HEAVY METAL AND RADIOACTIVE LABELING OF CELLS REACTING TO BRAIN TRAUMA IN MICE

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HEAVY METAL AND RADIOACTIVE LABELING OF CELLS REACTING TO BRAIN TRAUMA IN MICE

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INTRODUCTION

Repair of brain injuries has been the subject of neuropathological studies for nearly one hundred years. Gross examination of brains which had previously been injured revealed that scars had formed in the injured areas. The characteristics of the scars which formed in central nervous tissue depended not only on the extent of the injury; the length of time from injury to examination of the area also influenced the appearance of the scars. With time, scar tissue filled in the damaged area and appeared to wall off the area from the rest of the brain. The process of repairing the brain seemed, therefore, to be similar to the repair of injured areas noted in more easily accessible parts of the body. Thus, with the formation of a protective scar, the body could lessen or avoid further damage to the very vital nerve cells. The same mechanism that protected other areas of the body, namely scar formation, could protect the brain from infection or further loss of fluids.

Microscopic examinations of injured central nervous tissue revealed a changing picture within the damaged area too. At a short time after injury, great numbers of cells were to be found both in the wound areas and in the surrounding, seemingly unaffected, areas. During the period when scar formation was seen grossly, cells were noted to fill in and wall off the injured areas. Many of these cells persisted at long time intervals after an injury. Where had these cells come from? Had they been present in the brain prior to the damage and migrated to the injured area? Had precursor cells in the brain given rise to the great number of cells present at short time intervals?

Within the past decade these questions and others have been answered by means of the use of radioisotopes. Several investigators found that white blood cells, one of the body's defense systems, invaded the area surrounding

an injury to central nervous tissue. Great numbers of these cells accumulated in the area for some time after the injury. Many of these cells persisted in and around the wound for a long time. The white blood cells which persisted were the mononuclear leukocytes, the lymphoid cells. These findings suggested that the role the lymphoid cell plays in central nervous tissue injury repair should be further investigated. Clarification of this role was deemed necessary due to the limitations of conventional histological and special neuropathological techniques.

With conventional histological stains, it was determined that there were elements other than neurons comprising central nervous tissue. These other elements, the interstitial cells, were designated as neuroglia [G. neuron, nerve, + glia, glue] . The neuroglial cells were further classes as astrocytes, oligodendroglia and microglia, each having a characteristic nuclear morphology. Interest in these cells was stimulated by the study of brain injury repair, and the cellular processes which lead to repair after injury were studied by many. Early investigators, using conventional histologic stains, noted that in pathological conditions of central nervous tissue the neuroglia reacted by "proliferation;" i.e., there were an increased number of neuroglial nuclei located around the damaged area. Indeed, for many years neuroglial proliferative response was thought to be the primary reaction to central nervous injury (Adams, 1958). However, due to the limited number of mitotic figures seen in such areas, it was postulated that this proliferation took place by amitotic, or direct, division (Penfield, 1932).

With the development of special neuropathologic stains, the cellular morphology of the glia could selectively be studied. The glial cells were stained by the salt solutions of such heavy metals as silver and gold. Ramón y Cajal (1913) demonstrated the astrocytic cell body with gold chloride sublimate. Del Rio-Hortega (1919) described the cellular morphology of oligodendroglia and microglia with silver carbonate. Such staining techniques enabled neuropathologists to investigate the role of the neuroglia in scar formation. The astrocytes, stained with gold, were the neuroglial cells shown to take part in scar formation. Some investigators thought this response

to be a secondary reaction in brain injury repair (Hicks, 1947). Other authors proposed that astrocytic participation in the reparative process was more than a secondary reaction (Penfield and Buckley, 1928), and possibly, along with connective tissue elements, formed the protective scar which was seen grossly.

The major participants of the acute reaction following brain injury were considered to be brain phagocytes. In response to experimental punctures of the brain, these cells accumulated at the injury site and were responsible for ridding the area of necrotic debris. The reaction began shortly after injury and the period of most intense activity was during the first week after injury. The reaction then gradually subsided with some cells persisting for long periods of time after the injury. By means of conventional techniques, the brain phagocytes were variously described as leukocytes, mononuclear leukocytes, histiocytes, macrophages, compound granular corpuscles, gitter cells and microglia. Using the neuropathologic silver technique, some of the phagocytes were classified as microglia which, with time, were considered to form gitter cells (del Rio-Hortega, 1932). Thus, two neuroglial cell types were implicated in repair of brain injuries. The microglia phagocytized necrotic debris and astrocytes formed, or helped form, a protective scar.

The origin of the brain phagocytes still remained in question, however. Early investigators postulated a source extrinsic to the brain. Somewhat later investigators held that the phagocytes were derived from cells resident in the brain. During the past decade the issue has been resolved by the use of radioisotopes. Tritiated thymidine radioautography permitted the differentiation of cells undergoing premitotic synthesis of DNA from cells which were in a non-synthetic stage. With this technique, Smart and Leblond (1961) demonstrated a slow rate of DNA synthesis in the neuroglia of the normal adult rat brain. These authors classified the neuroglia undergoing DNA synthesis as astrocytes, oligodendroglia, microglia and spongioblasts on the basis of their nuclear morphology. Labeling hemopoietic tissues with tritiated thymidine prior to stab injury of the spinal cord, Adrian and Walker (1962) demonstrated that cells responding to the injury were mononuclear leukocytes. By means of the same labeling technique, Konigsmark and Sidman (1963) were able to show that at

least two-thirds of the phagocytes reacting to stab injury of the brain came from mononuclear leukocytes. Walker (1963) demonstrated in spinal cord that following tritiated thymidine injection, there was an increase with time of radioactive nuclei at injury sites. There was, however, no corresponding decrease in the grain count. This indicated an infiltration of cells into the area rather than reutilization of label already present at the site. Reutilization of tritiated thymidine in previous work had led to much weaker labeling of the new cells than was seen in the original population (Walker, 1963; Walker and Goldman, 1963). Also, Walker (1963) noted that the nuclear morphology of the radioactive cells was indistinguishable from glial nuclear morphology. From his investigations he concluded that in the wound, large lymphoid cells slowly hypertrophied into cells with nuclei resembling large oligondendroglia and astrocytes. Furthermore, astrocytes were known to aggregate around areas of central nervous tissue injury. This seemed to correspond to the layering of hypertrophied, elongated lymphocytes seen in response to skin homografts (Walker and Goldman, 1963) and tumor homografts (Walker, Yates and Duncan, 1964; 1965). The characteristics exhibited by lymphocytes, namely nuclear hypertrophy in central nervous tissue injury, and elongation and hypertrophy of cytoplasm in allograft beds, led to a further consideration. On the basis of nuclear morphology, lymphocytes were shown to hypertrophy and to resemble large oligondendroglia and astrocytes. Did these same hypertrophied lymphocytes exhibit a cytoplasmic affinity for the heavy metal glial stains? Were they, in fact, responsible for the astrocytic aggregation seen in repair of brain injuries?

The following experiment is concerned with the role lymphocytes play in repair of brain injuries and the morphological relationship between lymphocytes and neuroglia, primarily, the astrocytes.

The experimental design used to obtain information about these roles was simple stab wound in the cerebrum or allografts of spontaneous mammary tumor to the cerebrum in conjunction with tritiated thymidine injections at various time intervals. Portions of each injury or transplanted area would subsequently be processed either by radioautography and nuclear staining or heavy metal staining for neuroglial cellular morphology. This design was chosen

on the basis of the considerations discussed previously. Stab injury was shown by heavy metal stains to produce aggregations of astrocytes surrounding the injured area. Using radioautographic techniques in combination with nuclear stains, it has been postulated that lymphocytes slowly hypertrophy and, with time, come to resemble large oligodendroglia and astrocytes. In addition, the layering of hypertrophied lymphocytes seen in allograft beds in areas outside the cerebrum resembled the "felting" of astrocytes in brain repair described by Linell (1929). Spontaneous mammary tumor was used because allografts to areas outside the central nervous system evoked a lymphocytic response. This appeared to be the same reaction which had previously been described in homologous transplantation of spontaneous mammary tumor to the cerebrum (Murphy and Sturm, 1923). Although Smith and Walker (1967) were not able in tumor allografts to cerebrum to demonstrate cytoplasmic basophilia in the responding labeled cells by the usual histologic techniques, the features of allograft rejection seen in the investigation were similar to those seen in radioautographic experiments on grafting skin (Walker and Goldman, 1963) and grafting tumor subcutaneously (Walker et al., 1965). Furthermore, by injecting tritiated thymidine into juvenile mice, the glial cell population was labeled. Two months later, there was no decrease in grain count of the labeled cell types. This indicated that these cells form a fairly stable population and do not undergo repeated divisions. This population produced no detectable proliferation or migration in response to tumor allografts (Smith and Walker, 1967). Infiltrating lymphocytes from the blood were presumed to be responsible for the tumor allograft rejection. In electron microscopic examination of tumor homografts outside the central nervous system, the lymphocytes were seen to have elongated cytoplasmic expansions. The same mechanism could be expected in lymphocytes responding to tumor allograft rejection in the cerebrum with its very dense neuropil. Since infiltrating lymphocytes probably hypertrophy, their elongated cytoplasmic expansions might stain with the heavy metal stains, thus making their cellular morphology indistinguishable from the reactive astrocytes seen in neuropathological conditions. This experiment is concerned

with the role of lymphocytes in brain injury repair. The use of heavy metal glial stains in conjunction with radioautography should further define this role and the morphological relationship between reactive astrocytes and lymphocytes. If the two populations are indistinguishable on the basis of staining characteristics, a more plausible solution to the problem of the increased numbers of astrocytes noted in repair of brain injury would thus be presented, without resorting to the theory of amitotic division.

LITERATURE REVIEW

Reaction to injury

Neuroglial hyperplasia has long been considered the most characteristic response of nervous tissue to a great variety of injurious stimuli (Adams, 1959). The three types of neuroglia have all been implicated as responsible for the increased numbers of cells noted in and around areas of central nervous tissue damage.

Other cell types have also been commonly described as responding to central nervous tissue damage. Among these are fibroblasts, connective tissue elements, adventitial cells and vascular cells.

Tschistowitsch (1898) claimed that the glia cells swelled up and divided actively after the introduction of sterile, small celloidin tubes into the brains of mice. He felt that there was no evidence from his experimentation that the glia "added to the host of phagocytic cells which digest and remove the destroyed tissue," but he did not conceive it impossible. The phagocytic cells were thought to be derived from connective tissue cells of the pia and from the adventitia of the penetrating vessels, partly from the endothelial lining of blood vessels and leukocytes. Repair of the lost parts was considered to be made almost entirely by connective tissue. Nor did the glia take part in the formation of the cicatrix which replaced the destroyed elements. Rather, glial cells were reported to be merely concerned with the elaboration of a secondary zone of sclerosis around the scar.

Essick (1919), studying experimental puncture wounds in dogs, reported similar results. On gross examination two months after injury, there was solid replacement of destroyed nervous tissue by connective tissue. Microscopic examination of damaged areas of 2, 3, and 4 months duration showed that leukocytes had disappeared from the area, but phagocytic cells were still present in considerable numbers. Connective tissue had filled in the defect,

and between this replacement tissue and normal nervous tissue was a zone of reactive cells, which Essick termed a zone of gliosis. Healing was reported to be by the ingrowth of connective tissue because no glia cells could be found within the scar tissue itself.

Macklin and Macklin (1920) lesioned rat brains with hot and cold needles, then injected dye into the animals two days later to study vital staining of macrophages responding to the injury. Fully developed macrophages were found in the lesioned area from time intervals of 2 to 74 days after dye injection. The period of most intense activity was from 2 to 6 days after injection when vitally stained mononuclear cells containing much lipoid material were found in the inflamed region. However, when these cells were compared with macrophages of wounds of other parts of the body, they noted that the cells took up a lesser amount of stain and the duration of the staining period was shorter. The macrophages were thought to multiply by mitosis at the injury site and no transitional forms from lymphocytes were found. It was believed that the vitally stained cells were recruited in part from neuroglia, because hypertrophied neuroglial cells containing dye granules and other material were found in the area of inflammation. (This observation was made with hematoxylin and eosin stain.) In the healing process, macrophages were incorporated into the scar which had been formed by fibroblasts. There was no evidence in the investigation that the macrophages had actually proliferated to form the scar. When foreign tissue such as muscle, liver or spleen was introduced into the lesion in an effort to attract macrophages similar to those found in other areas of the body, negative results were obtained. The responding cells did not show transitional forms from lymphocytes, there were no greater numbers of cells, there was no increase in dye-uptake by the cells, nor were there more mitotic figures than observed in hot or cold needle lesions.

Penfield (1925) studied microglia and the process of phagocytosis in gliomas. He concluded that the microglial activity surrounding a glioma was similar to that described by del Rio-Hortega in other destructive processes of the central nervous system. They were seen by the author to act as scavengers of products of degeneration and cerebral destruction. In an area

surrounding a tumor, microglia took on ameboid forms; in areas of cerebral softening, more spherical and reticulated forms (Gitterzellen) were found. Regions of very active mitotic division were seen. The author stated that the function of microglia was to transport substances to outer surfaces of blood vessels, where they lost their granular and vacuolar appearance, decreased in size, and finally left the vicinity in characteristic form "for renewed phagocytosis." Penfield concluded that the presence of scavenger cells about blood vessels was indicative of the transfer and delivery of ingested substances rather than new formation of these cells from fibroblasts.

Bender (1925a, 1925b) noted "moderate to marked gliosis" in dog brains which had been experimentally punctured and injected with various extracts of nervous tissue. The observations were made in wounds of 4 weeks duration. The effect on the blood vessels was the same in all the experimental animals. Those vessels in and adjacent to the lesion were prominent due to a slight increase of endothelial and fibroblastic elements. Phagocytic reactions varied directly with the amount of hemorrhage and tissue destruction and inversely with the amount of encapsulation. Formation of a fibrous capsule was seen in three of five experimental groups: the injection of cerebrin, cholesterol and solid residue. Gliosis of the spider cell, fibril-forming type and cytoplasmic type, was noted adjacent to the lesion in all groups and ranged from "moderate to marked" reaction.

In a study of human puncture wounds, Wilson (1926) found connective tissue cicatrices in injuries 7 and $11\frac{1}{2}$ months old. Punctures made with a needle 1 mm in diameter showed no "gliosis" per se. In none of the needle tracks did the neuroglia appear proliferated at the edge, forming a limiting membrane; rather, the connective tissue elements seemed to blend with the neuroglial fibers at the edge. The reaction appeared more prominent in the gray matter than in white, but the author concluded that neuroglial elements played only a small part in repair of the damaged area.

Formation of a cicatrix in rabbit brain experimentally injured with a blunt needle was studied by del Rio-Hortega and Penfield in 1927. They felt that repair of lesions could be divided into 3 stages. The first stage was thought

to be characterized by microglial phagocytic activity which continued for a long time. Following this, the astrocytes were believed to become swollen and to undergo degeneration by "clasmatodendrosis." This activity was thought to set in motion the third stage which the authors described as being characterized by amitotic division of astrocytes. The cells produced by this hyperplasia were reported to become fibrous and also to arrange themselves in a radial manner around the wound. Astrocytes were stained with Cajal's gold chloride sublimate stain. Hortega's silver carbonate stain was used to demonstrate microglia. These cells were described as passing through transitional forms to become mature macrophages, compound granular corpuscles. At the center of the tracks, a strong connective tissue core was noted.

Because of the findings that occasional open tracks were found, without a connective tissue core and with little or no gliosis in the surrounding area, Penfield and Buckley (1928) injured dog brains with blunt and open needles. When blunt needles were used, all the injured tissue remained behind. This resulted in a closed track with a core of connective tissue which was firmly attached to the dura. The track was surrounded by a moderate gliosis in the cortical gray matter, while gliosis did not occur or there was even a decrease in astrocytes of the white matter. On the other hand, when punctures were made with a cannula of the same diameter as the blunt brain needle used, a gaping track resulted. This defect contained little or no connective tissue. It was surrounded by a moderate gliosis in the cortical gray matter and no gliosis or even a decrease in astrocytes was noted in the white matter. The authors contrasted the wounds made by both types of needles. About closed tracks, the astrocytes sent in their large expansions concentrically; about open tracks, these expansions were tangential to the canal. In general, the presence of damaged brain tissue in a wound produced a contracting cicatrix, gliosis in the contiguous areas and cerebral distortion. Clean removal of cerebral tissue resulted in little, if any, cicatrization, moderate gliosis in the gray matter and none in the white matter, slight superficial adhesions, and practically no distortion of the brain.

Globus (1928) reported that in chronic vascular disease of the brain, the changes in the astrocytes were progressive, with generalized hyperplasia, hypertrophy and transformation of the protoplasmic type into the fibroblastic variety. Oligodendrocyte changes in the investigation were mainly expressed in generalized hyperplasia and mild hypertrophy. The microglia were described as undergoing regressive changes to the final formation of compound granular corpuscles, gitter cells.

Cone (1928) concluded that there was a marked histologic difference in the acute responses which neuroglia and microglia underwent in central nervous damage. Astrocytes and oligodendroglia reacted in acute disease of the central nervous system by degenerating as did the nerve cells. Microglia responded in acute destructive lesions of the central nervous system by forming rod cells and then compound granular corpuscles. The author thought that metamorphosis from one cell group to another did not occur in acute changes. This study was done with the special glial stains.

Ferraro and Davidoff (1928) studied the reaction to removal of a portion of the cerebral cortex by Hortega's silver carbonate technique. They suggested that the oligodendroglia reacted by "acute swelling, atrophic degeneration, hypertrophy, hyperplasia and occasionally by the fusion of several cells into clusters." It was believed that oligodendroglia transformed into compound granular corpuscles.

In 1929, Linell investigated the neuroglial changes following cerebral trauma. In this experiment dog brains were injured with an open cannula and the neuroglial reaction was studied over a 2-month period by neuroglial stains. This reaction was reported to be a dual response, a microglial reaction and a macroglial reaction. The microglial reaction consisted of the transformation of microglia into compound granular corpuscles which in their fully developed condition, were large, round, probably ameboid cells, heavily loaded with lipoid material. This change took place rapidly, being well developed by 3 days after injury and reaching its maximum at 6 days after injury. Linell commented to the probable function of microglia and compound granular corpuscles: to phagocytize damaged nervous tissue and transfer of this degenerated tissue to the

blood stream for disposal. The macroglial reaction was characterized by later onset. Slight hypertrophy of astrocytes was reported in the wound area by 3 days, more marked hypertrophy surrounding the area of microglial reaction at 6 days after injury. At 14 days there was marked hyperplasia and hypertrophy of astrocytes reported by the author. He reported a definite orientation of strong astrocytic processes in a direction at right angles to the wound margin at this time. These processes reached the edge of the wound track. At 3 weeks, the astrocyte reaction was described as reaching its maximum. Some "felting" of astrocytic processes in the long axis of the wound to form scar tissue comprised of glial fibers was noted at 4 weeks. By 2 months, "the glial feltwork had thickened considerably and few cell bodies were observed in the glial 'mat'." The author considered the purpose of the reaction to be the formation of a protective scar. "The scar of a healed wound of the brain is the terminal result of the microglial reaction."

Russell (1929) studied experimental cerebral puncture reaction with trypan blue and the silver carbonate stain for microglia. Rabbits were used due to the ease with which microglia were demonstrated in this species by the silver carbonate stain. Intravital staining of the microglia was observed while there was "no evidence of the formation of Gitterzellen from neuroglia or that the neuroglia was able to take a vital stain." The ability of microglia to phagocytize dye granules led to the conclusion that microglia were a part of the reticulo-endothelial system.

Wells and Carmichael (1930) studied microglia in tissue culture. The silver carbonate method for staining the microglia of the brain also was effective in demonstrating cells present in cultures of tissue such as peritoneum and embryonic limb buds. These authors concluded from their in vitro experiments that microglia were of mesoblastic origin. This was in agreement with the conclusion of Hortega (1919) that microglia were a mesodermal element.

Rand and Courville (1932a, 1932b) described the histologic changes in the brain in cases of fatal injury to the head. Microglia, oligodendroglia and astrocytes were all considered by these investigators to undergo amitotic division. Mitosis was found only in "fully developed compound granular corpuscles." Microglia

were thought to react to local destruction and disintegration of tissue; oligodendroglia altered in response to general or widespread processes, particularly edema; and astrocytes reacted to local destruction and disintegration of tissue. The authors believed that gliosis only occurred as a result of tissue destruction arising regionally; generalized gliosis following head injury was thought not to occur. Microglia transformed to fully developed phagocytes, compound granular corpuscles. Oligodendroglia became enlarged and vacuolated almost immediately after injury. The authors claimed that astrocytes beyond the zone of necrosis were seen to actively proliferate. In the formation of glial scars, the astrocytes were considered to compensate for loss of tissue and to fill up the defect. At the margins of lacerations and in severely contused areas, microglia, oligodendroglia and astrocytes were thought to undergo destruction along with nerve cells.

