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Electron Microscopic Study of Anterior Pituitary Necrosis Caused by Hexadimethrine Bromide in Rats

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Intravenous administration of hexadimethrine bromide to rats causes infarction of the anterior pituitary (Kovács, Carroll and Tapp, 1964). As the pathogenesis of the lesion could not be fully elucidated by ordinary microscopic examination, it has been further investigated by means of the electron microscope. In the present work a highly active preparation of hexadimethrine bromide was used. Nevertheless, infarction of the pituitary occurred in only about three quarters of the animals and was of variable size, sometimes involving practically the whole lobe and sometimes only a small part of it. Four of the animals showed no lesions of the pituitary.

Material and Methods

The experiments were performed on 22 female rats weighing about 200 g and kept on a standard diet. Five mg of hexadimethrine bromide (Polybrene, Abbott) was administered in aqueous solution intravenously to each animal. In addition, one hour before the animals were sacrificed, India ink[1] (0.1 ml/100 g body weight) was also administered intravenously for a specific purpose which will be discussed elsewhere.

The animals were killed in pairs by decapitation $^1/_2$, 1, 2, 3, 4, 6, 8, 12 and 24 hours after the hexadimethrine bromide administration. Two rats received India ink only, and two others were not injected; these animals were used as controls.

The pituitaries were removed within 1 minute following decapitation. The posterior pituitary was discarded under the stereomicroscope. The anterior pituitary was bisected sagittally; one half was prepared for light microscopic examination and the other was cut into small pieces for electron microscopic studies. The tissues were fixed for 2 hours in 5 per cent glutaraldehyde in Millonig's phosphate buffer at pH 7.4. They were then washed with the buffer of the fixing solution and postfixed for 1—2 hours in a 1 per cent OsO_4 solution buffered at pH 7.4. After dehydration in serial alcohols the material was contrasted with uranyl acetate in 70 per cent alcohol and embedded in Araldit. A sample thick section at 1 or 2 μ was made from each block, stained with toluidine blue, and examined by phase contrast in order to select the most suitable blocks for electron microscopic studies. The ultra thin sections were "stained" with uranyl acetate and lead citrate. The electron microscopic examinations were made on a JEM-6C apparatus.

Results

Changes in the Course of the First 6 Hours

Light Microscopy. In a previous communication the histologic changes observed with the light microscope were described in detail (Kovács, Carroll, and Tapp, 1964). At 6 hours the first real signs of necrosis of the parenchymal cells become apparent: many cell nuclei are pyknotic, the cytoplasm is vacuolated, the granulation of the chromophil cells is blurred, and

[1] Pelikan Spezialtusche, C 11-1431a, Günther Wagner, Pelikan Werke, Hannover, Germany.

occasionally the cytoplasm has disappeared. In the present experiments the same changes were found, but with the 1 or 2 μ sections stained with toluidine blue the alterations could be recognised somewhat earlier and with more certainty than in the thicker paraffin sections. In the first hour after administration of the hexadimethrine bromide a few dilated vessels filled with erythrocytes and a slight loosening of the parenchyma can be seen. In the dilated vessels there is occasionally a homogenous palely staining substance in whose center some India ink granules can be observed. At 2—4 hours these changes become more prominent. In some of the vessels margination of granulocytes is occurring. In certain areas there are

