CCLXVII. IS THE ASCORBIC ACID CONTENT OF THE SUPRARENALS AND LIVER UNDER THE CONTROL OF THE NERVOUS SYSTEM?

By ALADÁR BÉLA LÁSZLÓ BEZNÁK
AND ZOLTÁN HARISS.

From the Institute of Experimental Pathology, University of Budapest.

(Received October 17th, 1934.)

FOR the sake of coordination and organisation various metabolic processes of the animal cell are under the control of the nervous system. To mention but one example, the manifold enzymic processes of cellular carbohydrate metabolism are governed by the autonomic nervous system. This circumstance gives rise to the question whether the vitamin C content of the cells in the animal organism is controlled by the nervous system. The experiments described in this paper are directed to decide this question.

Methods.

For the estimation of the ascorbic acid in the suprarenals and livers of cats, guinea-pigs and dogs [cf. Svirbely, 1933] the 2:6-dibromophenolindophenol titration was used. A solution of 0.02 % of the dye was freshly prepared and standardised against known concentrations of ascorbic acid. The tissue in question was quickly removed, weighed on the torsion balance, ground with quartz powder in the presence of a drop of trichloroacetic acid, extracted three times with 1 ml. of 3 % trichloroacetic acid, centrifuged and titrated to an accuracy of 0.01 ml.

Experiments.

First of all we wished to decide whether stimulation of the splanchnic nerve causes an increase in the ascorbic acid content of the venous blood of suprarenals. For this purpose dogs were narcotised with chloralose, the suprarenals of the left side found and a cannula inserted into the vein just above the entrance into the inferior vena cava; about 5 ml. of blood were drawn into 5 g. of 3 % trichloroacetic acid in a tared centrifuge-tube and weighed. Two resting samples were always taken. After this resting period the splanchnic nerves were stimulated just above the coeliac ganglion for 5 minutes with a faradic current. (Frequency 80, C.D.: 150 mm., source of current 4 v. accumulator.) That such a stimulation was effective could easily be seen from the increased blood flow through the suprarenals and from the marked vasomotor reaction in the area supplied by this nerve.

Similar experiments were carried out on cats under ether anaesthesia, but reliable information could not be obtained, since the trichloroacetic acid extracts of the blood of both animals yielded too low titration values.

This procedure having failed, we had to attempt an indirect line of attack. We estimated the ascorbic acid content of the right suprarenals of cats removed under ether anaesthesia. Then the splanchnic nerve to the left suprarenal was

stimulated as described above but for 2–10 minutes, the gland removed towards the end of the stimulation and its ascorbic acid content estimated. The results, compiled in Table I, show that the stimulation of the splanchnic nerves causes

Table I. *Ascorbic acid content of cats' suprarenals during rest and during splanchnic stimulation.*

Condition of the gland	Weight of gland mg.	Ascorbic acid mg./g.	Condition of the gland	Weight of gland mg.	Ascorbic acid mg./g.
Resting	184	0.91	Resting	213	1.05
Stimulated	205	0.86	Stimulated	231	1.09
Resting	149	1.36	Resting	235	1.19
Stimulated	155	1.07	Stimulated	240	1.17
Resting	212	1.07			
Stimulated	231	1.09			

no change in the ascorbic acid content of the suprarenal. They also confirm the results of Svirbely's estimations [1933] of the ascorbic acid content of the suprarenals.

In the third series of experiments the question was studied as to whether the degenerative section of the splanchnic nerve has an influence on the ascorbic acid content of the suprarenals. The splanchnic nerve on the left side above the coeliac ganglion was cut in cats with aseptic precautions under ether anaesthesia. After different lengths of time had been allowed for degeneration as shown in Table II the suprarenals were worked up as above.

