# CEREBRAL ISCHAEMIA AND ITS RELATION TO EPILEPSY.

by

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# INDEX - I.

# INDEX - II.

EXPERIMENTAL DATA.

# INDEX - III.

## PART II.

## MENINGO-CEREBRAL CICATRIX FOLLOWING CRANIOTOMY AND AFTER ELECTRICAL STIMULATION OF THE CORTEX.

INDEX -IV.

## INTRODUCTION.

During many years a number of observers have presented evidence suggesting that cerebral ischaemia, resulting from local vascular spasm, or from suddenly diminished blood supply to the brain as a whole, may be instrumental in producing an epileptic seizure. Investigators have also demonstrated that an epileptic discharge may originate in an area of cortex which has been pathologically altered by a previous ischaemia. In the investigation described here these two aspects of the problem of epilepsy have been studied.

The first part of the thesis deals with vasospasms and focal cerebral ischaemia produced by mechanical and electrical stimulation of pial and cerebral arterial vessels. In the second part of the thesis is described the histological changes in the cortex and meninges resulting from the above type of stimulation and also the changes following exposure of the brain under different conditions.

The purpose of the investigations recorded in part 1. of the thesis has been to determine the factors underlying the reactions of pial and cerebral vessels to stimuli applied directly to their walls. This opens up a problem which becomes ever wider with each new fact brought to light. In the past most of the work done on the reactions of pial and cerebral vessels has been directed toward the study of their neurovascular control and little attention has been paid to the inherent reactivity of smooth muscle itself. Fortunately the physiology of smooth muscle has been studied quite extensively in

other organs and in blood vessels elsewhere in the body. Many of the facts gathered from these studies will undoubtedly be found directly applicable to the smooth muscle in intracranial vessels. To separate the reactions inherent in the smooth muscle of pial vessels from those dependent upon the presence of neurovascular nerves is a problem which has only been started here; for Cannon has recently given us added insight into the complexity of the problem by demonstrating how smooth muscle becomes hyperirritable when deprived of its nerve supply. Such an observation may well lead to the discovery of mechanisms underlying many phenomena so far unexplained.

Although the methods of stimulation employed in the experiments described in this thesis, are grossly unphysiological the fact that they cause pial vessels to constrict would seem to be of practical importance, especially if, as a result, there occurred ischaemia in the underlying cortex; for the brain is frequently subjected to just such unphysiological stimulation in head trauma, embolism, electric shock, and electrical stimulation at operation and in the laboratory. Also if focal cerebral ischaemia could be produced in this manner the method might be used to investigate the old theory that local vascular spasm and focal ischaemia at times is the mechanism underlying epileptic seizures.

In part 2. of the thesis are described the histological changes in the brain and meninges resulting from mechanical and electrical

stimulation and from simple exposure at operation. An attempt has been made to determine which of these changes is due to drying, alterations in temperature, ischaemia, mechanical trauma or electricity

As mentioned in the preceeding lines the brain is frequently subjected to both mechanical and electrical stimulation. It is usually taken for granted that electrical stimulation as employed in physiological experiments has no injurious effect on the nervous system. To determine whether this is true is one of the objects of this investigation. As for trauma we know that a laceration or severe blow to the brain is injurious, but concerning the minimal amount of mechanical trauma required to produce histological change little investigative work has been done. This also holds true of the effect of drying and temperature changes on the brain and meninges. Such knowledge is of particular importance in operative neurosurgery. Craniotomies must be carried out and an attempt has been made here to determine how this may be done with a minimum degree of injury to the brain and meninges.

Unfortunately, in a problem so extensive, the present investigation has made only a meagre mark on the field to be covered. However for the writer new paths have been opened and the way seems clearer.

# PART 1.

## VASOSPASMS AND FOCAL CEREBRAL ISCHAEMIA PRODUCED BY MECHANICAL AND ELECTRICAL STIMULATION OF PIAL AND CEREBRAL ARTERIAL VESSELS.

### REVIEW OF THE LITERATURE

The literature directly related to the experiments described in the first part of the present thesis is scanty. It is concerned with investigations of the effect of mechanical and electrical stimulation on pial and cerebral vessels. However no phenomenon is an isolated event but is the result of a never ending chain of causes and effects. In studying the action of local stimulation on blood vessels, therefore, one must consider whether the stimulus acts on the vessel wall directly or on the nerves supplying it. This immediately brings up the question of the vessels anatomy, and in fact the physiology of smooth muscle in general. If vascular nerves play a part in the reaction then the whole problem of the vasomotor control of cerebral vessels must be considered. To cover such a literature would require more space than is necessary in this thesis. An attempt shall therefore be made to present the more important aspects of such a literature.

#### The Anatomy of cerebral blood vessels.

Bonnet (1896) felt that the wall of the cerebral arteries should be divided into six layers, as follows: (1) the endothelial tube; (2) the longitudinal fibre coat; (3) the elastica interna; (4) the layer of circular fibres; (5) the elastica externa; (6) the adventitia (or externa). He makes these divisions both on embryological and histological grounds.

Ranke (1915) agreed with this classification as have most other investigators on this problem.

Triepel (1897) and Cobb and Blain (1933) have shown that in the cerebral arteries the elastic fibres have a different arrangement and possibly are more numerous than in other parts of the body. Hackel(1927-28) pointed out that although the inner elastic layer varies in thickness, the thickness of the elastic membrane in relation to that of the muscle layer is in general greater in the brain arteries than it is in many other parts of the organism.

The transition of structure from that usual in extracranial arteries to that of the intracranial arteries was shown by Triepel (1902) in studying the arteries as they enter the posterior fossa of the cranium. In the transition there is an increase of the elastic tissue and more especially an aggregation of the elastic tissue into the inner elastic membrane, which assumes a greater thickness as the arteries enter the skull. Also there disappears from the muscle layers the irregularly grouped elastic fibres. Benninghoff (1930) has remarked that the intramural stretch imposed by the blood pressure seems to be adequately taken care of by the very strong internal elastic layer and by the media. According to Wolff (1938) the cerebral arteries would seem to be built to meet pressure requirements only from within; for both cerebral and dural arteries in passing into the region protected from outside pressure, reduce the adventitia and in man especially, the

elastic longitudinal fibres are almost completely lost.

According to Ranke (1915) the capillaries of the central nervous system have two unusual features. One is the connective tissue that lies between neighbouring capillaries, in the form of small bridges of adventitial cytoplasm. The other feature is that the capillaries of the cerebral cortex possess a special internal membrane. Such a membrane has never been observed by Cobb (1932) and according to him the capillaries in the brain appear much like the capillaries in other parts of the body.

The walls of the veins of the brain are extremely thin in relation to the large lumen, and they are composed largely of connective tissue. The small veins that run out of the capillaries are indistinguishable in their structure from the pre-capillary arterioles. In the larger veins that adjoin these there is an elastic membrane, but it is thin and as the veins become larger there is relatively less and less elastic lamina. This is especially true of the veins of the white matter. In the veins of the pia the elastic layer is reduced to a very thin ring lying just external to the endothelium (Cobb, 1932).

In summary one may quote Wolff (1938) - "The blood vessels of the brain differ in no histological essential from vessels elsewhere, though there are minor differences". And, as mentioned, Cobb (1932) feels that the capillaries in the brain appear much like capillaries elsewhere.

Angio-architecture and the question of end arteries in the brain.

These two problems are of particular importance in the present investigation where stimulation of various types has been applied to the pial vessels; for by this means it has been possible to produce cerebral ischaemia despite the rich anastamosis of vessels and sparsity of end arteries. Also, interesting areas of gliosis, following various types of stimulation, have been found in the cerebral gray matter, and the pattern of this gliosis has appeared to be dependent upon the anatomical arrengement of the cerebral vessels.

The cerebral arterial tree in man unlike that of many organs, has no hilum from which the vessels plunge into the body of the structure. On the contrary, the internal carotid and vertebral arteries are united by the circle of Willis, and its six large branches, which encircles the globoid hemispheres at their base. These six great trunks then divide into branches. A few enter the basal ganglia and choroid plexus, but for the most part, they spread themselves like a net in finer and finer branches over the surface of the cortex. Smaller arteries at innumerable points dive deeply into the cortical and sub-cortical tissues where, through their capillaries, they anastamose with one another and with others coming through the brain substance from the opposite surface of the hemisphere (Cobb, 1932).

This gives us a general picture of the gross anatomical arrangement of the vessels. Studies of the relative vascularity of various parts of the nervous system have been carried out on the rat by Craigie, 1920, 1921, 1930, 1931 and 1932, and on the cat by Campbell (1937-38) and Finley (1936). Craigie (1920-21) was the pioneer investigator to use quantitative methods in the study of the blood supply of the brain. A short history and a complete bibliography of the study of cerebral vascularity are included in his first paper (1920). His micrometric observations prove that the gray matter of the central nervous system is much more richly supplied with capillaries than the white matter. In fact in the regions studied the poorest part of the gray was nearly half as rich again in capillary supply as the richest part of the white matter; he found that lamina IV (granularis interna) was always the most vascular in the albino rat; laminae III (pyramidalis) and V (ganglionaris) were less vascular, while I and VI were very poor in capillaries. This held for specimens taken from the parietal, occipital, temporal, prefrontal and insular areas.

The angio architecture of the brain is closely connected with the cyto-architecture and myelo-architecture which have been so carefully studied by the Vogts. Pfeifer (1928) has studied the vascular anatomy in the cat and the plates in his monograph illustrate the correspondence of vascular to cellular histology. Some of his illustrations of injected preparations (for instance of the gyrus

lateralis of a cat) shows beautifully the dark strips made by the rich capillary bed in laminae 111, 1V and V as compared to the relatively lighter inner and outer layers of the cortex.

Cobb (1929) in preparations of rabbits brains made by injecting Berlin Blue intravitam showed essentially the same thing. He demonstrated that the neopallium (or isocortex) is generally supplied by surface vessels, small arteries plunging in from the pia, giving off a few arterioles and capillaries in lamina 1 and many more in laminae 11, 111, 1V and V; beneath this, lamina V1 is again less vascular; the white matter below resembling the white matter elsewhere.

As to the question of end arteries in the brain there has been some dispute. Cohnheim (1872) clearly annunciated the theory that infarcts could occur only where there was no anastamosis between arteries. He inferred that the arteries in the brain are end arteries and that emboli in the brain usually cause simple necrosis, while in the spleen and lung the infarcts are usually hemorrhagic.

Fay (1925) made injections of metallic mercury into human brains removed at autopsy and then made records of the injections by means of stereoscopic X-ray plates. The results are clear cut and prove that there are many anastomotic arterial trunks between the anterior, middle and posterior cerebral arteries. He believed that there is no evidence of anastamosis between the large vessels

supplying the subcortical white matter nor in the vessels of the basal ganglia.

According to Cobb (1931), Pfeifer in two monographs (1928 and 1930) proved beyond doubt that there are no end arteries in the brain. He showed by his injection preparations that the capillary bed of the whole cortex is one endless network and stated that a red blood cell, if given motive power, could travel through normal, open vessels from the occipital pole to the tip of the frontal lobe.

Lorente de No (1937) however denies ever having seen true artery anastamoses although both Lorente de No (1927) and Cobb (1931) have described free anastamosis in the capillary bed.

Wislocki (1937) and Lorente de No (1937) point out that Pfeifer in his profusely illustrated memoirs, brings no convincing evidence of arterial anastamosis, what he shows being, at most precapillary anastamoses. Although in the pia of the cat, arteries 10 to 15 mu diameter commonly anastamose with each other, Forbes (1937) and Campbell (1938) have observed in the cat a few anastamozing arteries in the basal ganglia; according to Wislocki and Campbell (1937) the vast majority of the arteries in the brain of cats, rats, rabbits and monkeys are end arteries, i.e. arteries which as such do not anastamose with each other.

Pfeifer (1928 and 1930) describes arterio-venous anastamoses in the pia, the arterial blood under high pressures supposedly hastening the blood flow in the veins. Wolff (1938) however states

that most workers have doubts as to the actual existence of such arterio-venous anastamoses and the general acceptance of such structures depends upon additional and more convincing evidence.

## Nerve supply of pial and intra-cerebral blood vessels.

### Anatomical evidence.

Contrary to the older conceptions it is now recognized that both pial and intracerebral vessels, in common with other tissues, are supplied with nerves. Nerves on the pial vessels were first described by Hüber in 1899. The older literature on this subject has been well reviewed by Stöhr in 1922 and by Dowgjallo in 1932.

In 1922 Stöhr gave a detailed and critical description of the innervation of the human pia and pial vessels. Little has been added to Stöhr's contribution. He used a modification of Schultze's sodium-hydroxide silver method and described an abundant nerve supply to the pial arteries made up of myelinated and unmyelinated fibres. These nerve fibres form superficial and deep plexuses in the adventitia of the arteries, the latter plexus lying just outside the media. He could rarely trace any fibres into the tunica media and could not demonstrate motor nerve endings there. Sensory endings in the adventitia of the larger pial arteries were described, but the most elaborate endings were found in the arterioles of the pia. Very rarely he found nerves loosely accompanying capillary vessels in the pia and giving off fine knob-like endings on their walls. Stöhr considered that these

were in direct functional relationship to the capillary walls. With his methods Stöhr was unable to find any nerves extending from the pia along intracerebral vessels.

In 1893 Kölliker described perivascular nerves on intracerebral arteries of 90 u in diameter, but gave no illustrations. Perivascular nerves in the medulla and spinal cord of cats and dogs were clearly demonstrated by Clarke in 1929. Kurusu and Hamada (1929) reported the presence of intracerebral vascular nerves in the adventitia and media of small arteries of dogs and monkeys. In 1932a , Penfield using a modification of the Gross-Bielschowsky technique demonstrated fine unmyelinated nerve fibres on intracerebral blood vessels and on vessels throughout the rest of the central nervous system in the cat, dog, monkey and in man. These nerves were found to be continuous with myelinated and unmyelinated nerves on the pial vessels and run in the perivascular sheath or in the adventitia and on smaller arteries run between adventitia and media. Chorobski and Penfield (1932) followed them onto arterioles as small as 25 to 30 u in diameter. No nerves were seen on intracerebral capillaries. Humphreys in 1939 using a protargol stain was able to demonstrate clearly fine unmyelinated fibres on the intracerebral capillaries. McNaughton (1938) has given an excellent review of this subject.

Vasomotor control of cerebral circulation.

Good reviews of the literature on this subject have been

given by Hürthle (1927), Forbes and Wolff (1928), Stöhr (1928), Penfield (1932a), Chorobski and Penfield (1932), Clark (1934), Wolff (1936), Bouchaert and Jourdon (1937), Cobb (1938) and Forbes and Cobb (1938).

The effects of cervical sympathetic stimulation on the cerebral vasculature have been thoroughly explored by various methods, and in essence the results agree. Direct observations were made through skull windows in cats, anesthetized (Forbes and Wolff, 1928) and unanesthetized (Thomas, 1935) as well as in the isolated perfused head (Pool, Forbes and Nason, 1934). The average constriction resulting from such stimulation is about 8 to 10 per cent. Experiments carried out by means of blood flow determination methods such as a constant pressure perfusion pump (Finesinger and Putnam, 1933), a thermocouple on the internal carotid (Gollwitzer-Meier and Eckardt, 1934; Schneider and Schneider, 1934), or a thermocouple in the substance of the brain (Schmidt and Pierson, 1934: Schmidt, 1934 and 35: Wolff and Cattell, 1935) reveal that stimulation of the cervical sympathetic nerves causes decrease in cerebral blood flow and constriction of the pial and intrinsic blood vessels of the cerebrum. As ascertained by blood flow methods, using a thermocouple in the brain substance (Schmidt, 1935) and a Rein Thermostromuhr on the internal carotid, common carotid and vertebral arteries, (Gollwitzer-Meier and Eckardt, 1934) the effects are said to be bilateral; by the skull window method, and by actual measurement of capillary filling in

both cerebral hemispheres (Talbot, Cobb and Wolff, 1929) the effects are shown to be distinctly unilateral. Moreover, stimulation of the cervical sympathetic nerve causes constriction of the dural arteries averaging 34 per cent. This contrasts with the average constriction of pial arteries which is 8 to 10 per cent, and the skin which is about 80 per cent (Pool, Nason and Forbes, 1934).

Vasomotor effects are not equally great in all parts of the neuraxis. The blood vessels in the medulla, for instance, are very little affected by stimulation of the cervical sympathetic. The arteries of the hypothalamus are affected somewhat more, and those in the cortex appear tobe most affected, although less than those of the pia (Schmidt, 1934, 1935). The latter in turn have less reaction than the vessels of the dura and skin (Pool, Nason and Forbes, 1934). Stimulation of the cervical sympathetic nerves commonly results in constriction of the arteries of the choroid (by direct observation, Putnam and Ask-Upmark, 1934).

The responses of the pial arteries to faradic stimulation in the thalamus and hypothalamus were also observed through skull windows. Constriction of the pial arteries results from stimulation of a hypothalamic area between the cerebral peduncle and the third ventricle. On the other hand, dilatation of pial arteries results from stimulation in a circumscribed area in the central portion of the tuber cinereum as well as from a more diffuse area in the thalamus (Penfield and Stavraky, 1935).

The results of vagus stimulation are definite and predictable, although interpretation of their nature is more complicated than in the case of the cervical sympathetic effects (Forbes and Wolff, 1928; Cobb and Finesinger, 1932). With the skull window technique in cats and in monkeys there was observed during the period of vagus stimulation, and while the blood pressure was falling, an average increase of 15 per cent in the diameter of the pial vessels in approximately three quarters of all experiments. The effects were bilateral. Similar results follow depressor nerve stimulation (Forbes, 1935).

The facial nerve is said to be the efferent pathway for this cerebral vasodilator effect (Cobb and Finesinger, 1932; Chorobski and Penfield, 1932), since dilatation of pial arteries is quite regularly obtained on vagus stimulation when the facial nerve is intact, but never when the latter is cut near its exit from the medulla.

There is no doubt that maximum dilator effects on vagus stimulation do usually accompany the great falls in blood pressure (Wolff, 1936). In fact according to Forbes when the major falls in blood pressure were prevented by clamping the abdominal aorta, dilatation occurred in somewhat less than a quarter of his experiments. However, several other satisfactory experiments (Finesinger and Putnam, 1933) indicated that the vagus-depressor nerve effects are not entirely the result of blood pressure changes. For instance when the perfusion pressure was kept constant (Finesinger and Putnam, 1933) the cerebral vasodilatation on vagus stimulation

still occurred. Moreover, experiments (Cobb and Finesinger, 1932) demonstrated that after cutting the facial nerve which contains the cerebral vasodilator fibres, cerebral vasodilatation on vagus stimulation was not observed. That the fall in blood pressure, though frequent, is not essential for the effect is further demonstrated by the fact that 15 per cent of the vagus vasodilator effects were without a concurrent fall in blood pressure (Forbes and Wolff, 1928).

In more recent vagus experiments (Forbes, 1935) vasodilatation occurred in 7 of 15 instances in which there was no fall in blood pressure. These data indicate that the fall in blood pressure, though possibly a factor in the cerebral vasodilatation observed to follow vagus stimulation, is not solely responsible (Wolff, 1936).

It has also been demonstrated that the effects of vagus stimulation occur after resection of the carotid sinus and depressor nerves in the cat (Forbes, 1935). Hence, the effect of the stimulation of the vagus nerve is not dependent upon the presence of the other afferents. Also resection of the cervical sympathetic nerve does not alter the effect of vagus stimulation (Wolff, 1936).

Vasoconstriction following direct stimulation of vessels.

The effect of direct stimulation on pial vessels was apparently first observed by Schultz in 1866 who found that these vessels constricted on electrical stimulation. Apart from his work little interest was taken in this particular problem until Florey's

paper appeared in 1925. Florey reported in detail how spasms could be produced in individual pial vessels by direct mechanical and electrical stimulation. He also showed that the cerebral arteries react to thermal and chemical stimuli by contraction and dilatation and studied the effect of drugs, asphyxia and sympathetic stimulation on these vessels. He concluded that "all the evidence given is therefore emphatically against the recognition of any effective intracranial vasomotor control, in spite of the fact that the vessels are capable, with appropriate methods of stimulation , of entering into strong contraction". Florey's observations are not entirely correct for we now know that in animals stimulation of the cervical sympathetic nerve results consistently in constriction of arteries in the pia (see Forbes and Cobb, 1938) even though this be slight. However what concerns us here is that Florey's observations led him to believe that the constrictions in pial vessels resulting from direct stimulation were not necessarily dependent upon a neurovascular mechanism. His evidence for this conclusion is based mainly on the fact that he was unable to discover any constrictor effect on the vessels from injections of adrenilin or pituitrin or from sympathetic nerve stimulation in the neck. In speaking of the pial vessels he pointed out that "it was thought that the localization of the contraction to the point stimulated indicated that the arteries were not innervated, but the messenteric vessels which certainly have a

vasomotor supply react in an identical way".

The findings presented in the present paper are in agreement with Florey that the constrictions produced by direct stimulation of pial vessels are probably largely independent of the vasomotor nerves which are present. However one of the arguements used in support of this contention is the one rejected by Florey; for Ricker and Chase (1938), have shown that an irritant applied to the mesentery causes not only a local traumatic constriction of an artery but that the constriction is transmitted instantly along the vessel and there occurs constriction of smaller muscular arteries and terminal arterial segments. This type of reaction has never been observed in the pial vessels where constriction remains localized.

The reactions of vessels elsewhere in the body to direct stimulation have been studied at length (see Krogh, 1930) and the findings are of help in analysing similar reactions in the pial vessels.

When the end of a flat ruler (2-3 cm. broad, smooth and with edges just rounded) is drawn steadily, but not roughly, across the human skin the area covered by the stroke becomes after 15-20 seconds distinctly paler than the surroundings, (see Krogh, 1930). Carrier, (1922) showed by microscopic observation that the blanching is due to the contraction of the skin capillaries and vessels and Heimberger, (1925) has shown further that a sharply

localized weak mechanical stimulus will generally result in a contraction of a length of capillary corresponding probably to one or two Rouget cells. Lewis (1926) refering to this phenomenon, remarked that "the contraction of the minute vessels in response to tension after a short period of latency, appears to be the direct result of stimulation of their walls. It is independent of nervous reflexes, central or local". Ebbecke (1917) saw it occur on skin to which the nerve supply had long been lost and Carrier (1922) on skin locally anesthetized. Lewis (1927) has seen it in skin to which the nerves had been cut surgically and allowed to degenerate, a white reaction being obtained across sensitive and insensitive skin.

The contraction response of the minute vessels according to Ebbecke (1917) can be produced similarly to that in the skin, on the surface of mammalian viscera such as the liver, spleen and kidney.

