THE LESIONS PRODUCED IN THE BRAIN OF RABBITS BY THE INJECTION OF INDIAN INK AND OF ARGYROL.

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It has been shown that after the injection of organisms into the general circulation of rabbits definite lesions are found in the nerve-cell laminæ of the brain¹. These lesions are necrotic, but all areas are not involved to the same degree. The fornix shows the maximum of damage; here the necrotic lesion is definitely ischæmic. In the cornu Ammonis the ischæmia is somewhat less absolute, while in the cerebral cortex groups of cells are necrotic, suggesting a partial local asphyxia, which is not so definite as that in the fornix or cornu Ammonis.

The reason for these three varieties of local necrosis is to be found in the difference in the local vascular supply of each area. In the fornix the vessels are terminal; in the cornu Ammonis there is a partial anastomosis; while in the cerebral cortex there is a looped system of vessels providing a rich collateral circulation to any given area. Hence interference with the local vascular supply in the cortex does not result in ischæmia, but in a partial asphyxia, which may be compensated to some extent by the collateral circulation.

EXPERIMENTS.

In this series of experiments (a) Indian ink granules and (b) argyrol were injected into the general circulation of rabbits. The brains were fixed in formalin (10 per cent.) and in formol-ammonium bromide. Serial sections (paraffin) were stained by toluidin blue and iron hæmatoxylin for the demonstration of the nerve-cell changes, and by Cajal's gold sublimate method for the neuroglial reaction.

RABBIT A.

Five c.c. of a 5 per cent. solution of Indian ink were injected into the vein of the ear, and the animal killed seven days afterwards. The brain and spinal cord were fixed in 10 per cent. formol saline.

There was an area of coagulation-necrosis in *each fornix* situated in the lateral curvature, midway between the frontal and the temporal cortex. On the right side the line of demarcation between the normal and the necrotic areas was very sharp, and the necrosed cells showed the characteristic shrinkage of the cell-body

and the homogeneity of the nucleus. On the left side the necrotic cells did not stain quite so intensely as those on the right side, thus indicating an effect of more recent date or of less intensity. In the upper and inner segment of the right fornix a small portion of the cell-layer showed such an increased affinity for the stain as to point to a certain degree of interference with its nutrition. The necrotic change affected the cells in the inferior portion of the fornix and the uncus of the temporal lobe.

In the cornu Ammonis no change was demonstrable in the granular layer, but in the pyramidal layer there were portions in which the cells stained more deeply than normal, both cell-body and protoplasmic processes showing an increased affinity for the dye.

RABBIT B.

Injection into vein of ear of 5 c.c. of a 5 per cent. solution of Indian ink. The rabbit died one and a quarter hours after the injection. The brain and cord were fixed in 10 per cent. formol saline.

FIG. 1.—At the upper margin of the figure the fornix cells are necrotic; in the white matter immediately below, there are scattered areas of œdema: the lower margin shows the lamina involuta, inferior to which lie the darkly stained necrotic cells of the granular layer of the cornu Ammonis. The vessels of the lamina involuta are filled with granules.

On the left side of the brain the entire fornix was necrotic, with the exception of a small segment adjacent to the superior cortex.

On the right side there was an extensive area of necrosis in the superior portion of the fornix. Immediately inferior to this necrotic area a considerable portion of the fornix layer was quite normal, but lower down where this structure bends towards the temporal lobe there was another necrotic area of considerable

extent. From this point onwards for some little distance the cell-band was quite normal, but at the inferior extremity of the fornix there was another necrotic area continued into the temporal lobe. In these necrotic areas the cells were hyperchromatic and shrunken, there was homogeneous atrophy of the nucleus, and considerable œdema of the tissues in the immediate vicinity.

The bilateral character of the lesions and their multiplicity are worthy of note in this case. The degree of œdema in the cerebral cortex was very slight; it was very evident, however, in the subcortical white matter.

RABBIT C.

Injection into vein of ear of 5 c.c. of a 5 per cent. solution of Indian ink.