Table II. *Ascorbic acid content of normal suprarenals of cats and after degenerative section of the splanchnic nerve.*

Condition of the gland	Weight of gland mg.	Ascorbic acid mg./g.	Days after nerve section
Normal	173	1.24	2
Degenerated	165	1.04	
Normal	270	1.30	
Degenerated	212	1.35	
Normal	147	1.18	
Degenerated	138	1.12	
Normal	224	1.27	29
Degenerated	245	1.10	
Normal	181	1.76	
Degenerated	179	1.60	
Normal	186	0.78	
Degenerated	183	0.76	
Normal	141	0.82	21
Degenerated	130	0.98	
Normal	79	1.16	
Degenerated	70	1.00	
Normal	156	1.13	
Degenerated	153	1.04	

It is evident from this table that the degenerative section of the splanchnic nerve causes no change in the ascorbic acid content of the suprarenal either as compared with the uncut side or as compared with that in normal cats.

These experiments warrant the conclusion, that the ascorbic acid content of the suprarenals is not under the control of the sympathetic nervous system.

This conclusion was put to the test of another type of experiment. Quite recently Feldberg and Minz [1933] have demonstrated that when splanchnic stimulation liberates adrenaline from the suprarenals it does so by the intermediation of acetylcholine. We therefore tried the effect of acetylcholine perfusion on the ascorbic acid content of suprarenals. Firstly the right gland was removed as normal control. The aorta was then ligatured just below the renal artery. The acetylcholine in Ringer solution was infused into the aorta through the mesenteric artery. Concentrations from 5×10^{-2} mg./100 ml. acetylcholine up to 5×10^{-1} mg./100 ml. were tried. The results recorded in Table III are those obtained with the maximum concentration.

Table III. *Ascorbic acid content of cats' suprarenals, normal and perfused with acetylcholine.*

Condition of the gland	Weight of gland mg.	Ascorbic acid mg./g.	Condition of the gland	Weight of gland mg.	Ascorbic acid mg./g.
Normal	186	1.88	Normal	403	0.59
Perfused	156	1.77	Perfused	360	0.52
Normal	178	0.90	Normal	196	1.29
Perfused	153	0.96	Perfused	193	1.21

It is evident from the table that this concentration of acetylcholine has no effect on the ascorbic acid content of the suprarenals.

It was stated by Euler [1933] that ascorbic acid is given up to the blood as a result of adrenaline injections. With the same perfusion method as described above, using 1:10,000 adrenaline-Ringer solution, we subjected this conclusion to reinvestigation with results shown in Table IV.

Table IV. *Ascorbic acid content of cats' suprarenals, normal and perfused with adrenaline.*

Condition of the gland	Weight of gland mg.	Ascorbic acid mg./g.	Condition of the gland	Weight of gland mg.	Ascorbic acid mg./g.
Normal	230	2.26	Normal	90	2.00
Perfused	269	1.89	Perfused	96	1.33
Normal	164	1.81	Normal	111	2.02
Perfused	146	2.06	Perfused	106	1.62

The ascorbic acid contents of the right and left suprarenals show greater variations in these cases than in any of the other experimental series. In three out of four cases there is a decrease of about 20 % of the ascorbic acid in the perfused glands. We do not feel however that the conclusion that ascorbic acid is expelled from the gland would be justified since in one case a difference of the same order of magnitude was found, but in this case the ascorbic acid content of the normal side was the lower one.

From the results of these experiments the conclusion was reached that the ascorbic acid content of the normal suprarenals is not under the control of the autonomic nervous system, and that perfusion with acetylcholine or adrenaline does not significantly alter the ascorbic acid content of the glands.

The total content of ascorbic acid in the liver is greater than that in the suprarenals. It was therefore interesting to try to find out whether the ascorbic acid in the liver is more mobile. Perfusion experiments were carried out on the liver of guinea-pigs in a manner exactly similar to that used in the case of the

suprarenals. The experimental animals had received a daily dose of 1 ml. lemon juice for a period of a month before the experiment in order to fill up their ascorbic acid stores. Table V shows the results of adrenaline perfusion.

It is clear that the ascorbic acid content of the liver shows no change on adrenaline perfusion.