Liebermann (1921) observed in frogs arteries that passing of a constant current for some seconds through unpolarizable electrodes would produce sharply localized constrictions at the kathode and dilatation at the anode. Ni (1922) obtained very sharply localized constrictions on capillaries in frogs webs, fins of fishes, wings of bats and fingers of human subjects by applying series of make and break shocks from either an induction coil or a battery. He showed that in some

cases the length of the constricted part seems to be no more than 2-4 u. Krogh remarks "it is inconceivable that such a constriction could be brought about indirectly. The stimuli must act either on a few fibrils in a single Rouget cell or on single endothelial cells". Heimberger (1926) finds on the nail capillaries of man that the constant current anode will bring the Rouget cells to contraction.

These references from the literature then show us that there are certain reactions in common between the vessels in the pia and elsewhere in the body. In the periphery local constrictions can occur as a direct result of stimulation of the vessel wall without any apparent participation of the vascular nerves which are present. As will be seen from the experiments described in this paper the same seems to hold true of pial and cerebral vessels despite the fact that these vessels likewise have an abundant nerve supply. However the fact that local constrictions occur without any apparent participation of nerves does not mean of course that these nerves do not play an active part under certain conditions.

There is no evidence in the literature at present to prove that human cerebral or pial vessels constrict on mechanical or electrical stimulation except a brief note by Riser et al (1931). They have recorded a single observation to the effect that mechanical stimulation of one pial vessel caused this to constrict

50% of its diameter. This observation was made during an operation for a cerebral tumor.

In the literature there are of course numerous observations and clinical analogies suggesting that spasm of cerebral vessels may occur under certain conditions (see Kennedy et al, 1938, and Cobb, 1938). Penfield (1933-38) has observed definite anular constriction in the pial vessels of epileptics following seizures. However spasms of the above type do not directly concern us here for the present paper deals with reactions to stimuli of a non-physiological nature. It is therefore dangerous to draw analogies between the spasms under discussion and those reported clinically.

There are other aspects of the literature that have some bearing on the findings to be reported in this paper but these shall be discussed later in their proper place.

# EXPERIMENTAL DATA.

## TECHNIQUE.

The majority of the observations included in Part 1. of the thesis were obtained in acute experiments on thirty cats. As there is considerable overlap between experiments, different observations were frequently carried out on the same cat. For instance, observations on the effect of electrical and mechanical stimulation were often made during one experiment. At the end of the experiment the cat was at times used to demonstrate focal cerebral ischaemia by intra vital staining. Also many observations included in Part 1. of the thesis were obtained during electrical or mechanical stimulation of pial vessels in cats (and one dog and one monkey) used in the sterile operations described in Part 11. of the thesis.

The animals were anesthetized with ether or with dial 0.5 c.c. of a 10% solution intraperitoneally.

A large decompressive bilateral craniotomy was performed. At times this was done at first on one side and a few hours later on the other. On opening the dura a petri dish was laid over the cranial defect and gauze soaked in physiological Ringer's solution closely applied to the edges of the dish to prevent the entrance of room air and drying of the cortex. In the centre of the petri dish was a small hole through which a glass stimulating rod or electrodes could be inserted. A

microscope was then mounted over the cortex and the vessels observed with high or low power magnification. When the latter was used it was necessary to remove the petri dish.

As the particulars of each experiment vary considerably, further details of technique will be included under the individual experiments.

## THE EFFECT OF MECHANICAL STIMULATION ON PIAL BLODD VESSELS.

### Observation on individual vessels in cats.

Preliminary observations on six cats confirmed the findings of Florey in their entirety.

It was found that gentle or heavy stroking of arterial vessels of all sizes with the tip of a glass rod produced vaso-constriction. (see Figs. 1 & 11). It was usually necessary to stroke the vessel at right angles to its long axis. By this means the vessel wall could be stretched and stretch appeared to be an adequate stimulus for constriction. The contraction occurred only over the area of vessel stimulated and was never propagated away for long distances as has been described in mesenteric vessels by Chase (1934,'38). However, constriction frequently occurred for a short distance on either side of the area stimulated. It was felt that this was due to the fact that the maximum stimulus was applied at the site of stimulation but that the vessel was stretched over a wider

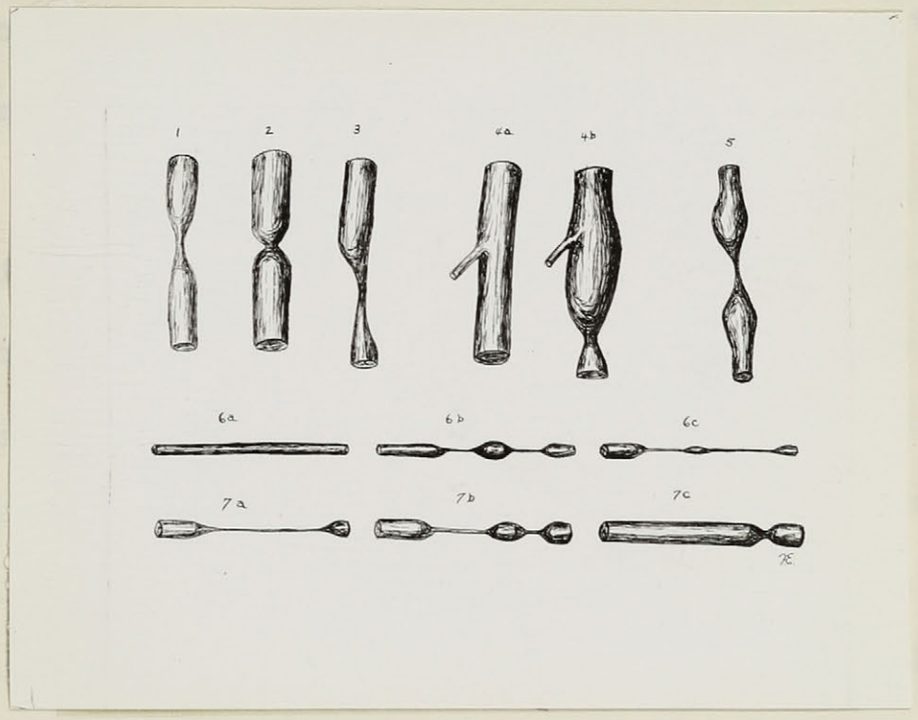
# FIGURE I.

Drawings1 to 5 were taken from photomicrographs of cat's pial vessels constricted by mechanical stimulation. The localized nature of the constrictions as well as other features mentioned in the text are well illustrated.

Drawings 6a to 6c represent the development of constrictions following stimulation of a vessel with bipolar electrodes leading to a thyratron, (intensity 5). An interval of 1¼ minutes elapsed between the time of stimulation and the degree of constriction present in drawing 6c.

Drawings 7a to 7c show the redilatation of a vessel after it had been constricted by running the electrodes along several mm. of its length. A period of 10 minutes elapsed while the vessel changed calibre from that shown in drawing 7a to 7c, (intensity 5 on thyratron dial - see text).

FIG. I.



area. This phenomenon was best demonstrated by looping a very fine silk thread (on a needle) about a pial artery and then, while observing it microscopically, applying traction on the thread. When the traction was released it was found that the vessel was frequently obliterated at the site of previous contact of the thread but that the vessel was also constricted for a considerable distance in either direction, the distance depending at least in part on the degree of vessel stretch.

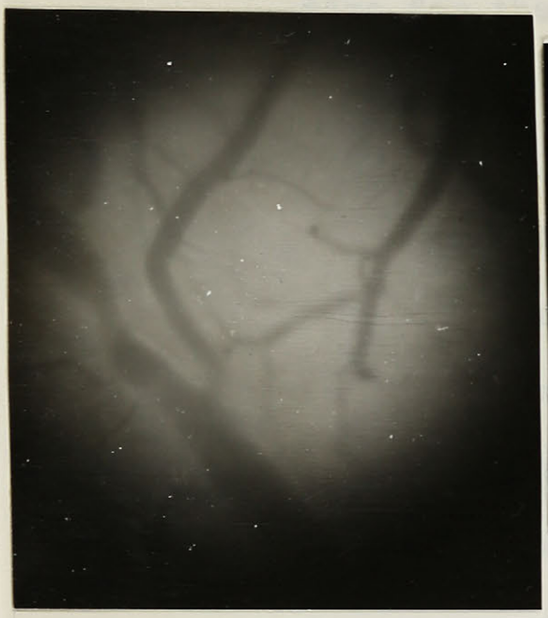
Frequently gentle stimulation produced complete obliteration of the vessel lumen and the movement of the corpuscles in branches of the main trunk leading into the constricted area ceased as far back as the first collaterals. This was seen microscopically in many cases. At other times stronger stimulation such as rolling the vessel back and forth with the tip of the glass rod was required to bring on the constriction. This was occasionally true in one portion of a vessel whereas on either side of this area in the same vessel, light stimulation caused a very localized constriction. Some vessels were completely refractory to stimulation or became so after a period of constriction. Occasionally, on gentle stroking of a vessel, dilatation rather than constriction resulted. However, despite the variability in sensitivity of various vessels to contract on stimulation the majority did so promptly when the cortex was first exposed and this remained true for hours if the brain was protected with a glass covering (see below).

FIGURE II.

The six illustrations are photomicrographs of cat's pial vessels constricted by mechanical stimulation. The bottom row of photomicrographs are of three normal vessels and the upper row are of the same vessels after mechanical stimulation.

FIG. II.

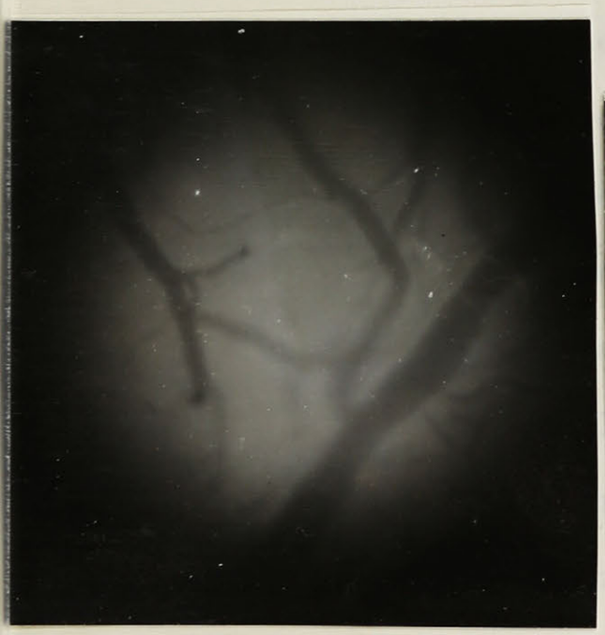
1



2

3

1a



2a

3a

The duration of the spasms was also variable lasting from several seconds to 12 minutes depending upon the sensitivity of the vessel and on the duration and strength of the stimulus. In a fresh cortex the average duration of a vessel spasm produced by quite vigorous stroking was about 3 minutes. The time for the contraction to reach its maximum varied from a few seconds to nearly a minute.

It has often been observed that a dilatation occurred on either side of an area of constriction. This dilation at times did not attain its maximum until an interval of one half to 2 minutes had elapsed.

If several mm. or more of a vessel was constricted by mechanical stimulation it usually did not redilate uniformly but in boloney-sausage fashion, areas of anular constriction alternating with areas of dilatation. When dilatation was complete there was at times a reactive hyperaemia, the vessel being increased in size by as much as 1/3 its normal diameter.

The above observations apply to arterial vessels of all sizes. The venous capillaries did not show any direct reaction to stimulation however and the arterial branches such as the middle cerebral were somewhat more refractory than the medium sized and smaller vessels. However trunks such as the basilar constricted promptly. The latter observation has been made in a large number of cats (10). In these the basilar was exposed through a burr hole in the base of the skull (during experiments

for a different purpose) and the vessel stimulated with a blunt metal rod. Gentle stroking of the vessel obliterated it almost completely.

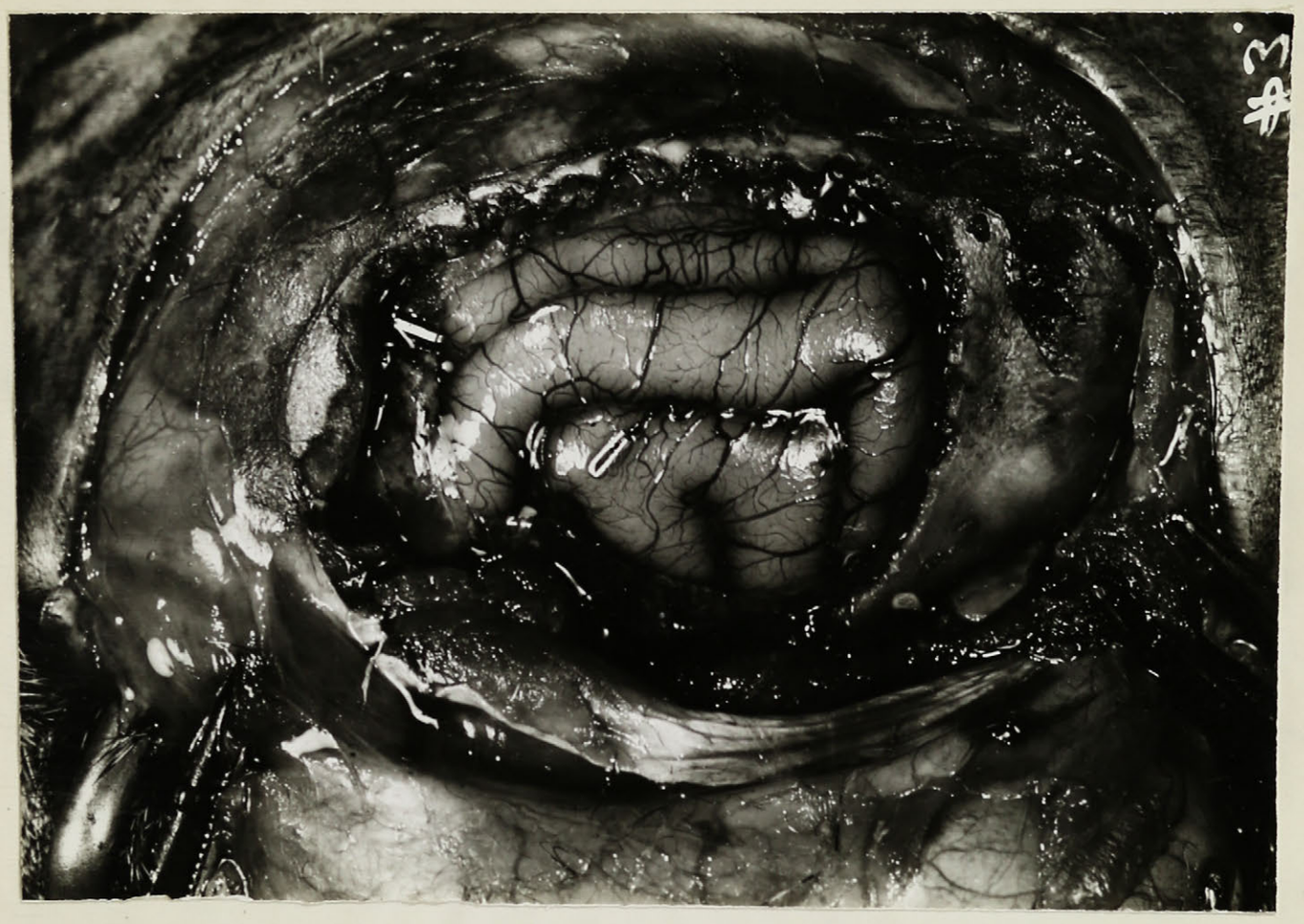
An interesting observation which we have been unable to explain is the following. If, as stated, the cortex was covered with a petri dish immediately after the dura had been opened, most vessels were found to constrict promptly to mechanical stimulation. However if the cortex was left exposed for a short time, even though it was irrigated with warm ringer, the vessels were frequently almost all refractory to even vigorous mechanical (or electrical) stimulation. On the other hand in many experiments at the end of several hours the vessels constricted as promptly if not more so than in the fresh brain. This has been true even after exposure of the brain to the air for 9 hours, the brain being irrigated from time to time with cool or warm ringer. These findings suggest that there are three stages in the excitability of the vessels to mechanical stimulation (and the same has been found to be true of electrical stimulation). An initial one when the cortex and its vessels are in approximately the normal state, a second when the vessels are refractory to stimulation due either to exposure to air or warmth (ringer) and a third when they are again irritable despite cooling and exposure.

## FIGURE III.

This is a photograph of a normal cat brain. It should be compared with Fig IV. which shows the appearance of the pial vessels in the same cat after electrical stimulation.

The craniotomy in this case is a little greater in extent than the ones described in Part II of the thesis.

FIG. III.



## ELECTRICAL STIMULATION OF INDIVIDUAL PIAL VESSELS.

In the earlier experiments an induction coil was used for stimulation but later a thyratron constructed by Mr. Cipriani, (similar to that described by Schmitt and Schmitt in 1932). This instrument produces a current which resembles the faradic current of an induction coil, but here the thyratron tube filled with mercury vapour acts as an interrupter. The current is thus constant and may be altered accurately in rate and intensity.

Both unipolar and bipolar electrodes were employed at different times. A bilateral craniotomy was performed as described under "mechanical stimulation of cerebral vessels" and a petri dish placed over the cranial defect as before. The animals (5) were anesthetized with dial 0.5.c.c. per Kilo intraperitoneally.

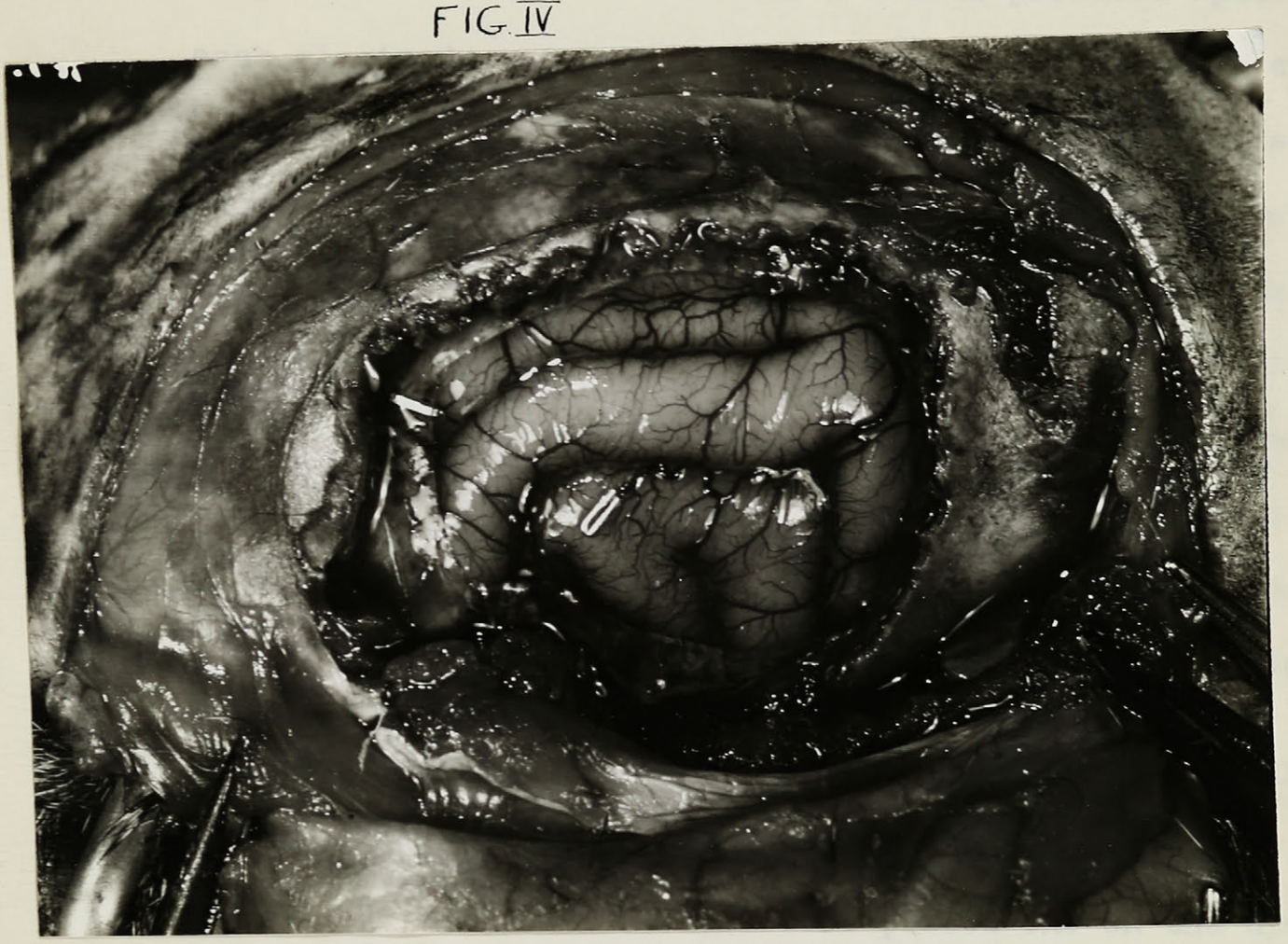
As with mechanical stimulation it was found that arteries, arterioles and capillaries (not venous capillaries) constricted promptly on electrical stimulation. (See Figs. lll & lV). With a weak unipolar stimulus it could be shown that the constriction was localized to the site of stimulation and was propagated for only a short distance, if at all, in either direction. The vessels contracted to complete obliteration with even a very weak stimulus but the degree of constriction varied with both the duration and strength of the stimulus.

In a fresh cortex protected from air, vessels usually

## FIGURE IV.

This is a photograph of the same cat brain as shown in Fig. III, taken immediately after the pial vessels had been constricted by electrical stimulation. Note that all the arterial vessels visible in Fig. III. have now disappeared or are seen as a faint gray line. Bipolar electrodes and a thyratron were used for stimulation (intensity 5). The vessels were constricted by gently moving the electrodes over all visible arterial vessels.

FIG. IV



constricted to obliteration on less than 1 seconds stimulation with the thyratron at 2 to 3 which is usually below the threshold for motor movement in the unanaesthetized cat's cortex.

With the thyratron at 5 (using a bipolar electrode) a single stimulation ofless than 1 second produced spasm at each electrode which advanced gradually, the maximum constriction being reached only after 1 to 2 minutes. The vessel did not usually contract uniformly but the whole area which had come under the influence of the stimulus showed beading, constrictions alternating with normal or slightly dilated vessel. Most of the dilated portions gradually gave way to constriction but only after a period of 1 to 2 minutes. With a decrease in the strength of the stimulus the effect on the vessel was less widespread and the constriction less marked.

