MICROGLIA : AN EXPERIMENTAL STUDY IN RABBITS AFTER INTRACEREBRAL INJECTION OF BLOOD.

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INTRODUCTION.

TILL within recent years knowledge of the interstitial cells in the central nervous system has advanced but slowly. Cajal divided the elements of the nervous system into three groups—nerve-cells, neuroglia, and adendritic corpuscles ; his gold-sublimate method of impregnation¹ resulted in the demonstration of an almost complete picture of neuroglia and of the morphology of the protoplasmic and fibrous neuroglia. In 1919 Del Rio-Hortega² first published his preliminary work upon adendritic corpuscles, in which he described the processes of these cells as shown after impregnation with silver ; with the silver method he was able to distinguish two types of cells, the oligodendroglia and the microglia. Further, he² brought forward considerable evidence for the mesodermal origin of the microglial cells and for their phagocytic properties. Though many workers have since agreed that compound granular corpuscles probably originate from microglial cells, it was for Pruijs³ to suggest that oligodendroglia may function as compound granular corpuscles. Still more recently Struwe⁴ has drawn attention to the presence of fat within astrocytes in pathological conditions. The function of supporting the brain structure also has been attributed to microglial cells by Schaffer⁵, but Reynolds and Slater⁶ believe they are chiefly concerned in the phagocytosis of particles of cerebral tissue. Jimenez di Asua⁴ was the first to bring forward evidence that microglial cells are part of the reticulo-endothelial system, though Raminex Corria⁷ had previously suggested such a relationship ; the former worker, on comparing the microscopic appearance of the cells in the spleen and kidney (after silver impregnation) with microglial cells found considerable similarity between macrophage cells and microglial cells ; failure, however, to stain the latter after intravenous injection of collargol and iron saccharate was explained on the basis that the cerebral vessels were impermeable to such substances.

The following work was undertaken to determine the rôle played by microglia and astrocytes in the removal of red blood corpuscles from brain tissue, as no literature upon the subject could be traced.

TECHNIQUE.

One half cubic centimetre of blood withdrawn from a rabbit's marginal ear vein was injected into the brain substance of the same rabbit under ether anaesthesia. In all, fifteen rabbits were so treated ; these were killed with chloroform or ether at the following intervals after injection, viz., 1, 2, 3, 4, 5, 6, 7, 8, 12, 15 or 22 days. Four other rabbits were killed on the third, fifth, eighth and fifteenth days. Immediately the animal was dead the head was

perfused with normal saline through either both carotids or the arch of the aorta. After thorough perfusion formol bromide was allowed to run through for ten to twenty minutes to ensure fixation. The brain was then removed, cut into thin slabs and placed in a sufficiency of formol bromide in an incubator at 37° C. for 48 hours. To obtain good sections of microglial cells an acid solution of formol bromide was found best, being made up as follows :—

Ammonium bromide		2 grm.
Formaldehyde (35—40 per cent.)	..	15 c.c.
Doubly distilled water		85 c.c.

Immediately previous to their being cut on a freezing microtome, the appropriate slabs of brain tissue were placed in fresh formol bromide for ten minutes in a paraffin oven at 50°C. Sections were then cut at 15 to 20 micra and placed in 25 c.c. of doubly distilled water, containing four drops of strong ammonia, for twenty minutes. After a very rapid wash in doubly distilled water the sections were placed in the silver bath in the incubator at 37° C. till they turned the faintest brown. The silver bath was made as follows : to 5 c.c. of a 10 per cent. solution of silver nitrate were added 15 c.c. of a 5 per cent. solution of pure sodium carbonate ; the precipitate was then re-dissolved by the addition of a few drops of strong ammonia and the resultant clear solution diluted to 75 c.c. with doubly distilled water. It is essential not to add too much ammonia ; the solution should be practically odourless. After the silver bath, sections were very rapidly washed in doubly distilled water and then placed in 10 per cent. formalin. They were then toned in gold chloride until blue-grey, fixed in 5 per cent. sodium hyposulphite, cleared and mounted in Canada balsam.