The superior portion of the fornix was quite normal, but in the lateral curvature of this structure on each side there was an extensive necrotic area in which the cells were hyperchromatic, shrunken, and stained as a dense homogeneous body in which the nucleus could, in the majority of instances, be barely recognised as a separate structure. The line of demarcation between the necrosed and the normal cells was very sharply defined. The lower portion of

FIG. 2.—Above, the fornix in which the necrotic cells (black) are prominent. Below, the lamina involuta, whose vessels are filled with Indian ink granules. Near the inferior margin note the deeply stained (necrotic) cells of the granular layer of the cornu Ammonis.

the fornix was free from necrotic change, but this was present amongst the cells of the uncus and the adjacent portion of the temporal lobe.

The vessels of the lamina involuta were dilated and there was considerable perivascular œdema. Small œdematous areas were present in both the fornix and the cornu Ammonis, and many cells in the pyramidal layer of the latter had undergone necrosis. The cells of the granular layer stained normally. In the cortex coagulation necrosis affected the pyramidal cells of the deeper layers, and there was present perivascular œdema of medium degree.

RABBIT D.

The left cervical sympathetic chain was divided, and Indian ink granules injected into the general circulation. An area of coagulation-necrosis was found in the lateral portion of each fornix. The cell bodies were hyperchromatic, shrunken, each one surrounded by a small area of œdema, and the nucleus was homogeneous and atrophic. The upper portion of the fornices close to the cortex was also necrosed. On following those necrotic areas through the series of sections towards the posterior aspect of the brain, it was found that their size varied from point to point until they gradually disappeared, thus demonstrating their ischæmic origin. The pyramidal cells of the cornu Ammonis were also necrotic.

FIG. 3.—Note necrosis of the fornix cells in the upper part of the figure. The necrotic change also affects the pyramidal layer of the cornu Ammonis (lower). The fine capillaries are seen filled with Indian ink granules.

RABBIT E.

The left cervical sympathetic was divided and Indian ink injected into the the general circulation.

There was an area of necrosis at the lateral angle of each fornix. In sections taken posterior to the necrotic area the lesion was found to disappear gradually. The cells of the granular layer of the cornu Ammonis were healthy with a few exceptions, but there was necrosis of the cells in the posterior portion of the pyramidal layer. There were no gross lesions in the cortex. The vessels of the entire brain were filled with granules, viz., in the cortex, choroid plexus, and lamina involuta, as were all the capillaries ramifying amongst the cells of the fornix and cornu Ammonis.

RABBIT F.

The left cervical sympathetic was divided and a 1 per cent. solution of argyrol injected into the general circulation.

In the anterior part of the brain there was a large necrotic lesion involving *almost the entire left fornix*, and the nerve-cells in both granular and pyramidal layers of the cornu Ammonis showed the same change. On the right side, at the same level, the fornix was quite normal, but in both granular and pyramidal layers of the cornu Ammonis there was a slight degree of necrosis.

Sections taken at a point more posterior, however, showed definite necrotic change at the *lateral angle of the right fornix* also, with a slight degree of necrosis of both cell-laminæ in the cornu Ammonis. The lesion was therefore bilateral, but involved one fornix much more than the other.

FIG. 4.—Note necrosis of the cells in the pyramidal layer of the cornu Ammonis. Each cell is shrunken and surrounded by a small area of oedema.

RABBIT G.

Intravenous injection of 10 per cent. argyrol. The rabbit died in three hours. Brain and cord fixed in formol saline.

There was a high degree of oedema present in the brain, affecting the cortex but not the subcortical white matter; the white matter superior and inferior to the fornix and the fornix itself. The perivascular spaces of the lamina involuta were widely dilated. There was a well-defined necrotic area in the *lateral curvature of each fornix*, and on the left side there was in addition one smaller in size, situated at some little distance from the mesial surface of the cerebral cortex. The white matter in the immediate vicinity of those necrotic areas was

markedly œdematous. Groups of the cells in the pyramidal layer of the cornu Ammonis were necrotic, but the lesion here was less marked than in the fornix. It is worthy of note that in spite of the highly œdematous condition of the nervous tissues the altered nerve-cells still stained very distinctly and showed the reaction characteristic of coagulation-necrosis to the iron hæmatoxylin stain. Another important point brought out by examining the sections in series was that necrotic foci appeared, and, after a varying number of sections, disappeared, to be replaced by others, thus showing that they were multiple.