Greenfield (1932), in describing the cellular response to nervous tissue injury, wrote that a response by mononuclear leukocytes was extremely common. He felt that these cells were not derived from the circulating blood, but rather, were formed through multiplication of the lymphoblasts already present in the perivascular space.

Dunning and Furth (1935), in studying the relationship between microglia, histiocytes and monocytes, demonstrated that histiocytes of chick embryo liver and peritoneal membrane possessed an affinity for the silver carbonate stain which revealed the microglia of the brain.

Hassin (1936) studied the reaction to implantation of a foreign body into central nervous tissue. He introduced cotton plechets into dog brains and followed the ensuing reaction for up to 6 months. The author concluded that there were 2 types of gitter cells: 1) ectodermal, which phagocytized destroyed nerve tissue and were formed by oligodendroglia and 2) microglia cells, which phagocytized non-nervous material. The types of cells which were seen responding to cerebral injury depended on the character of the injury and varied according to the type of foreign body present in the damaged area. Microglia were not the only source of gitter cells. Gitter cells were formed from microglia in acute, severe neuropathologic conditions in which not only nerve tissue, but

also the glia, succumbs. Gliogenous gitter cells occurred only in cases of isolated destruction of nerve fibers, the glia remaining unaffected. "When destruction of nerve tissue is not massive, but selective and limited to single nerve fibers as in multiple sclerosis, the reaction is also selective and is confined to the glia." In areas of secondary and primary degeneration, such as that seen in some cerebral lesions, the neuroglial type of gitter cells were reported. This observation "speaks against a vascular factor in their histogenesis, as a vascular factor produces massive destruction of the nerve tissue, with formation of microglial (mesodermal) types of such cells."

Carmichael, Kernohan, and Adson (1939) wrote that the primary response of nervous tissue to injury was that of phagocytes and the proliferation of fibrous tissue around the damaged area. The glial response was thought to be a secondary reaction.

Baggenstoss, Kernohan, and Drapiewski (1943) studied wounds made with a trochar in human material. The authors described changes seen in wounds of several hours to 7 years duration. In wounds of less than 5 days duration, there was acute swelling of oligodendroglia noted by the authors. Compound granular corpuscles were first seen 3 days after injury. Transitional forms from microglia to compound granular corpuscles were not found. The numbers of compound granular corpuscles were seen to increase through the second week, and many persisted in the area through 6 months duration. Hypertrophied astrocytes were noted at 4 days and persisted throughout 7 years. Scavenger cells appeared to be derived largely from the endothelial cells and adventitia of larger vessels as well as from blood mononuclear cells. In wounds of 8 to 10 days, significant numbers of fibroblasts and collagen fibrils were first described. Lymphocytes were seen in perivascular spaces in increased numbers; numerous transitional forms between lymphocytes and fully developed compound granular corpuscles were described by the authors. By 3 weeks, 3 zones were seen: a central zone of necrosis, partly filled with compound granular corpuscles; a capillary zone with fibroblastic proliferation and numerous lymphocytes; and a peripheral zone of hypertrophied astrocytes. An increased number of astrocytes over the previous period was not noted, nor did they appear to have a particular

arrangement or orientation to the wound as had been previously reported by Penfield et al. (1928) and Linell (1929). There was an increase in the number of astrocytes in the cortex around a wound of over 6 months duration. At 7 years, the wound center was loosely filled with collagen fibers. No phagocytes or lymphocytes were seen, the capillary zone had disappeared, however, the astrocytes persisted. The authors concluded that astrocytes play a minor role in the reparative processes.

Dougherty (1944) concluded that the first cells reacting to experimental brain wounds in the adult animal were lymphocytes which transformed to macrophages. Compound granular corpuscles arose from microglia and in imprint preparations were typical histiocytes which could not be differentiated from lymphogenous macrophages.

Dublin (1945) studied the histogenesis of compound granular corpuscles in response to heat cautery in the brain of rabbits. He concluded from his experiments that compound granular corpuscles appeared to arise principally by proliferation and differentiation of the adventitia of blood vessels, and less frequently from vascular endothelium. Microglia commonly showed some degree of swelling and loss of processes, but transformation to mature compound granular corpuscles were only infrequently seen.

In 1947, Silver and Walker studied thermal lesions of the cerebral cortex. Five days later with the use of colchicine, many mitotic figures were noted in cells associated with capillaries. They thought it possible that perivascular cells were the origin of compound granular corpuscles. There was no evidence noted of microglial proliferation over a 6 month period following injury.

Hicks (1947) studied experimental wounds of mouse brains with blunt, or occluded needles, open needles and punctures with an open needle through which was injected foreign material: bone particle suspension, various extracts of nervous tissue, and a suspension of avian tubercle bacilli. The gold chloride sublimate stain was not used in this study to demonstrate gliosis, defined by Hicks as a proliferation of fibrillary astrocytes and a laying down of their fibrils. In all experimental groups except that of the longest time interval of puncture with the introduction of avian tubercle bacilli, the results were the same. After

the first 48 hours, mononuclear phagocytes accumulated at the track margin and ingested red blood cells and cellular debris. This reaction reached its maximum by the end of the first week. Some phagocytes with debris persisted in the wound for as long as 270 days. Fibrous tissue proliferated along with the acute phagocytic response and there was conspicuous fibroblastic response from the injured meninges. By the second and third week, the track was filled with a loose fibrous core. Throughout the length of the experiments there was almost no proliferation of astrocytes and glial fibrils could be seen only extremely rarely. The narrowing of the track was attributed to the gradual disappearance of phagocytes and the shrinking and disappearance of fibrous tissue. On the other hand, the introduction of avian tubercle bacilli produced a different picture. At 48 days after injury, fibroblasts were in the track. Just at the outer edge of this zone, there were fairly numerous astrocytes with proliferated fibrils arranged in a somewhat radial fashion toward the wound track. The gliosis was attributed to the release of toxins into the tissue causing sublethal injury, but little necrosis. Hicks concluded that direct injury of astrocytes was necessary to stimulate gliosis, and that proliferation of astrocytes was a secondary healing mechanism.

Swank and Hain (1952) reported adventitial proliferation 1 week after injecting emboli into the arterial system of the brain. Experimental transient ischemia was studied in dogs by Lewis and Swank (1953). The authors reported an increased number of perivascular oligodendroglia.

Collias and Manuelidis (1957) described the histopathological changes observed in cat brains with implanted electrodes. In wounds of less than 1 week duration, the primary characteristic of the response was considered to be microglial proliferation and formation of gitter cells. From 1 to 2 weeks, hypertrophy of astrocytes, streaming of fibroblasts, and perivascular "round-cell infiltrates" were seen. At 1 month the microglial reaction had abated, but the astroglial reaction had greatly increased over previous stages. In wounds of 2 to 6 months duration, there was scar tissue formation "with an obvious tendency to encapsulate the implanted electrode." In general, they reported regressive changes in all types of responding cells.

Klatzo, Piraux and Laskowski (1958) described the reaction of central nervous tissue to edema caused by low temperature injury. Endothelial proliferation followed by an intense astrocytic gliosis was observed in these experiments. Studying stab wounds with the use of the electron microscope in 1959, Schultz and Pease were unable to identify the responding cells as lymphocytes. Following α-particle irradiation to produce lesions of the cerebral cortex, Maxwell and Kruger (1965, 1966), using the electron microscope, found hypertrophy and hyperplasia of pericytes, which were considered to develop into phagocytes. Vaughn (1965) also reported that phagocytes develop from pericytes and adventitial cells.

Dublin (1967, p. 214-217) discussed the stages of cellular reaction to traumatic intracerebral hemorrhage. The first response, during the first 2 days, was considered to be the infiltration of polymorphonuclear leukocytes. Lymphocytes appeared about 2 to 3 days after damage, which was thought to be followed by the entrance of macrophages into the area. In lesions of large proportions, " cells proliferate in great streams from the vascular adventitia, accompanied by mononuclears from the blood stream." Blood vessels and glia were considered by the author to enter the area more slowly than macrophages. "Especially in lesions which don't reach the cortical surface, healing which is in addition to vascular proliferation is mainly glial." If the cortical surface were broken in injury, the meninges entered the reaction, and connective tissue was extensively deposited.

Fuentes and Marty (1970) described the reaction to electrolytic lesions in the cerebral cortex of the cat by the use of the light and electron microscopes. They reported that at all time intervals after damage, reactive oligodendroglia were seen as hypertrophic and increased in numbers; there was also hyperplasia of astrocytes. The authors concluded that the neuroglial proliferation was produced by amitotic division.

Cavanagh (1970) studied the proliferation of astrocytes around a needle wound in the rat brain. Adult rats received experimental puncture wounds and groups were killed by perfusion at 1, 2, 3, 4 and 6 days after injury. Tissue sections were not stained with silver or gold neuroglial stains. Mitotic activity

was based on the number of mitotic figures seen in white and gray matter, and mitotic figures were classified according to size and phase. "Small" mitotic figures, measuring less than 8μ in diameter, were considered to be macrophages, microglia, oligodendroglia and other cells. "Large" mitotic figures, measuring more than 8μ and usually 12-15μ in diameter, were considered to be astrocytes. Some astrocyte nuclei in metaphase measured as much as 20-25μ in diameter. The author reported dividing astrocytes up to 1200μ from the wound edge, but that the number of "large" mitoses were always maximal between 200-400μ from the wound. The number of mitotic figures seen in immersion-fixed material was reported to be less than half the mean mitotic rate visible in perfusion-fixed material. This suggested that delay in the fixation of brains may have led to earlier reports of amitotic proliferation of astrocytes. When colchicine was injected directly into the brain wound, greater than twice as many colchicine-arrested metaphases were seen than when colchicine was injected intraperitoneally. The author concluded that there is no inherent resistance to colchicine in small and large glial cells of the brain; rather, colchicine given systemically does not have the ability to reach the dividing cells in the brain. There was an exponential decline of mitotic rates/unit area with distance from the wound edge. Away from the immediate vicinity of the injury, peak proliferation of all non-neuronal cells was reported to occur on the second and third day after injury. It was suggested that this may have been a response to the diffusion of materials in the escaped plasma from the region of vasular damage. Mitoses in astrocytes were thought to form up to one-fifth of the total cell divisions, and no evidence of an amitotic process of division could be detected.

Experimental allergic brain inflammation

Good (1950) induced acute non-allergic and allergic inflammations in the rat brain. He reported formation of phagocytes in central nervous tissue from hematogenous lymphocytes. In comparing allergic with non-allergic inflammatory states, it was found that allergic inflammation was more severe and the histological and characteristics differed. Numerous plasma cells, giant cells and eosinophils differentiated the allergic from the non-allergic state. A second challenge in a previously sensitized animal produced a heightened reaction.

Paterson (1960) demonstrated that transfer of allergic encephalomyelitis can be accomplished by injection of lymph node cells obtained from donor rats sensitized to spinal cord into rats pretreated neonatally with normal rat spleen cells. The recipients acquired immunological tolerance to donor cells by pretreatment and, thus, the donor lymph node cells could survive and function longer after transfer.

Studying lesions of experimental allergic encephalomyelitis in rats, Waksman and Adams (1962) observed that the lesions appeared to start with perivenous infiltration by mononuclear cells. These cells invaded the nervous parenchyma and seemed to destroy myelin directly. Plasma cells began to appear in the vascular cuffs, choroid plexus and meninges 2 to 3 days after the lesions first developed.

Levine, Hirano and Zimmerman (1965) studied hyperacute allergic encephalitis in rats with the use of the electron microscope. They described altered vascular permeability and leukocytic invasion of the parenchyma by escape through the walls of venules.

Lampert (1967) investigated "ordinary" and "hyperacute" experimental allergic encephalomyelitis (EAE) of rats with the electron microscope. He described the method of inducing lesions of both types of EAE and the characteristics of the resulting lesions. Early lesions of both ordinary and hyperacute EAE showed perivascular accumulations of mononuclear cells. The lesions appeared to be initiated by a few sensitized mononuclear cells which penetrate the walls of venules and destroyed oligodendroglia and myelin sheaths. Many non-specific mononuclear cells followed, stripped and digested the remnants of partially lysed sheaths and perhaps also the sheaths related to necrotic oligodendroglia.

Cammermeyer (1967) studied microglial cells in diffuse and granulomatous encephalitis in the rabbit. The author concluded from silver impregnated material that the increase in number of microglia is associated with mitotic division of a "juxtavascular histiocyte." There was no evidence suggestive of migratory activity or amitotic division of microglial cells. Transmissible mink encephalopathy was studied by Marsh and Hanson (1969). Intense astrocytosis was demonstrated with Cajal's gold chloride sublimate stain. Duckett and Pearse

(1969) described periventricular astrocytosis and acute necrotizing encephalopathy in rat brains. In brains with foci of necrosis, there was perivascular cuffing. The cells present in the cuffs and in the foci were mononuclear, and were considered to be small lymphocytes. The authors reported the areas adjacent to the ventricular system free from histologically appreciable evidence of inflammatory processes. However, there was marked periventricular gliosis. The "cellular response in the periventricular region was not evident on examination of sections stained with traditional methods, yet it was remarkably evident with the use of histoenzymatic techniques." The authors suggested that some of the cells of the choroid plexus contributed to the cells which became periventricular astrocytes.

Transplantation of tumor in the brain

Following Shirai's report (1921) that grafted heteroplastic tumor grew as well in alien host brain as in homologous host brain, Murphy and Sturm (1923) investigated the brain as a locus for transplantation. These authors found that transplanted mouse tumor grew well in rat, guinea pig, and pigeon brains if the tumor did not come into contact with a ventricle. A graft lying in the ventricle, or even coming into contact with the ventricle, led to a reaction in which the graft remnant was found embedded in a mass of reaction tissue similar to that which appeared "about an heterologous graft in subcutaneous tissue." This rejection phenomenon was characterized by the graft being surrounded by a zone of cells in which the lymphoid variety of cell was dominant. The intensity of the cellular reaction was seen to be roughly proportionate to the degree of relationship existing between the two species concerned in the experiment. The perivascular spaces of the blood vessels in the immediate vicinity of the heterologous transplant showed a pronounced collar of round cells, and the lumina of small vessels were often blocked with lymphocytes. As the tumor grew and reached the ventricle, the choroid plexus became enormously swollen and engorged with lymphocytes. The contact with this collection of lymphocytes, which frequently invaded the tumor first, caused necrosis of the portion of the tumor lying near the ventricle. This was followed by necrosis of the entire tumor.

When adult autologous spleen was introduced into a non-resistant animal in conjunction with heterologous tumor tissue, the tumors failed to grow. In a later study, Medawar (1948) found that homotransplants of the skin survived in the brain, if and only if the transplants failed to become vascularized.

The use of radioautography

Hughes et al. (1958) reported the use of a radioisotope of thymidine, which is a specific precursor of DNA, in an investigation of cellular proliferation in the mouse. They found the specific advantage of tritium for radioautography to be the very high resolution obtained. This was due to the very weak energy and, thus, the short range of its β radiation. "The maximum range in tissue of a tritium β-ray is only 6 μ and half of the β's will travel less than 1 μ." Tritiated thymidine was rapidly absorbed from the blood stream. The authors concluded that all labeling of nuclei occurred during the first hour after injection. Cronkite et al. (1959) investigated the use of tritiated thymidine in the study of DNA synthesis and cell turnover in hemopoietic tissues. They reported a very rapid incorporation of tritiated thymidine into the hemopoietic tissues throughout the body. Cells were present both in the bone marrow and in the peripheral blood which were capable of synthesizing DNA and were thus able to divide at least once again. The effective availability time of tritiated thymidine was believed to be less than 60 minutes, during which time it was either degraded or incorporated into DNA. When thymidine was injected subcutaneously, it remained in the body 30 minutes. The authors reported that in dogs, small lymphocytes with dense nuclei were rarely labeled as long as 36 hours after injection. In human beings only a rare labeled small lymphocyte was found in peripheral blood and then only after several days. However, large and medium-sized lymphocytes appeared promptly. Leblond, Messier and Kopriwa (1959) studied tritiated thymidine as a technique for investigation of renewal of cell populations. They reported the radiation damage to be less than that produced by phosphate-P^{32} and adenine-C^{14}. Because of this, it was possible to use doses of thymidine-H^{3} which allowed cells to be traced over periods of months in the body without apparent damage to these or other cells. Messier,

Leblond and Smart (1958), using tritiated thymidine, found a large number of labeled nuclei in the subependymal region of the lateral ventricle. Within 3 hours of injection, labeled neuroglial cells were found scattered throughout the brain. These cells were located at such a distance from the subependymal layer that the authors considered it unlikely that the labeled cells had migrated such a distance in so short a time. These findings indicated that there was another population of cells able to synthesize DNA in central nervous tissue in spite of the apparent absence of mitotic figures.

Further investigation of the uptake of tritiated thymidine by the neuroglial cells of normal central nervous tissue was reported (Hain, Rieke and Everett, 1960; Messier and Leblond, 1960; Smart, 1960, 1961; Adrian and Walker, 1962; Noetzel, 1962; Altman, 1963; Noetzel and Rox, 1964; Hommes and Leblond, 1967; Dalton, Hommes and Leblond, 1969; and Mori and Leblond, 1969a, 1969b, 1970.)

Smart (1961) reported that although there was mitotic activity within the subependymal layer of the adult rat, very few of these newly-formed cells migrate from the layer. In general, he proposed that most of the cells degenerated within the layer. Smart and Leblond (1961) studied neuroglial DNA synthesis in 3 ages of mice. They found labeled neuroglial cells in the normal brains of 3 gram, 18 gram and 28 gram mice. The cells were classified according to nuclear morphology. These were described as oligodendroglia, astrocytes, small dark and medium dark nucleated cells. The number and distribution of the different types of labeled neuroglial nuclei were then recorded. In both the 3 gram and 18 gram groups, the evolution of labeled nuclei after injection of tritiated thymidine was similar. On the basis of their work, the authors concluded that oligodendroglia and astrocytes develop from spongioblasts, that is, the small dark and medium dark nucleated cells, and that the normal brain neuroglial population turns over slowly with time. Adrian and Walker (1962) used tritiated thymidine to study DNA synthesis in glial cells of the normal adult spinal cord. They found so few labeled cells that the glial cells were not considered to constitute a renewal system, nor did the ependymal cells appear to be a stem cell population for neuroglia in the normal animal. In 1963, Altman used tritiated thymidine to study cell proliferation of rat and cat neuroglia. He found some

labeled neuroglial cells in all parts of the brain, suggesting a low rate of glial proliferation in the normal animal.

Mitotic division of neuroglia in the normal adult rat was studied by Hommes and Leblond (1967). They found labeled neuroglial cells throughout the brain. Labeled mitotic figures were also seen. They concluded from their work that the labeled cells of the brain arose locally and were not of hematogenous origin. The relatively small size of the labeled nuclei suggested that these were mostly oligodendroglia or other spongioblastic precursors of oligodendroglia. The authors combined radioactive labeling with a silver carbonate stain and found some labeled cells with cellular morphology characteristic of microglia. It was suggested that microglial mitoses make up about one-fifth (10-31%) of the dividing cells of the normal brain. Dalton, Hommes and Leblond (1969) used tritiated thymidine to study the correlation of glial proliferation to age in the normal adult mouse. They reported a small degree of glial proliferation in adult mice. They suggested that the glial population turned over slowly with the production of glial cells being balanced by the loss or degeneration of an equal number of glial cells. Continued activity of some of the brain nuclei was postulated to account for the glial proliferation observed throughout life.

Mori and Leblond (1969a, 1969b, 1970) investigated neuroglial proliferation in normal young rats using tritiated thymidine. Electron microscopic examination of the cerebral cortex and the corpus callosum revealed that, under normal conditions, microglia did not undergo mitosis, but astrocytes and some oligodendroglia were able to divide. Some degenerating astrocytes and oligodendroglia were described by the authors. They suggested that mitosis in 60-80 gram rats may be associated with growth and with myelination of brain tissue. The ability of these cells to divide could compensate for cells lost by degeneration and provide replacement cells in a population with a slow rate of turnover.