Many modifications of this method were tried, but this gave the most satisfactory and uniform results.

It was found that pure chemicals, doubly distilled water and chemically clean glassware were essential in order to obtain constant results. Several sections after impregnation were stained for fat with Scharlach R. Fresh sections were also impregnated by Cajal's gold sublimate method for protoplasmic astrocytes, while fourteen days later further sections were impregnated by the same method for fibrillary astrocytes.

MICROGLIAL AND ASTROCYTIC REACTION.

The normal microglial cell has an angulated or rod-shaped nucleus with very little protoplasm surrounding it ; passing out from the cell-body are fine processes which frequently branch and radiate in all directions. Microglial cells arrange themselves in proximity to the cells of the cerebral nuclei ; others lie along blood vessels, still others in the midst of the cerebral tissue or along the tracts of the brain substance. These cells with their processes are made visible only with special silver impregnation methods,

At the end of the first 24 hours following the injection of blood definite changes in the character and distribution of microglial cells were observed. In the vicinity of the mass of injected blood their number was in excess of normal. The nuclei were altered to an oval shape and were larger than in normal microglia. In no section had the nucleus become spherical. The protoplasm surrounding the nucleus was increased in amount and in more than one cell evidence of vacuolation was observed. The processes were shorter and fatter (Fig. 1), and a careful study of them furnished interesting facts. The first

FIG. 1.

apparent alteration in the process was a swelling of its substance, so that the usual fine wire appearance was replaced by that of a ribbon; but passing out from the edges were small irregular processes giving a ragged outline to each process. The shorter the processes the more regular was their outline. The substance of each process at this stage was fairly granular, with an occasional small clear area suggestive of a vacuole. Those cells in closest proximity to the foreign blood were almost globular, without any processes. These changes were assumed to indicate a withdrawal of the processes from the surrounding tissue towards the cell.

In two sections interesting phenomena were seen. At one place a few of the red corpuscles had become separated from the main mass of the blood and were lying loose in the cerebral tissue (Fig. 2). In this region microglial cells were seen with swollen processes; lying between the processes individual blood

FIG. 2.

corpuscles were found. The appearance suggested that the microglial cell was about to engulf the blood corpuscles. Evidence of such a possible function of the microglia was only discovered in one instance after a prolonged search ; in this case a red blood corpuscle was lying within the substance of a microglial cell (Fig. 3).

FIG. 3.

In sections stained with Scharlach R the microglial cells surrounding the mass of blood were found to contain multiple fat globules ; whilst those at a distance, showing only some swelling of the processes, were devoid of any fat globules. The origin of the fat will be considered later.

In order to determine how soon changes in the microglial cells might be observed, three rabbits were anæsthetised and their brains incised with a sharp scalpel. These animals were killed 6 hours, 12 hours and 18 hours after the operation. At the end of 6 hours changes in the shape of the microglia along the edge of the wound had taken place : this was quite evident at the end of 12 and still more so at the end of 18 hours. It is therefore apparent that microglia reacts to trauma very rapidly and that microscopic evidence of early changes may be found at the end of 6 hours.

Coinciding with the changes in the microglial cells the astrocytes were also altered. In a zone surrounding the mass of blood, very poor impregnation of the astrocytes was obtained ; the processes and cells appeared to have been completely broken up, leaving a debris of small granules of irregular shape and

size. Outside this zone the astrocytes showed swelling of their processes and their intracellular fibrils were barely distinguishable; the cell body was enlarged and the nucleus ill-defined. Surrounding these two zones which merged into each other the astrocytes appeared quite healthy.

Sections from the rabbits killed on the second and third days after injection of the blood showed a progressive increase in the numbers of microglial cells in the vicinity of the blood (Fig. 4). All forms, up to the swollen microglial

FIG. 4.

cell, were found. The origin of these new cells was sought and the presence of two or three microglial cells near the bifurcation of many capillary vessels appeared to offer a solution. At no place was the actual migration of a cell from within the blood vessel or from its lining to be seen. In the many sections examined only one mitotic figure was found; no amitosis was observed. It appears therefore that the increase in number of microglial cells is due to their migration from neighbouring blood vessels and not to their multiplication by fission in the immediate vicinity.