FIG. 5.—At the upper and left margins of the figure many deeply-stained necrotic cells are seen in the granular layer of the cornu Ammonis. Immediately inferior to this is a broad clear band of œdema: there is also generalised perivascular œdema.

RABBIT H.

Intravenous injection of 10 per cent. argyrol.

There was a high degree of œdema around the vessels of the lamina involuta, and in both white and grey matter of the cornu Ammonis, with considerable destruction of the tissue. The white matter generally throughout the brain showed the same change, and the vessels of the choroid plexus were markedly congested, while the plexus itself showed an acute cystic condition. Coagulation necrosis was present in the *fornix of both sides*. There was a very definite necrotic area in the lower portion where it becomes continuous with the temporal lobe, at the lateral portion, and in the pyramidal layer of the cornu Ammonis. In the necrotic areas the nerve-cell body was swollen and hyperchromatic. The nucleus was stained very deeply and homogeneously, and the tissue in the immediate neighbourhood of the necrotic cells was intensely œdematous,

SUMMARY OF EXPERIMENTS.

RABBIT A.—Indian Ink. Coagulation necrosis of each fornix at the lateral curvature: also a small necrotic area in the upper and inner segment of the right fornix: early necrosis of the cells in the pyramidal layer of the cornu Ammonis.

RABBIT B.—Indian Ink. On the left side almost the entire fornix necrotic: on the right side, three necrotic areas: (a) superior aspect; (b) towards the lower extremity; and (c) in the most inferior portion. Here the necrotic change is continued into the grey matter of the temporal lobe. In the white matter a considerable degree of œdema.

RABBIT C.—Indian Ink. Necrosis of the fornix at the lateral curvature on both sides. Granular layer of cornu Ammonis normal: many cells in the pyramidal layer necrotic. Considerable degree of œdema round vessels of lamina involuta, fornix, cornu Ammonis, and in the deeper layers of cortex.

RABBIT D.—Indian Ink. Left sympathetic divided: coagulation necrosis at lateral curvature of each fornix: at upper part of fornix near the cortex: pyramidal layer of cornu Ammonis necrotic: œdema round each necrotic cell.

RABBIT E.—Indian Ink. Coagulation necrosis at the lateral angle of each fornix: necrosis of pyramidal layer of cornu Ammonis: granular layer normal.

RABBIT F.—Argyrol 1 per cent. Left sympathetic divided. Almost entire left fornix necrotic. Necrosis of granular and pyramidal layers of cornu Ammonis: in the right fornix necrosis at lateral angle: necrosis slight in cornu Ammonis. A small degree of œdema round each necrotic cell.

RABBIT G.—Argyrol 10 per cent. A high degree of œdema in cortex and lamina involuta: lateral curvature of each fornix necrotic, another (small) necrotic area, on left side, of upper and mesial portion: groups of necrotic cells in the pyramidal layer of cornu Ammonis.

RABBIT H.—Argyrol 10 per cent. A high degree of œdema round the vessels of the lamina involuta, and in white and grey matter of cornu Ammonis: subcortical white matter œdematous: coagulation necrosis of both fornices at lateral angle, and inferiorly: pyramidal layer of cornu Ammonis necrotic. In the choroid plexus the vessels were congested, and the plexus itself was cystic.

DISCUSSION.