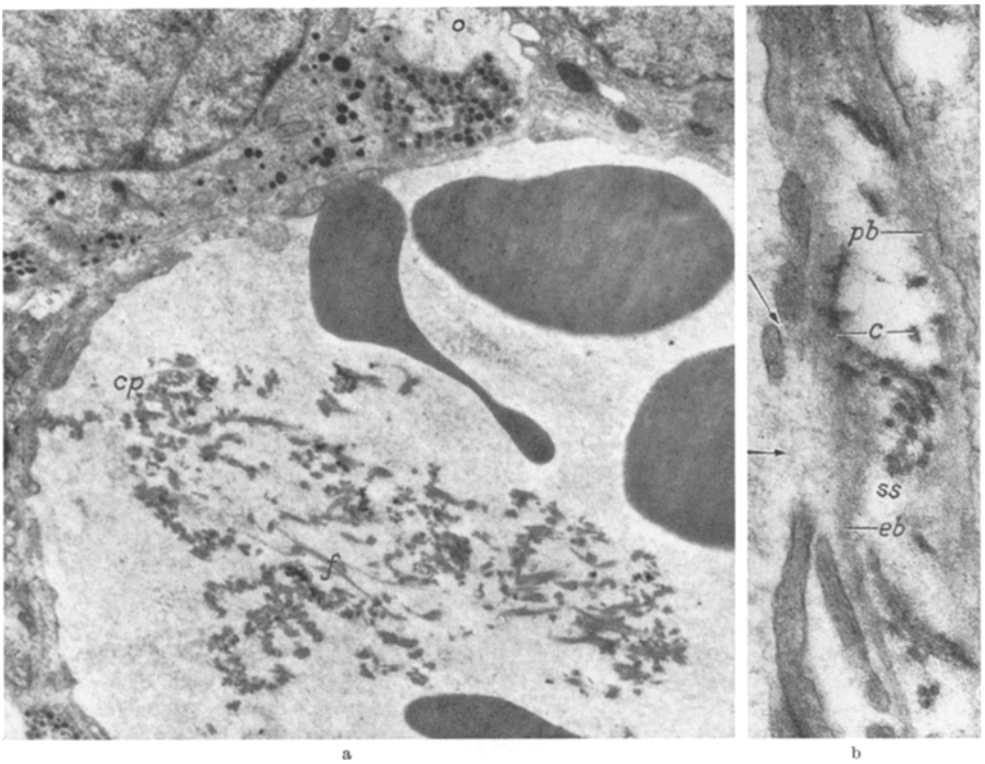


Fig. 1. a Dilated capillary in which precipitated fibrin (*f*) and India ink granules (*cp*) are seen. Thrombocytes are not present. In the upper part of the picture edema of part of a cell (*o*). Half an hour after treatment. 8,400 × b Ruptures (arrows) in the lining of the capillary endothelium. The endothelial basal membrane (*eb*) and the parenchymal basal membrane (*pb*) are occasionally blurred. The subendothelial space is dilated (*ss*), the collagen fibres (*c*) are separated. Half an hour after treatment. 39,520 ×. c Dilated intercellular spaces (*is*) filled with edema fluid, similar to the plasma in the capillaries (*bc*). The arrow points to an extracellular granule. Part of a LTH cell (A_2) at the upper rim of the picture is edematous. Half an hour after treatment. 3,895 ×

hemorrhages into the parenchyma and distortion of the architecture, and some of the cell nuclei show a dark colour and are shrunken. At 6 hours the hyperemia, dilatation of the vessels and hemorrhages are pronounced and the interstitial edema is very striking.

Electron Microscopy. At half an hour after the treatment most of the capillaries are of normal size, but here and there a few capillaries are moderately dilated. The lumen of these dilated vessels contains plasma and a few erythrocytes, and sometimes there is a small mass lying free in the lumen and consisting of filaments of fibrin and India ink granules (Fig. 1a). Occasionally the fibrin fibres adhere to the endothelial cells. None of the vessels are occluded.

In the endothelial cells of some of the capillaries ruptures of various sizes occur at the fenestrae (Farquhar, 1961). The bridging membrane normally has

interruptions 300—500 Å wide, but at these sites discontinuities a few hundred mμ wide are formed (Fig. 1 b). Under the ruptures the subendothelial basal membrane is normal at some places and blurred at others. At the ruptures of the vascular walls the subendothelial layer is often loose and widened, and its collagen fibres separated (Fig. 1 b); this is also seen occasionally around vessels whose walls are apparently intact. India ink granules are not present in the subendothelial layer.