The major definitive work that indicated mononuclear cells of the blood responded to central nervous tissue injury was done by Adrian and Walker (1962). Tritiated thymidine was injected to label hemopoietic tissue sufficiently in advance of injury such that no isotope would be available for DNA synthesis by cells proliferating in response to the injury. When the injury area was examined, many

labeled nuclei were observed. The radioactive cells were mononuclear and did not resemble any of the unlabeled glial cells in the adjacent tissues except for microglia. Since the responding labeled nuclei could not have arisen from cells resident in the injured tissue, they must have migrated into the tissue. The only large pool of labeled cells close to the injury was the blood. The invading cells were mononuclear and, therefore, were lymphoid cells. Walker (1963) demonstrated that when this same response is evoked and followed over the course of 32 days, the nuclear morphology of the labeled cells became similar to glial cells resident in nervous tissue. Radioactive small, spherical nuclei reached a maximum concentration at 5-9 days after injury and then tended to decrease. Other cells with radioactive, spherical to oval nuclei tended to increase with time and the largest were most plentiful at 32 days after injury. The author suggested that the radioactive cells with irregular nuclei which resembled microglia were initially monocytes. The other lymphoid cells of the blood, the large lymphocytes, were initially present in the injury area. The area these cells occupied at early time intervals "was later occupied by progressively larger and paler radioactive nuclei until, at 32 days, a large number of the radioactive nuclei showed morphology typical of neuroglial cells, especially of the large oligodendroglia and astrocytes." No evidence was seen for migration of radioactive cells from surrounding tissue, so the radioactive cells present at the injury appeared to be the same cells at all time intervals. The author proposed that nuclei of many of the large lymphoid cells at the wound slowly underwent pronounced hypertrophy into cells with nuclei resembling large oligodendroglia and astrocytes.

The technique of labeling hemopoietic tissues prior to injury was subsequently used to determine the origin of reactive cells in stab injury of the brain (Konigsmark and Sidman, 1963; Huntington and Terry, 1966; Roessmann and Friede, 1967, 1968), skin homograft reaction (Walker and Goldman, 1963) and tumor allograft reaction in the mouse brain (Smith and Walker, 1967). Konigsmark and Sidman (1963) reported that about two-thirds of the macrophages appearing about a stab injury to the brain were labeled leukocytes of the blood, and the authors thought that the cells were probably monocytes. It was concluded

that less than 50% of the macrophages appearing in the first 2 days after injury, and less than 30% thereafter, arose endogenously. The investigators injected thymidine-C^{14} after injury and stained sections with Cajal's gold chloride sublimate solution. The sections were then processed for radioautography. It was concluded that labeled cells with large nuclei did not take the stain, and conversely, no astrocytes, as defined by the staining results, were labeled.

Walker and Goldman (1963) demonstrated migration of medium and large lymphoid cells from the blood to the skin homograft and underlying tissue. Some round cells entered the donor skin. Most of the lymphoid cells remained in host tissue in areas underlying the graft. The authors reported that many of these cells hypertrophied and elongated, and their cytoplasm developed strong, ribonuclease-labile, basophilia. The number of lymphoid cells migrating to isografts and autografts was less and the cytoplasmic basophilia was weaker than that seen in homograft reactions. After all types of grafting, large, basophilic fibroblasts proliferated and became plentiful. The authors proposed that mitoses occurred rarely, if at all, in the hypertrophied and elongated lymphoid cell population.

Smith and Walker (1967) employed tritiated thymidine as a means of labeling 2 populations of cells to differentiate their responses. Tumor transplantation was used for immunological stimulation and brain injury. Glial cells in the adult animal were considered to proliferate at a very low rate; in juvenile animals, however, there was thought to be extensive DNA synthesis. These cells were labeled in juvenile animals, and most of them retained their label for a long period of time. Leukocytes, because of their repeated divisions, could be expected to lose their label over a period of time by dilution below radioautographically detectable levels. Tumors were allografted into adult animals with labeled mononuclear cells still present in the blood. An infiltration of these cells occurred in the area surrounding the tumors, which were rejected by 8 days. The authors suggested that some of these labeled cells were monocytes, but many of them were apparently medium-large lymphocytes. Glial cells were labeled in juvenile animals and tumors were allografted when the animals had matured. There was no evidence that astrocytes and oligodendroglia migrated

or proliferated in response to brain injury of immunological stimulation. The authors concluded that the cells infiltrating the cerebral tissue around tumor allografts must have come almost entirely from the blood.

Kosunen, Waksman and Samuelsson (1963) studied experimental allergic encephalitis with tritiated thymidine radioautography. A large portion of the cells of the perivascular infiltrates were found to be labeled by tritiated thymidine. Little label was taken up by the brain cells until the appearance of lesions. The authors concluded that the infiltrating cells were hematogenous and proliferated actively in the lesions. The cells were characterized cytologically as medium or large blood lymphocytes which evolved to become histiocytes during the evolution of the disease.

In a study of cell division occurring in injured spinal cord, Adrian (1968) used tritiated thymidine radioautography to differentiate 2 populations of cells. He concluded that although mononuclear leukocytes did proliferate in and around nervous tissue wounds, a population of cells originally present in the nervous tissue must also have proliferated in response to the injury. Reznikov (1968) reported almost no mitotic figures in glial cells after brain stab injury. He observed that both the intact and injured hemispheres of the brains of experimentally injured mice had a greater number of labeled glial cells than did uninjured animals.

Several investigators have dealt with neuroglial DNA synthesis in response to peripheral or cranial nerve injury (Sjöstrand, 1965a, 1965b, 1966; Watson, 1965; Kreutzberg, 1966; Friede, 1967; Friede and Johnstone, 1967; Olsson and Sjöstrand, 1969; Adrian and Smothermon, 1970).

Sjöstrand (1965a, 1965b, 1966), Kreutzberg (1966) and Adrian and Smothermon (1970) all demonstrated differences between the nuclei of intact or damaged cranial nerves (either the facial or hypoglossal). In the nuclei of damaged cranial nerves, an increase in mitotic activity of as much as 30 times that of the intact nerves was observed 4 days after injury (Kreutzberg, 1966). This acute reaction abated by the end of the first postoperative week (Sjöstrand, 1965a; Kreutzberg, 1966). The nuclei of most of the labeled cells resembled microglia; however, Sjöstrand (1965a) using Hortega's silver carbonate stain, found the

maximum response of microglia to be between the third and fifth postoperative week. He demonstrated a "remarkable hypertrophy of astrocytes in the regenerating nucleus" beginning on the third postoperative day by Cajal's gold chloride stain. The maximum astrocytic reaction was observed at 9-14 days postoperatively. After the third week, astrocytosis rapidly decreased and was negligible by 90 days. No significant change was observed in the oligodendroglial cell population. The heavy metal stains were not used on radioautographs in his investigation. Olsson and Sjöstrand (1969) found that leukocytes were able to enter degenerating portions of peripheral nerves in Wallerian degeneration. Since such cells were considered to be able to transform into macrophages, the authors concluded that part of the macrophage population seen in degenerating nerves was presumably hematogenous.

The homograft reaction

Within the past ten years, the rejection of allografts has been studied by radioautography and also by electron microscopy. Walker and Goldman (1963), using tritiated thymidine, traced labeled medium and large lymphoid cells from the blood to an area of skin homograft and the underlying tissue. Some of the cells were seen to invade the donor tissue, but most remained in the underlying host tissue. The authors reported that many of these cells hypertrophied and elongated. The cytoplasm of these cells developed strong, ribonuclease-labile, basophilia. Large basophilic fibroblasts also proliferated in the area. In electron microscopic studies of skin homograft and mammary tumor homograft rejection, Walker et al. (1964, 1965) described the cytoplasm of hypertrophied lymphocytes as containing many free ribosomes. Fibroblasts, on the other hand, had cytoplasm which was almost completely filled with granular endoplasmic reticulum. The authors suggested that the elongated, hypertrophied lymphocytes, rather than macrophages, were responsible for homograft rejection.

Wiener, Spiro and Russell (1964) studied epidermal homograft rejection by electron microscopy. They concluded that lymphoid cells were responsible for graft rejection. These cells were termed graft rejection cells.

Smith and Walker (1967) studied mammary tumor allograft to the cerebrum with radioautography. Although they were not able to demonstrate cytoplasmic basophilia in the hematogenous cells surrounding the allograft, medium and large lymphocytes were considered to be the causative agent in rejection of the tissue.

METHODS AND MATERIALS

Animals

C3H strain mice obtained from Jackson Memorial Laboratory, Bar Harbor, Maine were used as experimental animals. Adult animals weighing 23-28 g (average 25 g) received either stab injury or tumor transplantation. C3H strain mice weighing 6 g were obtained from our colony. All mice were maintained in plastic cages and received water and food ad libitum. The food was Old Guilford Laboratory Animal Diet, purchased from the Emery Morse Company, Guilford, Connecticut.

A/J strain mice with spontaneous mammary tumor were purchased from Jackson Memorial Laboratory. The tumors were maintained by isologous transplantation to the mammary area of other female A/J mice. Tumor derived from transplantation was used for brain implantation.

Radioisotope

The radioisotope used to label the DNA in this experiment was tritiated thymidine (thymidine-methyl-H^3, 1 mc/ml sterile water, specific activity 2.0 c/mM.) It was obtained from New England Nuclear Corporation, Boston, Massachusetts.

Stab injury technique

The animals were anesthetized with sodium amytal (160 mg/kg) given subcutaneously in 5% solution. The hair over the scalp was clipped and the skin swabbed with alcohol to remove any loose hair and debris. A midline incision was made in the scalp and the animal secured to a mouse board with the back up. The animal rested on small urethane-foam pads with holes cut for the abdominal and mouth areas to facilitate breathing. The edges of the incision were retracted to expose the right side of the calvaria, the underlying

connective tissue was cleared and the calvaria swabbed with alcohol. Under a dissecting microscope, a small hole was made in the skull cap by cutting out a piece of bone approximately 2mm x 2mm using a dental drill (Emesco 90N) with a milling bit. Care was taken not to drill too close to the sagittal sinus or the lambdoidal suture. After the piece of bone was removed, a No. 11 scalpel blade was used to make a longitudinal incision 2mm x 0.8 to 1mm deep in the right cerebrum. The piece of bone was replaced and secured by rotating it slightly. The scalp was closed using 9mm metal wound clips (Autoclips, Clay-Adams, Inc., N.Y.) and the animal allowed to recover.

Tumor transplantation technique

The host and donor animals were anesthetized with sodium amytal (160 mg/kg) given subcutaneously in 5% solution. The hair over the scalp of the host animal and over the tumor area of the donor animals was clipped and the skin was swabbed with alcohol to remove any loose hair and debris. The donor animal was secured to a mouse board stomach side up. A midline incision was made over the tumor area and the area was kept moist with a gauze pad wetted with normal saline. A midline incision was made in the scalp of the host animal and the animal was then secured to a mouse board as described in the stab injury technique above. The edges of the incision were retracted to expose the right side of the calvaria, the underlying connective tissue was cleared and the calvaria swabbed with alcohol. A piece of bone approximately 2mm x 2mm was removed from the skull as previously described. After the bone was removed, a No. 11 scalpel blade was used to remove a piece of cerebral cortex approximately 2mm x 2mm x 0.8 to 1mm deep. The meninges overlying the small area were removed with the cortical material. At this time a small piece of tumor was obtained from the donor animal and used to fill the defect in the host animal. The piece of skull bone was replaced and secured by rotating it slightly. The incision was closed as previously described and the animals allowed to recover.

Intracardiac perfusion technique

All animals from which analysis of radioautographs were made were perfused with 10% buffered formalin. A reservoir of 200-250 ml of fixative was placed 80cm above the level of the animal. The perfusate was delivered by means of a Cutter"28" Saf-T-Set (Cutter Laboratories, Chattanooga, Tennessee) to which a disposable 18 gauge, 1 inch hypodermic needle was attached. Perfusions were begun within 15 minutes of the onset of narcosis. The animal was anesthetized with sodium amytal (160 mg/kg) given subcutaneously in 5% solution, and laid stomach side up on a suitable surface.

A transverse incision was made in the skin and abdominal muscles just caudal to the xiphoid process. A curved hemostat was attached to the xiphoid process and the diaphragm was incised from the lateral and ventral portions of the rib cage, thus exposing the heart. At this time 0.03 ml of a dilute heparin solution (1000 units/ml) was injected into the left ventricle using a 1 ml disposable syringe with a 27 gauge disposable hypodermic needle. The pericardium was stripped away and the rib cage incised longitudinally on both sides just medial to the forelimbs. The chest plate was then lifted rostrally and held by means of the weight of the hemostat. The dorsal aorta was ligated at the level of the diaphragm. Grasping the right ventricle with a small forceps, perfusion was begun by forcefully thrusting the needle of the perfusion apparatus into the left ventricle. Care was taken to stop the thrust of the needle while it was within the ventricular cavity. Five to 10 seconds after maximum flow was established, the right atrium was incised to allow escape of the returning blood and perfusate. Perfusion was maintained for approximately 20-30 minutes, or until approximately 100 ml of the perfusate had run through the animal. At the end of this time, the brain was removed and placed in 10% buffered formalin.

Experimental design

All adult animals were given 3 subcutaneous injections of 100 μc of tritiated thymidine per injection at 4 hour intervals. Twenty-four hours after the first injection, the animals received either stab injury or tumor transplantation

as previously described. The control animals received the same dosage of radioisotope as the experimental animals, but did not receive any operative treatment.

Eight adult animals with stab injury and 4 controls were killed by perfusion with 10% buffered formalin at 3, 16, 32 and 64 days after injury (2 experimental animals and 1 unoperated animal at each time period.) Two adult animals with stab injury were killed 3 days after injury by perfusion with Cajal's formalin-ammonium bromide fixative (FAB) by the intracardiac perfusion technique described above. The brains were removed and put in FAB. Two adult animals with tumor transplantation and 1 unoperated animal were killed by perfusion with 10% buffered formalin at 8 days after transplantation. Juvenile animals weighing 6 g were given 6 subcutaneous injections of 30 μc of tritiated thymidine per injection at 4 hour intervals. Sixty days after injection, when the animals weighed 19-23 g, 2 received tumor transplants as previously described. Eight days later the 2 experimental animals and 1 unoperated animal were killed by perfusion with 10% buffered formalin.

Histologic technique

A. Processing of tissues

After 1 to 4 weeks of fixation in 10% buffered formalin, the brains were removed from the fixative and transected through the injured area, dividing them into a rostral portion, which was used for the collection of frozen sections, and a caudal portion, which was embedded in paraffin.

Transverse sections, 20 μ in thickness, were cut on a freezing microtome. Ten alternating serial sections were collected, rinsed twice in double distilled water and mounted on cleaned albuminized slides. After drying in a 37°C oven for 36-48 hours, the sections were coated with Kodak NTB2 nuclear emulsion according to the method of Walker (1959), omitting celloidin coating and subbing fluid from the procedure. The radioautographs were exposed for 8-12 weeks at 5°C, developed 5 minutes in Microdol X and fixed 5 minutes in acid fixer. They were then stained with hematoxylin and eosin (H and E) as described by Walker (1959), dehydrated in a series of graded alcohols, cleared in cedarwood oil and mounted in Permount.

Sections to be stained for neuroglia were collected in 10% ammonium hydroxide solution according to the Globus Modification of Cajal's gold chloride method (Penfield and Cone, 1961). After 24 hours in ammonium hydroxide, the sections were washed 3 times in double distilled water and then placed in 10% hydrobromic acid for 2 hours. This allowed the ammonium bromide to act as a mordant in the formalin fixed material. The stain for which formalin-ammonium bromide fixation was required could then be carried out. The sections were rinsed twice in double distilled water and then stained by Cajal's gold chloride sublimate method for astrocytes. A few sections from each injury area were stained for oligodendroglia and microglia as follows. The sections were collected in double distilled water and then pretreated according to the method of Wolman (1958): 20 minutes in 5% hydrochloric acid, rinsed in distilled water, placed in 0.01N sodium hydroxide solution for 4 hours. They were then stained by Penfield's Second Midification of del Rio-Hortega's Silver Carbonate Method. The stain gave inconsistent results and was not used for experimental determinations.

After staining, the sections were mounted on cleaned, albuminized slides. They were then dehydrated in alcohol, cleared in cedarwood oil and coverslipped with Histoclad.

Sections, 7 μ in thickness, were cut from the paraffin embedded material. These were put on cleaned, albuminized slides, deparaffinized and then coated with Kodak NTB2 nuclear emulsion according to the method of Walker (1959). Celloidin coating and dipping in subbing fluid were omitted from the procedure. The radioautographs were exposed 5 weeks at 5°C, developed 5 minutes in Microdol X and fixed 5 minutes in acid fixer. The slides were then stained with H and E, dehydrated in a series of graded alcohols, cleared in cedarwood oil and mounted in Permount. After drying, the slides were cleaned and used for analysis.

The brains that were perfused and fixed with FAB were treated somewhat differently than those described above. After 1 week of fixation, the brains were removed from the fixative. Frozen sections, 20 μ in thickness, were cut on a freezing microtome and 10 sections were collected for combined radioautographic

processing and heavy metal staining for neuroglia. Five sections were rinsed twice in double distilled water and then stained by Cajal's gold chloride sublimate method for astrocytes (Penfield and Cone, 1961) and mounted on cleaned, albuminized slides. After drying in a 37°C oven for 36-48 hours, the sections were coated with emulsion. The slides were exposed for 1 week, developed, and fixed. The remaining 5 sections were rinsed twice in double distilled water and mounted on cleaned, albuminized slides. After radioautographic processing, as described above, developing and fixing, an attempt was made to poststain the sections by placing them in Cajal's gold chloride sublimate staining solution.

Through the rest of the injured area, alternating serial sections, 20 μ thick, were collected for radioautographic processing or gold staining. Those sections to be processed for radioautography were treated in the same manner as the alternating serial sections from material fixed in 10% buffered formalin. The slides were developed and fixed after either 4, 8, 12 or 14 weeks exposure at 5°C. They were then stained with H and E, dehydrated, cleared and mounted in Permount. The alternate sections were rinsed twice in double distilled water and stained by Cajal's gold chloride sublimate stain, as above.

B. Cell Classification

Radioautographs stained with H and E were used to count and to classify nuclear types and to count silver grains. A nucleus which had at least 4 silver grains over it was considered radioactive. Nuclear counts were made with an A/O Spencer microscope using a 97X oil immersion objective and a 10X ocular lens which was fitted with a 1421 A Net Reticule (20mm dia. 15mm sq. ruled into 1.0mm sq's - American Optical Company, Buffalo, New York). The reticule measured 50 μ on each side when using the above optical system, and areas of 2500 μ^2 were utilized for counts. Nuclei were counted in gray matter along the edges of the stab injury or tumor and a corresponding area in the uninjured hemisphere of operated animals, in the corpus callosum of injured and uninjured hemispheres of operated animals, and in corresponding areas of unoperated animals. Nuclear counts were made in areas of 90,000 μ^2 (0.09mm^2) in the corpus callosum of each operated hemisphere. Areas of 40,000 μ^2 (0.04mm^2)

were counted in the corpus callosum of the uninjured hemisphere of operated animals and in unoperated animals. Counts were made in the gray matter along the edges of the wounds or tumors in an area 50μ wide, or a total area of 40,000μ^2. Areas of 40,000μ^2 were counted in corresponding gray matter of the uninjured hemisphere of the operated animals and in unoperated animals.

The nuclei of all cells other than neurons, ependymal cells, endothelial cells and polymorphonuclear leukocytes were counted. By excluding these cells, it was felt that the cells counted were glial cells and mononuclear cells which could not be distinguished from the glial cells.

The nuclear types were classified in the following morphological groups: 1) small, dark-staining, with nuclear diameter less than 4-5μ; 2) medium round, with nuclear diameter which was 5-9μ; 3) medium-sized elongated nuclei, whose smallest nuclear diameter was 5-9μ; 4) large, pale, oval-to-round nuclei, with a diameter of greater than 9μ and which were lighter-staining than other nuclei. In addition, there were some nuclei which were entered into an unclassified category because they did not fall into the above categories.

Gold stained sections 20μ in thickness were scanned using a 43X objective lens and a 10X ocular lens fitted with a 1421 A Net Reticule which measured 0.0169mm^2 in area with this optical system. The 43X objective lens was used to facilitate viewing the perikaryon and processes of the gold stained cells. At a higher power the cells appeared "grainy" and were difficult to define. Five consecutive areas of 0.0169mm^2 (0.0845mm^2 total) were scanned in the corpus callosum. Counts of astrocytes were not made in cortical gray matter due to the lack of stained cells in this area.