With regard to the mechanism of production of the lesions, it is evident that they show the characteristic morphology associated with arterial blockage. The nature and site of the lesion are similar whether Indian ink granules or argyrol be employed as the causative agent, and the extent of the damage varies according as the precapillary arterioles or the capillary

branches are obstructed. The size of the necrotic lesion also depends naturally upon the number of vessels involved in the ischæmic process. But while local necrosis is an effect common to injection of granules and to a much more particulate substance such as argyrol, there are also certain differences which seem to be associated with the degree to which the whole of the local vascular mechanism is disturbed. For example, œdema is not such a marked feature after injection of granules as after the injection of argyrol. In the case of the former, the œdema is not great and is purely local, but with the use of argyrol the œdema rises progressively with the increase in strength of the solution, and its effects are seen not only in the fornix and cornu Ammonis, but in the grey cortex and subcortical white matter also.

This gradual rise in the degree and extent of the œdema, which reaches its maximum with the injection of a 10 per cent. solution of argyrol, suggests involvement of the controlling nervous mechanism of the vessels, and that the degree to which this reacts depends upon the strength of the exciting agent. The first effect of the argyrol on the vessels must be one of stimulation, causing contraction and narrowing of the vessel lumen with local ischæmia; this is shown by the necrosis. But with the rise in potency of the exciting agent, or its more prolonged action, paralysis ensues with increased permeability of the vessel wall. Serum now readily escapes from the vessels, and by its pressure destroys the tissues in the neighbourhood of the ischæmic focus, thus extending and intensifying the effect of the necrosis produced by the initial asphyxia.

There are special points of interest to be noted in connexion with the necrotic lesions, viz.:—(1) The lateral aspect of the fornix is a very common situation, and the lesion is often bilateral. (2) The size of the lesion varies from point to point: it may even disappear at one level and reappear at another, being thus multiple. (3) Involvement of the entire fornix is due in all probability to the fusion of several discrete necrotic areas, as if the spastic process at first affected isolated points in the neurovascular mechanism, and then as the vascular spasm spread the necrotic areas became confluent, thus embracing the entire cell-layer.

In the neighbourhood of the damaged areas, the vessels in one of the experiments (argyrol) were observed to be thickened and hyaline, and there was a local reaction of the astrocytes (see fig. 6).

The lesions found to occur after the injection of Indian ink granules and argyrol are similar to those observed after the injection of organisms¹, and to those seen in certain clinical conditions.

They have been described by von Braunmühl in cases of acute excitement, and in puerperal insanity; and by Spatz and Neubürger in whooping-cough. These authors are of opinion that the lesion is due to

ischæmia, which commences with an angiospasm of the cerebral vessels, followed by paralysis, stasis, and congestion. The result is an asphyxiation of the nerve-cells, while the least injured glia-cells proliferate. Rickers⁴, discussing the pathogenesis of epilepsy, adopts a similar view, and points out that in the absence of any local organic cause the lesion must be functional in nature. The first stage in the process is vasomotor constriction and ischæmia, followed by paralysis, dilatation, and stasis. The nerve-cells then undergo the changes characteristic of coagulation-necrosis.

FIG. 6.—Note the acute hyaline thickening of the subcortical capillaries, and the astrocytic reaction. Inferiorly, a portion of the fornix is shown in which the cells are necrotic, and the adjoining white matter destroyed by acute oedema.

Finally it should be noted that the hyaline thickening of the smaller vessels of the brain following the injection of argyrol is an acute change, and has not been observed after the injection of either organisms or Indian ink.

CONCLUSIONS.

The lesion can be caused by mechanical blockage or neuromuscular spasm. It is always of the same nature, i.e., ischæmic and necrotic: oedema is a constant feature, but varies in degree with the potency of the causative agent. When the oedema is excessive, it is a sign of acute disturbance and,

added to coagulation-necrosis, materially augments the damage to the nerve-cell laminæ. It may even include the adjacent white matter.

The necrosis may be accompanied by local hyaline change in the vessel wall, and by a reaction on the part of the fibrillary neuroglia (see fig. 6).

REFERENCES.

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² SPIELMEYER, W., 'Die Pathogenese des epileptischen Krampfes,' *Zeits. f. d. Neurol. u. Psychiat.*, 1927, cix, 501.

² USHIMURA, quoted by Spielmeyer.

⁴ RICKERS, quoted by Spielmeyer.