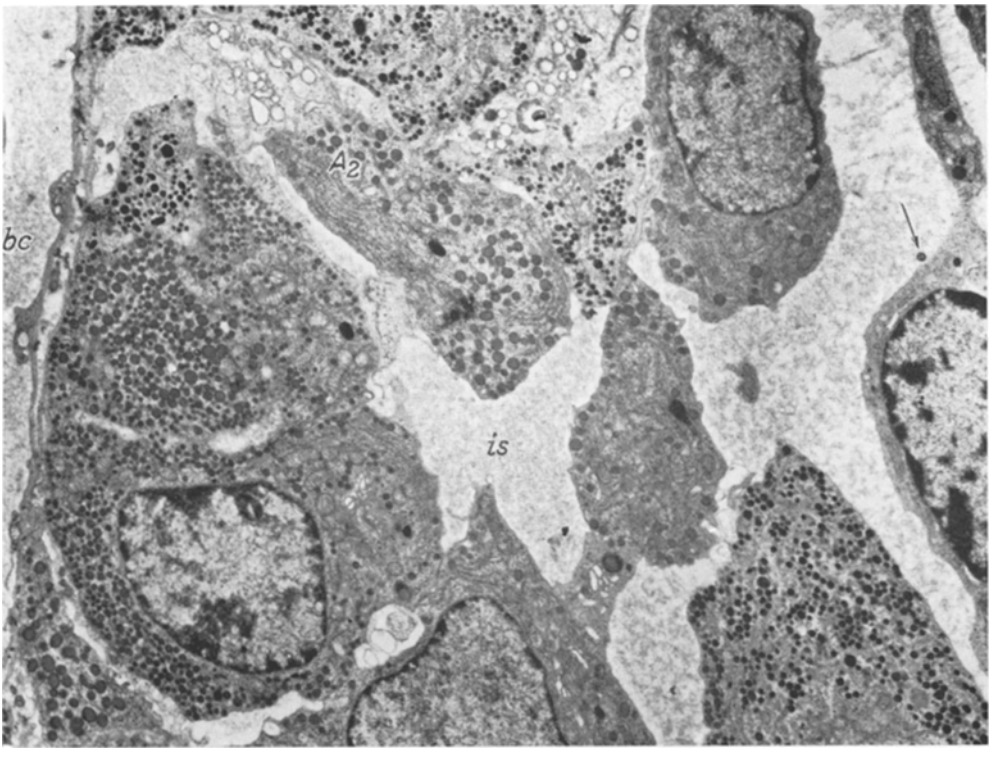
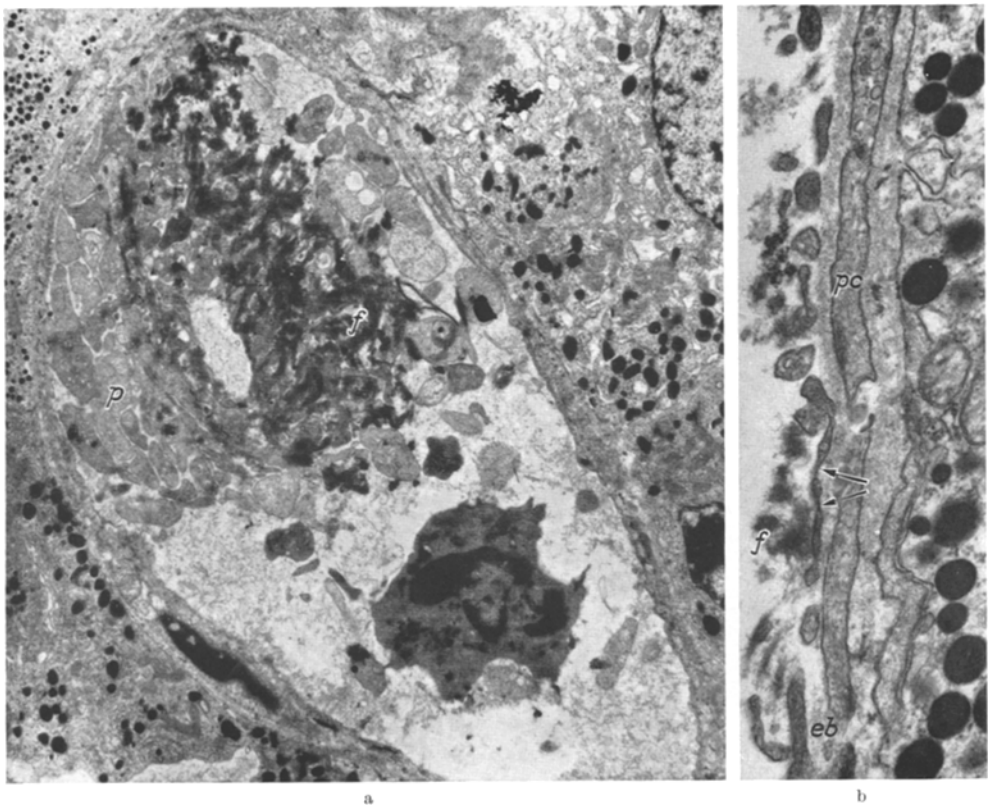


