CXXIV. THE ASCORBIC ACID CONTENT OF THE ADRENALS AND LIVERS OF DIFFERENT ANIMALS.

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EXPERIMENTS carried out in this laboratory showed that the ascorbic acid content of the adrenal glands of guinea-pigs decreased markedly if the animals were fed on a vitamin C-free diet. With regard to the biological importance of ascorbic acid it seemed worth while to estimate the ascorbic acid content of different animal organs under normal and varied experimental conditions. This estimation was done by titration with 2 : 6-dibromophenolindophenol blue. The results not only give an idea of the distribution of the acid but at the same time form a test of the reliability of the method of estimation if compared with the known data in literature on the vitamin content of animal organs.

The method was based on the original observation of Szent-Györgyi [1928] that hexuronic acid (ascorbic acid) readily decolorises indophenol blue. The reduction of dichlorophenolindophenol was later developed by Tillmans *et al.* [1932] for a method of estimating vitamin C. Tillmans's method was criticised by Zilva [1932] and later improved by Birch *et al.* [1933].

In the present study 2 : 6-dibromophenolindophenol blue was used which had been standardised against a known solution of ascorbic acid. The estimation was made as follows. The tissue was weighed and minced with sand, and trichloroacetic acid 1.5 % was added until the solution coloured thymol blue slightly red. The extract was filtered and titrated against the indicator to the first permanent trace of pink colour. The values obtained by this method are in fair agreement with biological tests so that on the whole the estimation seems to give reliable results. Glutathione and adrenaline do not disturb the results at this acid reaction. This also is in complete agreement with the results obtained by Birch *et al.* [1933].

It should be emphasised, however, that in exceptional cases the method may be misleading and should be employed critically. In an early paper of Szent-Györgyi [1930] mention is made of a reducing agent in the adrenal medulla.

Another reducing agent which reduces indophenol blue and is not identical with ascorbic acid is found in the juice of the onion. On evacuation of the pressed juice about one-half of its reducing capacity disappears, showing that the entire reduction could not have been due to ascorbic acid which is not volatile.

The cases cited above are those in which the method would give too high values for ascorbic acid and hence also for vitamin C. There are also cases in

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which without special precautions the method might give much too low values. Many plants contain enzymes which are capable of oxidising the acid very quickly and effectively in the juice or minced pulp. These enzymes are partly or wholly inactive in the uninjured cell. As an example the phenol oxidase may be quoted which, as found by Szent-Györgyi and Vietoris [1931], is greatly activated by the process of mincing and will in different plants oxidise ascorbic acid in a few seconds by means of the quinone which is produced. Peroxidase-containing plants, such as cabbage and carrots, also contain enzymes which oxidise ascorbic acid in the juice or minced pulp fairly rapidly in a reversible or irreversible manner. As Tillmans found, the reversible oxidised product is also very labile and prone to undergo irreversible changes. The enzymes of the cabbage have been studied by Szent-Györgyi [1931] and have been termed hexoxidases. Kohman *et al.* [1931] have found that shredding carrots and keeping them for 1-3 hours greatly diminished the vitamin content.

Apart from these special cases the reduction of indophenol under the described conditions might be taken as a fair estimation of the ascorbic acid content and hence of the vitamin. This method is of value if used extensively for a first orientation but final results should be checked by biological assay.

The results of the present study are given in Table I.

Table I.

Animal	Diet	Mg. ascorbic acid per g. adrenal	Mg. ascorbic acid per g. liver	Remarks
Pig	—	1.22	0.11	Adrenal, whole gland
		1.05	0.13	
		1.23	0.14	
		1.12	0.12	
		Average	1.15	
			0.12	
Ox	—	0.97	0.40	Adrenal, cortex only
		0.95	0.38	
		0.61	0.27	
		0.53	0.29	
		Average	0.76	
			0.31	
Calf	—	1.21	0.32	Adrenal, whole gland
		1.05	0.19	
		1.07	0.37	
		0.90	0.30	
		0.97	0.40	
		0.89	0.37	
		0.81	0.40	
		Average	0.99	
Sheep	—	1.56	0.51	Adrenal, whole gland
		1.35	0.40	
		1.32	0.51	
		1.23	0.46	
		1.39	0.42	
		1.14	0.48	
		Average	1.33	
			0.46	
Dog	Meat	1.56	0.10	—
		1.48	0.19	
		1.34	0.20	
		Average	1.46	
			0.16	

Table I (cont.).