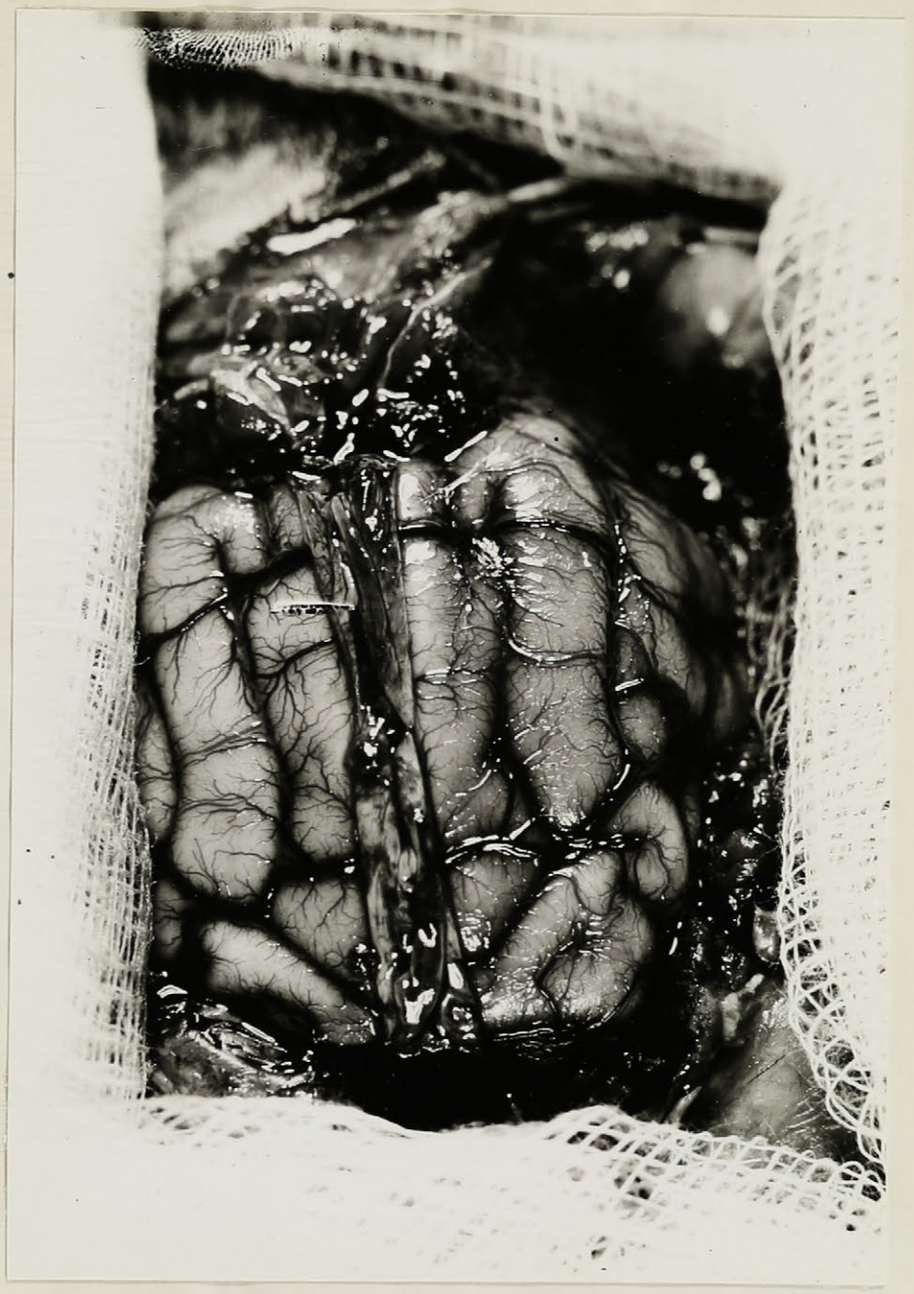
## THE EFFECT ON THE PIAL VESSELS OF PASSING 110 VOLTS THROUGH THE BRAIN.

In four cats electrodes were placed one on the upper lip and one in the temporal muscle overlying a cerebral hemisphere ( a bilateral craniotomy having been performed). The electrodes led to a plug which was connected to the 110 volt main. A current was then passed through the brain for 1 to 3 seconds by closing the circuit. The pial arterial vessels immediately became obliterated by constriction and for a few seconds were invisible to the eye. (see Figs. V & Vl.). Most of the veins

FIGURE V.

The photograph on the opposite page is of a normal cat brain. This picture should be compared with one of the same brain, shown in Fig. VI., after 110 volts had been passed through it.

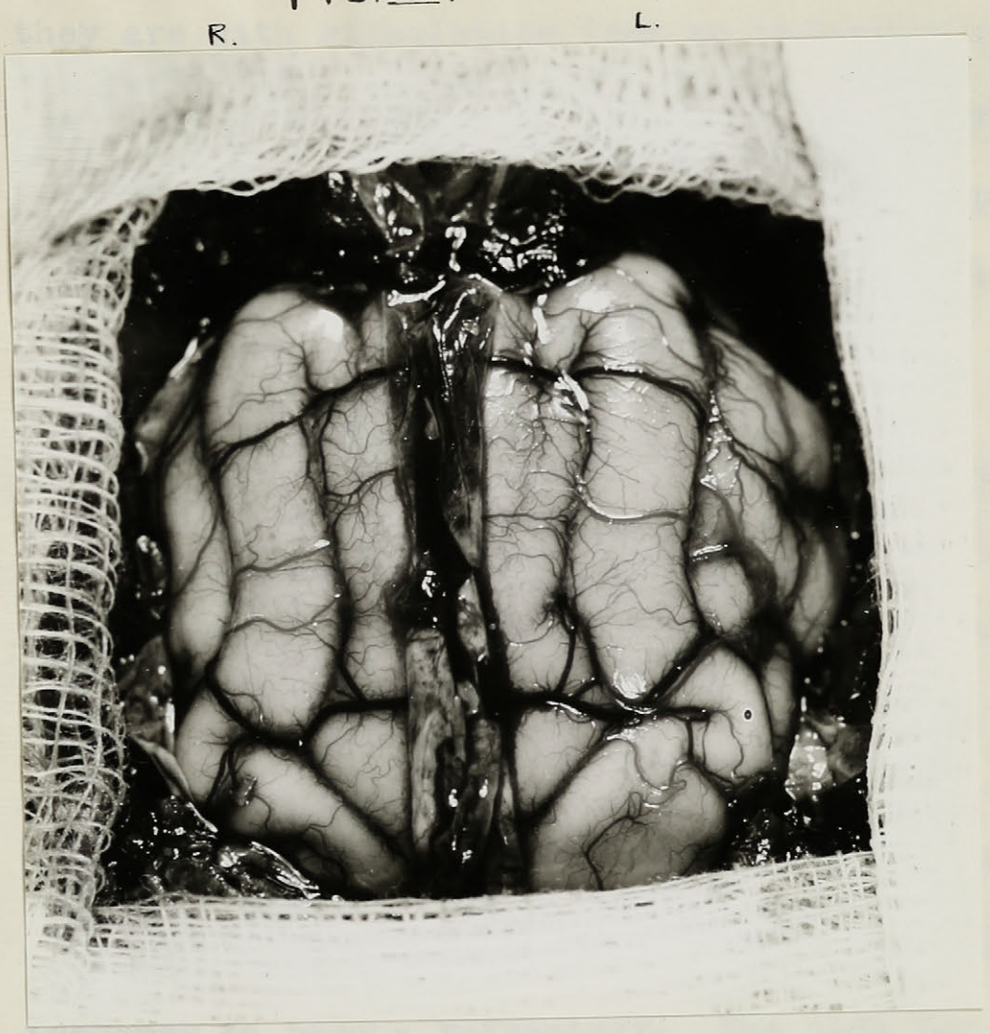
FIG. V.



## FIGURE VI.

This is a photograph of the same brain as shown in Fig. V., immediately after 110 volts had been passed through it for 3 seconds. One stimulating electrode was in the right temporal muscle, which was laid over the hemisphere and the other was on the left upper lip. The current was taken from the main.

FIG. VI.



also diminished in diameter but this may have been due to lessened arterial flow. At times pial arteries remained constricted for as long as three minutes. Constrictions from stimulation with 110 volts however were never as prolonged as they are with stimulation from an induction coil or a thyratron. The vessels in the hemisphere directly between the electrodes showed more constriction than those in the opposite hemisphere. This type of stimulation produces a violent tonic and clonic convulsive seizure in both cats or rabbits.

OBSERVATION ON INDIVIDUAL PIAL VESSELS IN DOGS AND MONKEYS.

Using both mechanicalaand electrical stimulation it was found that the pial vessels in dogs (one experiment) (anesthetized with dial intraperitoneally) showed the same type of spasms as described in the cat. However with mechanical irritation the stimulus had to be more forceful to produce obliteration of the vessel lumen and the vessels showed more tendency to become refractory. The same was true of electrical stimulation where a current of greater strength was necessary to constrict many of the vessels. Experiments were performed on several monkeys that were being operated on by Dr. Erickson. In these animals the cortex had been exposed for some time before stimulation was carried out and in all of them the vessels failed to constrict. Under dial anesthesia a craniotomy was then performed on a monkey and the cortex covered with a

petri dish immediately on opening the dura. The pial vessels in this animal also failed to contract on mechanical or electrical stimulation with the exception of one vessel which showed a feeble spasm. A similar experiment was then performed on a monkey under local anesthesia and light ether. When it had recovered from ether it was found that the vessels contracted on electrical stimulation. Repeated stimulation however was frequently necessary to bring about spasm. However after a vessel had once been partially constricted it became more sensitive for a time and it was then possible to keep most vessels over an area of cortex constricted at the same time. The conclusion from these experiments was that the pial vessels in the dog are somewhat less sensitive to mechanical and electrical stimulation than are the cat's and that the monkey's pial vessels are more refractory to direct stimulation than the dog's.

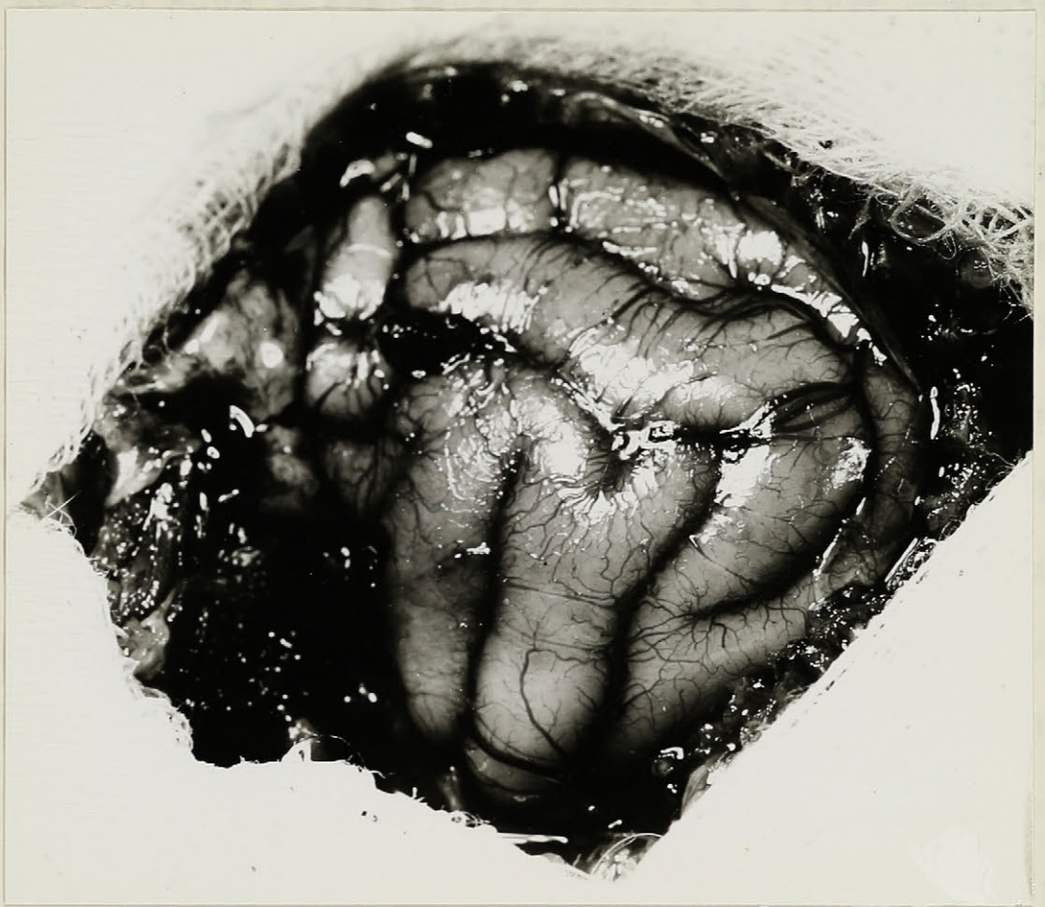
STIMULATION OF PARTIALLY DENERVATED PIAL VESSELS.

In two cats, using sterile technique, the middle cerebral artery was exposed low in the sylvian fissure through a small craniotomy. At this site the middle cerebral has already divided into three main trunks, two of which supply the sigmoid gyrus and the temporo-parietal region. The other runs posteriorly and supplies the temporo-occipital region. A silk ligature was carefully passed about the two anterior

## FIGURE VII.

The photograph is of a cat brain showing constrictions, in a number of branches of the middle cerebral artery, produced by electrical stimulation, (in the same manner as described in Fig. IV.). The middle cerebral artery of this cat was ligated low in the sylvian fissure 10 days previously. (See text).

FIG. VII



branches and tied tightly. The vessels ligated showed little if any change in calibre distal to the ligature and remained well filled with blood because of the abundant collateral anastamosis. The wound was then closed. At intervals of 8 and 10 days respectively after the first operation the wound of each cat was reopened and a wide craniotomy performed. The vessels which had been ligated were now stimulated distal to the ligatures with the thyratron using bipolar electrodes. It was found that the vessels contracted promptly on stimulation with the thyratron as low as 3. (see Figs. V11 & V111). This was true of vessels of all sizes in the peripheral distribution of the ligated vessels. The large trunk not included in the ligature also contracted promptly on stimulation and no definite difference in the stimulation threshold, latency, degree or duration of constriction could be made out between the ligated and normal vessel. Following this procedure each cat was killed and the vessels stained with Humphrey's Protargol method for nerve fibres by Francis McNaughton. It was found that only a few fibres remained on the large trunks of the vessels just distal to the ligatures. More peripherally in the smaller arterial branches only a few degenerated fibres were seen. On the normal vessels however, which in some cases were present in the same sections with the ligated vessels, many normal nerve fibres were present. Therefore although these experiments are not yet complete they suggest that

## FIGURE VIII.

This photograph was taken a half hour after that shown in Fig. VII. and is of the same brain. No stimulation was carried out in the interim and as is seen the vessels have redilated. A small traumatic subarachnoid hemorrhage has occurred in the interval between the two photographs.

FIG. VIII.

these constrictions resulting from mechanical and electrical stimulation are not dependent upon a neurovascular mechanism.

EFFECT OF DRUGS ON CONSTRICTED PIAL VESSELS.

In three experiments the effect of acetylcholine, on the constrictions described, was studied. To do this two vessels were observed microscopically. One was constricted to obliteration by electrical stimulation, the other was not stimulated and remained normal. Acetylcholine 0.2 mg/kilo was then injected I.V. The normal vessel dilated but the vessel constricted by electrical stimulation was not apparently affected by the acetylcholine. This observation was repeated a number of times on different vessels with the same result, except that in some vessels the constriction appeared to relax slightly more rapidly than is usual, following the injection of acetylcholine.

Inhalation of amyl nitrate for 10 seconds likewise appeared to have little or no effect in dilating constricted vessels although it caused prompt dilatation in neighbouring normal vessels. $CO_2$ inhalations also had no apparent effect on constricted vessels.

COMBINED ELECTRICAL STIMULATION OF PIAL BLOOD VESSELS AND OF THE CERVICAL SYMPATHETIC.

In one cat a pial vessel was stimulated with the thyratron at 4 for 2 seconds and microscopic readings taken during the

period of its constriction and redilatation. The procedure was then repeated but at the same time the sympathetic in the neck on the same side was stimulated constantly with electrodes leading from an induction coil. This experiment was carried out on a number of vessels and it was found that the time for the vessel to reach its maximum constriction and redilate was approximately the same with and without concurrent stimulation of the cervical sympathetic.

## VASOSPASM AND FOCAL CEREBRAL ISCHAEMIA.

So far in these experiments the effect of electrical or mechanical stimulation only on individual vessels has been described and this was as far as Florey went in 1925. As is well known, in the opinion of many it seems possible that a local vascular spasm, or more correctly a local cerebral ischaemia, may initiate a convulsion and also that repeated local vascular spasm may be part of the mechanism responsible for epilepsy (see Cobb, 1938). With the present technique it appeared that a method might be developed for testing this theory or at least for studying it. Until the present no adequate way has been described of producing temporary vascular spasm and focal cerebral ischaemia. Spasm of single pial vessels does not of course produce ischaemia because of the rich arterial anastamosis which is present. This has been shown by many investigators (see literature). The following experiments were therefore undertaken.

## A METHOD OF PRODUCING FOCAL CEREBRAL ISCHAEMIA.

Since arterial vessels will constrict promptly on electrical stimulation it appeared that focal cerebral ischaemia might be produced by stimulating all pial blood vessels over a small or large area of cortex. In a large group of cats (approximately fifteen in Part I. and II. of thesis), the pial vessels were stimulated over the parieto-occipital cortex. For stimulation a thyratron and bipolar electrodes were used. With a current which was subthreshold for the motor cortex in cats which had recovered from ether, it was found possible, by gently stroking the electrodes over the cortex to keep all the visible arterial vessels constricted over periods of half an hour or more. With currents of slightly greater strength it was even more simple to keep most visible vessels completely constricted during the period of stimulation. When stimulation ceased the vessels dilated in the usual manner, many showing boloney sausage constriction. The period for complete dilatation to normal size varied in different vessels and took anywhere from 1 minute to several hours. Of course the only vessels visible were those in the pia-arachnoid and one could not be certain that the blood flow in the intracerebral vessels was also cut off (either by constriction of pial vessels or of intracerebral vessels as well). Blood flow experiments were therefore carried out.

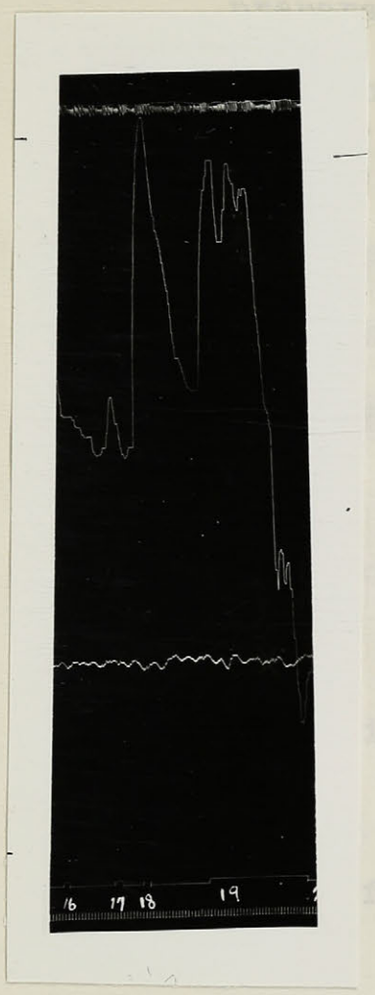
## THE EFFECT ON CORTICAL BLOOD FLOW OF FOCAL VASCULAR SPASM.

In three experiments two thermocouples leading to a

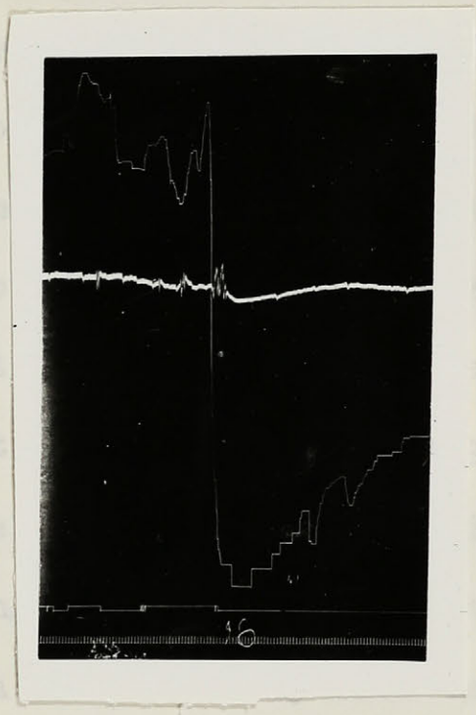
FIGURE IX.

Illustrations 1 to 3 show the effect on the blood flow in the cortex of constricting the blood vessels, by electrical stimulation, supplying an area of grey matter in which a thermocouple (leading to a thermo-electric blood flow recorder), has been inserted. Note that in each case when stimulation is first started there is a rise in blood flow followed by a rapid and marked fall. (In illustrations 1, 2 and 3 the periods of stimulation are shown by the indicator at numbers; 19 in illustrationl, 16 in illustration 2, and at 8 and 9 in illustration 3). See text.

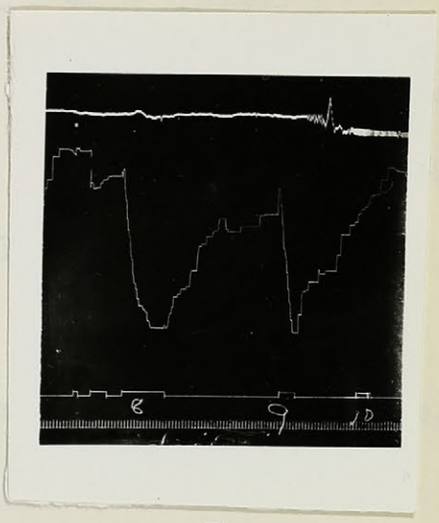
FIG. IX.




1.




2.




3.

thermoelectric blood flow recorder were inserted about 2 mm. diagonally into the cerebral cortex, one in the posterior sigmoid gyrus (Thermocouple 1.) and one in the middle supra-sylvian gyrus (Thermocouple 2.). The sensitivity of the preparation was then tested by injecting 0.25 c.c. of 1/10.000 solution of adrenilin into the fermoral vein and recording the effect on the blood flow. The vessels about thermocouple 2. or those leading to this area were now stimulated electrically with the thyratron at 3 to 7. (The threshold for movement in the motor cortex in cats recovered from ether is about 3 to 5). At the beginning of stimulation there was frequently a rise in blood flow at thermocouple 2. but as the vessels stimulated showed constriction there was usually a marked fall in blood flow without any significant change in B.P. (see Fig. 1X). As the vessels redilated the blood flow again increased. These findings were true, at times, when vessels several mm. to one and a half cm. away from the couple were stimulated: showing that the results were not due to an increase in heat of the cortex from the electrical stimulation. From these experiments it was obvious that vasoconstrictions brought about in this way resulted in a markedly diminished blood flow in the cortex if not a complete ischaemia.