Fig. 1 c

The parenchymal cells are separated to varying degrees by a finely granular edema fluid in which secretory granules are sometimes visible but fibrin is not present (Fig. 1 c). Some of these cells have a patch of cytoplasm which is swollen, pale and edematous, and contains swollen mitochondria, separated fibres of endoplasmic reticulum, and a few free ribosomes (Fig. 1 c).

Between the first and sixth hour the alterations gradually become more severe. At one hour after the treatment some capillaries contain true thrombi composed of thrombocytes and fibrin. The bulk of the thrombus consists of thrombocytes, degranulated to varying degrees. The fibrin component is less, and is generally located in the center of the thrombus (Fig. 2 a). India ink granules are enclosed between the fibrin fibres. At two hours there is a definite increase in the number of thrombosed vessels and in the size of the thrombi in them. Granulocytes also accumulate in the vessels, and some of them adhere to the endothelial cells. From two hours onwards many of the capillaries are enormously dilated and packed with erythrocytes.

Meanwhile the lesions of the capillary walls are progressing. These are essentially of the same type as seen at half an hour, but at one hour they are much more common and the ruptures are more extensive. In some vessels only fragments of the capillary endothelium remain (Fig. 2b). A number of endothelial cells show damage of their cell components: the mitochondria are swollen and the endoplasmic reticulum dilated. No definite opening-up of the intercellular junctions of the endothelium can be seen. Thrombocytes can often be found



a b

Fig. 2. a Thrombus consisting of thrombocytes (*p*) and fibrin (*f*) one hour after treatment. 3,572×. b Fragmented capillary endothelium to which precipitated fibrin (*f*) adheres. The membrane bridging the fenestrae of the endothelial cell remains in only two places (arrows). There is also a discontinuity of the endothelial basal membrane (*eb*). Between the endothelium and the parenchymal cell there is a pericyte process (*pc*). Two hours after treatment. 22,610×

adherent to the vascular walls (Figs. 2b and 4); this occurs only at the site of ruptures of the endothelial lining. Fibrin fibres are sometimes also attached to the ruptures. In the vicinity of the ruptures of the fenestrae the subendothelial layer are generally widened and are saturated with a plasma-like substance in which the basal menbranes of the vessel and of the cells appear blurred (Fig. 4). Similar alterations occur around some of the vessels in which the endothelial cells are normal, at least in the plane of the section. Occasionally the basal membrane is ruptured.

In some places, blood corpuscles escape from the vessels through the discontinuities in their walls (Fig. 3), and at 2 hours there are sometimes erythrocytes

in the cytoplasm of the damaged parenchymal cells or between them. Thrombocytes and India ink granules can also be seen extravascularly.