Animal	Diet	Mg. ascorbic acid per g. adrenal	Mg. ascorbic acid per g. liver	Remarks
Cat	Meat only	1.30	0.18	—
		1.45	0.15	
		1.06	0.19	
		1.07	0.15	
		1.31	0.19	
		1.13	0.17	
		Average	1.22 0.18	
Rabbit	Oats and dried clover	2.22	0.17	This diet was devoid of vitamin C
		2.08	0.07	
		1.41	0.08	
		1.12	0.06	
		3.06	0.13	
		3.08	0.15	
		Average	2.16 0.11	
Rabbit	Oats only for 10 days. Pre- viously on oats and dried clover	2.30	0.11	This diet was devoid of vitamin C
		1.62	0.07	
		1.48	0.08	
		2.85	0.07	
		1.68	0.07	
		1.97	0.09	
		Average	1.83 0.08	
White mouse	Bred for several genera- tions on oats, corn, and moistened bread	3.10	0.38	This diet was devoid of vitamin C
		2.30	0.62	
		2.10	0.42	
		2.20	0.36	
		Average	2.40 0.45	
Rat	Same as for mouse	5.10	0.20	This diet was devoid of vitamin C
		5.30	0.17	
		4.40	0.16	
		6.70	0.20	
		4.80	0.31	
		Average	5.20 0.21	
Guinea-pig	Sherman's basal diet and excess spinach for 3 weeks	1.29	0.32	—
		1.35	0.24	
		1.27	0.26	
		1.14	0.11	
		Average	1.24 0.23	
Guinea-pig	Sherman's basal diet for 3 weeks	0.10	0.02	Severe scurvy in all animals
		0.07	0.02	
		0.06	0.02	
		0.06	0.02	
		Average	0.07 0.02	
Guinea-pig	Sherman's basal diet and excess spinach for 4 days	1.50	0.20	—
		1.70	0.20	
Guinea-pig	Spinach for 4 days and basal diet only for 24 hours	1.60	0.11	—
		1.46	0.09	
Guinea-pig	Spinach for 4 days and basal diet only for 72 hours	0.78	0.04	—
		1.24	0.06	

From this the following conclusions may be drawn.

(1) The ascorbic acid content of the adrenals, based on 1 g. of tissue, is much higher in every animal than that of the liver.

(2) The amount of ascorbic acid in the adrenals and liver is in fair agreement with the known antiscorbutic activities of these organs [Birch *et al.*, 1933; Mills, 1932; Vedder, 1932].

(3) Certain animals, principally the rat and mouse as well as the rabbit, are able to store ascorbic acid in the adrenals and liver even if fed on a vitamin C-free diet. Dogs and cats were likewise able to do so when they were fed on meat which is relatively low in vitamin C. These results are likewise in agreement with that of Birch *et al.*

(4) Guinea-pigs fed first with liberal amounts of spinach and then placed on a vitamin C-free diet showed a decrease in the ascorbic acid content first in the liver and then in the adrenals, indicating that the liver serves as a reserve store for the ascorbic acid.

In order to complete this work an estimation of the ascorbic acid content of the various organs of the guinea-pig was made and the results are given in Table II. As seen from the table the ascorbic acid content is highest in the adrenal and lowest in the muscle, results which are in agreement with previous knowledge.

Table II. *Guinea-pigs fed on sugar beets and basal diet for 2 weeks.*
No signs of scurvy on autopsy.

Ascorbic acid in mg./g. of tissue.					Average
Adrenal	0.18	0.20	0.43	0.19	0.25
Liver	0.016	0.013	0.026	0.02	0.02
Kidney	0.04	0.02	0.02	0.01	0.02
Heart	0.006	0.005	0.007	0.005	0.005
Spleen	0.05	0.08	0.16	0.02	0.08
Leg muscle	0.001	0.002	0.01	0.001	0.005

SUMMARY.

A study is made of the reducing capacity, and hence of the ascorbic acid content, of the adrenals and livers of different animals. The technique of estimating the ascorbic acid is described and discussed.

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