CLXVI. VITAMIN C IN THE ADRENAL GLANDS AND THE HYPOPHYSIS CEREBRI OF THE OX.

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SEVERAL attempts have been made lately to ascertain where the ascorbic acid is located in the tissue of the adrenal glands [Bourne, 1933, 1, 2; Giroud and Leblond, 1934].

The ability of ascorbic acid to reduce a solution of silver nitrate even in an acid medium has been used to demonstrate the occurrence of vitamin C in the tissues.

The experiments have been carried out in the following way:

- (1) The fresh organs are frozen and cut in slices 2-3 mm. thick.
 - (2) Treatment with 0.4 % AgNO_3 (p_{H} 4-4.5 by acetic acid or HNO_3) for 5-10 minutes.
 - (3) Washing in water.
 - (4) Treatment with 5 % $\text{Na}_2\text{S}_2\text{O}_3$ made acid by NaHSO_3 for 5-10 minutes.
 - (5) Washing in running water for 12 hours.
 - (6) Hardening in alcohol and preparation of paraffin sections.
- (2), (3) and (4) are carried out in darkness; finally staining with eosin or haematoxylin-eosin.

Macroscopically the vitamin C-containing tissues are coloured black through an impregnation with free metallic silver in the places where the ascorbic acid has reduced the acid silver nitrate.

This impregnation begins immediately in the fresh tissue and is found only in the most superficial cell layers; this certainly depends upon a coagulation of the albuminous fraction of the tissue caused by the silver nitrate and upon a precipitation of AgCl . These substances are supposed to form a barrier for any further progress of the silver impregnation of the deeper cell layers.

I. *The adrenal glands.*

The colouring begins rapidly in the cortex and is followed by a slower black impregnation of the medulla which finally turns completely black.

Microscopically (Fig. 1, Plate VII) the cortex shows in particular a staining of the cells of the zona fasciculata and reticularis.

The impregnation is seen as black granules of nearly equal size evenly dispersed in the cell cytoplasm but not appearing in the nucleus. In the sections stained with eosin the place of the nucleus is seen as an empty spot, free from granules.

Furthermore black granules are deposited in the intercellular tissue either arranged like strings of pearls along the interior of the lymph vessels and in the intercellular spaces or diffusely scattered over the tissue.

This arrangement will be discussed later.

Fig. 1. Suprarenal bodies from ox stained with AgNO_3 and eosin.

Fig. 2. Hypophysis from ox stained with AgNO_3 and eosin.

The medulla, which is built up by cells not so densely packed and more irregularly arranged than in the cortex, is completely covered with small silver granules staining both the cells and the intercellular tissue. The staining here looks very different from that of the cortex, the granules of the medulla being of a smaller size and covering the medullar tissue quite evenly so that this tissue looks as if it were covered with a grey carpet.

The sections stained with haematoxylin show that the silver impregnation is most pronounced in the cells in which the cytoplasm has a greater affinity to the acid than to the basic components of the stain solutions.

II. *The hypophysis cerebri.*

Only the anterior lobe is stained.

Here the cells are not so densely packed as in the cortex of the adrenal gland and the staining extends to a greater depth from the surface.

Microscopically (Fig. 2, Plate VII) the cytoplasm of the cells is seen uniformly filled up with black granules of equal size. The granules, as with the suprarenal cortex, are not to be found in the nucleus.

In the sections stained with haematoxylin-eosin the silver staining is more pronounced in the cells with greater affinity to the acid than to the basic components of the stain solution.

As found in the adrenal cortex a number of black granules are also to be seen in the intercellular spaces, the lymph vessels and the connective tissue in the hypophysis cerebri.

It is very probable that these extracellular deposits of silver granules are also due to reduction of AgNO_3 by ascorbic acid which has passed the cell membranes either on its normal way from the cells to the tissues in other parts of the body and *vice versa* or through a postmortally injured cell membrane.

REFERENCES.

- Bourne (1933, 1). *Nature*, **131**, 874.
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