During the first 6 hours fluid accumulates between the parenchymal cells; it is seen as a finely granular substance, in which there are scattered a few loose secretory granules, mitochondria and endoplasmic reticulum membranes. The cytoplasmic change in some parenchymal cells which was observed at 1 hour is rather more common at 2 hours, but still involves only a few cells in small foci.

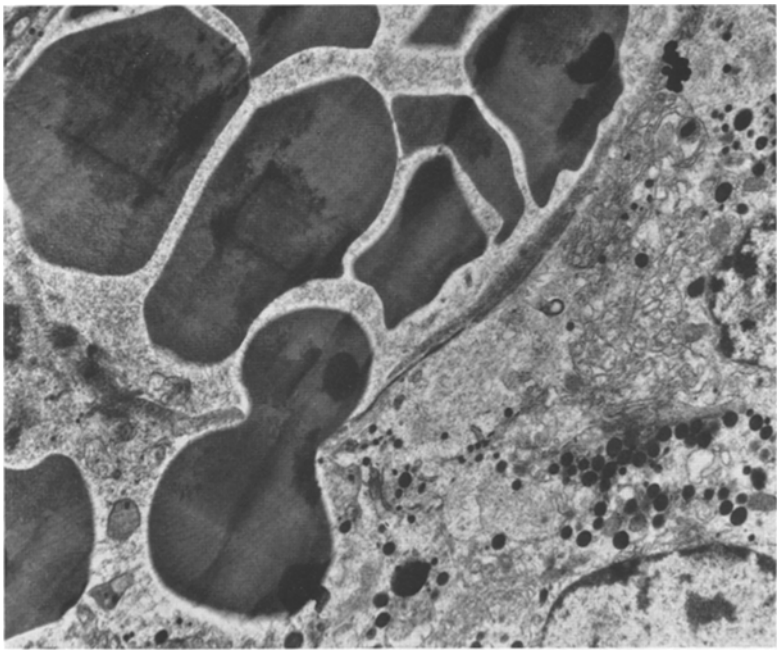


Fig. 3. Diapedesis of erythrocyte at an opening in the capillary wall. Four hours after treatment. 5,700×

Fat droplets appear in the cytoplasm of some of the cells. Among the edematous cells there are also a few cells which are denser than normal. After the appearence of the haemorrhages and the severe edema of parenchymal cells the walls of many of these cells become ruptured, and in some areas the cell boundaries disappear so that the cytoplasm of neighbouring cells merges. In the third hour nuclear changes corresponding to chromatolysis and pyknosis develop (Fig. 5); subsequently these alterations become more frequent and more severe.

Changes between the 6th and 24th Hours

Light Microscopy. From the 6th hour onwards the necrotic phenomena progress rapidly, and the zones characteristic of infarcts begin to develop (SHEEHAN and STANFIELD, 1961). In the margin zones the nuclei show signs of lysis, rhexis and pyknosis. The cytoplasm becomes detached from the nuclei, and its granulation is not obvious. The vascular walls gradually become necrotic. Dehemoglobinisation of the erythrocytes starts. Here and there leucocyte infiltration may be seen.

At 24 hours the typical picture of infarction is to be seen. The extent of the necrosis varies, but even in the largest infarcts a peripheral rim of a few cell always remains alive.