DEMONSTRATION OF FOCAL CEREBRAL ISCHAEMIA BY MEANS OF INTRA-VITAL STAINING.

By stimulating electrically, in the manner described, the

FIGURE X.

This photograph was taken of a cat brain one hour after the termination of an experiment in which the arterial vessels were constricted over the left supra-sylvian gyrus and during the period of constriction 60 c.c. of a 1% solution of gentian violet was injected into the femoral vein. The whole brain, except for the left suprasylvian gyrus, turned a deep blue. The gyrus stimulated remained a whitish-pink colour. The cat was killed immediately and the brain removed (see text). Bipolar electrodes leading to a thyratron (intensity 6), were used.

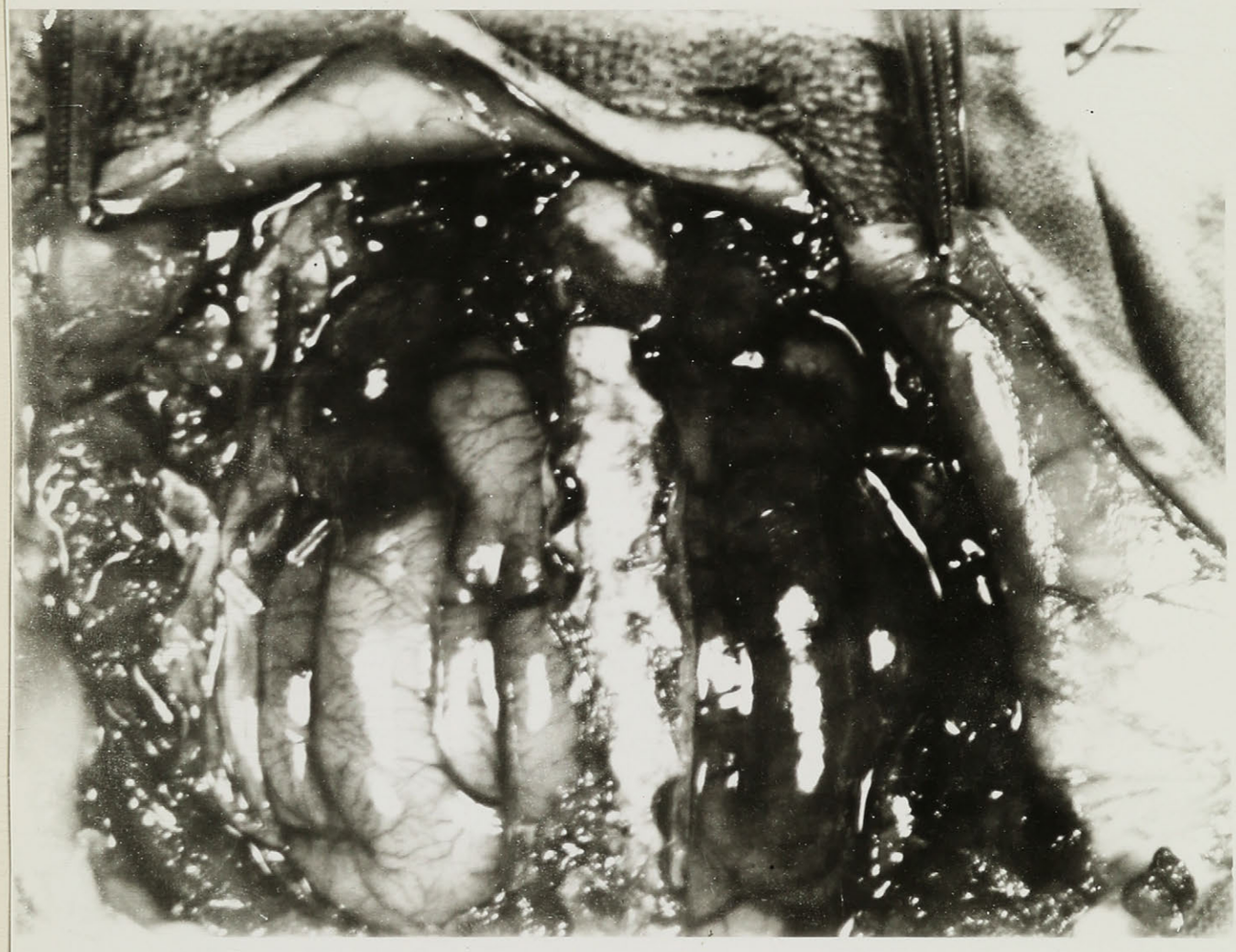
FIG. X

vessesl over one middle suprasylvian gyrus of a cat, and those immediately surrounding it, were constricted. (A bilateral craniotomy having been performed). During the period of stimulation 60c.c. of a 1% aqueous solution of gentian violet was injected into the femoral vein. The brain promptly turned a deep blue except in the area where the blood vessels had been constricted. (see Figs. X & Xl). This area remained white. During this period the cat continued to breathe actively and the heart, although rapid in rate, beat forcefully. The animal was then killed by injection of formalin 10% into the femoral vein and by a stab wound in the heart. The brain was promptly removed and about one hour later was photographed. This experiment was repeated in three cats with the same result. Coronal section of the brains showed that ischaemia had been almost complete through the whole depth of cortex in a large portion of the area stimulated and that even the immediately underlying white matter was a considerable paler blue than the surrounding brain. It was interesting (see later histological findings) that the area of ischaemia was frequently in the form of a triangle as shown in Fig. Xlll.C. Similar results were obtained in two cats by substituting chlorazol sky blue (10c.c. of a 14% aqueous solution) for gentian violet. (see Fig. Xll.).

FIGURE XI.

The experiment in this case was identical to that described in Fig. X. except that the arterial vessels were constricted over the whole left cerebral hemisphere. The photograph shows that the gentian violet injected in the femoral vein has turned the right cerebral hemisphere blue, whereas the left hemisphere, over which the vessels were constricted, remained whitish-pink.

FIG. XI.

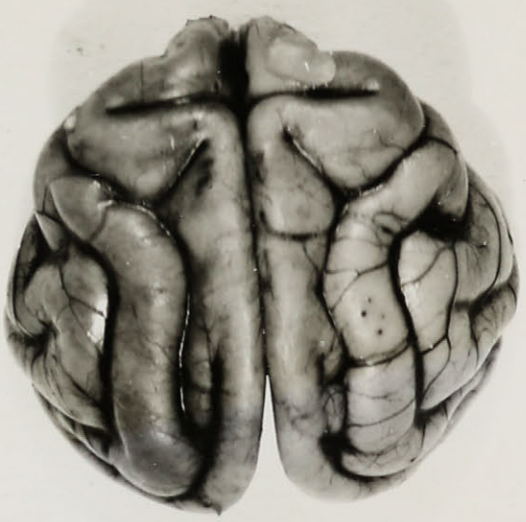


L R

## FIGURE XII.

The same experiment was performed on this cat as described in Figs. X. and XI. except that in place of gentian violet, chlorazol sky blue, 10 c.c. of a 14% solution, was injected into the femoral vein. Before and during the injection the vessels over the left suprasylvian gyrus (and immediately surrounding regions), were constricted by electrical stimulation with bipolar electrodes leading to a thyratron (intensity 8). Note that the vessels are clearly demonstrated over the right suprasylvian gyrus but are barely visible over the same gyrus on the left side. Colored photographs of this brain gave a better picture. Note that there is swelling of the left suprasylvian gyrus following electrical stimulation Photograph taken 2 hours after completion of the experiment.

FIG. XII.



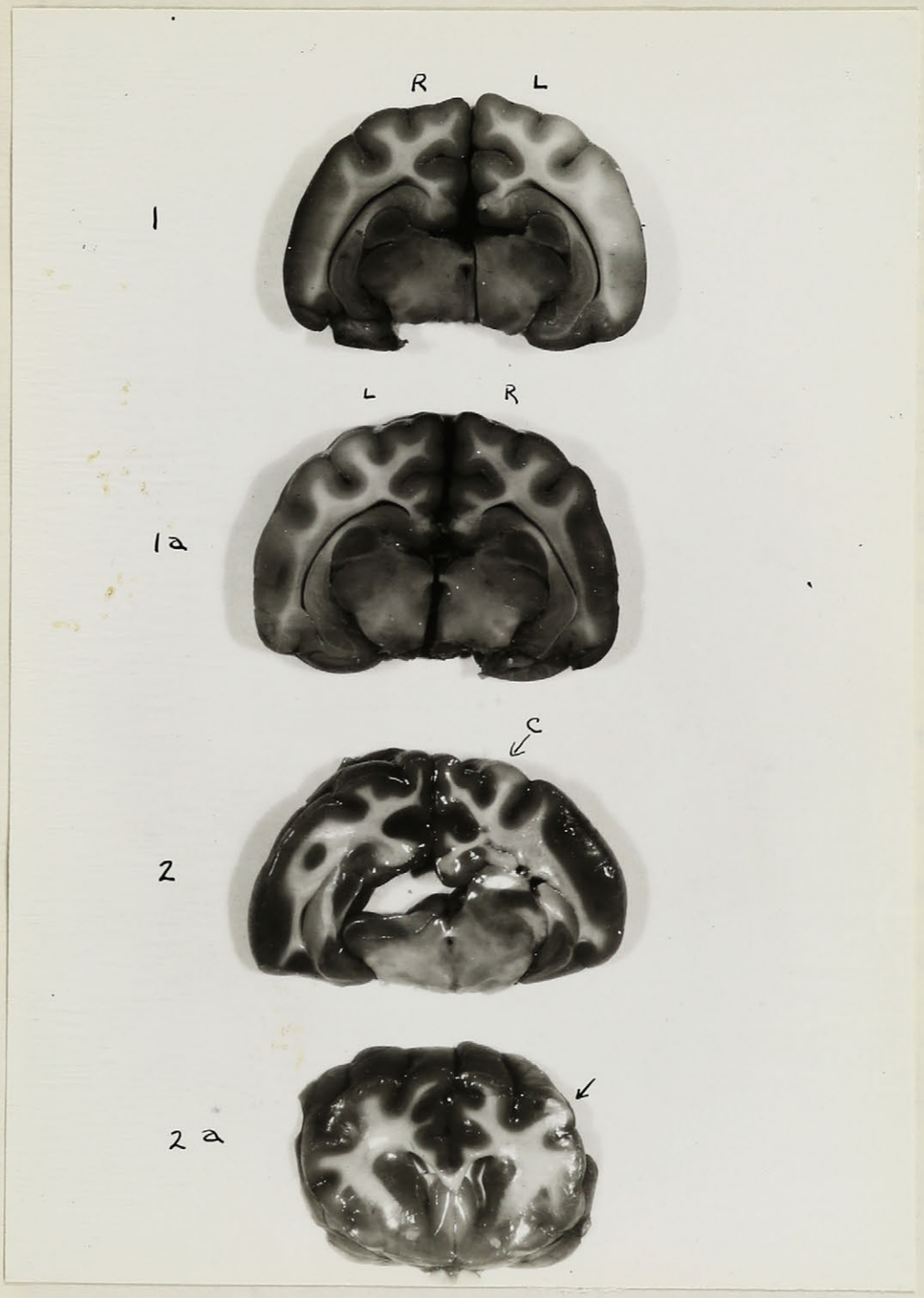
## FIGURE XIII.

The photographs shown here were taken of the brains described in Figs. X. and XI. after these were cut.

Photographs 1 and 1a are of the brain shown in Fig. XI. twenty-four hours after the experiment. The brain had been in formalin 10% in the interval and the aqueous solution had caused partial diffusion and washed out some of the gentian violet from the brain. Note, however, that the grey matter over the left hemisphere is paler than that on the right.

Photographs 2 and 2a are of the brain shown in Fig. X., one hour after the experiment. The arrows point to the patch of ischaemia in the suprasylvian gyrus. Note that this patch is in the form of a definite triangle.

FIG. XIII.



## THE EFFECT OF FOCAL CEREBRAL ISCHAEMIA ON CATS RECOVERED FROM ETHER.

To study the physiological effect of focal cerebral ischaemia on the cortex, five experiments were done on cats recovered from ether. Under sterile technique a craniotomy was performed in the usual manner and the sigmoid gyrus exposed. To do this necessitates opening the frontal sinus. Therefore in the hope of preventing infection the sinus was swabbed out with mercuroehrome and the passage leading into the nose blocked with bone wax.

The motor cortex was then stimulated with bipolar electrodes leading from a thyratron and the threshold stimulus for motor movement determined. The strength of stimulus was then diminished to a subthreshold level for the motor cortex and this strength of current used to stimulate the cerebral vessels. It was first tried out on the vessels in the post-parietal cortex. When these were found to constrict promptly the vessels over other areas were stimulated.

It was found that constriction of all or any vessels could be produced over small or large areas of temporal, parietal or occipital cortex without any apparent clinical effect on the animal (which had recovered from ether) i.e. no motor movements occurred. When the vessels were constricted over the sigmoid gyrus, however, motor movements occasionally resulted and at times these were clonic in nature and persisted

for a short time after stimulation had ceased. It was felt that these movements were probably the result of direct electrical stimulation of nerve cells but the point could not be proved.

## FOCAL CEREBRAL ISCHAEMIA PRODUCED BY MECHANICAL STIMULATION OF PIAL VESSELS.

Like electrical stimulation gentle mechanical stimulation can produce complete obliteration of pial vessels over wide areas of cortex and over small areas vessels can be kept continually constricted for periods as long as half an hour. When stimulation is stopped dilatation of most vessels is usually complete in 3 to 4 minutes.

Similar experiments have been carried out with this type of stimulation on three cats as described with electrical stimulation. Thus diminished blood flow in the cortex has been demonstrated with the blood flow recorder on mechanical stimulation of vessels surrounding the thermocouple and small patches of ischaemia have been shown to be present in the cortex (following mechanical constriction of vessels ) on intravital injection of gentian violet. However the degree of diminished blood flow and the areas of ischaemia have been less marked and more difficult to produce than when the vessels were constricted electrically.

FIGURE XIV.

This is a photograph of a cat's brain. One hour and a half before the photograph was taken the dura was opened bilaterally. The brain exposed on the left side was left in free contact with the room air whereas, immediately on opening the dura, the brain on the right was covered by a watch glass surrounded with gauze soaked in Ringer's solution. The watch glass was removed just before taking the photograph.

Note that there is engorgement of the vessels over the left hemisphere. The vessels over the right hemisphere appear the same as when the dura was first opened. At operation the left cerebral hemisphere appeared diffusely pink in contrast to the white appearance of the opposite side. This is not very apparent in the photograph.

FIG XIV.

L. R.

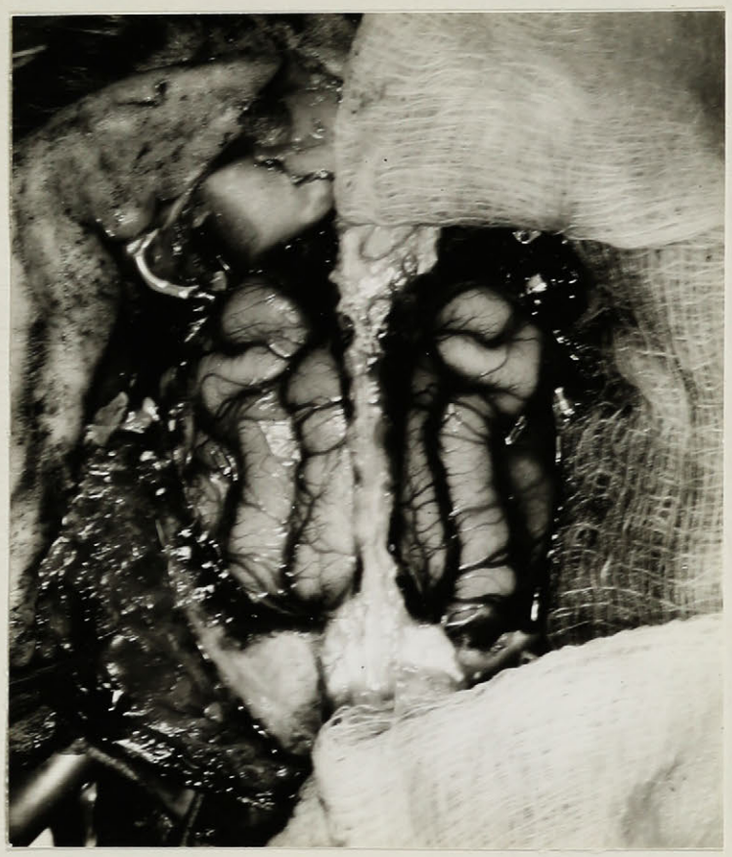
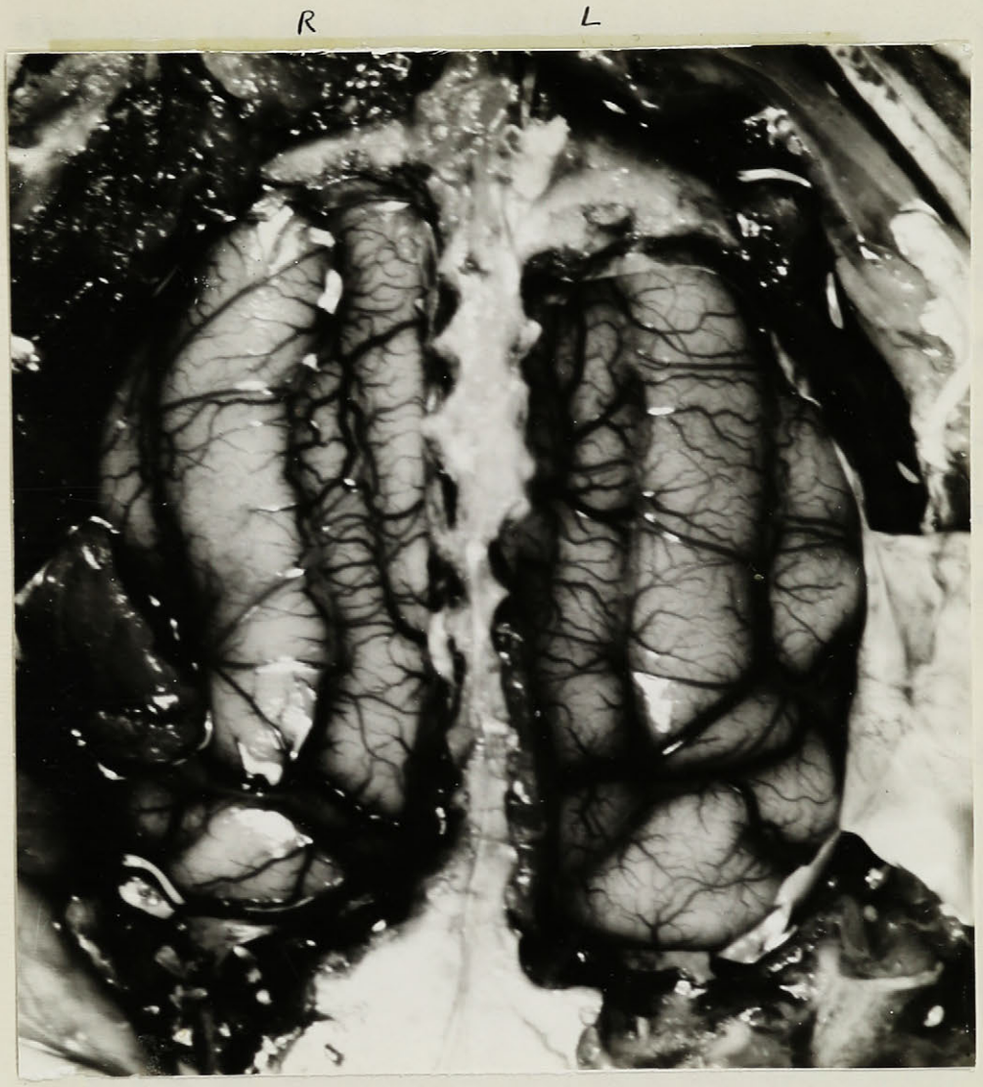


FIGURE XV.

This photograph is of a cat's brain treated in an identical manner to that described in Fig. XIV. Note that the brain on the left which was exposed to air for one hour and forty-five minutes is darker than that on the right and that there is a definite engorgement of pial vessels over the left hemisphere. The brain exposed on the right side was covered (as described in Fig. XIV) until the photograph was taken.

FIG. XV.



## THE EFFECT ON THE PIAL VESSELS OF EXPOSURE TO AIR AND OF IRRIGATION WITH RINGER'S SOLUTION AT BODY TEMPERATURE.

In seven cats the effect on the pial vessels of simple exposure to air was studied (see Part 2. of thesis). In these experiments a bilateral craniotomy was performed on cats that had been anesthetized by dial intraperitoneally. Over the craniotomy defect on one side a small watch glass was placed immediately after opening the dua. Strips of gauze soaked in Ringer's solution were placed about the edges of the watch glass so that any air entering would be saturated with moisture. The cortex of the opposite hemisphere was left exposed to the room air.

It was found that the cortex which had been covered by a watch glass remained normal in appearance even after two hours of exposure. The cortex remained a fresh greyish white color and the arteries and veins showed little evidence of change in color or diameter. In some experiments there was slight dilatation of the arteries.

The cortex of the opposite hemisphere, however, which had been left exposed to room air showed definite and consistent changes (see Figs XlV & XV). In about half an hour after the dura had been opened the arteries on this side became slightly dilated. This dilatation gradually advanced, the arteries becoming full and bulbous and standing out on the surface of

the brain. The veins likewise showed some dilatation. At the same time the smaller arteries and arterioles increased in diameter so that many which were not originally visible to the naked eye became so after about 1 hour of exposure. By the end of one hour the cortex exposed to room air showed a diffuse pinkish blush as compared to the white appearance of the opposite side. The dilatation of vessels reached its maximum at about 2 hours after exposure. Thereafter the dilatation became less noticeable and the cortex took on a more greyish pink color in contrast to the previous brighter pink.

It was found that a similar type of vascular engorgement, to that described, resulted if the cortex was irrigated with Ringer's solution at body temperature. This dilatation of vessels came on almost immediately after irrigation. At times it occurred on irrigation with cool or cold Ringer's but usually it did not. This type of dilatation seemed to be an active response of the vessels to a thermal stimulus. On the other hand the slow dilatation resulting from exposure to room air appeared to be more of a fatigue phenomenon or vasoparesis resulting from the noxious stimulus of drying.

THE EFFECT ON THE CORTEX OF STRONG ELECTRICAL STIMULATION.