Table V. *Ascorbic acid content of guinea-pigs' livers, normal and perfused with adrenaline.*

Condition of the liver	Weight of sample mg.	Ascorbic acid mg./g.	Condition of the liver	Weight of sample mg.	Ascorbic acid mg./g.
Normal	270	0.077	Normal	221	0.140
Normal	230	0.081	Normal	233	0.140
Perfused	255	0.083	Perfused	294	0.140
Perfused	251	0.068	Perfused	264	0.150
Normal	293	0.063	Normal	182	0.090
Normal	274	0.070	Normal	244	0.090
Perfused	230	0.078	Perfused	292	0.080
Perfused	235	0.084	Perfused	231	0.090
Normal	215	0.078			
Normal	276	0.070			
Perfused	217	0.090			
Perfused	271	0.078			

Table VI. *Ascorbic acid content of guinea-pigs' liver, normal and perfused with acetylcholine.*

Condition of the liver	Weight of sample mg.	Ascorbic acid mg./g.	Condition of the liver	Weight of sample mg.	Ascorbic acid mg./g.
Normal	242	0.070	Normal	293	0.094
Normal	247	0.090	Normal	238	0.130
Perfused	283	0.090	Perfused	183	0.130
Perfused	291	0.090	Perfused	237	0.092
Normal	286	0.090	Normal	258	0.062
Normal	246	0.090	Normal	252	0.072
Perfused	256	0.090	Perfused	268	0.075
Perfused	294	0.090	Perfused	266	0.090
Normal	261	0.090	Normal	236	0.098
Normal	246	0.090	Normal	236	0.091
Perfused	250	0.090	Perfused	174	0.116
Perfused	240	0.090	Perfused	256	0.108

Table VI shows the results of the acetylcholine perfusion experiments. These results show quite conclusively that acetylcholine perfusion has no effect on the ascorbic acid content of the liver. It is concluded that the ascorbic acid content of the liver is just as immobile as is that of the suprarenals.

DISCUSSION.

These experiments prove that the stimulation or degenerative section of the splanchnic nerve supply to the suprarenal has no effect on its ascorbic acid content, and that perfusion with relatively great amounts of adrenaline or acetylcholine is also without effect. The same applies to the conditions in the liver.

Although the ascorbic acid content of the blood of the efferent vessel coming from the gland could not be estimated and consequently direct evidence is lacking, it is believed that the great constancy of the ascorbic acid content allows correct conclusions regarding the rôle of the suprarenals and liver as stores for ascorbic acid.

If, during any stimuli tried, ascorbic acid is liberated and given up into the efferent blood, its amount must be less than the experimental error of our estimation, this being $\pm 15\%$. Taking 200 mg. as the average weight of cats' suprarenals, the ascorbic acid content is about 0.2 mg. Consequently the amount which might have escaped must be less than 0.03 mg. This is a very small quantity indeed considering what a large supply of ascorbic acid the organism needs. It is therefore unlikely that the suprarenals act as a store for ascorbic acid. A similar conclusion has already been reached by Harris and Ray [1933] on different grounds.

Undoubtedly during the action of the four investigated factors profound changes are taking place in the metabolism and consequently in the biochemical structure of the gland, and it is remarkable that these changes leave the ascorbic acid content unchanged. Since our method measures the total ascorbic acid content and since ascorbic acid is not synthesised by the body, it is unlikely that the level of the ascorbic acid content is kept up as a dynamical equilibrium by continuous new formation. This great constancy of the ascorbic acid content probably indicates that its rôle in the cell interior is rather to maintain some important general standard, such as reduction potential, than to take actual part in any of the metabolic reactions.

SUMMARY.

1. The ascorbic acid content of the suprarenals of cats and of the liver of guinea-pigs as estimated by the 2 : 6-dibromophenolindophenol method shows no change during stimulation or degenerative section of the splanchnic nerve.

2. Perfusion of these organs with adrenaline or acetylcholine in Ringer solution causes no change in their ascorbic acid content.

It is concluded that the ascorbic acid contents of the different organs and the transport of ascorbic acid in the organism are not under the control of the nervous system and that neither the suprarenals nor the liver act as a store for vitamin C.

REFERENCES.

- Euler (1933). *Naturwiss.* 236.
Feldberg and Minz (1933). *Pflüger's Arch.* 233, 657.
Harris and Ray (1933). *Biochem. J.* 27, 2006.
Svirbely (1933). *Biochem. J.* 27, 960.