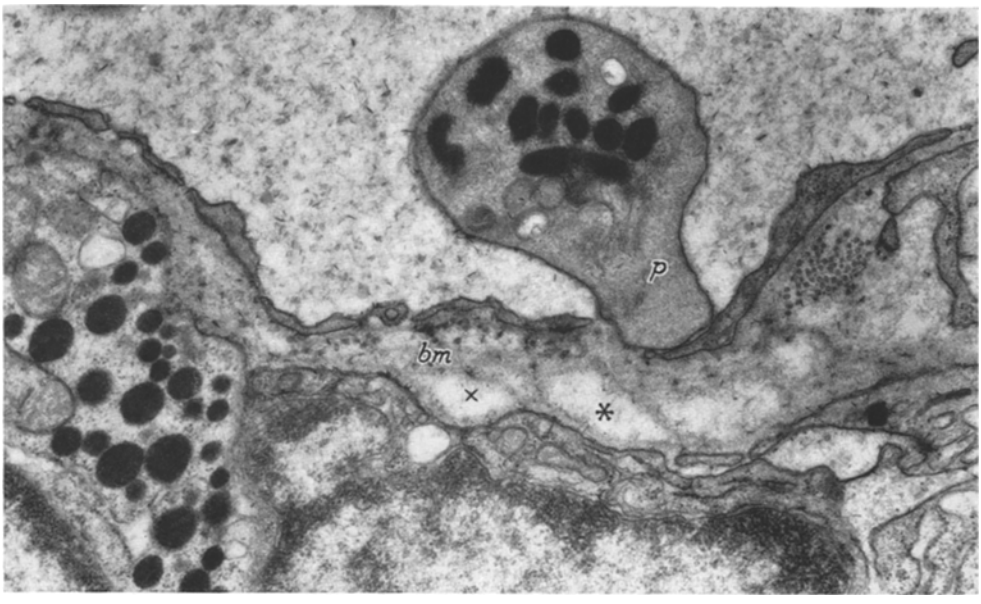


Fig. 4. Thrombocyte (*p*) adherent to the rupture of the capillary wall. The subendothelial layer is less dense in places (asterisk), and the basal membranes (*bm*) are blurred. Four hours after treatment. 18,430×

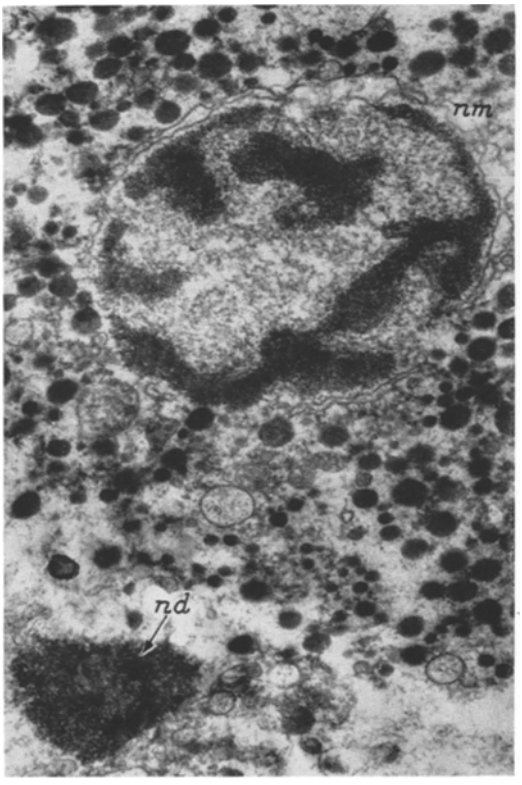


Fig. 5. Parenchymal cell with disorganized cytoplasm and loss of cell membrane. The pynknotic nucleus is detached here and there from the dilated perinuclear cystern (*nm*). At the lower part of the picture a nuclear fragment (*nd*) can be seen. Three hours after treatment. 13,400×

In the infarcted area the cell contours are blurred, and generally the cell nuclei do not stain. Occasionally nuclear remnants are visible, sometimes forming hematoxilinophil clumps.

Electron Microscopy. In electron microscope sections the magnification is too great to allow recognition of the gross pattern of the infarct, and in particular it is difficult to differentiate the various margin zones from each other or even from the central area of the infarct. But this deficiency is not important as the present paper is concerned essentially with the pathogenesis as studied in the first 6 hours.

Discussion

The main purpose of the present investigation has been to try to ascertain what happens to the blood flow during the first 6 critical hours.