In two cat, Nos. P.2178 and P.2184, a gyrus (in one case the sigmoid and in one the middle suprasylvian) was stimulated for twenty minutes with the thyratron at 9, which is close

to the maximum stimulus for the thyratron. In both cases about fifteen minutes after onset of stimulation it was noted that there was quite marked swelling of the gyrus stimulated. This swelling was present when the gyrus was very pale from vasoconstriction of all visible arterial vessels. The swelling therefore could not have been due to increased blood supply to the gyrus. It became even more marked when the blood supply returned to the gyrus. In cat P.2178 the swelling was still very evident at autopsy (verified by Dr. Pudenz and Dr. Erickson) 48 hours later. This partly disappeared after 24 hours in formalin when the photograph was taken. Cat P.2184 was not killed for 15 days after operation and the swelling by this time had been replaced by definite atrophy of the stimulated gyrus. Particulars of the experiment on cat P.2184 will be given in Part 2. of the thesis.

These observations are included here, as the second part of the thesis is confined to the description of autopsy findings and histo-pathological changes rather than to observations at the time of operation. The significance of these observations, however, will be discussed in Part 2. of the thesis in so far as they are related to the histo-pathological findings.

## DISCUSSION AND SUMMARY.

Among the observations recorded here and by Florey (1925) there are a number worthy of discussion.

It will be remembered from the review of the literature presented in the first part of this paper that Lewis (1926) and Krogh (1930) are of the opinion that certain constrictions of vessels in the skin brought about by mechanical and electrical stimulation are purely local in character and apparently not dependent upon a nerve supply to the vessels. Pial vessels, as reported in this thesis, react in an apparently identical way to similar stimulation. The constrictions produced are frequently strictly localized and are not propagated. The time relations for their developement (the latency) and their duration are practically the same in the skin and pia when mechanical stimulation is used. Also the pial vessels which had been partially denervated (or in some cases possibly completely denervated) still contracted promptly on stimulation.

It has been found in this investigation that the most effective type of mechanical stimulus to produce contraction appeared to be stretch of the vessel wall; as, for instance, rolling it from side to side or stretching it by means of a silk thread looped about the vessel. Lewis (1926) likewise found this to be true of vessels in the skin. This seems to be an important point for by this means pial vessels might be able to maintain their tone by a purely local mechanism, i.e. intra-

arterial pressure (or stretch). This might explain Forbes, Nason and Wortman's observation in 1937 that pial vessels dilate when the blood pressure falls to a critical level for the individual animal (usually about 60mm. of mercury) - and conversely when the pressure rises the dilated arteries constrict and soon regain their normal calibre. They point out that Bayliss' observation might be applied here. He suggested that "relaxation of arterioles in response to a fall of blood pressure (as well as contraction in response to increased tension) might be explained as a characteristic of smooth muscle, for it is known to react in a similar manner in other organs". Wolff in 1936 has remarked that, "Great elevations in systemic arterial blood pressure cause slight and even moderate increases in diameter of cerebral arteries when observed in a properly prepared skull window experiment. However, it is striking that such dilatation is far from maximal, is usually of short duration and is followed by constriction while the blood pressure remains at a constant high level".

Wolff (1936) also says "The aortic and carotid afferents, by preventing excessive blood pressure, conserve cardiac energy. Through those nerves arterial hypertension produces peripheral vasodilatation while hypotension produces peripheral vaso-constriction. Now it is regularly observed that the pial vaso-dilatation on stimulation of these afferents starts late, - beginning 15 to 90 seconds after onset of stimulation and

usually when the systemic depressor effect has begun, or the fall in blood pressure is well under way. However, the delay in onset of the dilator effect should not be taken too seriously, because the time of onset of dilatation represents merely the time when dilatation first becomes apparent in the vessels under direct inspection. Since the blood pressure on vagus stimulation usually begins to fall almost at once, the cerebral arteries at first would tend to collapse passively. In fact, in some instances, there is, at first, an actual narrowing which is later followed by dilatation. It is therefore quite likely that the cerebral dilatation occurs simultaneously with or only slightly after, the general vasodilatation" etc.

These two observations quoted from Wolff (1936) might be interpreted as evidence of a reflex vasomotor control of cerebral vessels. On the other hand the fact that vasoconstriction of cerebral vessels occurs with a rise in general blood pressure and that the pial arteries dilate when systemic blood pressure falls is strictly in keeping with the proposition, set forth in this thesis, that a rise in intra-arterial tension (i.e. rise in blood pressure), by stretching the vessel wall would cause it to constrict and conversely that a fall in intra-arterial tension (i.e. a fall in blood pressure), by diminishing the stretch would cause the vessel to dilate. It is interesting that there is a considerable latent period before the above dilatation or constriction occurs for Bayliss notes "Smooth

muscle is caused to enter into contraction by stretching. The rate of contraction of the smooth muscle is usually slow, compared with that of striated muscle; it has also a longer latent period". It will be remembered that a similar latent period and slow contraction of smooth muscle was noted in the experiments reported in this thesis where vessels were stimulated mechanically and electrically.

Vessels in various parts of the body do not react in the same way to a local stimulus and it has been shown here that pial vessels in different animals also vary in their response. The reactions may be of two main types either local, as described above, or apparently definitely dependent upon a neurovascular mechanism. (As described by Chase, 1934).

In considering the apparently local reaction it has been found that the dog's pial vessels are less sensitive to mechanical and electrical stimulation than the cat's and the monkey's are even less so than the dog's. This would lead one to believe that in man constriction would be even more difficult to produce by this method of stimulation. In support of this it is significant that despite the volume of neurosurgery carried out in recent years there is apparently only one observation recorded in the literature to suggest that spasms in pial vessels of humans can be produced by local stimulation. This is the one, already mentioned, by Riser et al in 1931 who described the constriction of a single pial vessel (to 50% of

its original diameter) on mechanical stimulation.

Spasms which are dependent upon a neurovascular mechanism are quite different in type, at least at times, from those described in this thesis. The theories of Ricker regarding the reaction of vessels to noxious stimuli (embolism etc.) are based upon the participation of a neurovascular mechanism. Chase, one of the exponents of Ricker's theories in this country, has shown (1934-38) that an irritant (such as an air embolus) applied to a mesenteric vessel causes a local traumatic contraction which rapidly spreads peripherally to the terminal segments of the vessel, i.e. to the capillaries. Due to this constriction the capillaries fatigue and dilate (constrictor paresis), dilatation then occurs in arterioles, the proximal artery remaining constricted. This leads to slowing of capillary current and exudation of fluid and later red blood cell diapedesis. Although diapedesis of red cells may occur from cerebral vessels as the result of embolism it would seem that the mechanism whereby this is brought about cannot be the same as implied in the theory of Chase; for, as has been shown here, pial vessels (and hence probably intracerebral vessels) in animals do not respond to an irritant in the same way as the mesenteric vessels. It is true they will contract locally but the constriction is not propagated and apparently not dependent upon active neurovascular control.

Also, as pointed out, spasm in human pial vessels is

apparently less likely to occur on local irritation than it is in cats. Therefore, although the pial and cerebral vessels in man are probably under some constrictor (tone) influence, it would seem rather dangerous to theorise about what happens in human cerebral vessels from observations on vessels where a neurovascular mechanism plays an active part (as in the mesentery of rabbits described by Chase, 1934-38).

Although cerebral and pial vessels are under some neural control, evidence that this is less than elsewhere in the body has been well demonstrated. Forbes, Finley and Nason (1933) on stimulation of the cervical sympathetic found that the pial arteries constricted less than those in the dura. Using similar stimulation, Pool, Forbes and Nason (1934) observed the constriction in the vessels of the ear to be 8 to 10 times as much as in the pia. McNaughton, (1938) suggested that while it remains unproven it seems possible that there are quantitative differences in the nerve supply to the blood vessels of the pia, dura, and skin corresponding to their differences in Physiologic response.

In 1937 Villaret et al made some direct observations on the effect of air emboli on pial vessels. They found that the air was not a sufficiently intense stimulus to cause any contraction in the pial vessels. However on injecting solid emboli such as ground up pumis stone they obtained marked

constrictions. The reason for these findings may well be that the air embolus can be compressed into a very thin column which might not cause much stretch of the vessel wall. Since in the present experiments stretch appeared to be an adequate stimulus for spasm none would occur. With a solid embolus like pumis stone however a particle larger than the normal diameter of the vessel would stretch the vessel wall and constriction would result.

The fact that, as shown in this investigation, the pial arteries in the cat constrict so promptly on electrical stimulation and that as a result focal cerebral ischaemia was produced with ease is important to remember in stimulation experiments on the cat's cortex and also on the dog's and monkey's cortex, even though the pial vessels do not constrict so easily in the latter. If this is not taken into consideration, which it rarely seems to be, it would appear that errors might be introduced in studying fatigue of nerve cells to electrical stimulation and in determining thresholds, facilitation, extinction, blood flow and so in the cortex. This observation might partly explain why the excitability of the cortex diminishes on repeated electrical stimulation. Also in these days when electrical exploration of the human cortex is carried out so frequently at operation it should be considered: for it is perhaps possible that strong stimulation might cause spasm in the intra cerebral vessels even if it

isn't observed in the pial ones, (which of course are exposed to air and as a result might conceivably have become refractory). It has been shown that pial vessels react only under certain conditions and the conditions may not have been optimum in observations carried out on the human cortex. For instance in the cat, pial vessels respond promptly if the brain is protected by a glass covering immediately on opening the dura. Frequently, however, they show a period when they are refractory if left exposed (without a glass covering) and then usually become sensitive to stimulation again even if open to air for long periods. During the period when the pial vessels are refractory, the intra cortical vessels of course may still be reacting to the stimulation.

In the experiments described in this paper the observation was made that temporary focal ischaemia from vascular spasm did not produce clinical evidence of epileptiform cortical discharges in cats recovered from ether. This statement deserves some comment. In the first place electrical stimulation was used. By this method the blood supply to large areas of temporal, parietal or occipital cortex was almost completely cut off without producing any clinical effect on the animal. However this does not prove that subclinical eleptiform discharges were not taking place. Also if the stimulation was carried forward to the sigmoid gyrus (motor cortex), movements of the animals

extremities frequently clonic in nature resulted and it could not be proved whether these were due to the direct effect of the stimulation on the motor nerve cells or whether ischaemia played a part. Despite these drawbacks to the method it would seem that it could be used to advantage in conjunction with electroencephalographic recordings from the cortex. For instance the recording electrodes could be placed over a gyrus and ischaemia, relative at least, of this gyrus produced by constricting all the vessels leading to it, without directly stimulating the gyrus itself. In this manner one might be able to test (at least in part) the belief long held that temporary focal ischaemia from vascular spasm may give rise to an epileptic seizure.

Finally if one could regard the reactions of the cat's pial and cerebral vessels as representative of what happens in the human brain then we might have a partial explanation for the transitory paralyses following electrical stimulation and exposure of the brain at operation, and of the transitory paralyses following trauma, of the so called "punch drunk" syndrome from repeated trauma, part of the mechanism concerned in electric shock and even perhaps of "commotio cerebri"; for severe head trauma in the cat as well as electric shock does undoubtedly cause widespread vasoconstriction of cerebral vessels and exposure of pial vessels will produce a degree of

vasoparesis. Unfortunately what happens in the human under these circumstances is still not completely solved and it would appear that spasms in human cerebral vessels are less likely to occur as a result of trauma and electrical stimulation than in animals.

## SUMMARY AND CONCLUSIONS.

1. The effect of mechanical and electrical stimulation on the pial vessels of thirty cats has been studied.

2. The pial vessels in animals contract vigourously on mechanical or electrical stimulation.

3. The dog's pial vessels are not as sensitive to this type of stimulation as the cat's and the monkey's are less so than the dog's.

4. Such evidence as there is, suggests that these spasms are not dependent upon a neurovascular mechanism. They resemble closely the local spasms in peripheral vessels described by Thomas Lewis and others as being muscular and not neuromuscular. They are localized to the site of stimulation and are not propagated as in the neurovascular reactions described by Chase and Ricker in the mesenteric vessels of rabbits.

5. In the experiments mechanical stretch of the vessel wall appeared to be an adequate stimulus to produce vasoconstriction. It is therefore suggested that the tone of a pial vessel, at least in the cat, might be partly maintained by a purely local mechanism, i.e. intra arterial pressure (or stretch). This hypothesis might in part explain the fact that pial vessels constrict with a rise in blood pressure and dilate with a fall.

6. Diminished blood flow in the cortex has been demonstrated as a result of these vasoconstrictions by thermo electric blood flow recording. Areas of focal cerebral ischaemia have been shown to be present, following constriction of pial vessels by electrical or mechanical stimulation, by intravital staining. It has been demonstrated that these areas of focal ischaemia are frequently in the form of a triangle corresponding to the anatomical distribution of the cortical vessels.

7. No clinical evidence was found that temporary focal cerebral ischaemia from vasospasm in the temporal, parietal or occipital cortex of animals recovered from ether gave rise to any epileptiform neuronal discharge. That subclinical neuronal discharges occurred as the result of ischaemia could not be ruled out with the methods employed.

8. The fact that electrical stimulation causes focal cerebral ischaemia through spasm of vessels should be taken into consideration in certain physiological problems where the effect of electrical stimulation on nerve cells is being studied.

9. A theoretical discussion of the findings as regards their application to human vessels is presented, especially in relation to their possible significance in electrical stimulation of the brain at operation, in electric shock, and in head trauma.

# PART II.

## MENINGO-CEREBRAL CICATRIX FOLLOWING CRANIOTOMY AND AFTER ELECTRICAL STIMULATION OF THE CORTEX.

### INTRODUCTION.

In the second part of this thesis (on Dr. Penfield's suggestion) an attempt has been made to determine if the brain or meninges are injured in any way by exposure at operation and whether electrical stimulation has any harmful effect upon them.

It is usually taken for granted that electrical stimulation, as employed in physiological work and in stimulation of the brain at operation has no injurious effect upon the tissues. Since, as shown in the first part of the thesis, electrical stimulation causes cerebral ischaemia through vasoconstriction of pial blood vessels, one would expect to find pathological changes as a result of such ischaemia. This has been found to be true in cats. The lesions have been typical and could be graded in severity according to the duration and intensity of the electrical stimulation, i.e. to the duration and degree of cerebral ischaemia.

Interestingly enough lesions have been found, following simple exposure of the brain at operation, which from their nature, and the similarity of their anatomical distribution

to those produced by electrical stimulation, appear undoubtedly to be due also to cerebral ischaemia.

These findings seem of importance, for judging from the literature, or rather the lack of it, on this aspect of the problem, one gets the impression that the dura may be opened and the brain left exposed during a prolonged operation without any damage to the cortex or subsequent development of adhesions over areas of brain that have not been grossly traumatized.

We know of course that severe injury to the brain gives rise to a meningo-cerebral cicatrix (Hortega and Penfield, 1927, Penfield, 1924 and 1927a, and others): and we also know from Sayad and Harvey's (1923) work that if the dura is opened and closed again immediately adhesions between pachy- and lepto-meninges do not develop providing the arachnoid is not injured. What we do not know, however, is the degree of injury the arachnoid is capable of withstanding and, in the performance of a simple craniotomy, what comprises an injury to the brain or meninges.

The experiments described in the second half of this thesis were in part suggested by observations made at the Montreal Neurological Institute. Clinically it has been the impression here that patients showed less reaction after a small craniotomy than when a large flap was turned. This has

been particularly evident following operations where, after a large craniotomy was performed, the brain was left exposed for long periods and the cortex explored electrically. A number of these patients developed hemiplegia and aphasia 6 to 48 hours after operation even when no cerebral substance was removed.

It is true that the pathological changes producing these signs and symptoms must have been largely reversible for the latter were transitory. However, the fact that in some cases they persisted for several weeks suggests that some tell tale pathological change might have been left behind and it is felt that the histopathological lesions described in this thesis support this possibility.

## REVIEW OF THE LITERATURE.

In the present study of the histological changes in the brain, resulting from operative exposure and electrical stimulation, the interstitial cells (particularly the astrocytes) are found to play a most important part. It seems advisable therefore that the literature concerning these cells should receive some attention.

Excellent reviews of the literature on the interstitial cells of the central nervous system have been given by Penfield in 1924, Penfield and Cone in 1926(a) and (b), Penfield in 1927(a) and (b), Hortega and Penfield in 1927, Penfield and Buckley in 1928, Penfield in 1928 and 1932(b). These papers contain the best work on this subject in English. The review of the literature that follows is largely borrowed from these papers.

Virchow (1846) apparently first distinguished a ground substance in the nervous system and he came to recognize neuroglia cells and fibres. Kölliker (1893) and Andriezen (1893a) subdivided astrocytes into two types, protoplasmic cells and fibre cells. In 1913(a) and (b) Cajal described his gold chloride sublimate method for staining astrocytes. With this method he was able to demonstrate that the astrocyte was a unit, independent of syncytial connections (refuting the theory (Held, 1903) of a glial syncytium). Cajal now separated

off from clasical neuroglia a large group of non-nervous cells, which were unstained or incompletely stained by all the neuroglia methods then in use. He called these cells the "third element" in the nervous system.

Del Rio-Hortega (1919, 1921b) was able to stain and described microglia. He showed that microglia was morphologically and biologically and developmentally different from neuroglia. Under the latter he grouped astrocytes and oligodendrocytes (oligodendroglia).

The morphology of astrocytes has been minutely described by Cajal (1913b). He divides them into fibrous and protoplasmic types according to the presence or absence of fibres in the cell cytoplasm. Normally astrocytes are all protoplasmic in the grey matter of the brain and spinal cord, while in the white matter, including the subpial or plexiform layer of the cerebrum, the astrocytes are fibre formers. The protoplasmic astrocyte is provided with expansions which pass out in all directions and branch with the greatest frequency. The fibrous astrocyte expansions are much straighter, less numerous and longer. Both types are attached to small blood vessels by one or many perivascular feet.

For a further detailed account of the morphology of astrocytes see Penfield (1932b).

The attraction which blood vessels, pia and connective tissue has for astrocytes is a very interesting phenomenon

and not yet completely explained. The perivascular foot (sucker process, foot plate, trumpet, podic) was described by Golgi (1885). Numerous other workers have given detailed reports of the perivascular foot plates of astrocytes and how they wrap themselves around a capillary wall (or small vein or artery). See Penfield 1932b. He remarks "The Virchow-Robin space is demonstrated clearly in pathological conditions when it is seen to be filled with leucocytes or with red blood cells, etc. Under such circumstances the foot process of the astrocytes are external to the space and doubtless make up, at least in part, this outer wall. It does not necessarily follow from this, however, that there are no cells of mesodermal parentage beyond this membrane".

The application of the astrocyte foot process to the pia of the cerebrum, cerebellum and spinal cord has been described by Andriezen (1893b) and many others. Similarly in the development of astrocytes the manner in which spongioblasts send out expansions that terminate at the external limiting membrane or pia mater has been described in detail by Lenhossék (1895) and Cajal (1909).

Further evidence of the influence of an attracting surface on these cells is seen when the nervous system is invaded by the vascular tree. At this stage spongioblasts are still migrating from the ependymal epithelium, only now the peripheral expansion (applied to the pia) becomes attached to a vessel

rather than ot the superficial pia mater. Penfield (1928b) notes "the invasion of the nervous system by the vascular tree with its investment of pia has provided a new attraction surface for the peripheral pole of the spongioblast".

Connective tissue likewise offers an attracting surface for astrocytes. Penfield (1932b) says, "In very old brain scars such as appear long after birth injury tubes of tissue are formed lined about with connective tissue. Within these tubes are to be found fibrous astrocytes many of whose expansions terminate at right angles upon the inner surface of the lining membrane. Thus it is a characteristic typical of astrocytes to line the adjacent surface of a well-organized connective tissue wherever found, although they do not react so to unorganized strands of connective tissue".

"Held (1909) described a "membrana neurogliae superficiales" and "perivascularis", a limiting membrane made up of neuroglia which was said to separate mesodermal from ectodermal tissue of the nervous system. This membrane was contributed to by the perivascular and subpial foot plates of the astrocytes which thus formed a sort of mosaic barrier". Whether the attractions of astrocytes to the various surfaces described can be accounted for on Held's theory seems still undecided. For a further discussion of this important point see Penfield (1932b).

Oligodendroglia (oligodendrocytes) were first demonstrated by Robertson (1899, 1900), under the name of mesoglia, by means of his platinum method. Del Rio-Hortega (1921b) independently re-discovered the cells by means of a more reliable and complete staining method. There is much in common between oligodendrocytes and astrocytes. The nuclei are similar, those of the former being rounder and smaller. They both have centrosomes, have similar Golgi apparatus, similar fuchsinophile granules, similar gliosomes. The outstanding morphological differences are: first, the absence of perivascular feet in oligodendroglia; second, the absance of fibres; third, the smaller cell body with smaller, more delicate expansions. (Penfield 1932b).

Oligodendroglia is found in either the white or gray matter. In the former it is interfascicular and in the latter frequently lying as a perineuronal satellite. "Oligodendrocytes, wherever found and whether interfascicular or satellite, have roughly the same morphology". Penfield (1932b).

In 1919-1921, Del Rio-Hortega stained microglia selectively with his silver carbonate method. He described its morphological and structural characteristics, its morphological evolution to form rod cells and fat granule cells, its motility and phagocytic functions and finally its histogenesis and general distribution in the nerve centres. Later investigators have added little to the fundamental normal and pathological characteristics of

microglia. The microglia represents the reticulo-endothelial system in the central nervous system, in the broadest sense. Penfield (1932b). It is generally accepted that microglia is of mesodermal origin in contradistinction to neuroglia which is of ectodermal origin.

Cerebral cicatrix.

In 1927 Del Rio-Hortega and Penfield studied the histological changes in the brain following simple punctures with a blunt trocar and also after more extensive wounds. They demonstrated most beautifully, by the then recent staining technique, the reactions in the neuroglia and microglia. They found that as early as the first day after an aseptic stab wound of the brain the microglia cells near the lesion had already changed their form to that of amoeboid cells. By two days many microglia cells were present about the lesion with a predominance of the amoeboid and rounded forms. In the early days after the stab, all transitions from the ramified spiderlike microglia cells of the normal cerebrum to compound granular corpuscles could be seen in a zone about the wound. Mitotic division of these granular corpuscles at the wound margin was seen also as early as the third day.

In some large wounds, 10 to 20 days old, all the central area was filled with scattered granular forms of microglia. In wounds of longer duration there were still to be seen rounded microglia cells in the central part, but the separation from

normal tissue was less sharp. Spider-like forms of microglia cells were already seen nearer the wound. In older scars, for example, on the fifty-second day after a small stab wound had been made in a rabbit's brain, microglia cells with expansions almost as complicated as those present in the normal cortex were found. As long as there were products of destruction to be cleared away, compound granular corpuscles were present. As soon as these products had disappeared, the ramified microglia cells again made their appearance and were found in the scar itself.