Two cell categories (Figures 6 and 7) were used: 1) large, well-stained cells with thick processes and 2) small, more lightly-stained cells with more delicate looking processes. Cell bodies which lacked definite processes and processes which were not seen to extend from a definite cell body were not classified or counted.

To determine the frequency of large gold stained cells in 0.0845mm^2 of corpus callosum, 20μ thick sections stained with H and E were used to count the

total number of nuclei in 0.0845mm^2. The frequency of large gold stained cells was then expressed as a ratio of these cells per total nuclei in 0.0845mm^2.

RESULTS

A. Unoperated animals and the uninjured hemisphere or operated animals

On gross examination, the brains of all unoperated animals appeared normal. There was no evidence of infection around the scalp wound of injured animals. Transverse sections of these brains were scanned and their architecture compared with reference plates in an atlas of the mouse brain (Kovak and Denk, 1968). Gray matter and white matter could readily be distinguished in sections stained with H and E, and with Cajal's gold chloride sublimate stain. It was also possible to identify the corpus callosum and hippocampus as discrete structural entities with these stains.

Sections which were stained with gold chloride sublimate showed a predominance of small, lightly-stained cell bodies with lightly-stained processes (Figure 6). There were from 0 to 3 large gold stained cells per $0.0845mm^2$ of the corpus callosum of unoperated hemispheres, whereas there was an average of 30 small, lightly-stained cells counted in the same area. The corpus callosum of the uninjured hemisphere from operated animals demonstrated a similar preponderance of small cells (average: 33) over large cells (average: 2) per $0.0845mm^2$. In $0.0845mm^2$ of the corpus callosum of unoperated hemispheres, the large gold stained cell population was less than 1.5 percent.

Analysis of radioautographs

Nuclear counts were made in the cortical gray matter and the corpus callosum of unoperated animals and in the uninjured hemisphere of operated animals injected with tritiated thymidine 1 day before operation. Table 1 shows the data obtained from these counts. These data are also represented in the bar chart of Figure 1. In addition, 2 animals were injected with tritiated thymidine 60 days before tumor transplantation. The data obtained in counts from the uninjured areas of these individuals are listed in Table 2 and are

represented in the bar chart of Figure 1. An unoperated animal injected at the same time interval showed numbers of labeled cells which correlated with counts made previously on unoperated animals of this age (Smith and Walker, 1967) and these data are not included in Table 2 or Figure 1.

Table 1

Frequency of labeled nuclei in 0.04mm^2 of uninjured areas of operated animals

Animals were injected with 100 μc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours before stab injury or tumor transplantation and killed at each of the designated time intervals.

Time after operation(days)	Animal	Cortical gray matter			Corpus Callosum		
		Total number of nuclei	Total number of radioactive nuclei	Percent labeled	Total number of nuclei	Total number of radioactive nuclei	Percent labeled
3	a	70	0	0	207	5	2.4
	b	73	1	1.4	180	3	1.7
	Control	73	0	0	188	2	1.1
8	a*	49	4	8.2	140	4	2.8
	Control	62	2	3.3	261	6	2.3
16	a	25	0	0	100	2	2.0
	b	65	2	3.1	142	2	1.4
	Control	62	0	0	182	0	0
32	a	60	0	0	184	2	1.1
	b	48	1	2.1	150	2	1.3
	Control	63	1	1.6	125	0	0
64	a	68	3	4.4	214	1	0.5
	b	56	0	0	152	1	0.6
	Control	78	2	2.6	159	4	2.5

*Tumor transplantation

Table 2

Frequency of labeled nuclei in 0.04mm^2 of the cortical gray matter and of the corpus callosum in the unoperated hemisphere

Juvenile animals were injected with 30 µc of tritiated thymidine 6 times at 4 hour intervals 60 days before tumor transplantation. They were killed 8 days after operation.

Animal	Cortical gray matter			Corpus Callosum		
	Total number of nuclei	Total number of radioactive nuclei	Percent labeled	Total number of nuclei	Total number of radioactive nuclei	Percent labeled
a	72	7	9.7	166	16	9.6
b	70	3	4.3	207	18	8.2

Figure 1

Percent labeled nuclei in 0.04mm^2 of the cortical gray matter and of the corpus callosum of unoperated animals and of the uninjured hemisphere of operated animals.

The animals were injected with 100 μc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours before operation. Each pair of bars represents a different mouse. The last pair at each time interval is from an unoperated mouse unless designated otherwise. Animals designated 8* received 30 μc of tritiated thymidine 6 times at 4 hour intervals 60 days before tumor transplantation.

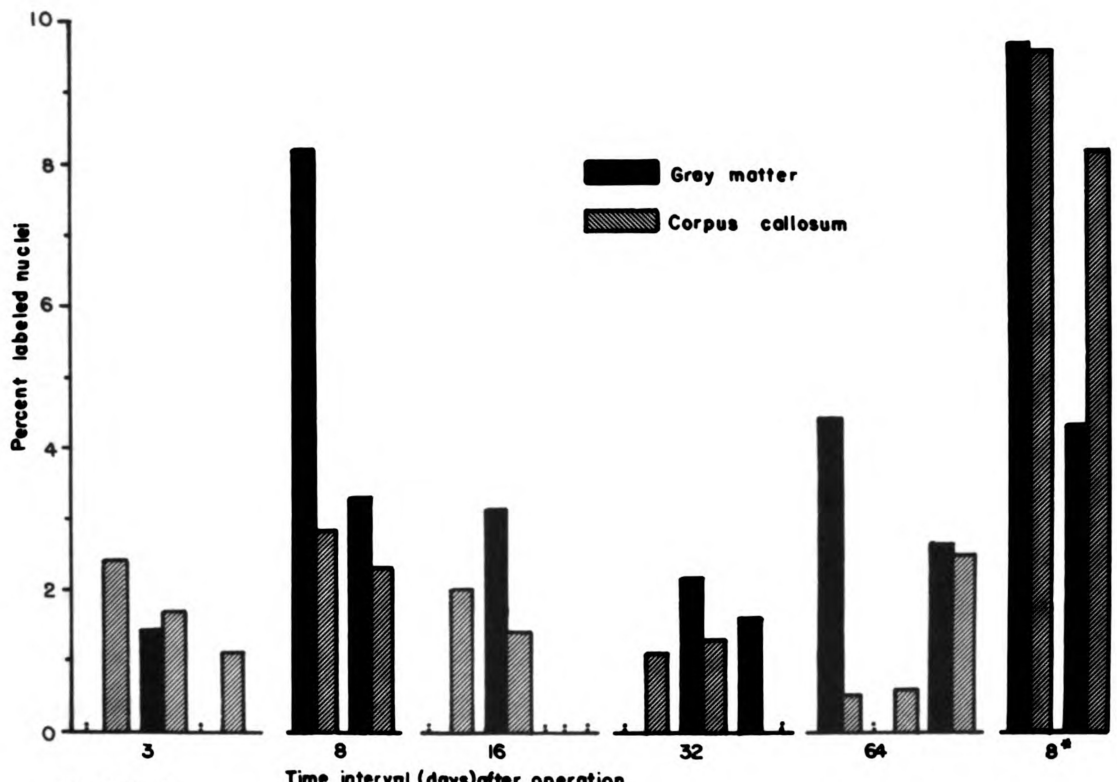
10
8
6
4
2
0
Percent labeled nuclei
Gray matter
Corpus callosum
3
8
16
32
64
8*
Time interval (days) after operation

FIGURE 1

B. Operated animals

Description of the wound

In all groups of animals injured by stabbing, the wound was found to pass through the cortical gray matter to the corpus callosum. In a few animals, the wounds extended deeper and were found to pass through the corpus callosum into the white matter of the hippocampus. All stab wounds contained necrotic debris. Those which extended into the white matter of the corpus callosum and hippocampus retained their hemorrhagic character throughout the length of the experiment. Some wounds had longitudinal expansions which ran parallel with the fibers of the corpus callosum. These expansions contained necrotic debris.

In animals which received mammary tumor allograft, the tissue mass was seen to lie in the cortical gray matter with its deepest portion abutting or just interrupting the white matter of the corpus callosum. These wounds did not appear to retain as much hemorrhagic material as did the simple stab wounds.

In gold stained sections, the injured hemispheres of experimental animals showed a predominance of large, round to somewhat rectangular cell bodies (Figure 7). The processes of these cells were darker, more heavily stained, and appeared thicker than those of the large gold stained cells seen in the uninjured hemisphere.

In 2 animals sacrificed 3 days after stab injury, a large collection of erythrocytes and necrotic debris occupied the central part of the lesion. In sections stained with H and E, many dark-staining pyknotic nuclei were present in and around the wound. Polymorphonuclear leukocytes were rarely seen. Mitotic figures were found in both the gray and white matter near the lesion at this time. Gold stained sections showed large cells in the corpus callosum around the lesion and its expansions (figure 8).

Four animals received tumor allografts to the brain. Two animals were injected with tritiated thymidine 1 day before transplantation and 2 were injected with tritiated thymidine 60 days before transplantation. Eight days after grafting the tumors to the brain, the animals were killed. The 2 animals injected 1 day before transplantation will be described first.

In one individual, on sections which were stained with H and E, the tumor appeared necrotic in certain areas. Many cells with medium-round nuclei were seen within and surrounding the graft. Figure 9 shows labeled glial-like nuclei in the tissue surrounding the tumor allograft. Figure 10 shows radioactive medium-round and medium-elongated nuclei in the cortex within 100 μ of the tumor allograft. Polymorphonuclear leukocytes and mitotic figures were not seen at this time or at subsequent time intervals. Hematoxylin and eosin stained sections from the second individual did not reveal any allografted material. It appeared that when the brain of this individual was transected prior to paraffin embedding, the transverse cut was made just caudal to the allograft rather than through it. For this reason, analyses of radioautographs were not made on this individual, and data for the tumor allograft reaction were collected from only 1 individual. Gold stained sections from both individuals with tumors were obtained however. These sections, as well as those from the other animals which received tumor allograft, showed many large, well-stained cell bodies with thick processes. They were located primarily in the white matter of the corpus callosum and hippocampus. Some of the cells could be seen in the gray matter surrounding the unstained tumor mass, but these were not as well stained, nor did their processes appear to be as large, as those found in the white matter (Figures 11 and 12). Hematoxylin and eosin stained sections from the animals injected with tritiated thymidine 60 days before tumor allograft to the brain also showed the tumor to be necrotic in certain areas. Many cells with medium-round and medium-elongated nuclei were seen within and surrounding the graft. Polymorphonuclear leukocytes were not seen. One mitotic figure was noted in 1 of the animals.

Sixteen days after stab injury, necrotic debris was still present in the central part of the lesion toward the lower end of the wound track. Many large, pale to medium-dark nucleated cells appeared to fill the upper portions of the track. These cells seemed to be contiguous with the cells of the meninges. Gold stained sections showed many large well-stained cell bodies with large processes in the white matter of the corpus callosum and hippocampus. A few of these cells were observed around the lesion in the gray matter adjacent to the corpus callosum (Figure 13).

At 32 and 64 days after stab injury, H and E stained sections showed cells with medium-round nuclei, medium-elongated nuclei and large, pale nuclei present in and around the wound. Small dark nucleated cells containing phagocytosed material were present in expansions of the wound track in the corpus callosum. Gold stained sections looked similar to those of the animals sacrificed 16 days after injury (Figures 14 and 15).

Analysis of gold stained sections

Table 3 shows the number of large, well-stained cells in the corpus callosum around the lesion area for all experimental animals injected with tritiated thymidine 1 day before injury. The total number of nuclei in the same area were counted in 20μ thick sections stained with H and E. These data are also presented in the bar chart, Figure 2. The χ^2 statistic as described by Croxton (1953) was used to test the hypothesis that the ratio of the number of large well-stained cells to the total number of nuclei in 0.0845mm^2 of corpus callosum did not change significantly with the type of injury or with the time of sacrifice after injury. Tables 4 and 5 list the χ^2 and p values calculated from these data for animals within the same treatment groups, and for groups killed at different time intervals after stab injury or tumor transplantation. No significant differences were seen between the 2 individuals killed at each of the designated time intervals or between the groups killed at various time intervals after stab injury or tumor transplantation. It should be noted here that the designations "a" and "b" were arbitrarily assigned to the individuals within a group. These were used to facilitate the collection of data from individuals and the computation of the χ^2 and p values.

Table 3

Frequency of gold stained cells in 0.0845mm^2 of corpus callosum of the operated hemisphere

Animals were killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation (days)	Animal	Total nuclei	Large gold-stained cells	Small, lightly-stained cells	Percent large gold-stained cells
3	a	316	36	15	11.4
	b	368	35	10	9.5
8	a*	397	42	15	10.6
	b*	420	47	12	11.2
16	a	368	35	17	9.5
	b	328	28	5	8.5
32	a	593	68	20	11.5
	b	387	36	2	9.3
64	a	444	52	4	11.7
	b	500	48	5	9.6

*Tumor Transplantation

Figure 2

Percent large gold stained cells in 0.0845mm^2 and radioactive nuclei in 0.09mm^2 of the corpus callosum of the operated hemisphere.

Animals were injected with 100 µc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours before operation. Each pair of bars represents a different mouse. Animals designated 8* received 30 µc of tritiated thymidine 6 times at 4 hour intervals 60 days before tumor transplantation.

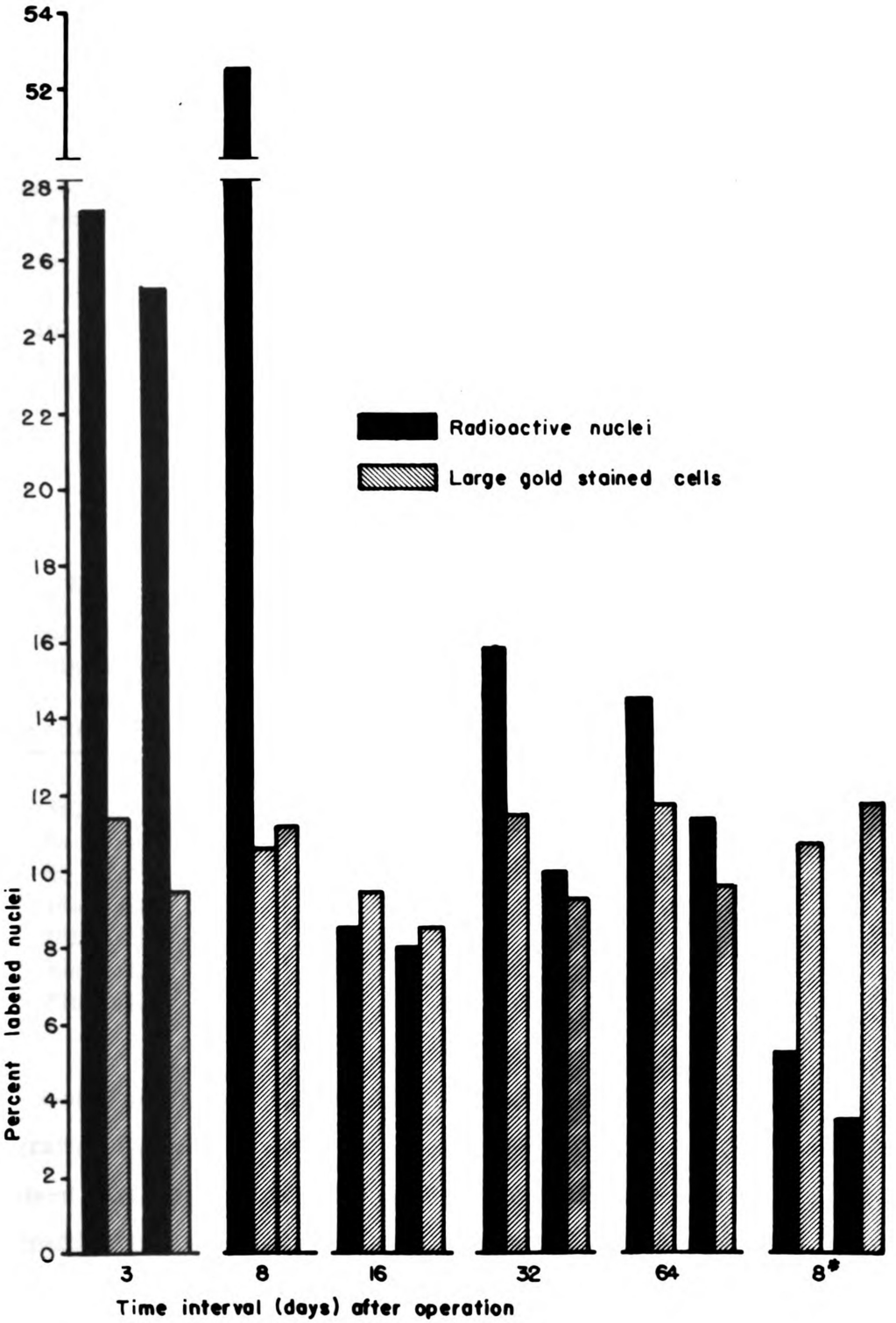
54
52
28
26
24
22
20
18
16
14
12
10
8
6
4
2
0
Percent labeled nuclei
Radioactive nuclei
Large gold stained cells
3
8
16
32
64
8*
Time interval (days) after operation

FIGURE 2

Table 4

Statistical results for gold stained cells

χ^2 and p values of differences between the two individuals killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation (days)	χ^2 value	p value
3	0.6467	$>.30$
8	0.0783	$>.75$
16	0.1998	$>.50$
32	1.1567	$>.25$
64	1.1071	$>.25$

Table 5

Statistical results for gold stained cells

χ^2 and p values of differences between the groups killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation (days)	χ^2 value	p value
3a, 8a	0.1192	$>.70$
3b, 8b	0.5934	$>.30$
8a, 16a	0.2407	$>.50$
8b, 16b	1.4377	$>.20$
16a, 32a	0.9079	$>.30$
16b, 32b	0.1276	$>.70$
32a, 64a	0.0147	$>.90$
32b, 64b	0.0225	$>.80$

Gold stained sections from 2 additional animals which received tumor allografts were also scanned for large gold stained cells. These animals had received different pretreatment (injection of tritiated thymidine 60 days before allografting) than the animals listed in Table 3. In both groups the time interval between allografting and killing was 8 days. The ratios of large gold stained cells to the total number of nuclei in 0.0845mm^2 of corpus callosum were 47 per 433 (10.8%) and 53 per 452 (11.7%). No significant differences were seen between the individuals of this group ($p > .80$), or between this group and the animals listed in Table 3 ($p > .50$).

Analysis of radioautographs

Table 6 shows the total number of radioactive nuclei counted in 0.09mm^2 of the corpus callosum around the lesion for all experimental animals injected 1 day before injury. These data are presented as a bar chart in Figure 2. The χ^2 statistic showed that in 1 case there were significant differences between the 2 individuals that were killed at each time interval and in 3 instances between the groups that were killed at different time intervals after stab injury or tumor transplantation. Tables 7 and 8 list the χ^2 and p values calculated from these data.

Table 6

Frequency of radioactive nuclei in 0.09mm^2 of the corpus callosum of the operated hemisphere

Animals were injected with 100 µc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to operation. They were killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation (days)	Animal	Total number of nuclei	Total number of radioactive nuclei	Percent labeled
3	a	246	67	27.3
	b	278	70	25.2
8	*	512	268	52.5
16	a	328	28	8.5
	b	373	30	8.0
32	a	535	85	15.9
	b	568	57	10.0
64	a	612	89	14.5
	b	563	64	11.4

*Tumor transplantation

Table 7

Statistical results for radioactive nuclei

χ^2 and p values of differences between the two individuals killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation (days)	χ^2 value	p value
3	0.2740	$>.50$
16	0.0561	$>.80$
32	10.9674	$<.001$
64	2.6097	$>.10$

Table 8

Statistical results for radioactive nuclei

χ^2 and p values of differences between the groups killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation (days)	χ^2 value	p value
3a, 8	42.47+	$<.001$
8, 16a	168.1+	$<.001$
16a, 32a	11.7729	$<.001$
16b, 32b	1.0651	$>.30$
32a, 64a	1.0665	$>.30$
32b, 64b	0.5254	$>.30$

Table 9 lists the number of each nuclear type counted in 0.09mm^2 of corpus callosum of the operated hemisphere in animals that were injected 1 day before injury. These data are presented as a bar chart in Figure 3. Tables 10 and 11 give the χ^2 and p values calculated from these data. Significant differences were found in each nuclear category between the 2 individuals killed at each of the designated time intervals and between the groups killed at each of the designated time intervals after stab injury or tumor allograft.

