depend entirely on the pre-experimental diet. Thirdly, if the vitamin were held as a reserve we should expect it to disappear from the suprarenal during the early stages of a vitamin C-free regimen and no scorbutic symptoms to develop until such reserves had been drawn upon. This is what happens with vitamin A, no ill-effects beginning to appear until the very last traces of vitamin A have been used up from the liver. But with vitamin C on the contrary we find that the amount in the suprarenal diminishes gradually during the whole course of scurvy. At the tenth or twelfth day when teeth lesions are already far advanced there may still be considerable amounts of ascorbic acid in the suprarenal; and again, at death from scurvy it is not unusual to find small amounts of ascorbic acid still present in the organ.

2. The suprarenal and the synthesis of vitamin C by certain species. In an earlier paper [Harris and Ray, 1933, 2] we mentioned that the suprarenal of the rat was considerably richer in vitamin C even than that of the ox or normal guinea-pig, judging from the titration result, the activity being ten times greater than that of orange juice, or 6 mg. of ascorbic acid per g. Biological tests (curative method) have confirmed this conclusion, both for normal rats' suprarenals and for the suprarenals of rats which had been kept for long periods on a vitamin C-free diet (Fig. 2).

Since the rat is known to synthesise its own vitamin C (as the above test further demonstrates), and one of us has shown the same to be true also of the dog [Harris, 1931], it seemed a possibility that the suprarenal might be the site

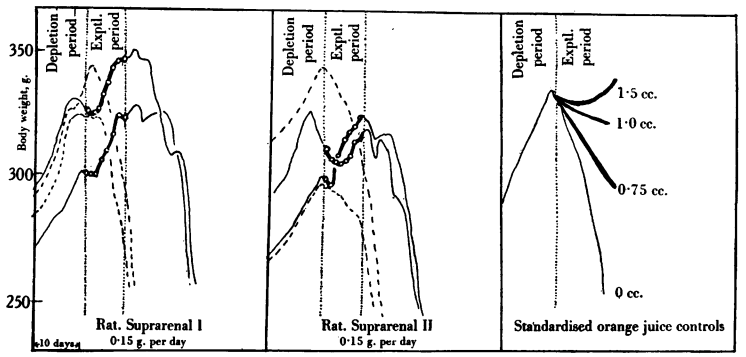

Fig. 2. Curative tests on guinea-pigs, with rat suprarenals from rats (I) fed on vitamin C-free diet and (II) fed on the vitamin C-free diet *plus* cabbage.

▬▬ experimental curative period; ——— vitamin C-free diet; — — — negative controls.

of the synthesis in such species. In order to try and test this working hypothesis, a number of rats were submitted to adrenalectomy[1] and then placed on a vitamin C-deficient diet, consisting of

Bran	...	...	80 parts by weight
Oats	...	...	720 ,,
Egg-yolk	...	...	40 ,,
Salts	...	...	8·4 ,,
Cod-liver oil		...	1 %

(Provision of eucortone or adrenaline appears to be unnecessary for this species.) If the suprarenal were in fact concerned in the synthesis of vitamin C in the rat, we had hoped that symptoms of vitamin C deficiency might appear. However, the rats seemed to thrive normally after the operation and on the vitamin C-free diet. Unfortunately this finding can only be regarded as a negative result and cannot be taken as final and conclusive evidence of lack of suprarenal synthesis; since two objections might legitimately be raised—first, that small amounts of accessory tissue had escaped removal; and, secondly, that there had occurred some regeneration of new tissue after the operation. We did in fact observe in three out of four animals killed after four weeks what appeared to be rudiments of such new tissue. Taking all considerations into mind, however, the weight of evidence seems to be definitely opposed to the theory that the synthesis of vitamin C, which is known to occur in species like the dog or rat, has its seat in the suprarenal.

If, as appears likely, the physiological properties of ascorbic acid depend essentially on its specialised reducing properties, it may be supposed that the large amounts in the suprarenal cortex are involved in a system needed to maintain adrenaline-like substances in a reduced condition. Work in another connection [Harris and Fish, 1928–29; Harris, 1933, 2] has shown that in certain other sites vitamin C seems to be needed primarily for maintaining the functional

[1] We are indebted to Prof. J. H. Burn for kindly demonstrating the technique.

activity of certain types of cells and preventing their premature degeneration, *e.g.* osteoblasts, odontoblasts, ameloblasts, cementoblasts (so that vitamin C in this respect may be contrasted with vitamin D which does not act directly on cell structure in the bone and teeth but through phosphate and calcium metabolism). In tissues such as the tooth pulp and growing bone, however, in contrast with the suprarenal (and certain other organs) in which vitamin C appears to play an equally important rôle, it may be noted that there is no appreciable increased local concentration of vitamin C.

Summary.

1. The medulla as well as the cortex of the suprarenal is intensely rich in vitamin C.

2. The cortex (in the ox) is about thrice and the medulla about twice as potent as orange juice (or standard lemon juice), weight for weight.

3. The biological results agree precisely with the chemical titration, showing that ox suprarenal medulla contains about 1·1 to 1·2 mg. of ascorbic acid per g.

4. A negative silver stain is no certain guide as to the presence or absence of vitamin C.

5. The significance of the localised concentration of vitamin C in the suprarenals is discussed. It is shown that it cannot be regarded as a reserve store for the body, comparable for example with the vitamin A resources of the liver. It is thought more probable that it is needed for protecting the normal functional activities of the organ. To test the theory that the suprarenal is engaged in synthesising the vitamin in such species as are known to make their own (such as dogs and rats), adrenalectomised rats were fed on vitamin C-free diet for periods of several weeks to see whether scurvy developed, but with negative results.

6. Rat suprarenal has the extremely high activity of about three times that of the ox or normal guinea-pig, or ten times that of orange juice, as determined biologically and by titration (6 mg. ascorbic acid per g.).

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