These observations have been recorded at length as they serve as an accurate index of what one should find in any traumatic destructive lesion of the brain. Del Rio-Hortega's and Penfield's observations on neuroglia will also be recorded at length for similar reasons.

They found that within twenty-four hours after a stab wound had been made in the brain, the astrocytes, close to the margin of the central blood clot, had become irregularly swollen. The expansions had become fat and the cytoplasm granular. At three days, the zone about the wound in which there were altered astrocytes had increased in width. Close to the centre of the stab were to be seen neuroglia cells whose cytoplasm was much swollen and whose expansions were fragmented. They appeared to be undergoing a process of destruction such as is seen in the amoeboid change of Alzheimer (1910) or clasmatodendrosis of Cajal (1913b). The fragments of expansions (Füllkörperchen)

clustered about the cells from which they had broken off. These fragments and the cell bodies were seen to be coarsely granular. A second type of change was noted in the astrocytes a little further from the wound. These astrocytes showed swelling but their expansions were not destroyed.

Fibrillation or a third type of change was also described. They noted that during the process of swelling, glia fibres became invisible owing to turbid protoplasm which surrounded them. After a stage of swelling and multiplication, however, the cells laid down fibrils and became robust fibrous neuroglia. From this point of view scars in the grey matter may come to have the same appearance as those in the white matter. In these preparations, Rio-Hortega and Penfield found multiplication of the astrocytes by amitotic division as early as the third or fourth day.

Acute swelling of oligodendroglia described by Penfield and Cone (1926a) does not apparently appear in this type of injury to the nervous system.

Del Rio-Hortega and Penfield (1927) found that when a connective tissue core forms at the centre of a stab wound, connective tissue collagen fibrils are laid down and the wound contracts. In stabs where no connective tissue was present there was no tendency for a radial arrangement of the astrocytes and no evidence of contraction.

Penfield in 1927(a) summarizes his views on the mechanism

of cicatricial contraction in the brain. He points out that meningo-cerebral adhesions form when the pia mater is injured and cerebral tissue exposed. An anastamosis is thus established between cerebral and meningeal vessels by way of the adhesions or scar. In such a scar are to be found astrocyte expansions attached to vessels well out in the connective tissue, while the astrocyte cell body remains beneath the pia. This is taken as evidence of superficial contraction of the cicatrix which pulls a vessel once beneath the pia through that membrane into the scar, thus carrying with it the attached astrocyte expansions which evidently hypertrophy under tension. He also says that contraction of a conneetive tissue cicatrix within the brain seems to draw towards it the vaso-astral framework. In support of this he shows that in the presence of a cerebral scar there is migration of the ventricle toward it.

In areas of pial adhesion where no break was visible in brain structure, he found a marked hypertrophy of the subpial astrocyte expansions in the direction of the adhesive pull, while their other smaller expansions radiated in all directions as usual.

In 1928 Penfield and Buckley published a report on the histological picture resulting from puncture of the brain with a hollow and with a blunt needle. The findings were similar to those reported by Hortega and Penfield in 1927 in their study

of hollow and closed puncture wounds of the brain. When the core of injured tissue was removed there was less gliosis in the surrounding brain and the astrocytes arranged themselves tangentially to the tract, rather than in a radiating fashion as found in the closed tracts. It is interesting that the lower ends of even the blunt stabs were sometimes partially empty, in which case they resembled the tracts made by a hollow cannula.

Foerster and Penfield in 1930(a) and (b) made a histological study of scars removed from a group of patients suffering from traumatic epilepsy. The scars resulting from gunshot wound are perhaps the most illuminating as far as the present thesis is concerned. Foerster and Penfield found that an outstanding feature of all these scars was the rich plexus of newly-formed vessels in and about the cicatrix. This vascular plexus anastamosed very freely with the large vessels which entered the scar from without. It also anastamosed freely with the intracerebral vessels.

These findings were similar to the ones reported by Penfield and Buckley in 1928 as a result of deep puncture wounds of the brain. They found that in addition to the new collagen which forms so rapidly in a closed brain wound there is a very rich plexus of vessels which anastamose with the surrounding vessels of the brain. The vessels in the collagen tract in turn anastamose with the meningeal vessels. Also, from their

illustrations, it will be noted that the rich plexus of vessels in the brain radiate away from the puncture tract. The radiating arrangement of the astrocytes corresponds to that of the vessels. In the hollow tracts there was no radiation of vessels and little sign of gliosis although the traumatic damage to the brain surrounding the tract must have been about the same in the two types of puncture.

Penfield (1925a and 1932b) suggests that this reactive gliosis about puncture wounds may be due to some stimulating effect from the destroyed cerebral tissue, for when the core of the tracts is removed, little gliosis results. The radiating arrangement of the astrocytes he feels is due to the traction exerted by the contracting scar.

In 1938 Evans and McEachern reported some very interesting findings brought to light in their study of cerebral cicatrization. This work was published in more detail by Evans in the form of a thesis to McGill University in 1937. They feel that there are two chief factors involved in the mechanism of cerebral scar formation: (a) the relative degree of anaemia in the affected zone and (b) the absence or presence of free blood in the cerebral tissues. They studied the late result of a sudden, complete vascular occlusion in the monkey. They found that this resulted in a typical lesion, namely a large cyst surrounded by a minimal zone of glial and connective tissue hypertrophy. They argued that this lesion is the result of a

sudden massive necrosis, the result of anaemia followed by liquefaction. Lesions of another type seen clinically - resident for example in the course of the middle cerebral artery and characterized by abundant scar formation - were interpreted as being due to a partial reduction in blood supply in the distribution of the blood vessels concerned. They feel that the anaemia may be severe enough to result in loss or severe damage to neurones, but of a degree adequate to stimulate glial and connective tissue hypertrophy. Escape of blood through vessel walls, they believe, may also play a role in such processes.

Evans and McEachern are of the opinion that free blood in the cerebral tissue may also have been operative in their second group of cases. They state "in other words red infarction may have occurred to complicate the picture, but if so it no doubt occurred in association with a slowed circulation in the distribution of the involved artery - probably because of an occlusive process in the vessels. That diapedesis of red blood cells does occur when circulation is slowed has been amply demonstrated by the work of Ricker (1919) and Chase (1938)".

Evans and McEachern's hypothesis that relative ischaemia of the cerebral tissues may stimulate growth on the part of the astrocytes is an interesting conception and is upheld by the findings in the present investigation. No evidence that diapedesis of red blood cells occurs when the circulation is slowed has been found however. It is of interest also that

despite slowing of circulation, (of all degrees) even sufficient to cause complete destruction of cerebral tissue, Evans and McEachern found no diapedesis of red cells in their monkeys. Also the work of Chase (1934 and '38) was done on the mesenteric vessels of rabbits and the danger of inferring that similar phenomena may occur in human vessels has been pointed out in the first part of this thesis.

Hypertrophy of astrocytes, and other reactions, following the injection of whole blood into the cerebral hemisphere was studied by Carmichael in 1929. His findings were essentially the same as those of Evans and McEachern who performed similar experiments.

Putnam and Alexander (1938) have made essentially the same observations as those recorded by Evans and McEachern above. They state "The gross and microscopic picture of extensive tissue destruction which follows sudden closure of large arteries is so well known as to require little description. The parenchyma of the anaemic territory rapidly becomes necrotic. There is a loss of minerals. The necrotic tissue is phagocytized by cells of mesodermal and glial origin, and the debris transported to vessels in the vicinity. Glial fibrosis usually stops at the edge of the area of complete destruction as if lacking an ectodermal framework on which to grow. The end result is usually a cyst of yellow fluid, in which a spiderweb-like framework of vessels and connective tissue may float. The details of the

process are superbly described, for example in the text books of Spielmeyer and of Jakob; and Evans and McEachern have recently restudied it from the experimental point of view". - "The process may take a wholly different form if collateral circulation maintains even a small irrigation through the partially infarcted area as has been shown by the pathological studies of Alexander and Newbill. Such partial infarctions are often at some distance from the point of obstruction. Under these circumstances the alterations in the gray matter are much like those already sketched as resulting from temporary interruption of circulation; pyknosis, later necrosis of the ganglion cells, satellitosis, hypertrophy of glia, a variable amount of proliferation of connective tissue".

From the above observations then, we may conclude that a reduction in circulation to the brain if sufficient (and yet not so complete as to cause widespread destruction) will stimulate hypertrophy of astrocytes. This point has a most important bearing on the present investigation and will be treated in more detail in the final discussion.

Rand and Courville (1936) have described the architectural disruption that occurs in cases of cerebral laceration. Most of these changes have been covered in the literature already discussed.

Cerebral contusion is another form of brain injury which has

received considerable attention in the literature. Evans and McEachern (1938) define this state as one which is characterized by multiple, petechial perivascular haemorrhages. These haemorrhages, they point out, may be the result of actual traumatic rupture of the continuity of blood vessels, but they believe on theoretical grounds that the haemorrhages more often represant the profound circulatory changes - slowing of circulation - which accompanies this condition and which may be severe enough to lead to widely distributed perivascular haemorrhages (after the theories of Ricker (1919) and of Chase (1938).).

Alexander and Putnam (1938) have described perivascular haemorrhages in the brain both as a result of traumatic endothelial tears in venous and arterial vessels and also as a result of stasis. They present an excellent analysis explaining why in certain cases the haemorrhages are predominately in the white matter and in certain others in the grey.

The Regeneration of the Meninges and Meningo-cerebral cicatrix.

Under this heading the more important literature on regeneration of the meninges following injury and on meningeal adhesions will be reviewed. The literature on cerebral cicatrix has been discussed in the preceeding pages and will only be mentioned here in so far as the meninges play a role in its formation.

In 1923 Sayad and Harvey showed that the dura mater when injured, heals rapidly without the formation of adhesions to the underlying structures. They believed that the mechanism of repair of this membrane is similar to the repair of an injury elsewhere in the body with the exception that the external surface of the arachnoid acts as a limiting membrane beyond which no further reaction takes place and against which the inner lining of the dura is formed. Also, this impregnability of the arachnoid surface to the reaction of repair in the dura prevents the formation of adhesions and acts as a mould for the reconstruction of the dura.

Lear and Harvey in 1924 carried the above investigation further by studying the effect of injury on the pia-arachnoid alone without injury to the overlying dura. The experiments were carried out on dogs. A decompressive craniotomy was performed and a large curved flap of dura turned down. Two series of experiments were carried out. In one, the pia-arachnoid underlying the dural flap and far away from the suture line, was touched with a red hot spatula. In the other series instead of using the hot spatula the pia-arachnoid was injured by plunging a mosquitoe forcep into the brain, and spreading the points apart on withdrawal. In both series the dura was closed tightly immediately after the injury to the pia-arachnoid, care being taken throughout not to injure the dura except along the suture line.

The conclusions from these experiments were that where there is an injury to the pia-arachnoid and cortex, even though the overlying dura is uninjured, dense adhesions between all three layers of the meninges and the cortex are formed. As early as eight days following the injury, the dura is sufficiently adherent to tear away with it portions of the underlying cortex. In certain of these experiments there were adhesions about the sutures placed in the dura. This finding led them to the belief that injuries much less severe than the ones they used would probably lead to adhesions. They felt therefore that "the essential point in the formation of the adhesions found in these experiments is the injury to the outer cells of the arachnoid". These authors also found that blood could be left in the subdural space without subsequent formation of adhesions - so long as there was no injury to the arachnoid and dura. This latter observation is different from the findings of Penfield and Norcross in 1936. These authors found subdural blood and adhesions in cats previously injured by a hammer blow on the head. They attributed the adhesions to the irritative effect of the subdural blood.

Because of the difference in the reactions of the leptomeninges from the pachymeninges in the above experiment, Harvey and Burr in 1925 were led to study the origin of these membranes. Two series of experiments were performed. In one

series a portion of the nervous system (of amblystoma punctatum) was transplanted without neural crest cells; in the second series a portion of the nervous system was transplanted with neural crest cells. The following conclusions were drawn from these experiments. "The view of His and Kölliker, supported by Salvi, Sterzi, Weed and others, that the pachymeninx and leptomeninx have a common origin in a primitive mesenchyme derived from a specific germ layer, the mesoderm, is incorrect.

Certain ectodermal elements derived in large part from the neural crest are contributed to the mesenchyme and take part in the formation of the leptomeninx.

These facts have been demonstrated experimentally in Amblystoma and are confirmed by observations in the chick and pig embryo.

Such origin suggests that the cells of the leptomeninx may have certain characteristics of their own, apparent in their reaction to injury and in the neoplasms arising in them".

Penfield (1927a) referring to Lear and Harvey's work on the regeneration of the meninges (1924) remarks that "This difference in reaction is not necessarily evidence of a difference in the origin of the pachy- and lepto-meninges, however. Injury to pia-arachnoid breaks the barrier formed by avascular arachnoid and necessarily removes the protective covering of the nervous system. Such a break calls forth an energetic connective tissue response, whether the nervous tissue is exposed in the central

or peripheral system, as though nervous tissue were indeed a foreign body".

Flexner in 1929 repeated Harvey and Burr's experiments on transplants of portions of the nervous system with and without neural crest cells. He found that the presence of neural crest cells failed to lead to observable differences in the meninges of the transplants.

Bagley in 1928 published two papers on the functional and organic alterations in the central nervous system and meninges due to the presence of blood in the subarachnoid space. The first paper was on the experimental results of injections of blood into the subarachnoid space of animals. The second paper was on the findings in clinical cases of subarachnoid haemorrhage. In both the experimental animals and in humans he found the blood gave rise to an inflammatory reaction in the meninges, cellular changes and destruction in the cortex, meningeal and meningo-cerebral adhesions and finally ventricular dilatation. These pathological changes were accompanied by severe neurological signs and symptoms varying from slight differences in behaviour to sever convulsive seizures and even death.

Penfield in 1924 published an excellent paper on experimental meningo-cerebral adhesions. He found that "superficial adhesions result from slight injury and extend some little distance over the surface of the brain from the point injured. These adhesions are composed of an interlacement of connective tissue

fibrils from the dura with the fibrils of a superficial gliosis.

The thickness of the new or adventitious dura, formed after removal of the old, varies according to the available blood supply. Movement of foreign bodies are also factors to be considered. The dura is thick after a decompression operation and thin beneath a bone flap. It is thicker over a brain laceration.

Placement of the least irritating foreign bodies, as celloidin, on the surface of the brain causes a very thick, hard connective tissue envelope to form all about it. The results are the same even when there is no wound of brain and when the foreign body lies beneath a relatively non-vascular bone flap. The layers of this envelope form a bursa-like cavity, preventing direct adhesion to the scalp or skull. Adhesion between brain wound and lower layer of the envelope forms, of course, as well as adhesion between upper layer and skull or scalp.

With this paper of Penfield's (1924) the summary of the literature is concluded. There are of course a number of other excellent works on the nature of meningo-cerebral adhesions but it is felt that the more important facts relating to this subject have already been adequately reviewed here.

# EXPERIMENTAL DATA.

## TECHNIQUE.

In the investigation described in Part II of this thesis the pathological changes in the brains and meninges of eighteen cats, one dog and one monkey were studied at different intervals after these had been exposed to air under various conditions or stimulated electrically at operation.

All the animals were operated on under sterile technique. They were anesthetized with dial 0.5. c.c. of a 10% solution per kilo intraperitoneally.

A large bilateral, or in some cases unilateral, craniotomy was performed on each of the animals. This was either in the form of a decompression or an osteoplastic craniotomy, (for particulars see tables of experimental data). In the large majority of the cats the supra sylvian gyrus was exposed with only a small portion of the surrounding cortex. The bone defect was always considerably larger than the opening made in the dura.

The dura was then opened and gently turned down over the temporal muscle, its inner surface being exposed. Great care was taken not to touch the arachnoid at any time.

Experiments of different types were carried out, the exposed cortex of one hemisphere usually being treated differently from that on the opposite side (see tables of experiments for

particulars).

In some cases the exposed brain was covered by a watch glass on one side or by a petri dish if both operative sites were to be protected from room air. If this was to be done gauze soaked in Ringer's solution was laid about the margins of the wound (as described in Part I of the thesis). The watch glass or petri dish was placed on this and its edges sealed with moistened gauze so that any air reaching the covered brain would be saturated with moisture.

The brain was stimulated electrically in a number of experiments. This was carried out with bipolar electrodes leading to a thyratron as described in Part I of the thesis. The brain was stimulated either when covered with a petri dish or exposed to air. If the former was done, the electrodes were inserted through a small hole in the petri dish. The duration of stimulation varied as did the intensity, the latter ranging from 3, (3 to 5 is about the threshold for the motor cortex in unanesthetized cats) to 9. In all cases, however, the stimulating electrodes were applied to the cortex at 2 second intervals, the duration of each individual stimulation being 2-3 seconds.

In certain experiments the exposed brain was irrigated continually. This was done by allowing Ringer's solution, at body temperature in an intra-venous infusion flask, to flow over the cortex. The rubber tubing leading to the flask was run through a large basin of water (also at body temperature).

In one experiment, using a similar set-up, iced Ringer was allowed to flow over the operative exposure.

During the operation in each case a drawing was made of the exposed brain and in some cases a photograph taken of the operative site.

At the termination of each experiment the dura was closed tightly with fine silk, the bone flap was wired in place and the scalp closed in layers with silk, dermal being used for the skin.

The animals were then allowed to live for periods varying from 24 hours to three and one half months.

All animals were killed in the same manner. They were anesthetized with ether. A canula was then inserted in one carotid artery. When this was in place the animal was killed by a stab wound in the heart. The external jugular vein, opposite to the carotid containing the canula, was then cut and 200 c.c. of a 10% formalin solution injected slowly into the carotid. After a period of 20 minutes the old cranial wounds were carefully opened. The superficial layers of the temporal muscle were cut away by sharp dissection until the bone defect could be seen. If this was a decompression the dura was incised about its margins so that any adhesions between dura and pia-arachnoid would remain undisturbed. The bone over the remainder of the vault was then ronguered away and the brain removed. In this way the dura and a portion of the overlying temporal muscle was left attached to the removed brain over the site

previously exposed at operation. (see photographs). Where an osteoplastic flap had been turned this was carefully elevated. If it seemed impossible to do this without disturbing underlying adhesions, a saw cut was made directly through the whole skull in the manner described by Penfield (1924) and a block of brain, bone and temporal muscle removed intact.

The brain was then placed in 10% formalin. In several cases the brain was cut immediately and blocks placed in 95% alcohol for subsequent Nissl staining, in Zenkers for Mallory's phosphotungstic acid staining or in formalin ammonium bromide for silver and gold staining.

In most cases the brain was left in formalin 10% for 3 to 4 days and then photographed, cut and rephotographed. Blocks for paraffin sections were left in formalin for another 24 to 48 hours, then washed overnight and run through the alcohols in the usual manner. P.T.A. staining was done on Zenkerized paraffin sections. Penfield's second modification of Rio-Hortega's silver carbonate method was used for combined oligodendroglia and microglia staining. Excellent staining was obtained with gold chloride sublimate for astrocytes by cutting frozen sections directly from formalin fixed material, even when this was old. The sections were received in 1% formalin, washed rapidly in two changes of water and placed directly into the gold bath. The material fixed in F.A.B.

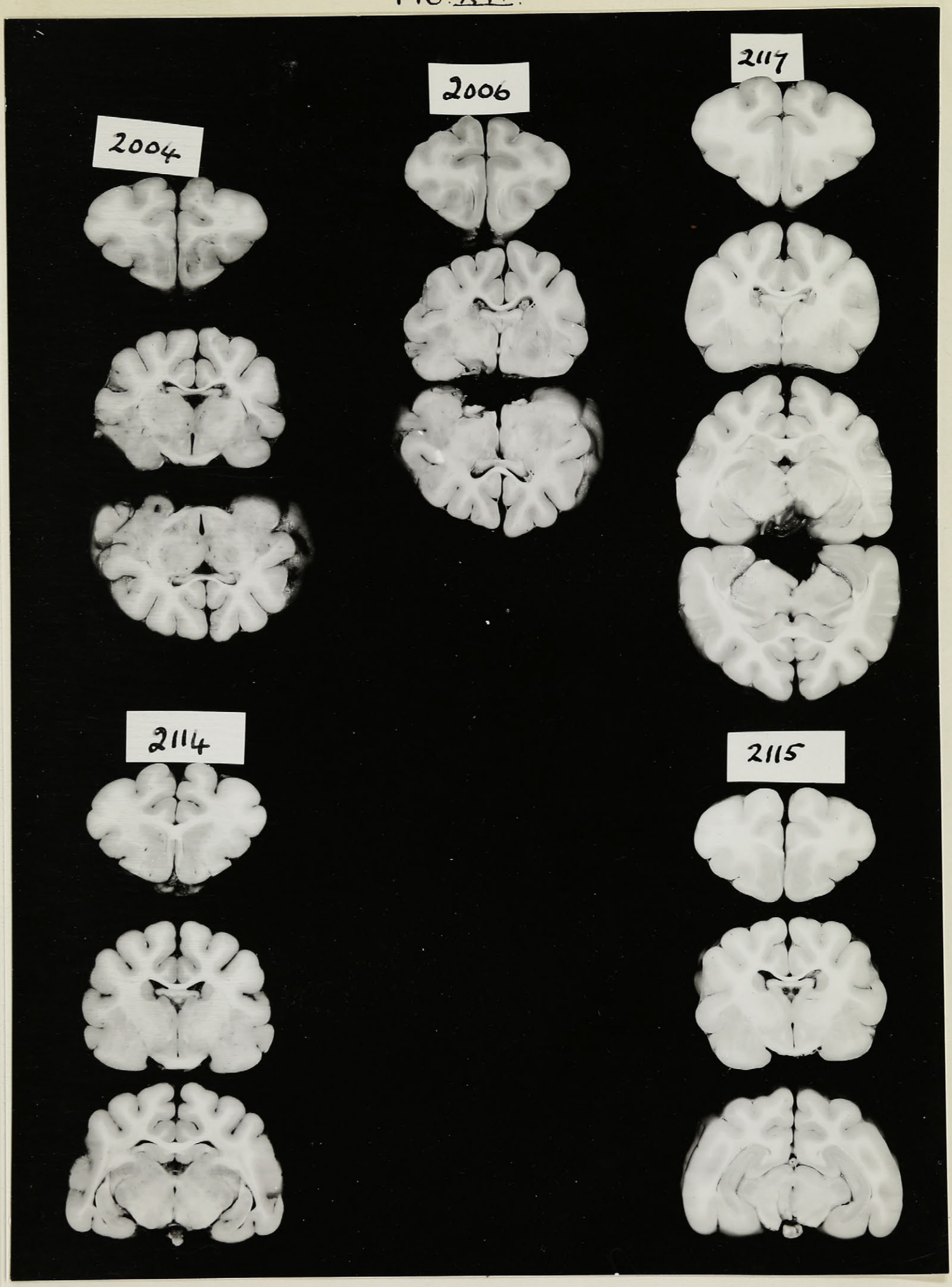
did not give as good results nor did that treated with the Globus procedure.