Table 9

Frequency of radioactive nuclear subgroups in 0.09mm^2 of corpus callosum in the operated hemisphere

Animals were injected with 100 µc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to stab injury or tumor transplantation. They were killed at each of the designated time intervals after operation.

Time after operation (days)	Animal	Nuclear subgroups: Total number of nuclei	Small dark	Medium round	Medium elongated	Large, pale, oval - to - round	Unclassified
3	a	246	7	33	21	0	6
	b	278	14	25	29	0	2
8	*	512	55	142	47	12	12
16	a	328	0	15	7	4	1
	b	373	0	10	7	12	1
32	a	535	1	18	25	41	0
	b	568	4	12	23	19	4
64	a	612	0	34	4	45	6
	b	563	0	4	15	44	1

*Tumor transplantation

Figure 3

Percent labeled nuclei of each nuclear type in 0.09mm^2 of corpus callosum of the operated hemisphere.

Animals were injected with 100 µc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to stab injury or tumor transplantation. Included for comparison are the percent gold stained cells in 0.0845mm^2 of corpus callosum of these animals.

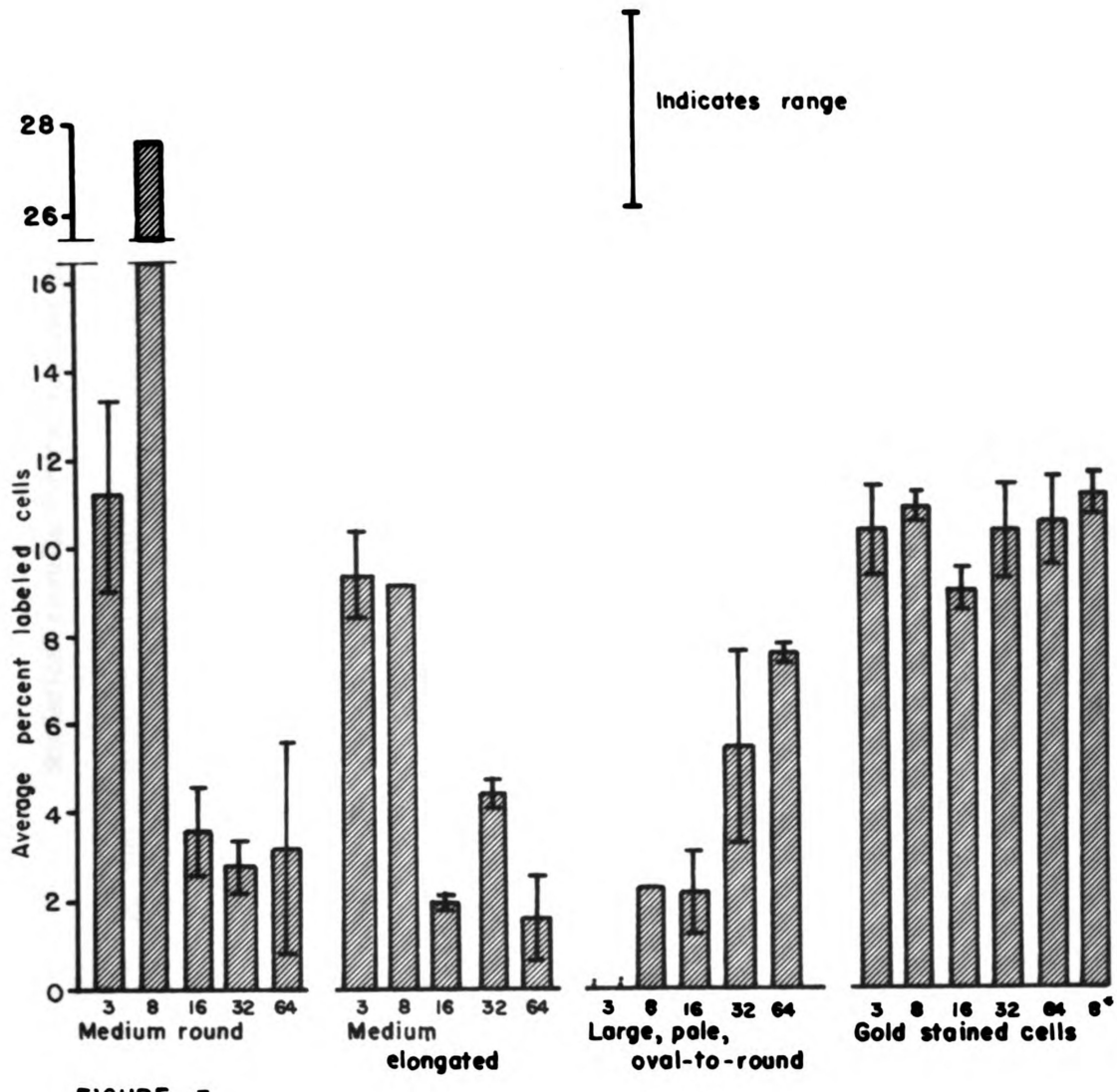
Indicates range
28
26
16
14
12
10
8
6
4
2
0
Average percent labeled cells
3 8 16 32 64
Medium round
3 8 16 32 64
Medium elongated
3 8 16 32 64
Large, pale, oval-to-round
3 8 16 32 64 8*
Gold stained cells

FIGURE 3

Table 10

Statistical results for radioactive nuclear subgroups

χ^2 and p values of differences between the two individuals killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation(days)	Medium round nuclei		Medium elongated nuclei		Large, pale, oval-to-round nuclei	
	χ^2 value	p value	χ^2 value	p value	χ^2 value	p value
3	2.8368	> .05	0.5430	> .30	*	*
16	1.8169	> .10	0.0590	> .80	3.1224	> .05
32	1.6248	> .20	0.3346	> .50	9.1642	< .005
64	21.9961	< .001	7.4518	< .01	0.0896	> .75

*Values were not calculated because there were no cells with large, pale, oval-to-round nuclei observed in the animals killed at 3 days.

Table 11

Statistical results for radioactive nuclear subgroups

χ^2 and p values of differences between the groups killed at each of the designated time intervals after stab injury or tumor transplantation.

Time after operation(days)	Medium round nuclei		Medium elongated nuclei		Large, pale, oval-to-round nuclei	
	χ^2 value	p value	χ^2 value	p value	χ^2 value	p value
3a, 8	19.18+	<.001	0.0842	>.75	*	*
8, 16a	70.57+	<.001	16.4975	<.001	1.3521	>.20
16a, 32a	0.8588	>.30	3.9076	<.05	16.2775	<.001
16b, 32b	0.3183	>.50	3.4430	>.05	0.0114	>.90
32a, 64a	3.3554	>.50	19.3575	<.001	0.0005	>.95
32b, 64b	3.9858	<.05	1.6701	>.10	10.7408	<.005

*Values were not calculated because there were no cells with large, pale, oval-to-round nuclei observed in the animals killed at 3 days.

Table 12 lists the frequency of labeled nuclei in 0.04mm^2 of cortical gray matter along the wound in animals injected with tritiated thymidine 1 day before operation. These data are presented as a bar chart in Figure 4. This figure also includes data obtained from the corpus callosum which are listed in Table 6. The data for the nuclear subgroups listed in Table 12 are represented as a bar chart in Figure 5.

Table 13 lists data obtained from counts of radioautographs of 2 individuals injected with tritiated thymidine 60 days before tumor transplantation. These data are included as a bar chart in Figure 4. In the corpus callosum and the cortical gray matter along the wound, some of the cells of the medium-round and medium-elongated nuclear subgroups were labeled. No large, pale, oval-to-round radioactive nuclei were found in the corpus callosum. In the corpus callosum of 1 of the animals, 3 labeled nuclei were found in the area subjacent to the tumor. Two labeled medium-round nuclei and an unlabeled mitotic figure were seen in the gray matter immediately above the corpus callosum and within 200μ of the wound. The sections from the other animal did not demonstrate labeled nuclei within 200μ of the wound either in the gray matter or the corpus callosum. Most of the labeled cells counted in the corpus callosum were found at distances greater than 250μ from the wound.

Table 12

Frequency of radioactive nuclei in 0.04mm^2 of cortical gray matter in the operated hemisphere

Animals were injected with 100 μc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to stab injury or tumor transplantation. They were killed at the designated time intervals after operation.

Time after operation (days)	Animal	Total number of nuclei	Total number of radioactive nuclei	Percent labeled	Nuclear subgroups			
					Small dark	Medium round	Medium elongated	Large, pale, oval-to-round
3	a	87	27	31.0	2	18	7	0
	b	92	28	30.4	0	15	13	0
8	*	72	54	75.0	1	25	17	7
16	a	126	42	33.3	4	19	7	11
	b	103	27	26.2	1	10	5	11
32	a	136	29	21.3	0	11	10	8
	b	190	41	21.6	0	15	4	16
64	a	181	35	19.3	0	7	13	15
	b	268	82	30.6	2	7	27	46

*Tumor transplantation

Table 13

Frequency of radioactive nuclei in 0.04mm^2 of cortical gray matter along the wound and of the corpus callosum in the operated hemisphere

Juvenile animals were injected with 30 µc of tritiated thymidine 6 times at 4 hour intervals 60 days prior to tumor transplantation. They were killed 8 days after transplantation.

Animal	Cortical gray			Corpus callosum		
	Total number of nuclei	Total number of radioactive nuclei	Percent labeled	Total number of nuclei	Total number of radioactive nuclei	Percent labeled
a	109	3	2.8	505	27	5.3
b	137	2	1.5	484	17	3.5

Figure 4

Percent radioactive nuclei in 0.04mm^2 of the cortical gray matter along the wound and in 0.09mm^2 of corpus callosum in the operated hemisphere.

Animals were injected with 100 μc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours before stab injury or tumor transplantation. Each pair of bars represents a different mouse. Animals designated 8* received 30 μc of tritiated thymidine 6 times at 4 hour intervals 60 days before tumor transplantation.

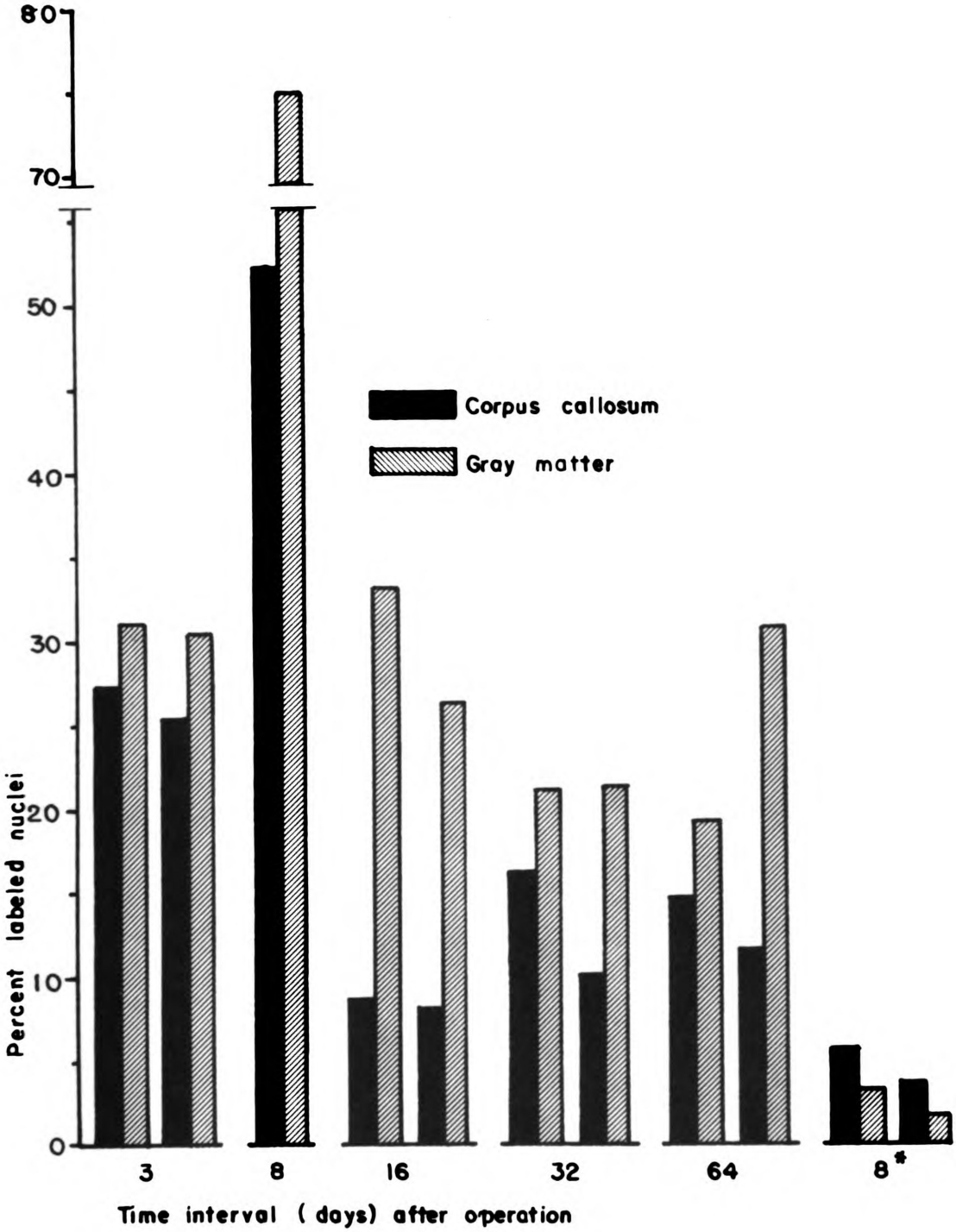
80
70
50
40
30
20
10
0
Percent labeled nuclei
Corpus callosum
Gray matter
3
8
16
32
64
8*
Time interval (days) after operation

FIGURE 4

Figure 5

Percent radioactive nuclei of each nuclear type in 0.04mm^2 of cortical gray matter along the wound in the operated hemisphere.

Animals were injected with 100 µc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to stab injury or tumor transplantation.

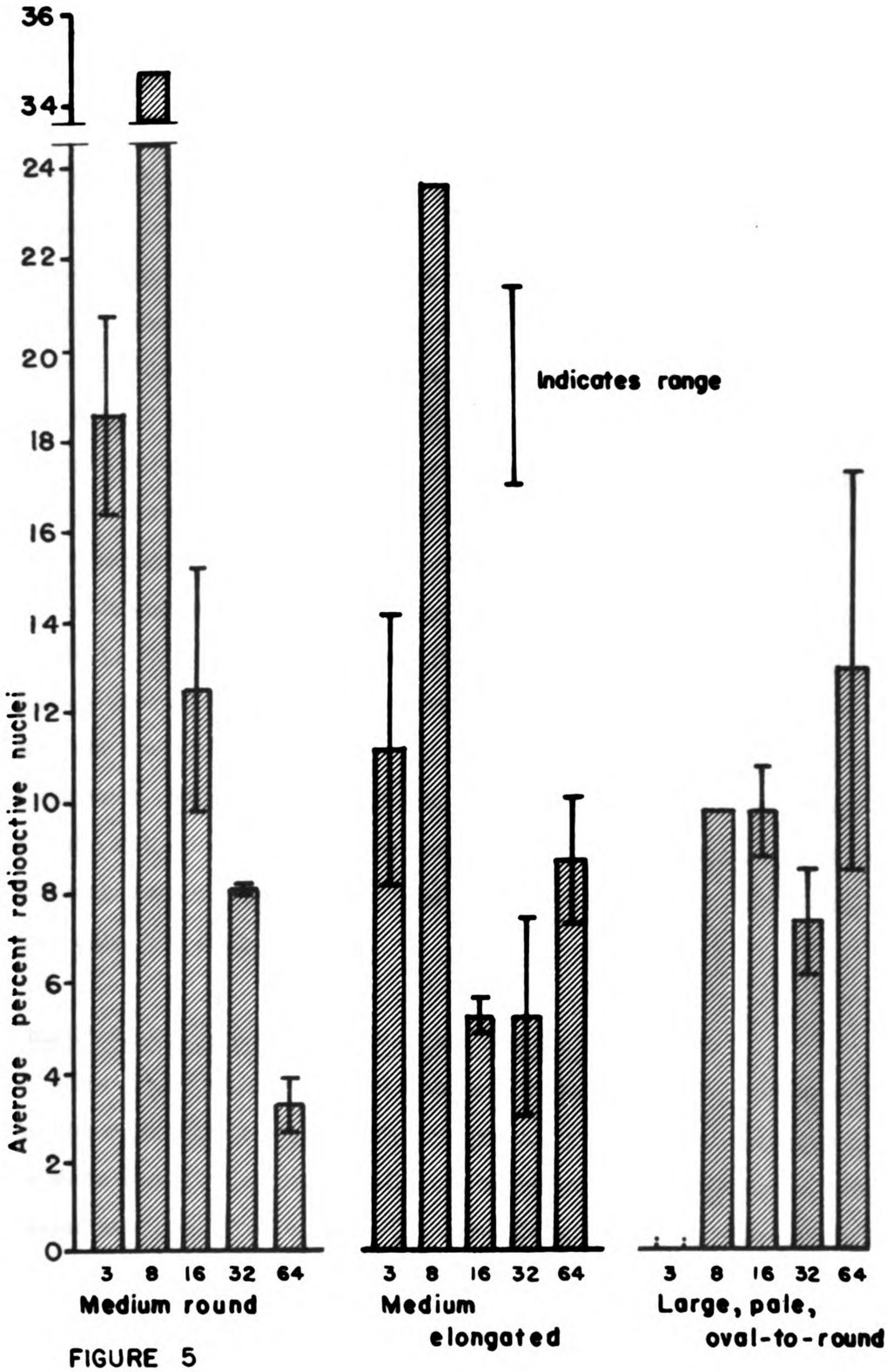
36
34
24
22
20
18
16
14
12
10
8
6
4
2
0
Average percent radioactive nuclei
Indicates range
3 8 16 32 64
Medium round
3 8 16 32 64
Medium elongated
3 8 16 32 64
Large, pale, oval-to-round

FIGURE 5

Mitotic figures

A search was made for mitotic figures at each time interval. In the animals injected with tritiated thymidine 1 day before injury, mitotic figures were seen only in the sections from animals killed 3 days after stab injury. Thirteen mitotic figures were counted in 0.270mm^2 of corpus callosum (0.045mm^2 in each of 6 sections). There were 3 prophases, 8 metaphases and 2 anaphases. All of the figures were considered radioactive except 1 prophase which had 3 silver grains overlying it. Eight mitotic figures were seen in 0.110mm^2 of corpus callosum (0.0275mm^2 in each of 4 sections). There were 2 prophases, 4 metaphases and 2 anaphases. Of these, 1 prophase, 1 metaphase and 1 anaphase were not radioactive. One mitotic figure in metaphase was seen in 0.04mm^2 of cortical gray matter of 1 animal injected 60 days before tumor transplantation. It was not radioactive.

Radioautographs of 20μ thick sections showed various degrees of labeling. Some sections appeared devoid of labeled cells in the tissue surrounding stab injury or tumor transplantation. Other sections showed labeled nuclei, but none of the sections showed lesioned areas with heavily labeled cells ($>$20 grains per nucleus). No quantitation of labeled nuclei was done on the slides from any of the animals because of the variability of the technique. These sections were used, rather, to count the total number of nuclei per 0.0845mm^2 of the corpus callosum (Table 3).

Formalin-ammonium bromide perfused and fixed material

Five slides which were prestained with gold chloride sublimate were viewed under the microscope before radioautographic processing. Large gold stained cells were seen in the corpus callosum of the injured hemisphere. After the emulsion was developed and fixed, the emulsion over the entire surface of the slides appeared discolored, the sections had turned a dark brown color and the cells could not be distinguished from the background. The slides that were processed for radioautography before staining with gold discolored when they were placed in the gold bath, and neither silver grains nor cells could be demonstrated.

Large, well-stained cells were demonstrated in gold stained sections collected in the alternating serial section method. These cells were found in the corpus callosum of the injured hemisphere. The alternating sections which were processed for radioautography demonstrated no silver grains over the tissue up to and including 14 weeks of exposure at 5^{o}C.

Figure 6

Three small, lightly-stained astrocytic cell bodies are seen in the center of the photomicrograph. Small pieces of fibrous material and cell bodies which did not appear to have processes were not counted. Unoperated animals and the uninjured hemisphere of operated animals showed a predominance of these cells. Cajal's gold chloride sublimate stain for astrocytes. X 640.