Contents of Vessels. When a normal animal is killed and the pituitary is removed, most of the blood escapes so that in histologic sections the capillaries are almost empty. In the present experiments the capillaries already at *half an hour* show a different appearance. Some of them are empty, but others are moderately distended by precipitated plasma and contain a few red corpuscles and small wisps of loose fibrin. The fibrin deposition is certainly not of post-mortem development, as similar fibrin masses were not found in any of the control animals.

Obviously the blood in the capillary is stagnant, and the fibrin is deposited only from this stagnant blood.

An other problem is to explain why a number of the capillaries at half an hour are moderately distended with plasma, and why most of the plasma did not escape from these capillaries after death as in a normal animal. This might be due to a loss of tissue tension on the part of the surrounding parenchyma or to some paralysis of the capillary wall.

At *one hour*, platelets accumulate around the fibrin coagulum, and a few polymorph leucocytes and platelets become attached to the wall of the capillary. These platelets must have come from blood in the proximal part of the capillary. A few capillaries are distended by a packed mass of red corpuscles. This phenomenon must be due to an inflow of blood into a damaged capillary, with loss of the plasma into the surrounding tissues. The mass of red corpuscles was unable to escape after the animal was killed, and presumably therefore could not move forward during life. In other words the red corpuscles in these particular capillaries are in stasis.

During the period from *one to six hours* there is a continuation of the processes which have given rise to the appearances at one hour. The platelet thrombi increase in number and become more occlusive. An increasing number of capillaries become distended by red corpuscles in stasis.

From *six hours onwards* there is certainly no circulation through the area, as the parenchyma and the vessel walls undergo ordinary infarction.

In the late stages, at *8, 12* and *24 hours*, there is still no evidence of re-establishment of circulation through the infarcted area, though the surviving areas of parenchyma at a distance from the infarct enjoy an apparently normal circulation.

It is not possible to say exactly what the circulatory condition is during the first six hours. The most attractive theory is that there is no blood flow through the area during that period; the blood in the vessels is stagnant, though there is

an occasional slight ooze of plasma containing platelets and polymorphs into some of the capillaries and a slight ooze of ordinary blood into other capillaries. The oozing does not amount to a re-establishment of the circulation.

Capillary Walls. The changes in the capillary walls consist essentially of a rupture of the fenestrae. For the capillaries in endocrine organs (FARQUHAR, 1961). The rupture seems to take place by the opening-up of gaps at the intercellular junctions between endothelial cells. There is remarkably little deposition of fibrin at the ruptures. The only real attempt to close the breach is by the attachment of platelets.

The aetiology of these ruptures of the fenestrae is not very clear. One possibility is that the hexadimetrine bromide might become attached locally to the vessel wall. HUGGINS and SUGIYAMA (1965) considered that in the adrenal vessels this substance, being a cationic polymer, becomes linked to the negatively charged capillary wall, and that in turn the negatively charged platelets adhere to the surface of the polymer. An alternative view is based on the work of KIMURA et al. (1961, 1962), who showed that hexadimethrine bromide causes degranulation of mastocytes and liberation of histamine and serotonin. MAJNO and PALADE (1961) studied the local effects of injections of histamine and serotonin. They demonstrated openings of the intercellular junctions between the endothelial cells in the vessels of non-endocrine tissues. It is possible that some such mechanism could be responsible for the rupture of the fenestrae also in the pituitary vessels. Nevertheless it is difficult to account for the fact that the resultant lesion is an infarction of a patch of parenchyma and not of the entire gland. A third possible explanation is that the rupture of the fenestrae might be a result of a local ischemia in the pituitary capillaries due to an arrest of the blood flow at some more proximal site. As previous studies by light microscopy have not shown any thrombosis of the portal vessels in the stalk during the first 6 hours only some vasospasm could be considered.

The evidence from the electron microscope studies is not sufficient to determine which, if any, of these three possible explanations is correct. Nevertheless it is quite clear that in the very early phase the capillary lesions and the necrosis of the parenchyma can not be ascribed to capillary thrombosis. Later on the platelet masses in the capillaries certainly seem to be occlusive. However, it seems probable that the parenchymal cells have been killed by ischemia before this local capillary obstruction could play a significant part.