Using the above methods the following staining techniques were completed on each of twenty brains. 1. Haematoxylin Van Geisen (Weigert's). 2. Nissl's method for nerve cells modified (Thionin). 3. Cajal's gold chloride sublimate method for astrocytes (modified). On a large number of these brains where the research indicated it the following staining methods were used. 1. Mallory's phosphetungstic acid method. 2. Bodian's method for axis cylinders. 3. Loyez's method for staining Myelin sheaths in Paraffin sections. 4. Del Rio-Hortega's silver carbonate method for oligodendroglia. 6. Penfield's second modification of the above method - for combined oligodendroglia and microglia.

FIGURE XVI.

These photographs are of brains described in the text and were taken after the brains had been in formalin for three days or more.

FIG. XVI.



## FIGURE XVII.

In this Figure are shown photographs of brains described in the text. In brains 2004 and 2006 note that there is roughening of the pia-arachnoid over the left hemisphere from which the adherent dura (at the site of the previous operation) has been stripped. There is slight atrophy of the left suprasylvian gyrus in brains 2004 and 2006.

FIG. XVII.

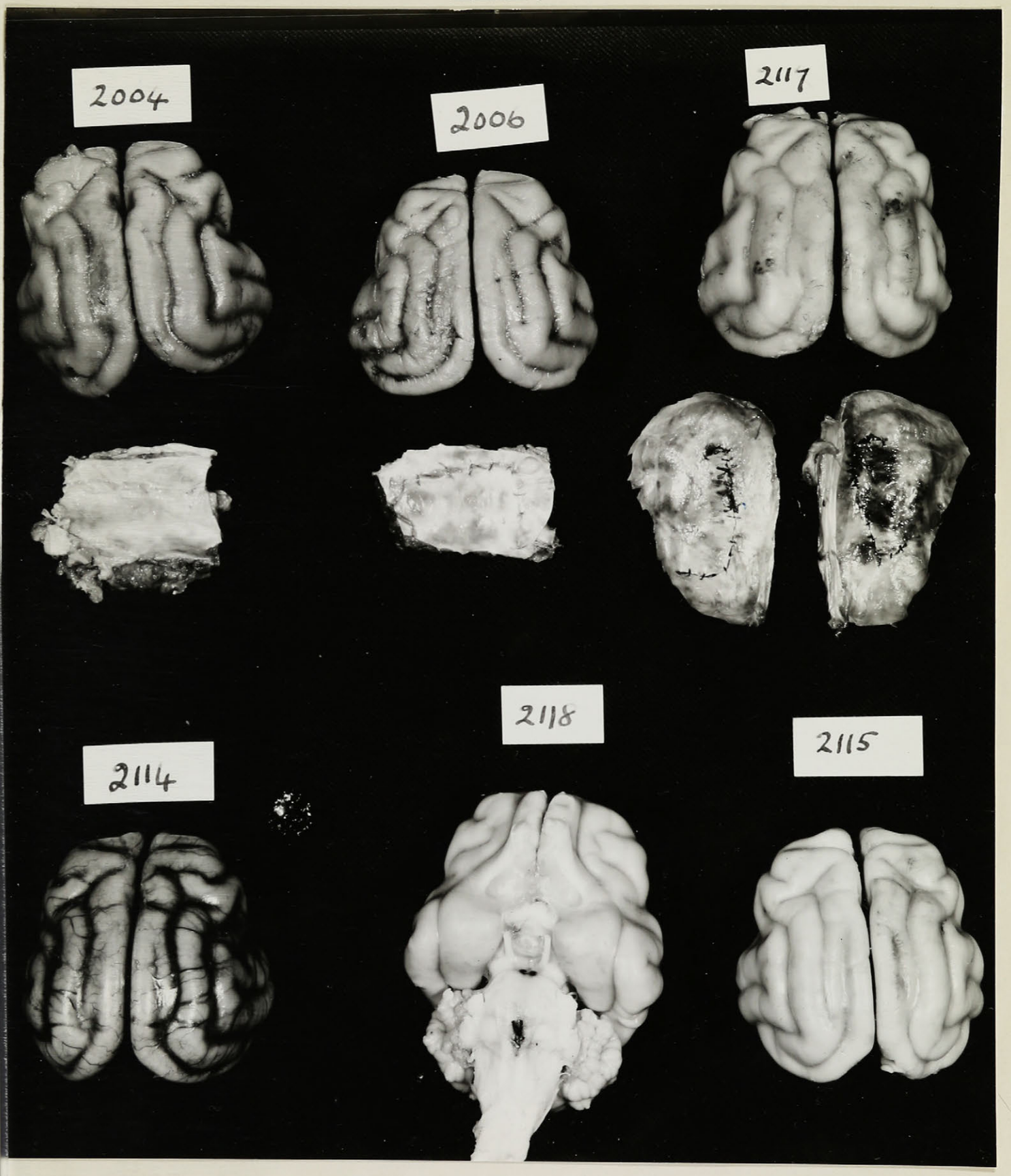
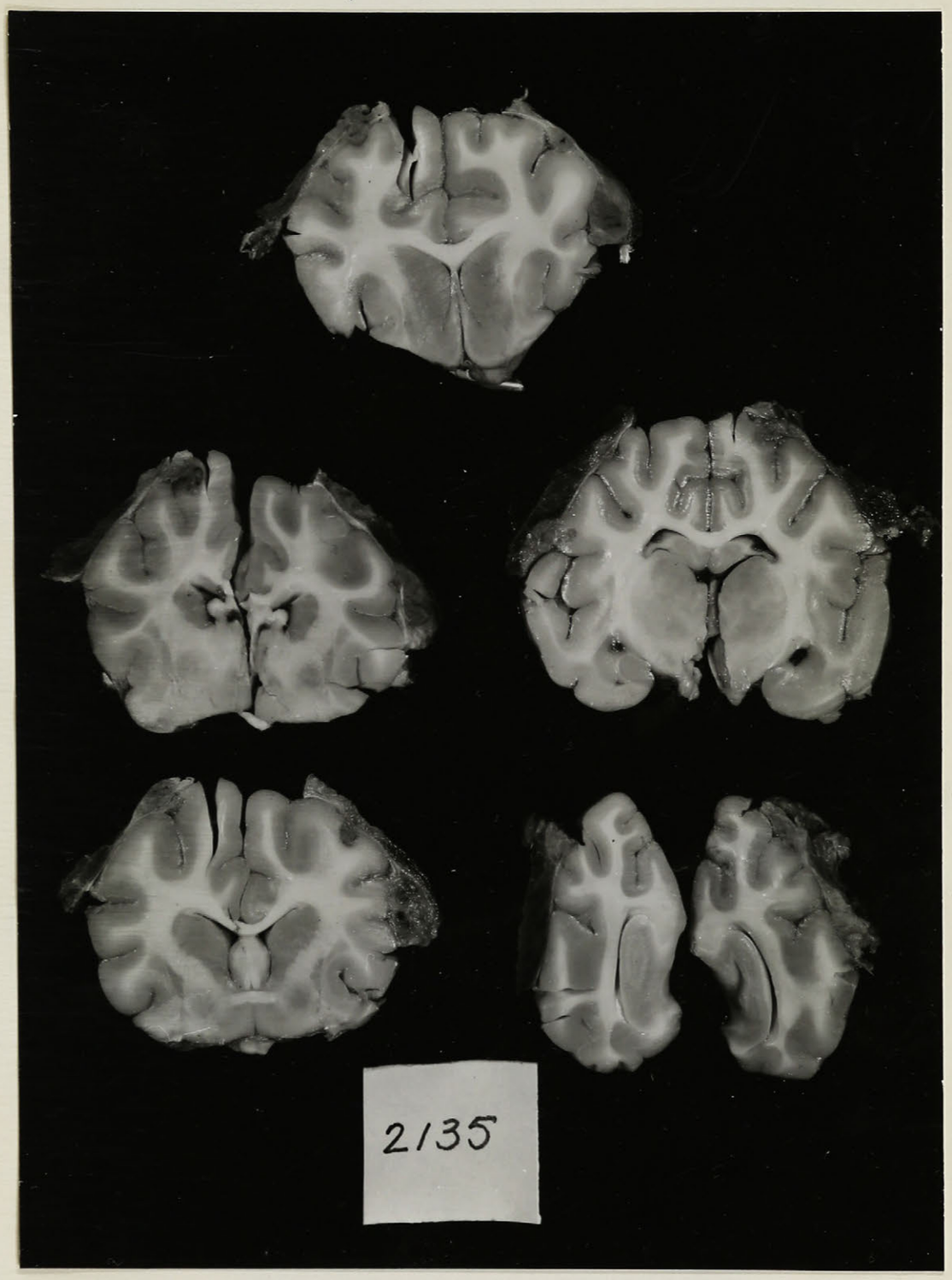


FIGURE XVIII.

This photograph is of a dog's brain (No. P.2135) described in the text. The animal was killed 105 days after operation. Note how densely adherent and thickened the dura is in this case. In places there is some convolutional flattening. This was more evident than in any other brain. See tables of experimental data for particulars of experiment.

FIG. XVIII.

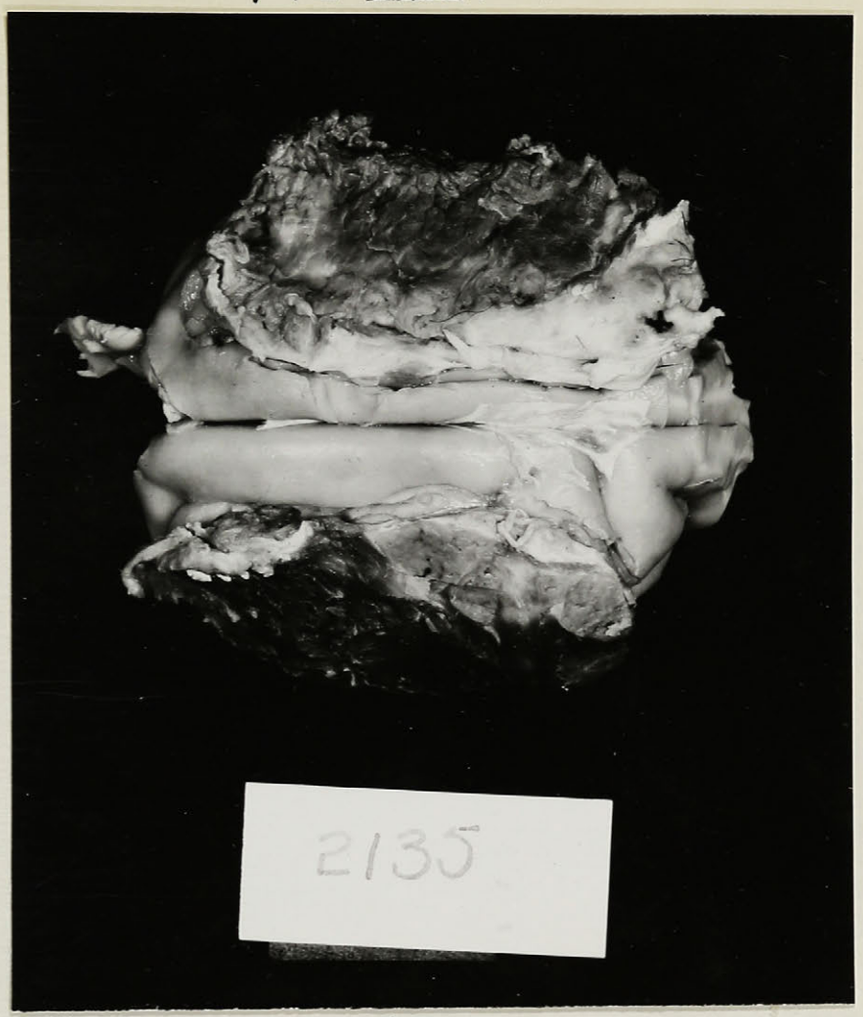


## FIGURE XIX.

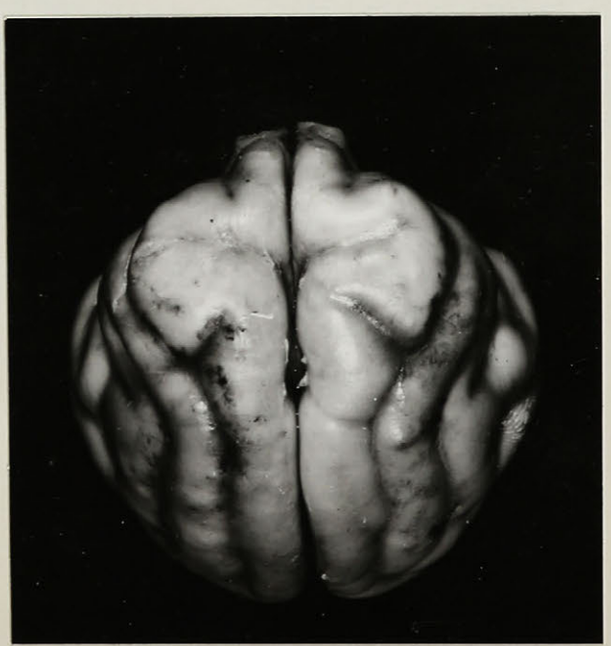
The photograph marked 2135 is of the same dog's brain shown in Fig. XVIII. The adherent dura with some overlying temporal muscle is well seen. The extent of the previous craniotomy can be judged from the area of brain covered by adherent dura.

Photograph B. is of the brain of Cat P.2178. The sigmoid gyrus and immediately adjacent cortex of this cat had been stimulated for ½ an hour. (Thyratron - intensity 9). Note that there is swelling of the left sigmoid gyrus. This brain had been in formalin 10% for 24 hours before the photograph was taken and some of the swelling had already subsided.

FIG. XIX.




A



B

## FIGURE XX.

This photograph is of the brain of monkey P.2137. The animal survived 27 days after a left osteoplastic craniotomy. For particulars of experiment see tables of experimental data.

Note that there is little thickening of the dura and that it is not adherent to the temporal muscle. Compare this brain with those in which a decompression had been done as shown in Figs XIX., XXII., XXIII., etc.

FIG. XX.

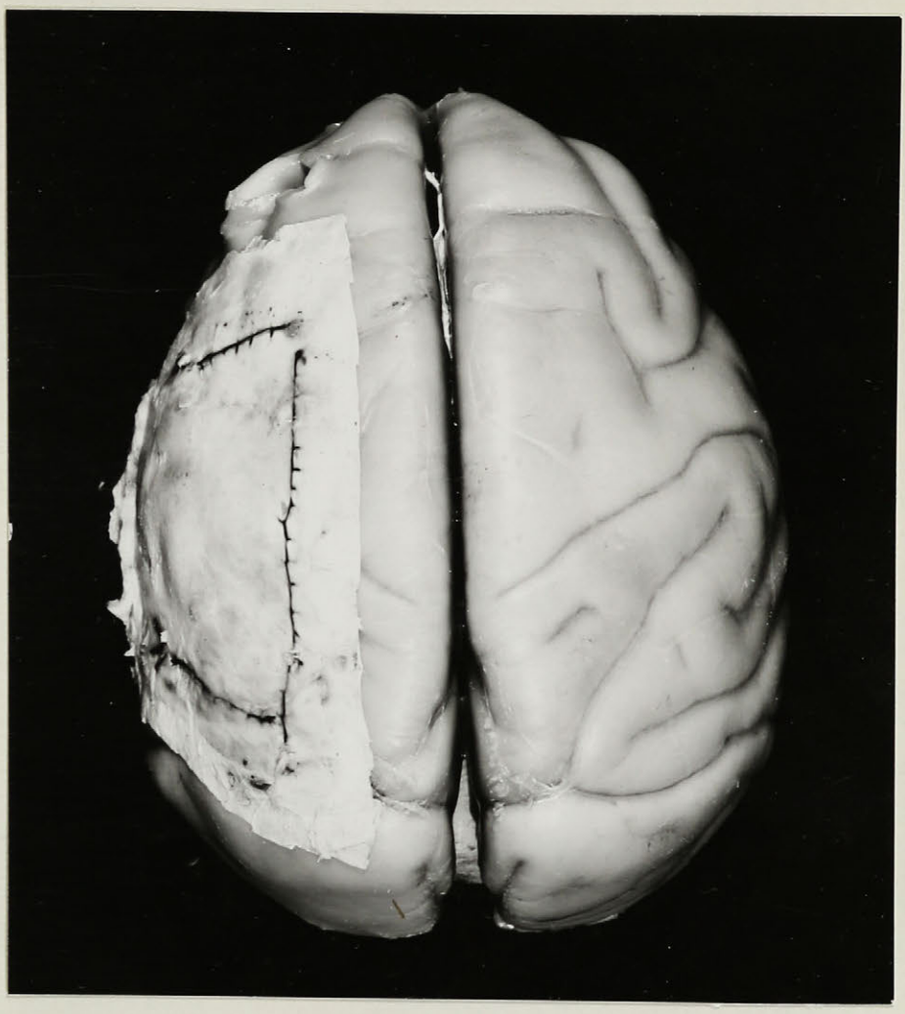


FIGURE XXI.

The photograph is of a monkey brain P.2137, the same brain as shown in Fig. XX. Note that there is little or no thickening of the dura, which is, however, adherent to the pia-arachnoid at the site of the previous operative exposure.

FIG. XXI.

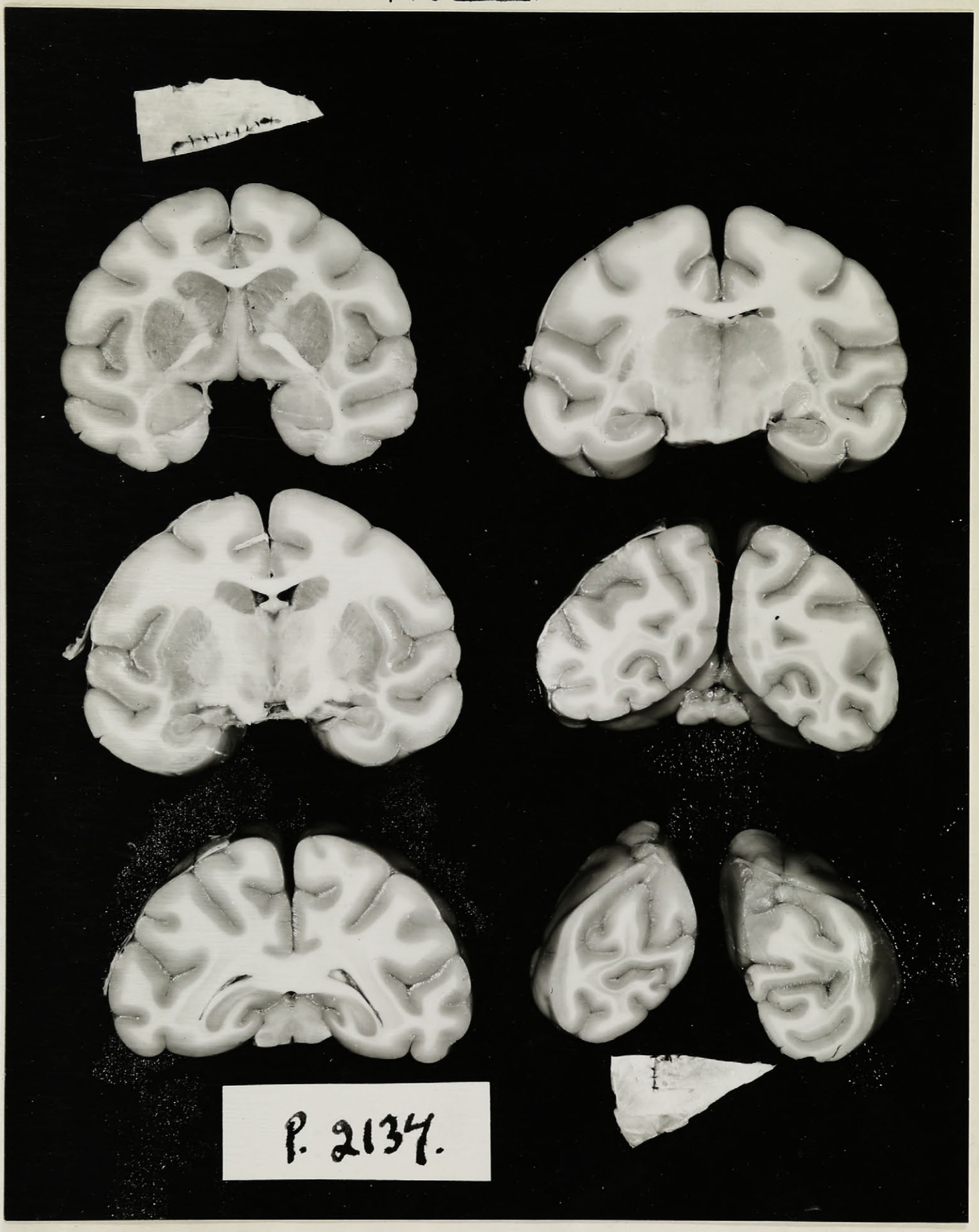
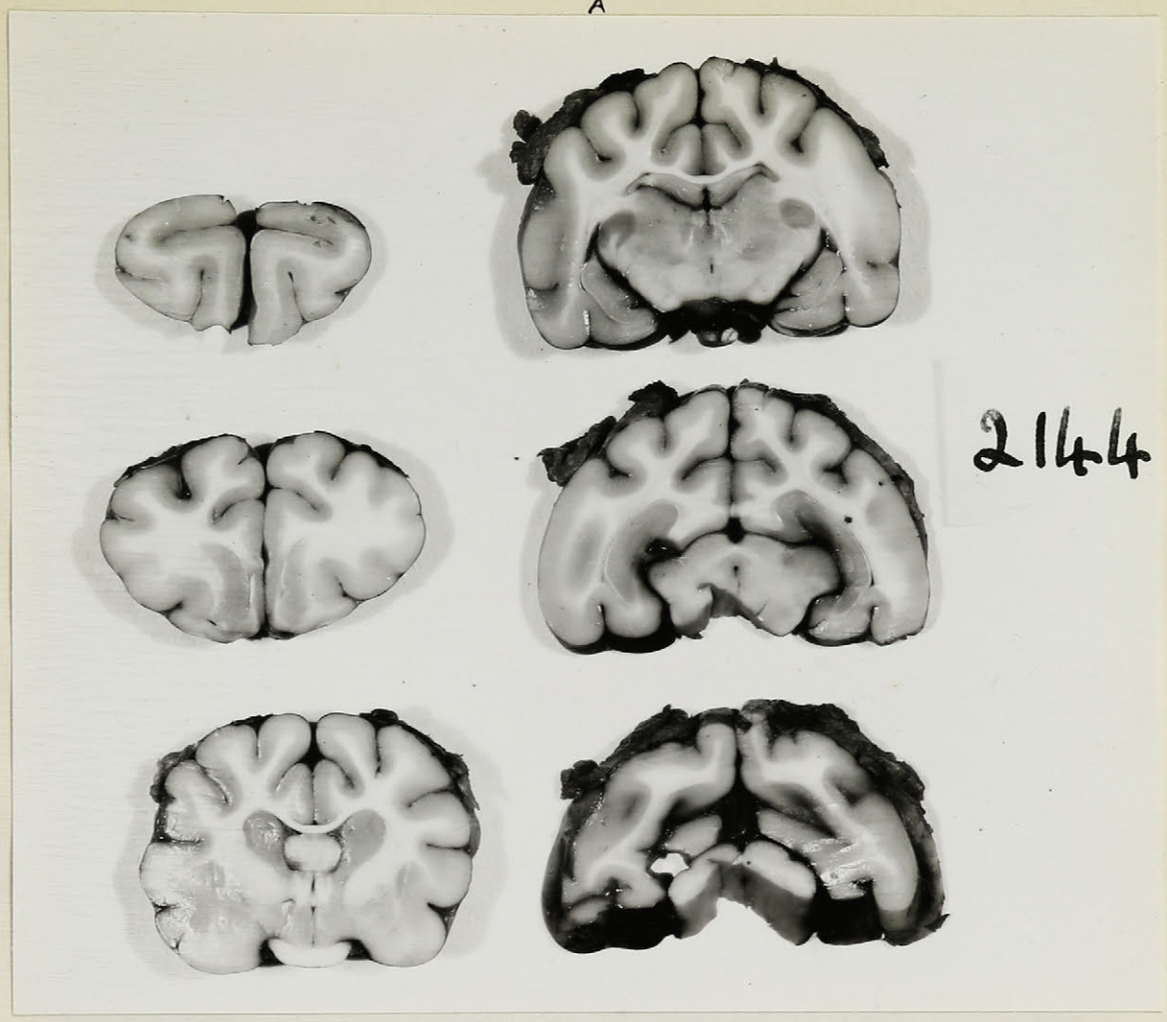


FIGURE XXII.