Four days after the injection, the microglial cells were still increasing in number. New cells now made their appearance. These cells were large, with an oval pale staining nucleus; they were not numerous and were scattered evenly in the tissue round the foreign blood. A close scrutiny of many sections failed to provide evidence as to their origin. Histologically they appeared to be large macrophage cells, closely resembling those derived from the reticulo-endothelial system. Sections stained with Scharlach R revealed microglial

cells, crowded with fat globules, around the blood. At this stage there appeared little alteration in the character of the injected blood; the red corpuscles still remained intact and stained well with eosin. It seems possible therefore that the microglial cells may have derived their fat globules from damaged brain tissue rather than from breaking-down blood corpuscles.

By the seventh day the maximum increase of microglial cells had taken place, for on the following and succeeding days they were fewer in number. Though the latter had ceased to increase, changes in the astrocytes were observed. On the fifth day following the injection of blood these cells were altered at the periphery of the damaged area. The zone containing swollen astrocytes was still present, but outside it they were dividing, as shown by the presence of several amitotic figures. Further, processes were seen extending from the cells in this region towards the mass of blood. On succeeding days the astrocytes of this zone were increased in number and the processes extending towards the region of the blood were very numerous. By the twelfth day they had walled off the damaged area; the cells involved in this were large, with a fair amount of perinuclear protoplasm and with very thick processes.

Difficulty was encountered when an estimate of the fate of the injected red blood corpuscles was attempted, as it was impossible to be certain that sections from each rabbit were strictly comparable. No apparent diminution of the number of red blood cells was noticed up till the fifth day. From then onwards they were found to be decreasing in numbers. Sections from the rabbit killed on the eighth day showed only a few whole red blood corpuscles, while in sections from the rabbit killed on the twelfth day they were entirely absent. At this stage the region of the wound was occupied by large cells having an oval, deeply staining nucleus; in the protoplasm of these cells fine highly refractile crystals were scattered (Fig. 5). These crystals were yellowish

FIG. 5.

in colour and were similar to those found in nerve cells following intracerebral hæmorrhage. They were, therefore, taken to be end-products of red blood corpuscles. The cells which contained pigment were in many respects similar to globular forms of microglia, as the nuclei were of the same shape, size and staining properties. They differed from the large macrophage cell in that the nucleus was more deeply stained. Along with the appearance of these cells containing crystals new vessel-formation was noticed in the centre of the wound ; at first consisting of only a few capillaries, by the fifteenth day the vessel-formation was more definite and a venous channel with some perivascular fibrous tissue was present.

On the twenty-second day few microglial cells were found in the wound ; round it the microglia was of the normal resting type. The astrocytes in the same region were still large, with thick processes passing down to the central vessel ; the debris of the damaged astrocytes had been removed. In the wound a few cells containing crystals were found, and in sections stained by Scharlach R only a few fat-containing cells were present. The most striking feature of the picture was the excess of fibrillary astrocytes walling off the wound from the surrounding structures (Fig. 6).

FIG. 6.

Throughout this research careful attention was paid to the question of the possibility of the astrocytes acting as removers of effete products. In no section were fat globules observed in any astrocytes ; further, no cells of this type were seen to take any part in repair other than in the walling off of the wound produced by the injected blood.

CONCLUSIONS.

- (1) Microglial cells are the chief elements concerned with the phagocytosis of damaged brain tissue and in part with the removal of red blood corpuscles, lying free in the brain substance.
- (2) Large monuclear cells, probably derived from the reticuloendothelial system, are also concerned with the removal of red blood corpuscles.
- (3) Microglial cells commence to remove products of degeneration within a few hours of wounding.
- (4) Microglial cells may engulf red blood corpuscles in the early stages and contain pigment in the later stages.
- (5) Astrocytes degenerate rapidly and only commence to recover and to wall off the wound at the end of five days.
- (6) Astrocytes do not act as phagocytic agents.
- (7) Red blood corpuscles may remain as such in the cerebral tissue for not longer than twelve days.

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