Figure 7

Large, well-stained astrocytic cell bodies are demonstrated in the upper left quadrant of the photomicrograph. The processes of these cells appear thicker and more heavily stained than those of the small astrocytic cell bodies. Fragments of fibrous material and cell bodies which did not appear to have processes were not counted. A predominance of large, well-stained cells was found in the corpus callosum of operated hemispheres. Cajal's gold chloride sublimate stain for astrocytes. X 640.

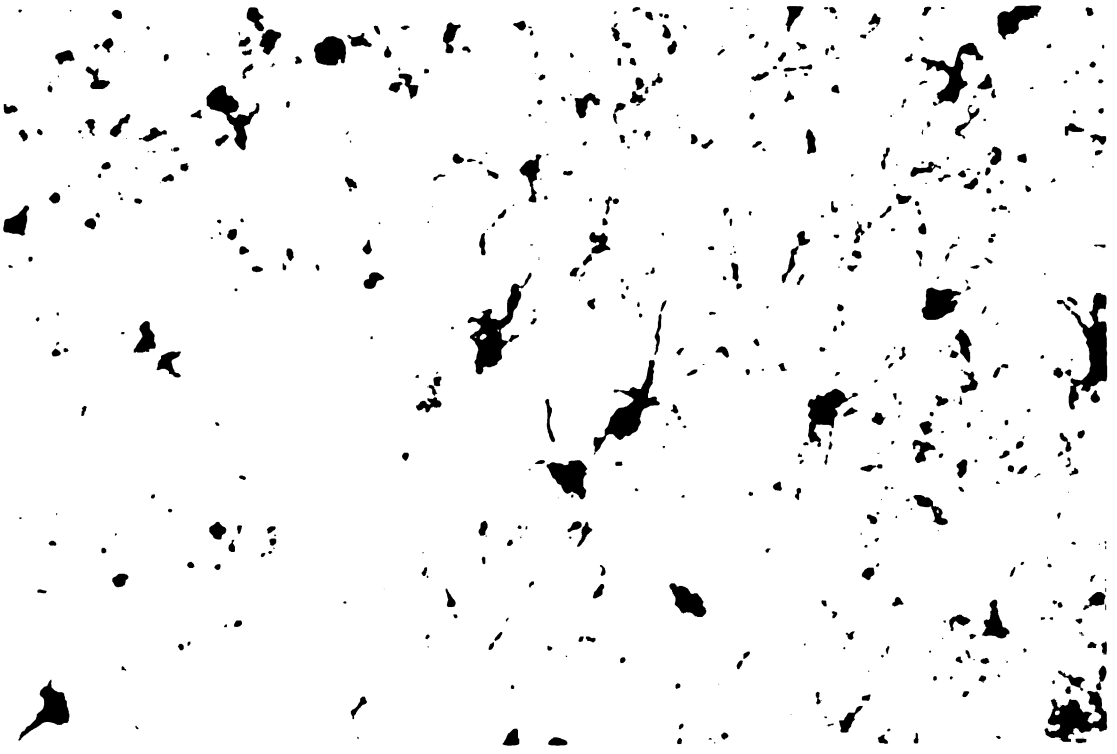

Figure 6. Small gold stained cells in unoperated hemisphere

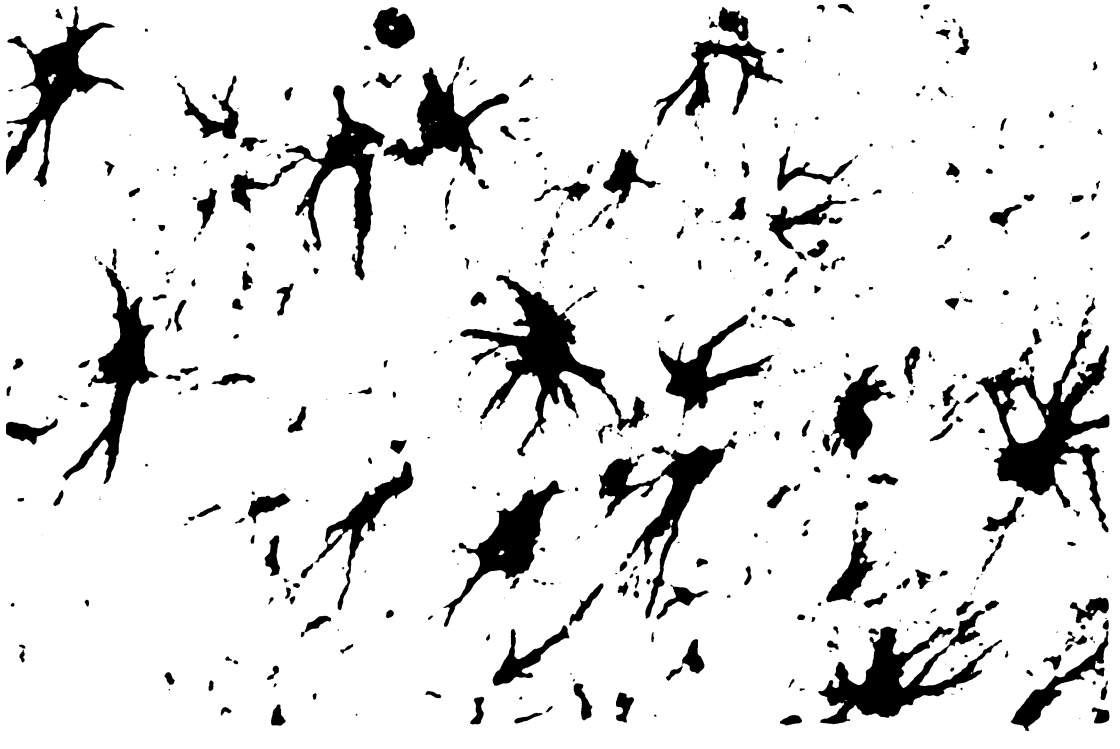

Figure 7. Large gold stained cells in operated hemisphere

Figure 8

Large, well-stained cells are located in the corpus callosum and hippocampus, in the lower one-third of the photomicrograph. The lesion is located at the upper left and cortical gray matter occupies the remaining upper two-thirds of the photomicrograph. Cajal's gold chloride sublimate stain for astrocytes. X 280.

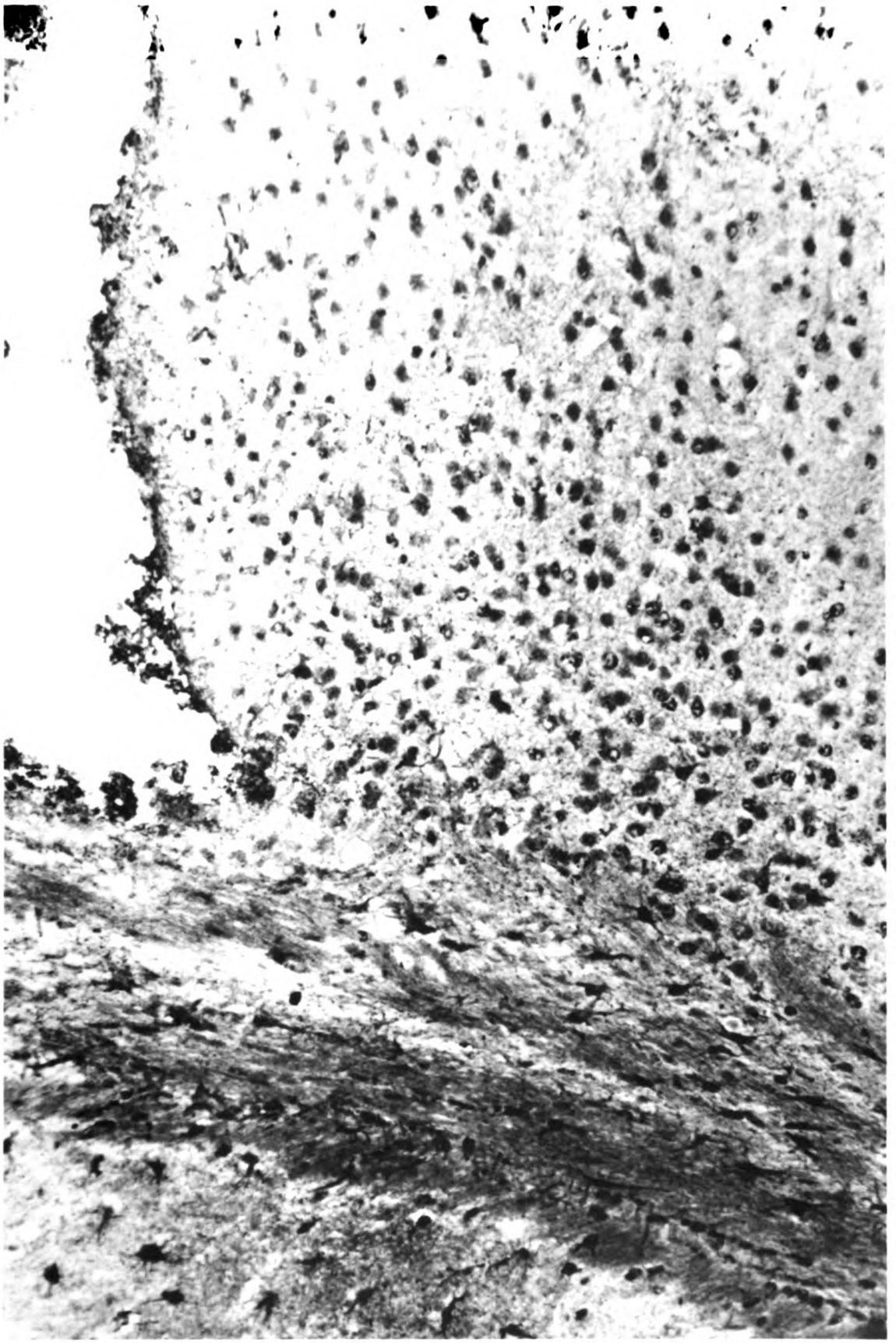

Figure 8. Large gold stained cells in the corpus callosum of the operated hemisphere 3 days after stab injury

Figure 4

Percent radioactive nuclei in 0.04mm^2 of the cortical gray matter along the wound and in 0.09mm^2 of corpus callosum in the operated hemisphere.

Animals were injected with 100μc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours before stab injury or tumor transplantation. Each pair of bars represents a different mouse. Animals designated 8* received 30μc of tritiated thymidine 6 times at 4 hour intervals 60 days before tumor transplantation.

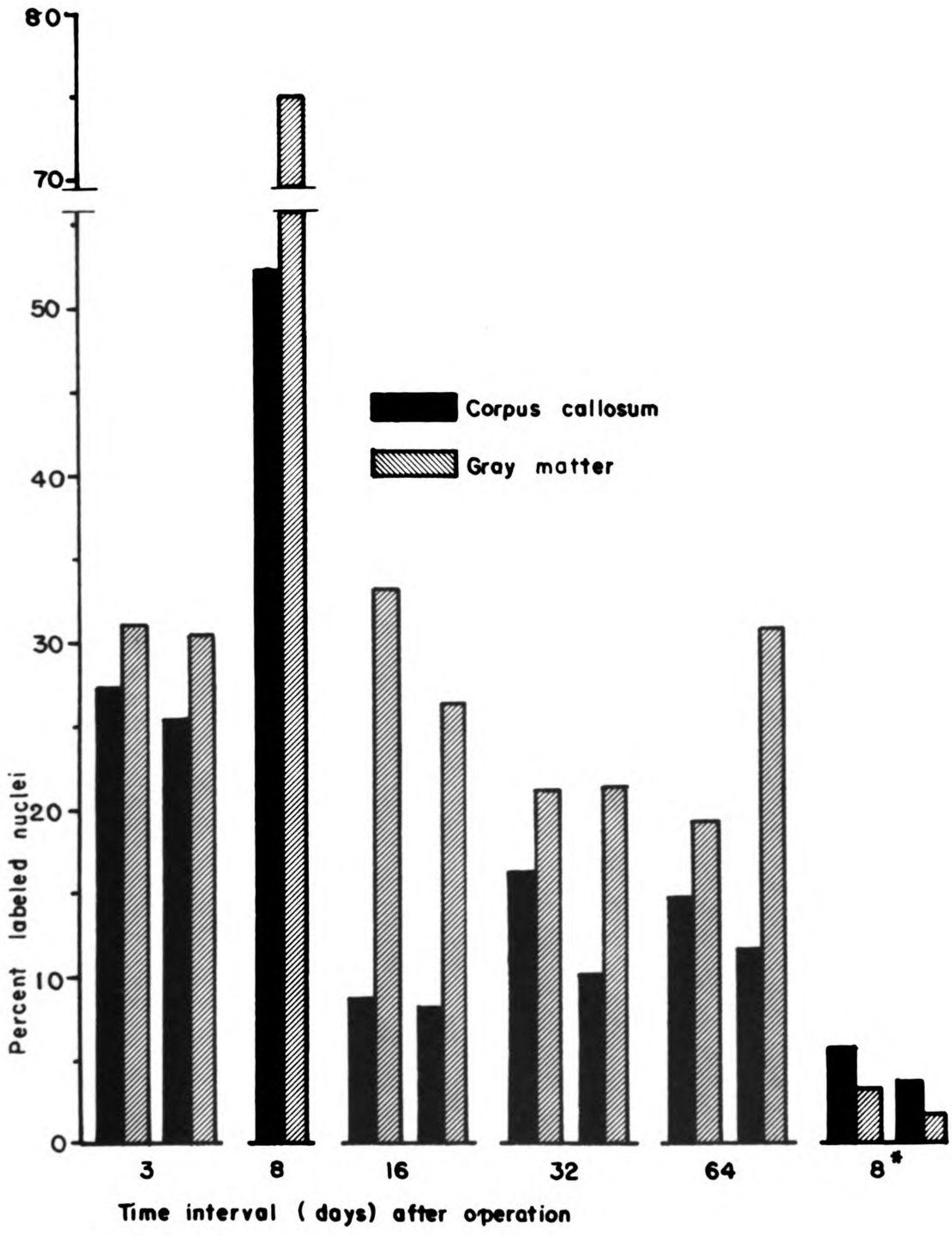
80
70
50
40
30
20
10
0
Percent labeled nuclei
Corpus callosum
Gray matter
3
8
16
32
64
8*
Time interval (days) after operation

FIGURE 4

Figure 5

Percent radioactive nuclei of each nuclear type in 0.04mm^2 of cortical gray matter along the wound in the operated hemisphere.

Animals were injected with 100 µc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to stab injury or tumor transplantation.

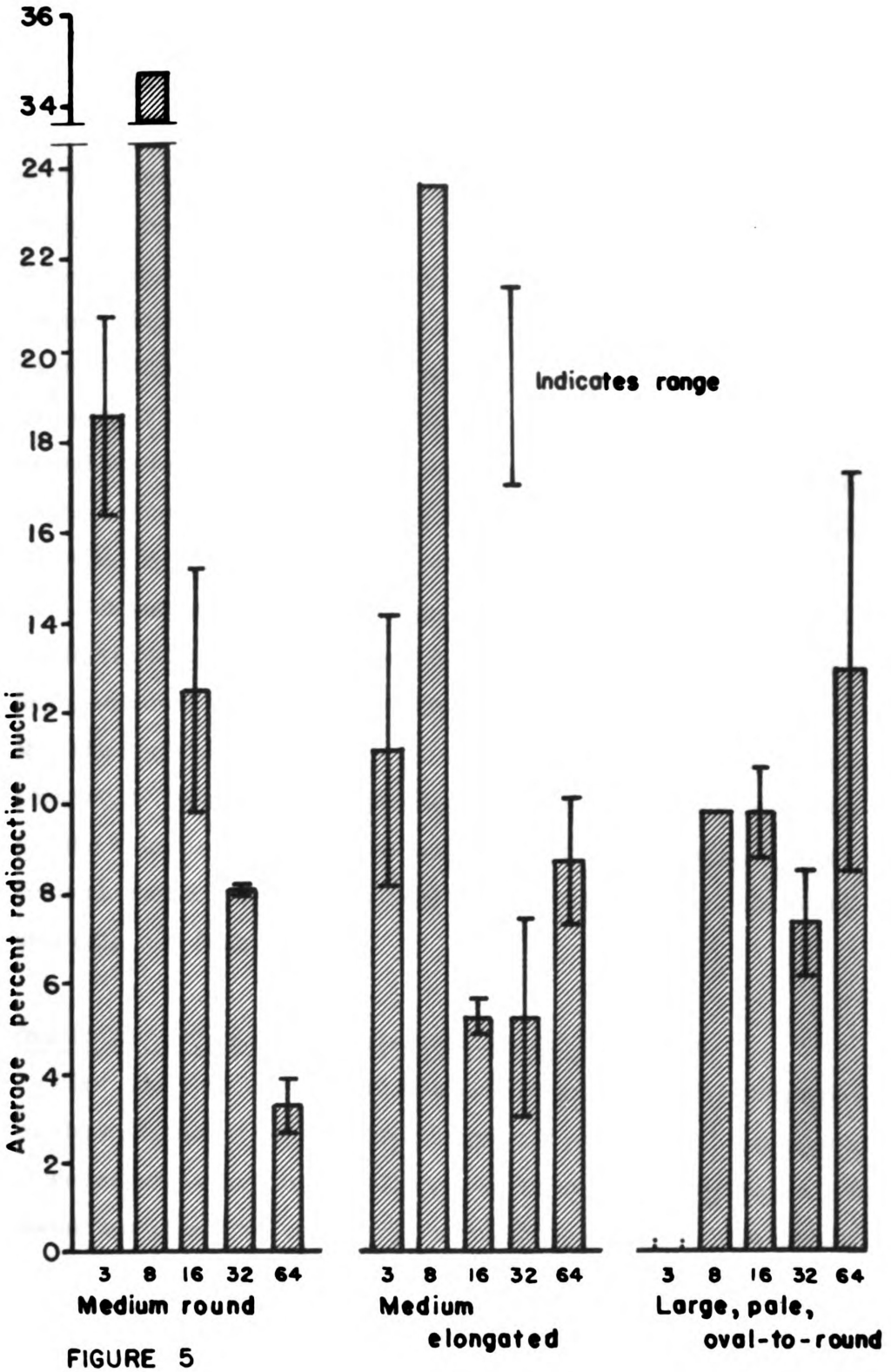
36
34
24
22
20
18
16
14
12
10
8
6
4
2
0
Average percent radioactive nuclei
Indicates range
3 8 16 32 64
Medium round
3 8 16 32 64
Medium elongated
3 8 16 32 64
Large, pale, oval-to-round

FIGURE 5

Mitotic figures

A search was made for mitotic figures at each time interval. In the animals injected with tritiated thymidine 1 day before injury, mitotic figures were seen only in the sections from animals killed 3 days after stab injury. Thirteen mitotic figures were counted in 0.270mm^2 of corpus callosum (0.045mm^2 in each of 6 sections). There were 3 prophases, 8 metaphases and 2 anaphases. All of the figures were considered radioactive except 1 prophase which had 3 silver grains overlying it. Eight mitotic figures were seen in 0.110mm^2 of corpus callosum (0.0275mm^2 in each of 4 sections). There were 2 prophases, 4 metaphases and 2 anaphases. Of these, 1 prophase, 1 metaphase and 1 anaphase were not radioactive. One mitotic figure in metaphase was seen in 0.04mm^2 of cortical gray matter of 1 animal injected 60 days before tumor transplantation. It was not radioactive.

Radioautographs of 20μ thick sections showed various degrees of labeling. Some sections appeared devoid of labeled cells in the tissue surrounding stab injury or tumor transplantation. Other sections showed labeled nuclei, but none of the sections showed lesioned areas with heavily labeled cells ($>$20 grains per nucleus). No quantitation of labeled nuclei was done on the slides from any of the animals because of the variability of the technique. These sections were used, rather, to count the total number of nuclei per 0.0845mm^2 of the corpus callosum (Table 3).

Formalin-ammonium bromide perfused and fixed material

Five slides which were prestained with gold chloride sublimate were viewed under the microscope before radioautographic processing. Large gold stained cells were seen in the corpus callosum of the injured hemisphere. After the emulsion was developed and fixed, the emulsion over the entire surface of the slides appeared discolored, the sections had turned a dark brown color and the cells could not be distinguished from the background. The slides that were processed for radioautography before staining with gold discolored when they were placed in the gold bath, and neither silver grains nor cells could be demonstrated.

Large, well-stained cells were demonstrated in gold stained sections collected in the alternating serial section method. These cells were found in the corpus callosum of the injured hemisphere. The alternating sections which were processed for radioautography demonstrated no silver grains over the tissue up to and including 14 weeks of exposure at 5^{o}C.