Parenchyma and Interstitium. There is clear histologic evidence that plasma fluid, containing apparently the same amount of protein as in the lumen of the capillaries, escapes through the ruptured fenestrae into the interstitial tissue of the capillary wall and then into the spaces between the parenchymal cells. This occurs as early as half an hour and becomes progressively more severe. It is a curious fact that no fibrin is deposited in these pools of extravasated plasma.

The extravasated plasma fluid initially enters only one part of a parenchymal cell, producing a localised edema of the cytoplasm. Subsequently the parenchymal cells lose their outlines. Even red corpuscles may enter the cytoplasm.

Relation to Human Pathology. The light microscopic picture of the pituitary necrosis induced experimentally by hexadimethrine bromide administration (KOVÁCS et al., 1964) is identical to that of human post partum pituitary necro-

sis (SHEEHAN and STANFIELD, 1961). Electron microscopic examinations of human pituitary necrosis have not yet been carried out, but it is possible that vascular changes similar to those described in the present paper may play a part in the development of human pituitary necrosis.

Summary

Intravenous injection of hexadimethrine bromide in rats causes necrosis of the anterior pituitary. As early as after half an hour there is dilatation of the vessels, slight intravascular fibrin precipitation, rupture of the vessel walls, and leaking of plasma into the spaces between the parenchymal cells. At one hour, platelets accumulate around the small fibrin coagula and also become attached to the ruptures of the vascular walls; these may occlude the capillary lumen. The necrosis of the parenchyma becomes recognizable later, and is apparently secondary. The necrosis is thus considered to be the result of a local circulatory disturbance.

Elektronenmikroskopische Untersuchungen der Nekrose des Hypophysenvorderlappens, hervorgerufen durch Hexadimethrin-Bromid bei Ratten

Zusammenfassung

Schon nach 30 min findet man eine Erweiterung der Gefäße, geringe intravasculäre Fibrinniederschläge, Einrisse der Gefäßwände und Austritt von Plasma in die Zwischenräume zwischen die Parenchymzellen. Nach 60 min sammeln sich Thrombocyten um die kleinen Fibrincoagula an und haften an den Stellen der Gefäßeinrisse. So dürfte es zu einem Verschluß der Capillarlichtungen kommen. Die Parenchymnekrose wird erst später erkennbar und ist offenbar sekundärer Natur — sie wird also als Folge der örtlichen Kreislaufstörung aufgefaßt.

References

FARQUHAR, M. G.: Fine structure and function in capillaries of the anterior pituitary gland. Angiology **12**, 270—292 (1961).

HUGGINS, C. B., and T. SUGIYAMA: Production and prevention of two distinctive kinds of destruction of adrenal cortex. Nature (Lond.) **206**, 1310—1314 (1965).

KIMURA, E. T., P. R. YOUNG, and G. H. BARLOW: A study with low and high molecular weights of hexadimethrine-bromide — an antiheparin agent. Proc. Soc. exp. Biol. (N.Y.) **111**, 37—41 (1962).

— —, and R. K. RICHARDS: Interaction of histamine, serotonin and heparin with hexadimethrine-bromide, a mast cell fragmentor. Proc. Soc. exp. Biol. (N.Y.) **107**, 19—23 (1961).

KOVÁCS, K., R. CARROLL, and E. TAPP: Experimental hexadimethrine necrosis of anterior pituitary. Lancet **1964II**, 919—921.

MAJNO, G., and G. E. PALADE: Studies on inflammation. I. The effect of histamine and serotonin on vascular permeability: an electron microscopic study. J. biophys. biochem. Cytol. **11**, 571—605 (1961).

SHEEHAN, H. L., and J. P. STANFIELD: The pathogenesis of post-partum necrosis of the anterior lobe of the pituitary gland. Acta endocr. (Kbh.) **37**, 479—510 (1961).

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