Figure 6

Three small, lightly-stained astrocytic cell bodies are seen in the center of the photomicrograph. Small pieces of fibrous material and cell bodies which did not appear to have processes were not counted. Unoperated animals and the uninjured hemisphere of operated animals showed a predominance of these cells. Cajal's gold chloride sublimate stain for astrocytes. X 640.

Figure 7

Large, well-stained astrocytic cell bodies are demonstrated in the upper left quadrant of the photomicrograph. The processes of these cells appear thicker and more heavily stained than those of the small astrocytic cell bodies. Fragments of fibrous material and cell bodies which did not appear to have processes were not counted. A predominance of large, well-stained cells was found in the corpus callosum of operated hemispheres. Cajal's gold chloride sublimate stain for astrocytes. X 640.

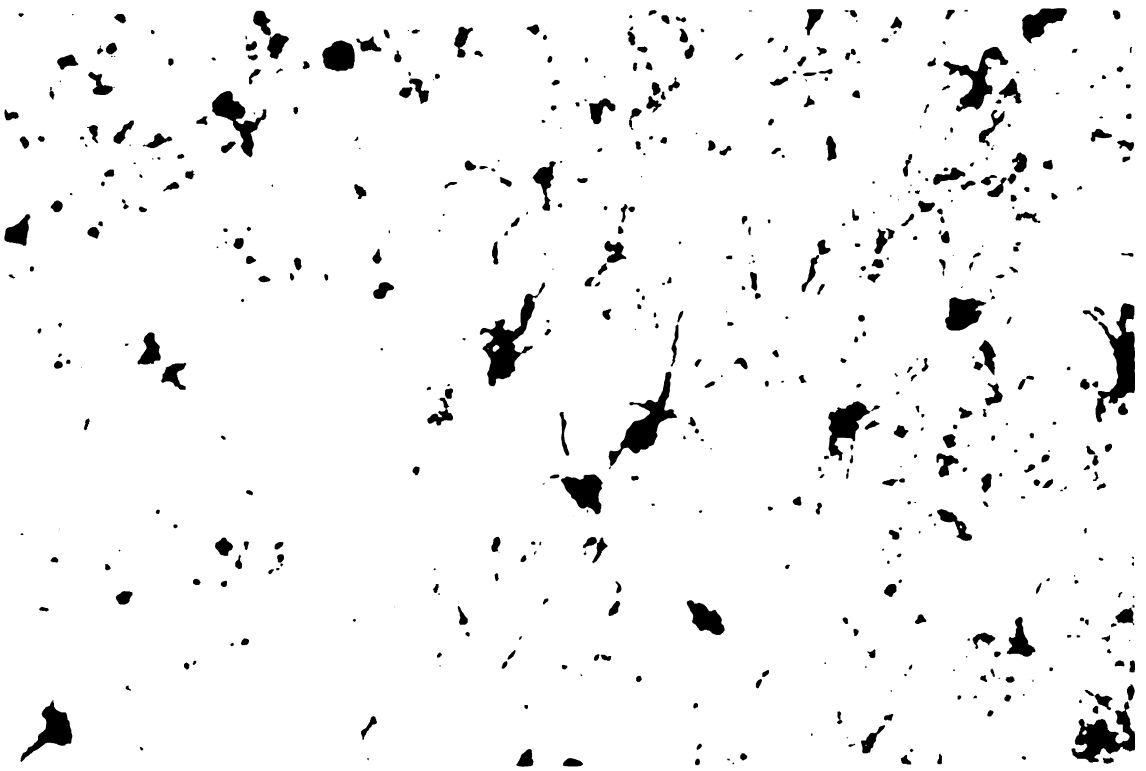

Figure 6. Small gold stained cells in unoperated hemisphere

Figure 7. Large gold stained cells in operated hemisphere

Figure 8

Large, well-stained cells are located in the corpus callosum and hippocampus, in the lower one-third of the photomicrograph. The lesion is located at the upper left and cortical gray matter occupies the remaining upper two-thirds of the photomicrograph. Cajal's gold chloride sublimate stain for astrocytes. X 280.

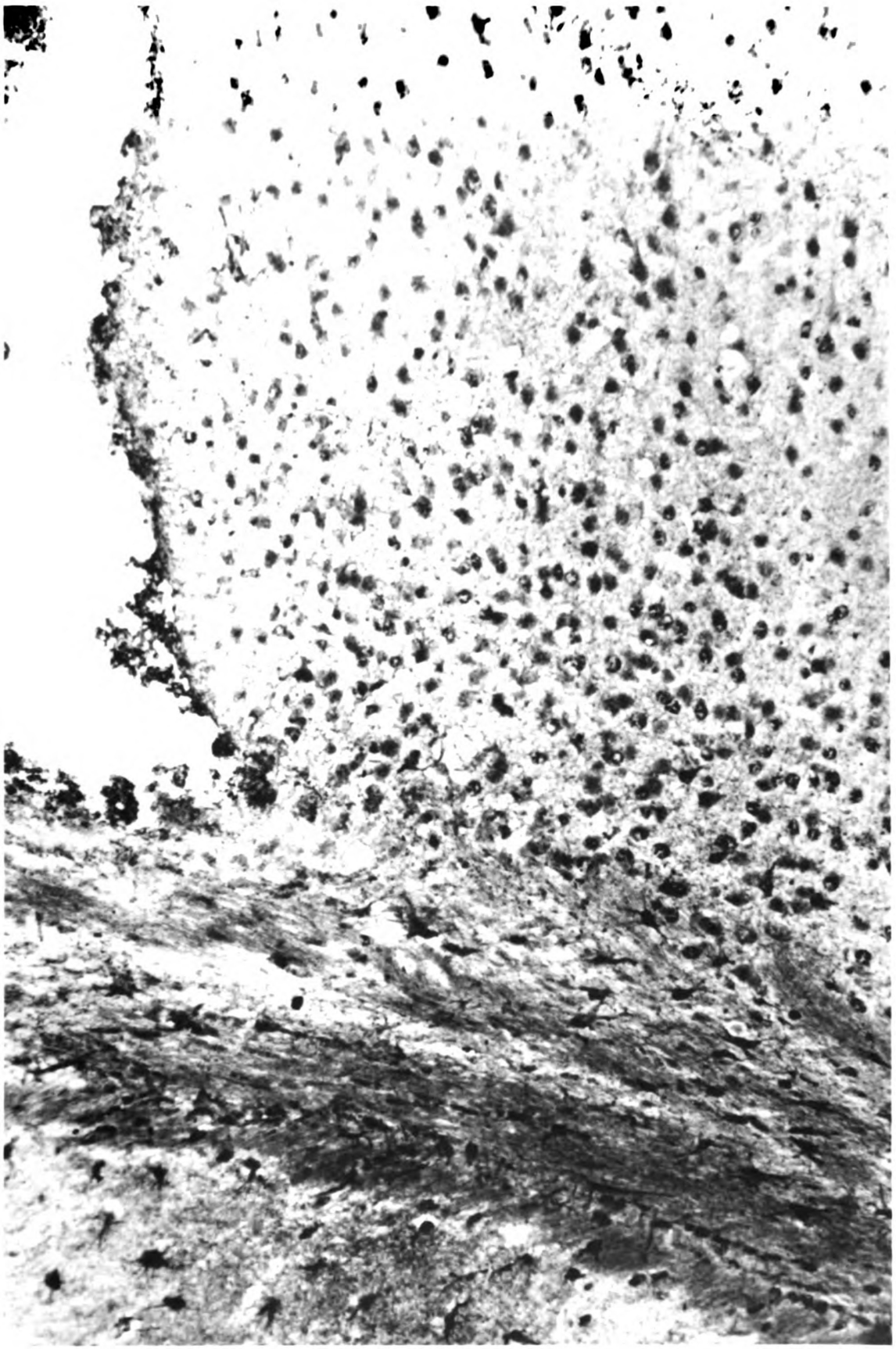

Figure 8. Large gold stained cells in the corpus callosum of the operated hemisphere 3 days after stab injury

Figure 9

Radioactive nuclei located in the cortical gray matter 50μ - 100μ from the edge of tumor 8 days after allografting. The animal was injected with 100μc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to tumor transplantation. The photomicrograph demonstrates labeled glial-like nuclei which are derived from the mononuclear leukocytes of the blood. Hematoxylin and eosin. X 720.

Figure 10

The section demonstrates radioactive medium round and medium elongated nuclei located in the cortical gray matter 100μ - 150μ from the edge of the tumor. The animal was injected with 100μc of tritiated thymidine 3 times at 4 hour intervals beginning 24 hours prior to tumor allograft and was killed 8 days later. Hematoxylin and eosin. X 640.

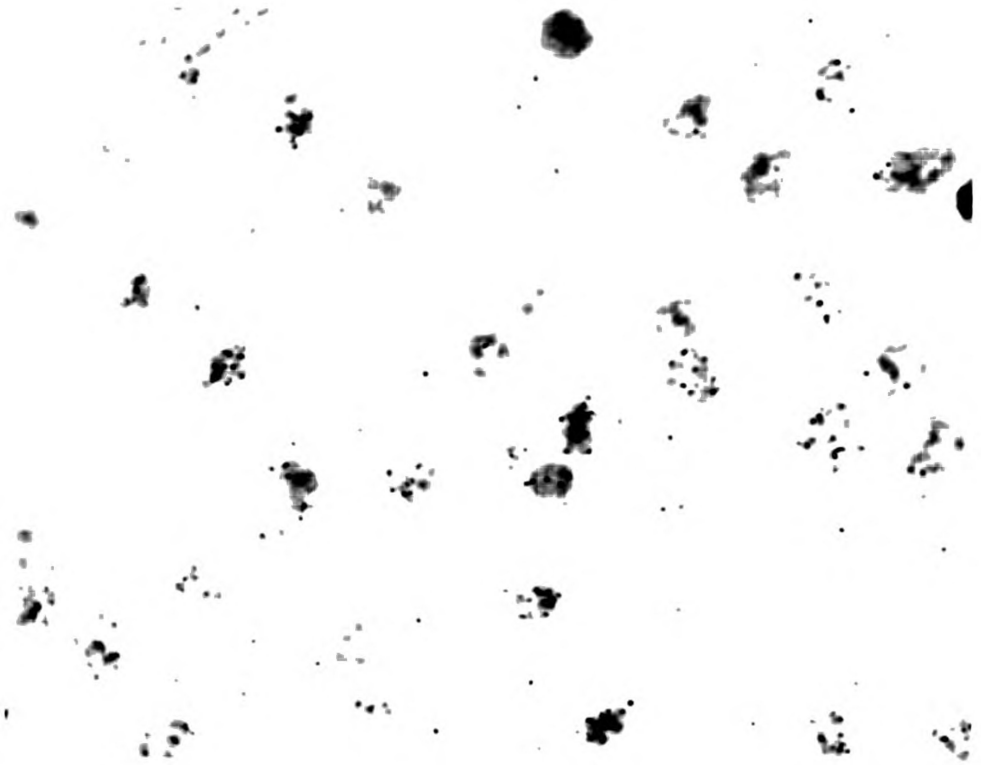

Figure 9. Radioactive nuclei in the cortical gray matter

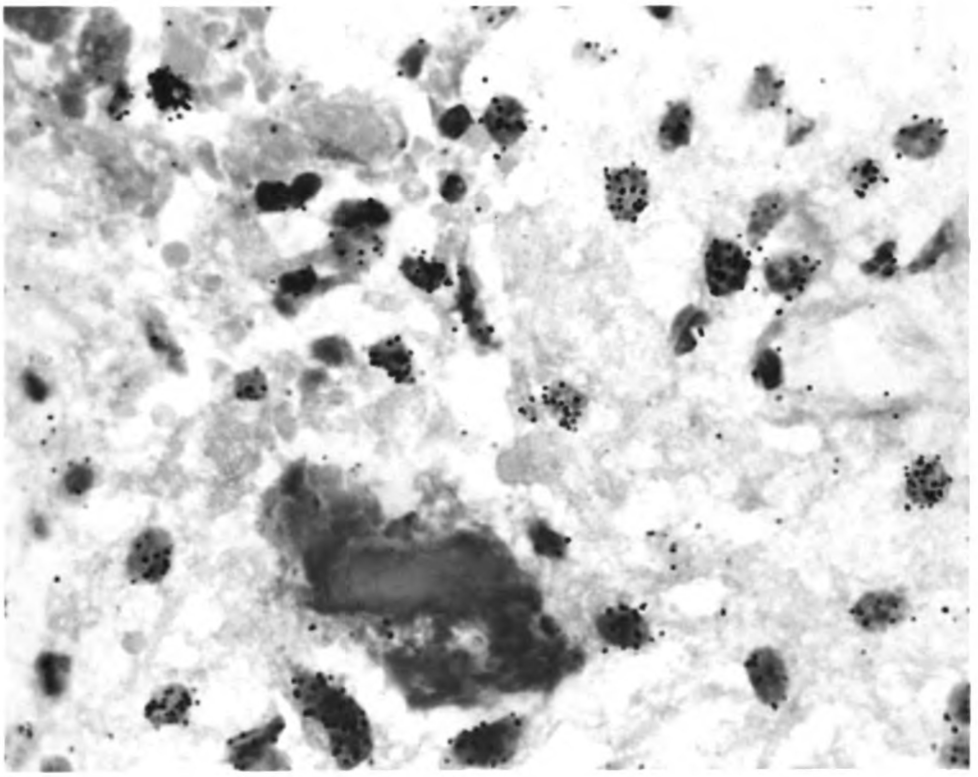

Figure 10. Radioactive nuclei in the cortical gray matter

Figure 11

Large, well-stained cell bodies are located in the corpus callosum and in the cortical gray matter lying just above it. The unstained tumor mass occupies the upper left quadrant of the photomicrograph; the corpus callosum is located in the lower right corner. Cajal's gold chloride sublimate stain for astrocytes. X 240.

Figure 11. Large gold stained cells in the corpus callosum of the operated hemisphere 8 days after tumor allografting

Figure 12

The unstained tumor mass lies in the left one-third of the photomicrograph. The large, well-stained cells in the corpus callosum and the cortical gray matter lying just above it are located in the lower one-half of the photomicrograph. The animal from which this section was taken was injected with tritiated thymidine as a juvenile animal. The animal from which the section shown in Figure 11 was taken was injected with radioisotope 1 day prior to transplantation. Cajal's gold chloride sublimate stain for astrocytes. X 280.

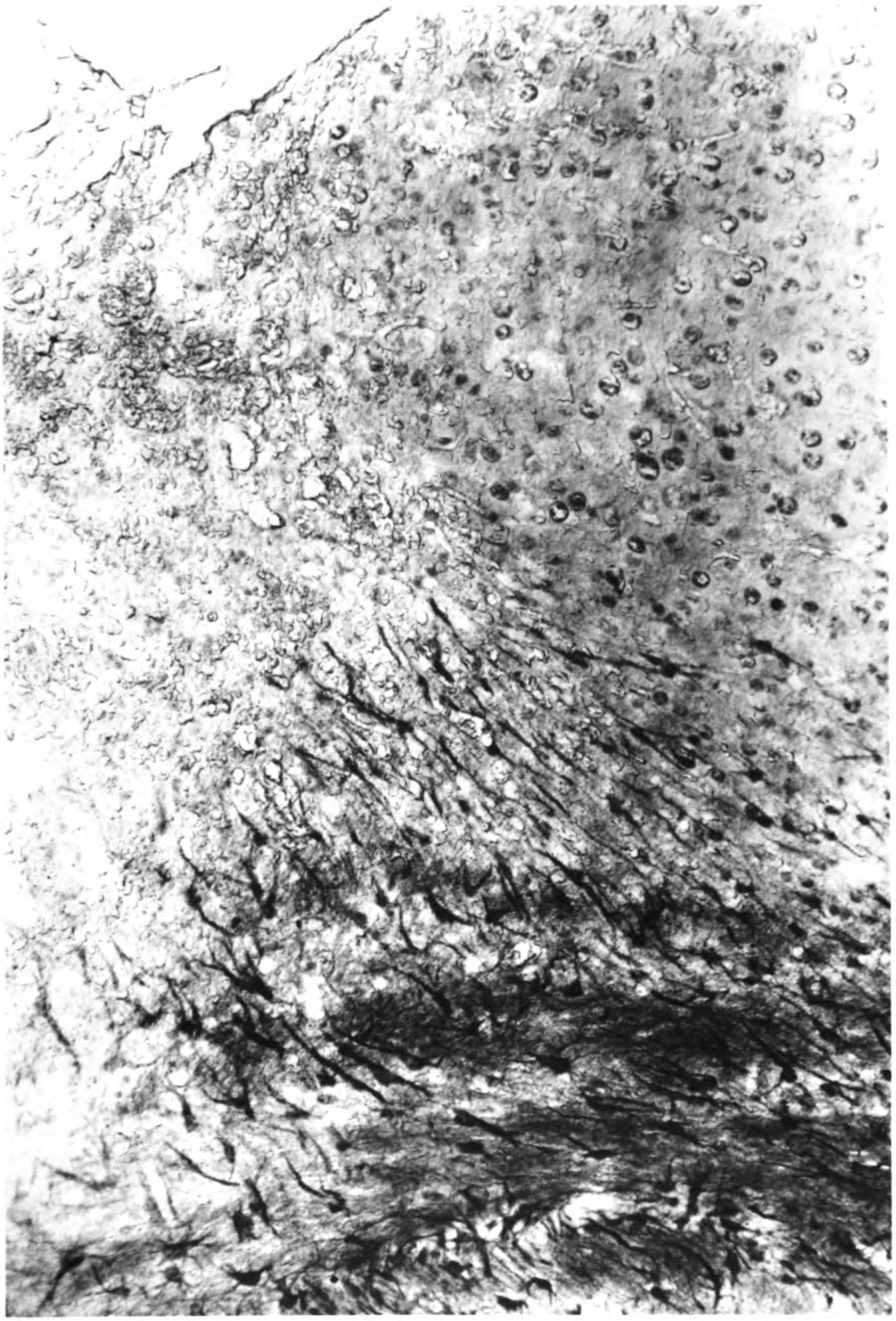

Figure 12. Large gold stained cells in the corpus callosum of the operated hemisphere 8 days after tumor allografting

Figure 13

Large, well-stained cell bodies located in the corpus callosum around the lesion 16 days after stab injury. The lower part of the lesion appears filled with debris. The corpus callosum can be seen at the lower right corner; cortical gray matter occupies the remainder of the photomicrograph. Cajal's gold chloride sublimate stain for astrocytes. X 280.

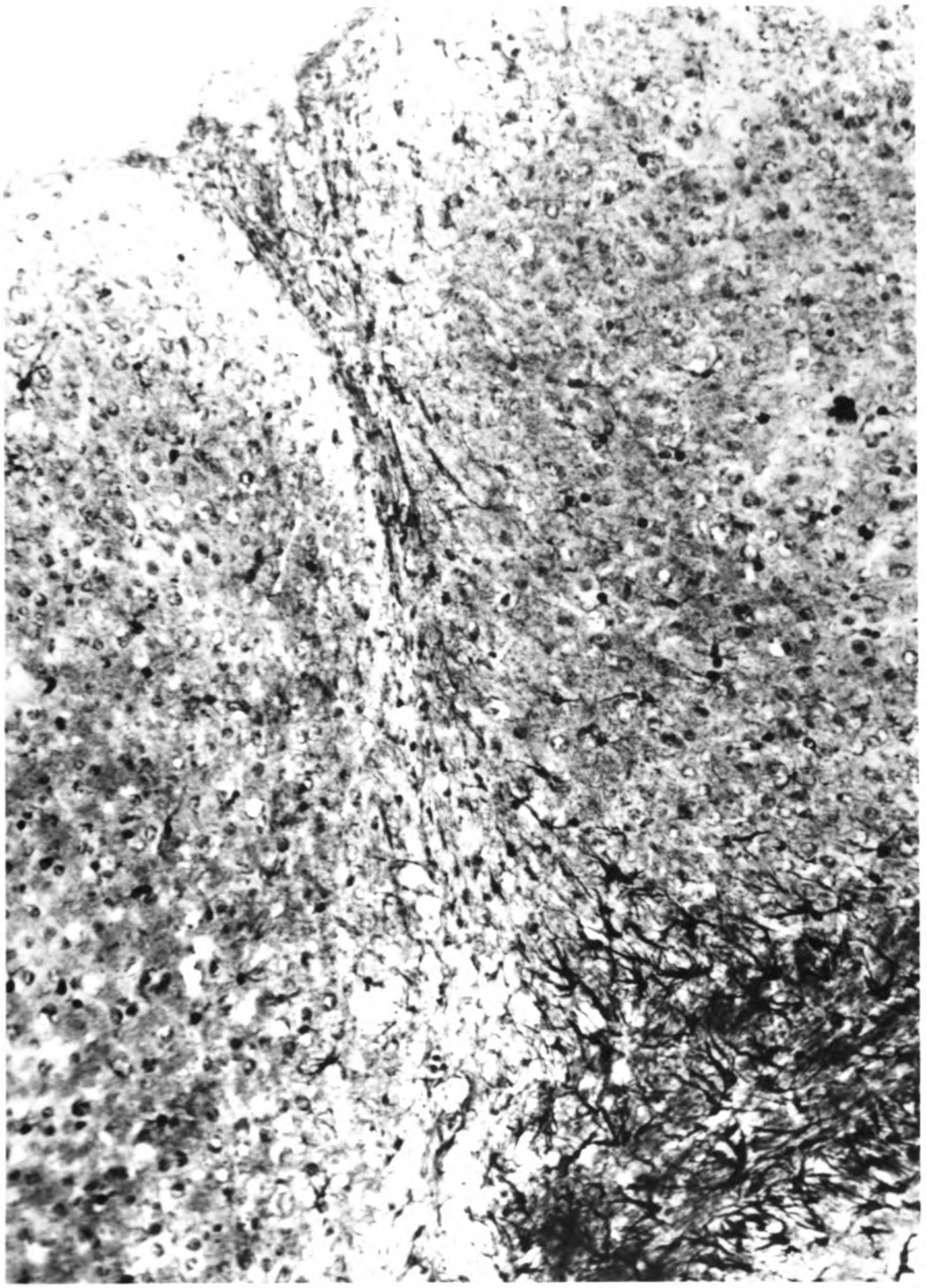

Figure 13. Large gold stained cells in the corpus callosum of the operated hemisphere 16 days after stab injury

Figure 14

Large, well-stained cell bodies can be seen in the corpus callosum at the bottom of the photomicrograph. The lower portion of the wound track is almost entirely filled with necrotic debris. A few astrocytes can be seen in the cortical gray matter just above the corpus callosum on both sides of the lesion. This wound extended deep into the hippocampal region. Cajal's gold chloride sublimate stain for astrocytes. X 280.

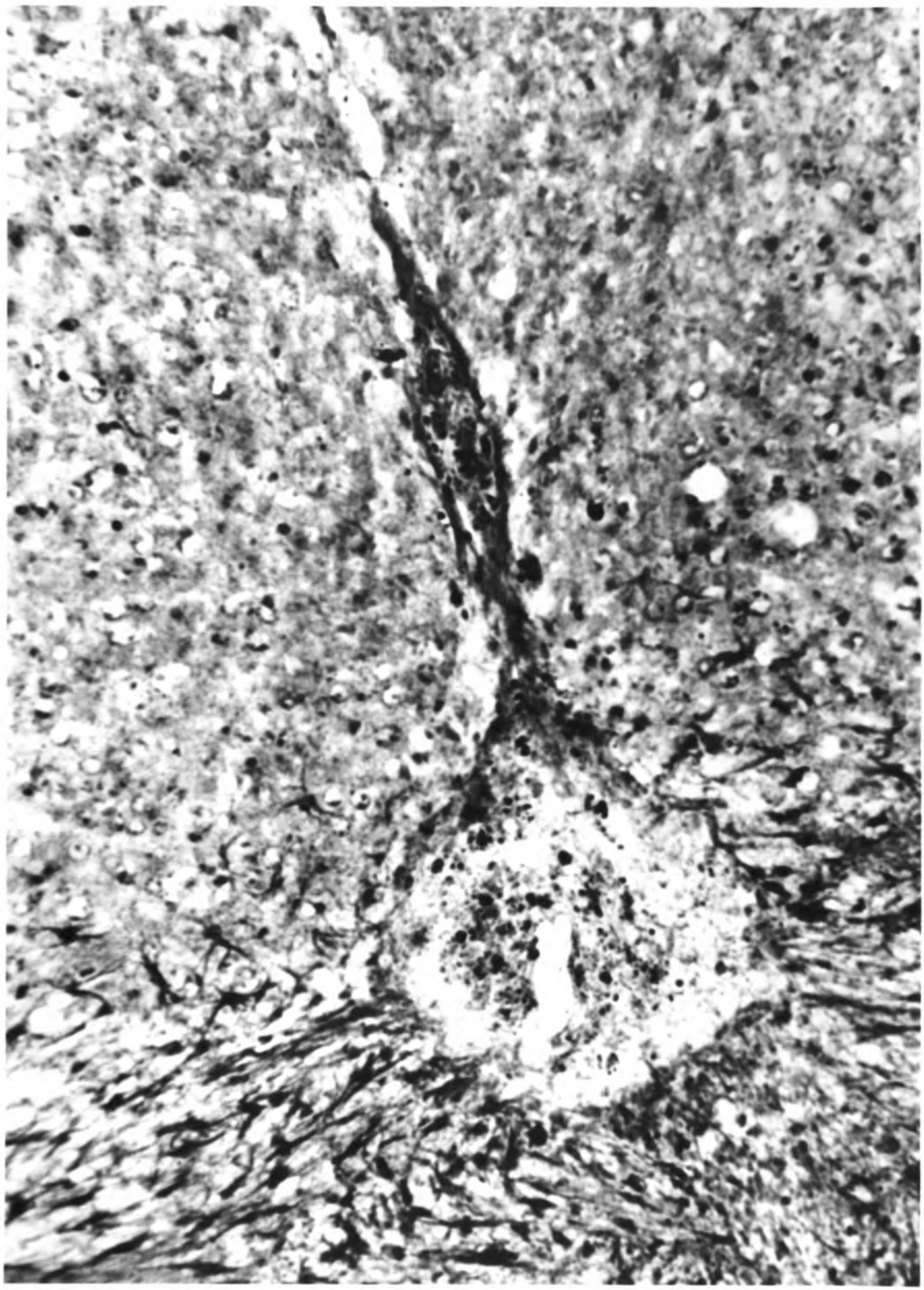

Figure 14. Large gold stained cells in the corpus callosum of the operated hemisphere 32 days after stab injury

Figure 15

Large, well-stained cell bodies located in the corpus callosum in the lower left corner of the photomicrograph. Cajal's gold chloride sublimate stain for astrocytes. X 280.

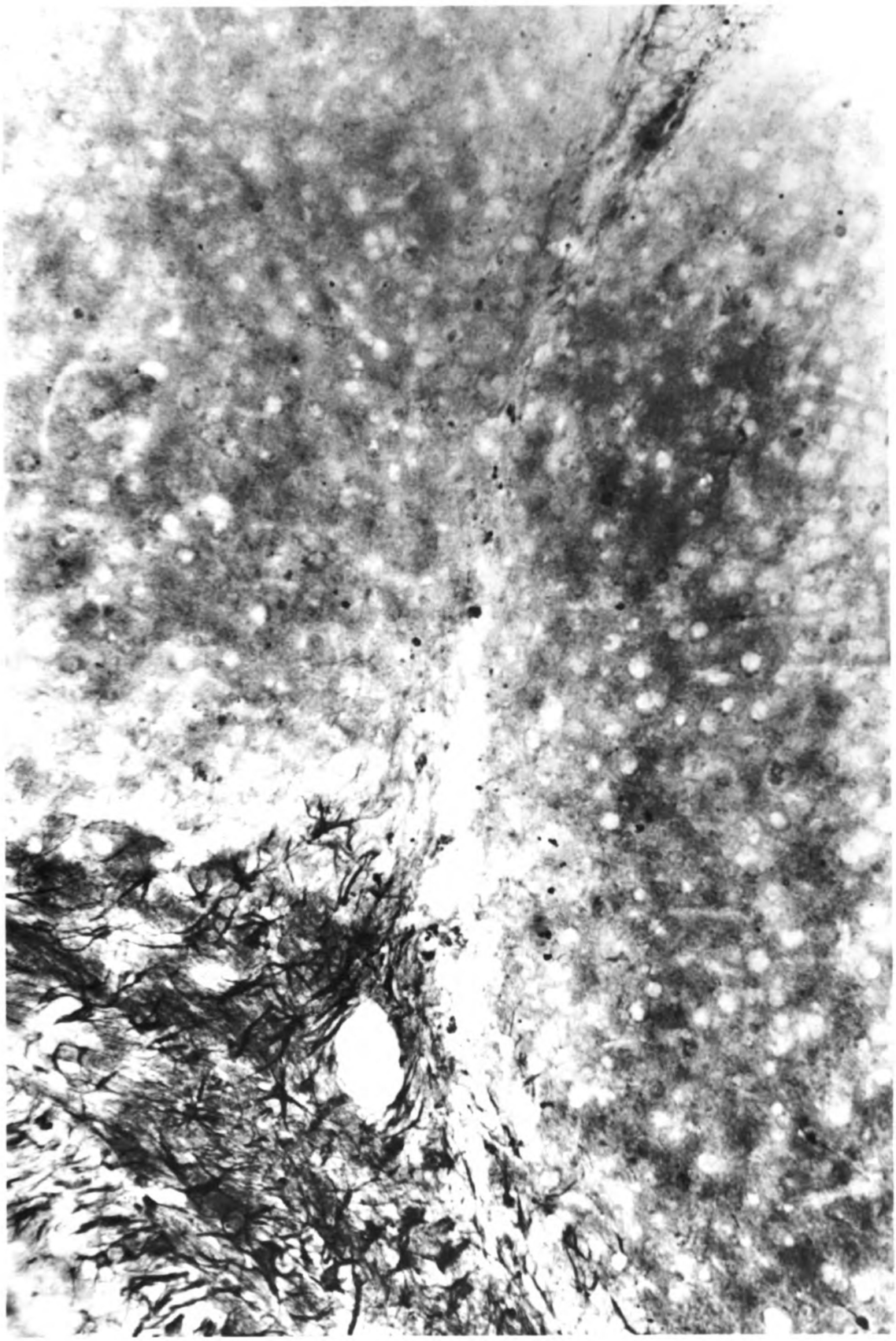

Figure 15. Large gold stained cells in the corpus callosum of the operated hemisphere 64 days after stab injury

DISCUSSION

The experiments described above were carried out to determine the answer to the question: Do the mononuclear leukocytes of the blood that respond to injuries of central nervous tissue give rise to cells that can be stained with gold chloride sublimate and which might, as a result, be mistaken for glial cells taking part in scar formation?

Mononuclear leukocytes are known to infiltrate central nervous tissue in response to injury (Adrian and Walker, 1962). Monocytes are reported to respond to stab injury of central nervous tissue (Konigsmark and Sidman, 1963; Huntington and Terry, 1967). Lymphocytes have been suggested as the agent responsible for rejection of allografted material in central nervous tissue (Murphy and Sturm, 1923; Smith and Walker, 1967) as well as in non-nervous tissues. Since lymphoid cells have been shown to enter an area of allografted tissue in non-nervous tissue with hypertrophy and formation of multiple, long thin cellular processes occurring in the area underlying the graft (Walker and Goldman, 1963; Walker, et al., 1964; 1965), it would not be unreasonable to expect this to happen in nervous tissue. The matrix of central nervous tissue is the dense neuropil with its highly interwoven glial and axonal network. Hypertrophy of the cytoplasm of lymphoid cells with attenuation of the cytoplasmic expansions due to the density of the neuropil could be expected. The attenuated cytoplasmic expansions might have an increased affinity for heavy metal stains. If so, the morphology could suggest a likeness to the astrocytic glia, cells indigenous to the central nervous system. In non-nervous tissue, hypertrophied lymphoid cells have been reported to form a layer underlying an allograft. This description is not unlike the "felting" of astrocytes around wounds of central nervous tissue reported by Linell (1929). Thus, if lymphoid cells demonstrated an affinity for heavy metal staining, they could be the cells which have been reported to take part in glial scar formation. Hypertrophy and the formation of

cytoplasmic processes could so change cellular morphology that the only means of identifying the origin and fate of these cells would be radioautographic techniques and the specific heavy metal stain for astrocytes.

Attempts to study injured central nervous tissue with the combined techniques of radioautography and specific heavy metal stains have given inconsistent results (Konigsmark and Sidman, 1963; 1964; Hommes and Leblond, 1967). Konigsmark and Sidman (1963) reported a combined gold stain-radioautographic technique in which the brain of an animal injected with thymidine-C^{14} was fixed in formalin-ammonium bromide. Sections, 20μ in thickness, were processed for radioautography after being prestained by Cajal's gold chloride sublimate stain. They reported labeled cells with large nuclei that did not take the astrocyte stain and no cells which were stained were labeled. At a later date (1964), they reported that, whereas radioautographs stained with toluidine blue showed 100 labeled cells per area, those stained with gold showed only 10-20 labeled cells in the same area. Hommes and Leblond (1967) reported that none of the well stained astrocytes appeared to be labeled in their study utilizing combined radioautography and Cajal's gold chloride sublimate stain. They too used 20μ thick sections, but their material had been fixed in 10% formalin.

In the present study, the radioautographs of material perfused and fixed in Cajal's formalin-ammonium bromide (FAB) solution demonstrated complete inhibition of the emulsion reaction to the β-particles from the tissue. No silver grains were seen over the radioactive tissue. Sections prestained or poststained with Cajal's gold chloride sublimate stain resulted in the loss of definition of gold stained cells. Because there was no consistent method found to combine the gold stain with the radioautographic technique, and since there was variability noted in radioautographs of 20μ thick frozen sections of material fixed in 10% formalin, a radioautographic technique that had given reproducible results in the past was used for analysis in this study. Evaluation of the role that lymphoid cells play in the gold stained cell reaction to central nervous injury had to be inferred from studies of the two populations.

The experimental approach was based on the hypothesis that if the population of gold stained cells responding to central nervous tissue injury were derived from the population of mononuclear leukocytes, then, within a defined area, the two populations would be expected to react similarly. If, on the other hand, the two populations showed dissimilar responses to injury, stab wound and the immunological stimulus of tumor allograft, then the hypothesis would be considered false.

Large numbers of labeled cells, over 50% of the total number of cells, were seen in the cortical gray matter and the corpus callosum surrounding the tumor allograft. Over 20% of the cells surrounding a stab wound 3 days after injury were labeled. These labeled cells were known to have infiltrated the lesioned area from the blood because the radioisotope was injected 1 day prior to injury. Thus, none of the label was available to cells synthesizing DNA in response to the injury. Injection of radioisotope into unoperated animals would have been expected to label extremely few cells in the nervous system (Table 1; Smart and Leblond, 1961; Adrian and Walker, 1962). This method of tracing labeled leukocytes has been used in the past to study injured muscle (Bintliff and Walker, 1960), spinal cord (Adrian and Walker, 1962; Walker, 1963; Adrian, 1968) and brain (Konigsmark and Sidman, 1963; Huntington and Terry, 1967; Smith and Walker, 1967).

Fluctuations in the labeled cell population were noted at different survival intervals after stab injury, and a large influx of labeled cells occurred in response to tumor allograft (Tables 6 and 12, Figure 4). These fluctuations in the labeled cell population are consistent with the theory that mononuclear leukocytes migrate in and out of a lesioned area. Macrophages from the blood infiltrate the area, phagocytize necrotic debris and many leave the area. Lymphoid cells would be expected to migrate into an area in response to antigenic stimulation. This population of cells would be expected to remain in the area until the stimulation subsided. Over 2 months after stab injury, many labeled cells were still seen to surround the lesion (Tables 6 and 12). Significant differences were noted in the labeled cell populations at different time intervals after stab injury and after tumor allograft (Table 8).

The large gold stained cell population of the corpus callosum, on the other hand, reacted differently than the labeled cell population. Large fluctuations in the size of this population were not seen, either in response to stab injury or to tumor allograft (Table 3, Figure 2). No significant differences were demonstrated between individuals killed at the same interval or between animals killed at different time intervals after stab injury (Tables 4 and 5, Figure 2). Significant differences were not seen between the two individuals that had received tumor allograft (Table 4). Nor were there significant differences noted in the population of gold stained cells between animals which received stab injury and those which received tumor allograft to the brain (Table 5). Large gold stained cells were not seen in the cortical gray matter surrounding the lesion. Comparison of the labeled cell population with the large gold population in the corpus callosum showed that, within approximately the same area ($0.0900mm^2$ and $0.0845mm^2$), the two populations do not respond similarly to either stab injury or tumor allograft (Tables 3 and 6, Figure 2).

To determine whether one of the subgroups of labeled nuclei rather than the total labeled population influenced the gold cell population, the latter was compared with the subgroup populations. Each of the nuclear subgroups fluctuated with the type of stimulus and the survival interval after stab injury, just as had been noted for the total labeled cell population (Tables 9 and 12, Figures 3 and 5). Significant differences were noted for each nuclear type between the individuals with the same survival time and between animals with different survival intervals after stab injury (Tables 10 and 11). The results of this comparison demonstrated that these subgroup populations respond differently than does the gold cell population. Therefore, the gold cell population is not influenced either by the total labeled cell population or a subgroup thereof.

Furthermore, when the glial population was labeled in the juvenile animals, there was no evidence of migration of these cells to an area of tumor allograft. This finding was consistent with previous work (Smith and Walker, 1967). There was a decrease in the frequency of labeled nuclei 8 days after allografting, but this could be explained by the number of infiltrating mononuclear luekocytes, which would not have been radioactive due to dilution of label below detectable levels.

Lymphoid cells do infiltrate central nervous tissue in response to stab wounds and tumor allografts, but these cells do not take on the gold stain. Considering the behavior of the gold stained cells of the corpus callosum, it is reasonable to accept the classical definition that these are astrocytes and that they had hypertrophied in response to the stab wound or tumor allograft. Lymphoid cells may hypertrophy after infiltrating central nervous tissue, but they don't contribute to the gold staining cell (astrocyte) population of the corpus callosum.

Nuclear hypertrophy was noted in response to stab injury of the brain (Tables 9 and 12, Figures 3 and 5). This finding is consistent with previously reported work (Walker, 1963) which suggested that lymphocytes infiltrated an area surrounding stab injury in spinal cord and their nuclei hypertrophied into glial-like nuclei. In the present study, the labeled medium-round and medium-elongated nuclei decreased in frequency between 3 days and 16 days after stab injury. The population of large, pale, oval-to-round nuclei increased in frequency over the length of the experiment, as had been reported previously (Walker, 1963).

Labeled mitotic figures noted at 3 days after stab injury indicate that the mononuclear leukocytes which infiltrate an area of central nervous tissue injury continue to divide as reported by Konigsmark and Sidman (1963) and Smith and Walker (1967).

SUMMARY AND CONCLUSIONS

Tritiated thymidine radioautography and Cajal's gold chloride sublimate stain for astrocytes were used to determine if the mononuclear leukocytes which invade central nervous tissue in response to injury might be stained with gold, and thus, might be mistaken for the gold stained cells which are reported to take part in glial scar formation. Unoperated adult animals had a low frequency of labeled cells in the brain. Unoperated hemispheres of brains of injured animals also showed a low frequency of labeled cells in the brain. In animals injected with tritiated thymidine 1 day before operation, large numbers of labeled cells were seen in the corpus callosum and the cortical gray matter along the wound 3 days after stab injury and 8 days after tumor transplantation. Because of the time of labeling, these cells were known to be mononuclear leukocytes. The labeled cell population fluctuated in response to the type of stimulation, stab injury or the immunological stimulation of tumor allograft, and with survival time after stab injury. Furthermore, when the cells were classified as to nuclear type, the subgroups of the labeled cell population also fluctuated with the type of stimulation and with survival time after stab injury.

The role of glial cells in the response to tumor transplantation was evaluated by injecting juvenile mice with tritiated thymidine (6 gram body weight) 60 days before operation. Injured hemispheres of these animals showed no evidence for migration of labeled cells to the area of the tumor allograft.

The gold cell population of the corpus callosum remained stable with both types of stimulation and with time of survival after stab injury. These cells were not seen in the cortical gray matter along stab wounds or surrounding tumor allografts.

Because the mononuclear leukocyte population fluctuated in response to injury or transplantation and the gold cell population remained stable under the

same conditions, it was concluded that the two populations were independent and that the hypertrophied lymphoid cells were not stained by Cajal's gold chloride sublimate stain for astrocytes. The gold stained cell population of the corpus callosum (astrocytes) hypertrophied in response to stab injury or tumor transplantation.

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