The two photographs shown here are of the same brain - cat P.2144. Note the thickened adherent dura over both hemispheres. Some temporal muscle is adherent to the outer surface of the dura. See the text and tables of experimental data for further particulars.

FIG. XXII

A



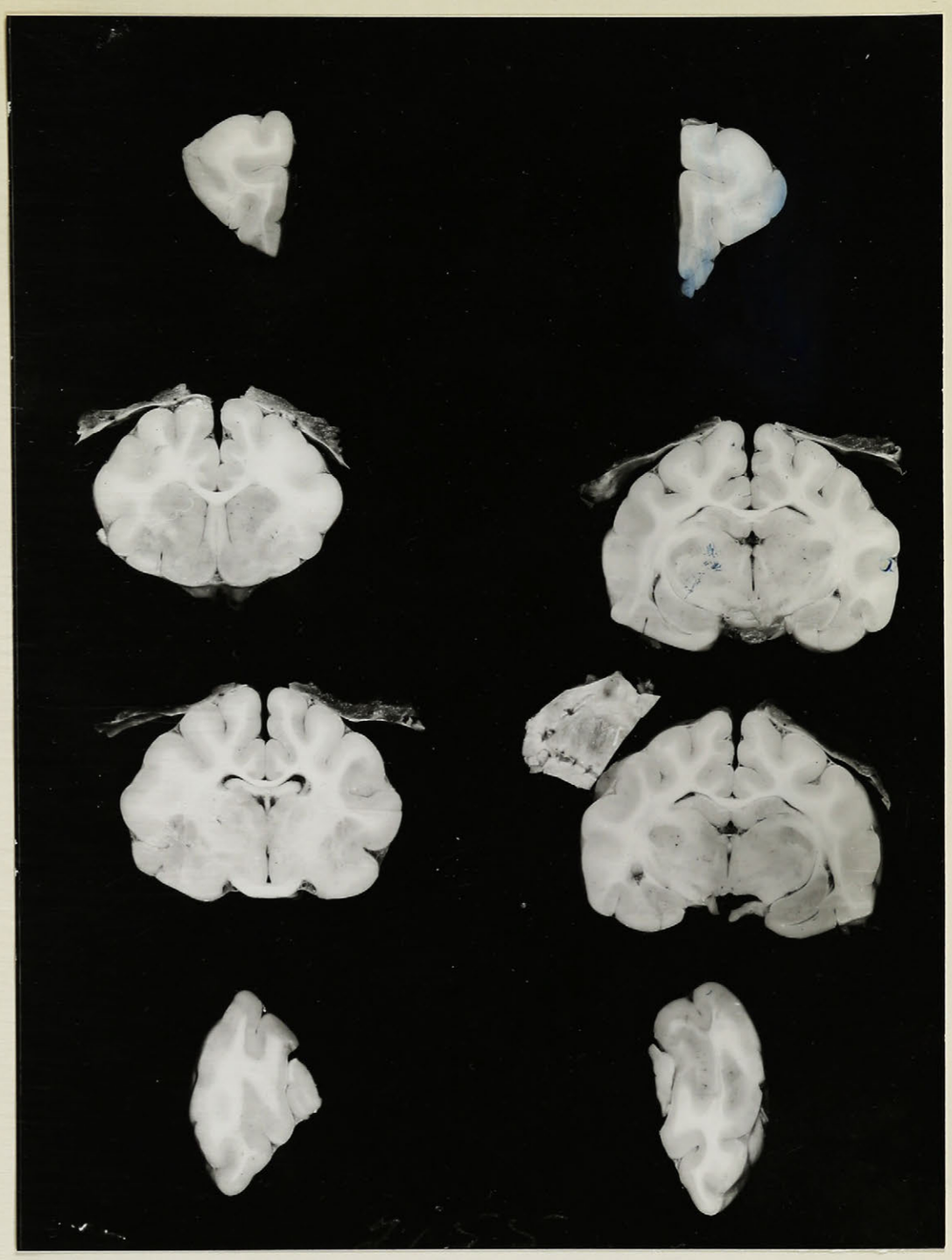
2144

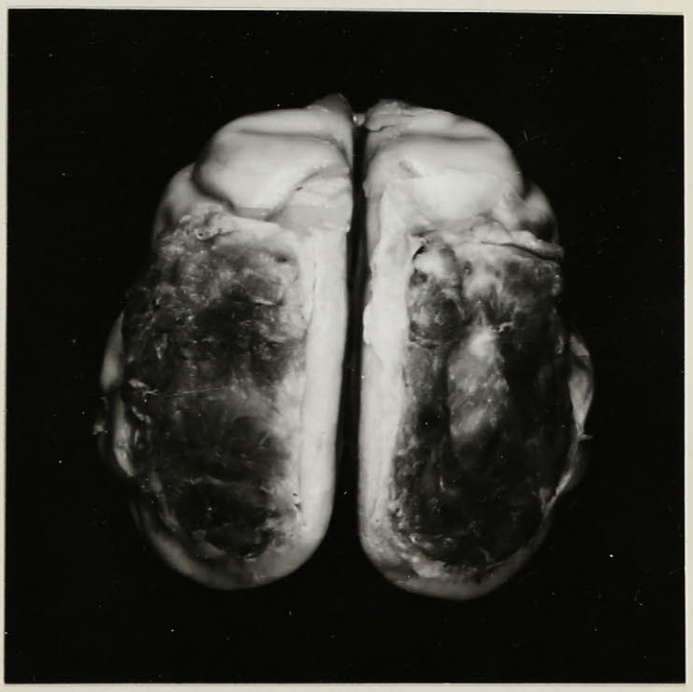
B

FIGURE XIII.

The two photographs on the opposite page are of the brain of cat No.P.2155. In the upper photograph it will be seen that the dura has been elevated to demonstrate its point of maximum adherence to the pia-arachnoid. This is at the suture line in the dura and over the suprasylvian gyrus in each hemisphere. In the lower photograph the extent of the craniotomy (decompression) can be judged by the extent of the temporal muscle adherent to the dura. The actual opening in the dura at operation, however, was considerably smaller than this, exposing mainly the suprasylvian gyrus, but also a portion of the lateral and ectosylvian gyri. The extent of the craniotomy and dural opening in this experiment is about the same as performed on most of the other cats in this investigation.

FIG. XXIII.





## FIGURE XXIV.

Both photographs on the opposite page are of the brain of Cat No.P.2168. In the upper photograph the dura has been stripped from the brain and the extent of the dural opening can be judged by the line of silk sutures in the dura. Note that there is some flattening of the convolutions bilaterally in this brain.

The lower photograph shows essentially the same features as described in the lower photograph of Fig. XXIII.

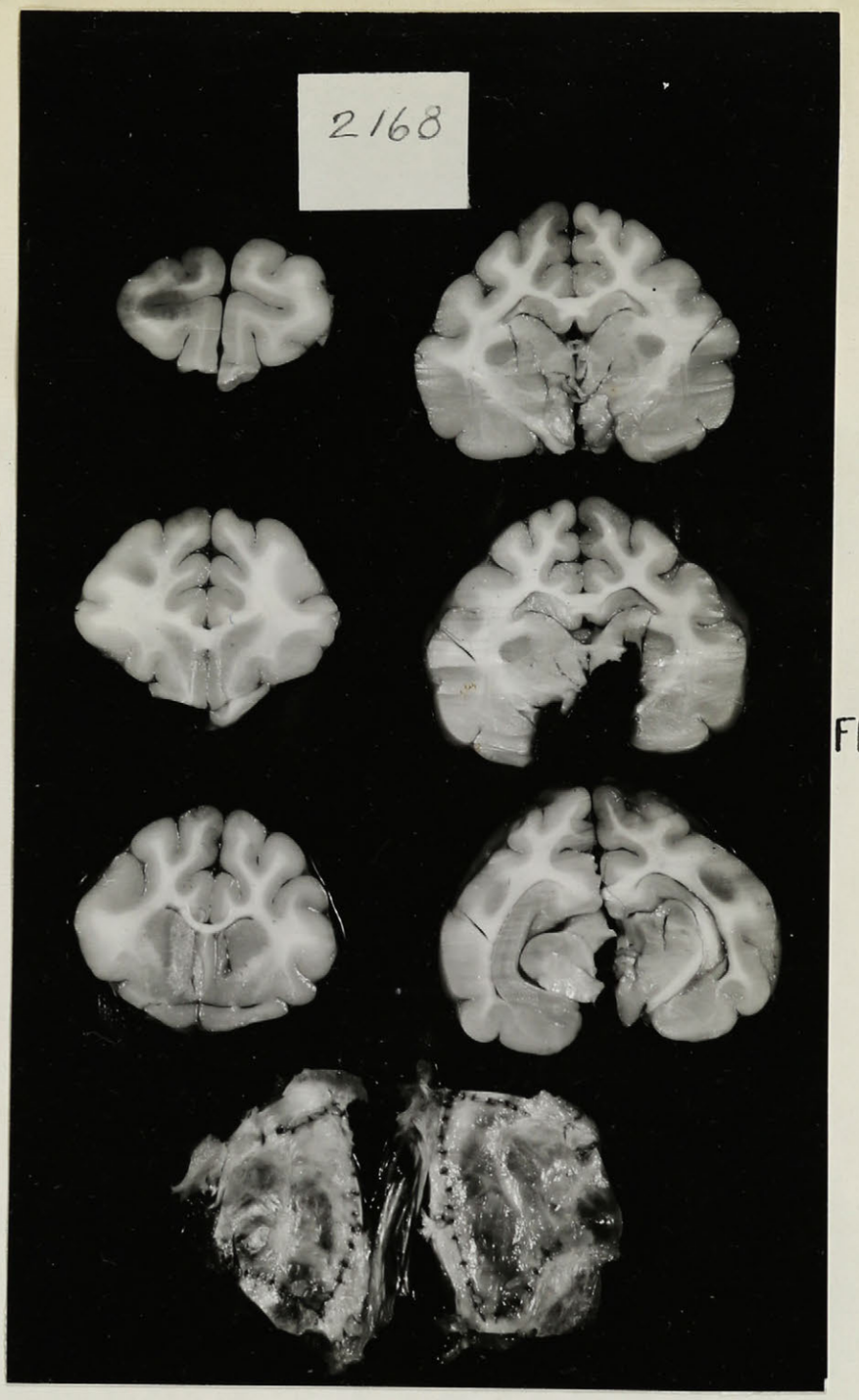



FIG. XXIV.

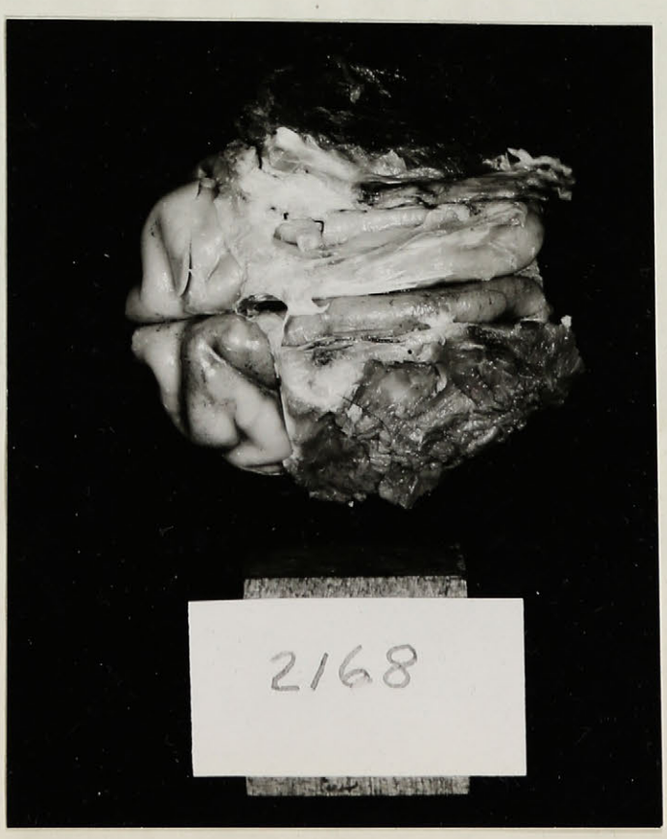


FIGURE XXV.

The brains shown on the opposite page have been described in the text. Brains 2186 and 2184 show adherent dura bilaterally and in places this has been elevated to show the point of maximum adhesions.

Brain 2194 shows no evidence of adherent dura, (see text and tables of experimental data).

Brain 2189 - the osteoplastic craniotomy as seen, was exceptionally small in this case.

Brain 2173 is from another experiment not included in the thesis.

FIG. XXV.

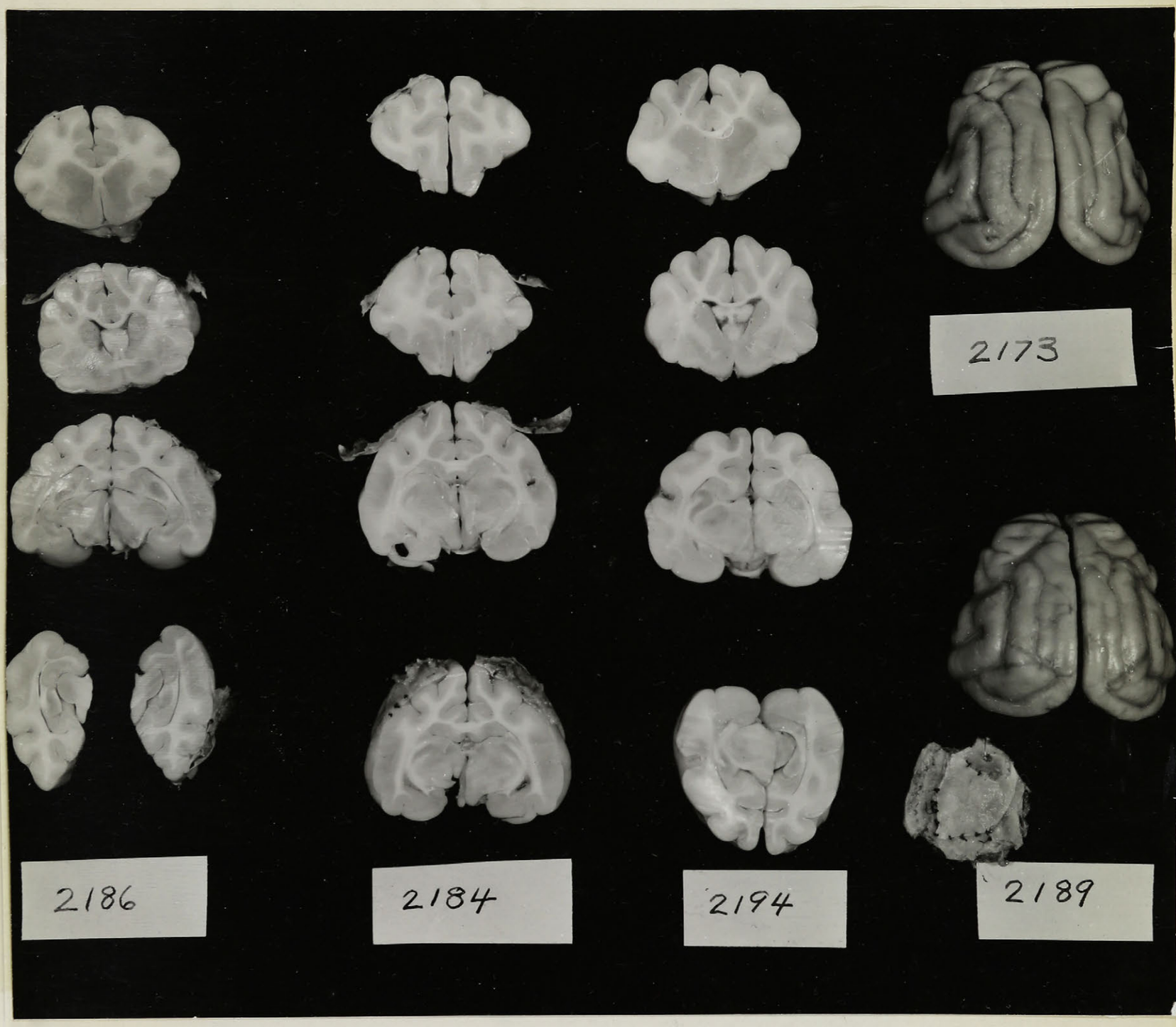
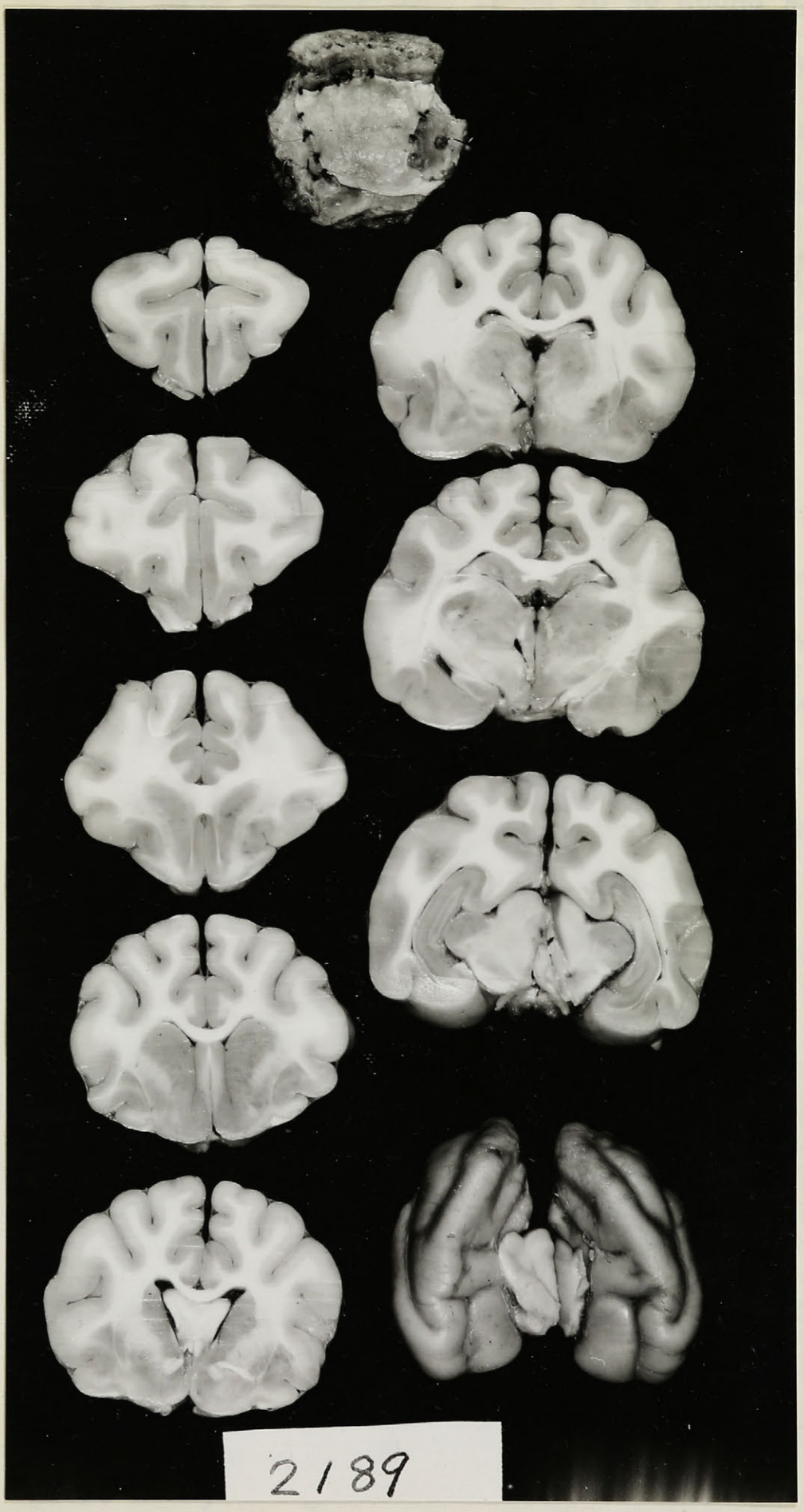


FIGURE XXVI.

This photograph is of the brain of cat P.2189 - already shown in Fig XXV. The dura in this case was adherent to both the arachnoid and the bone flap and came away from the brain when the flap was elevated.

FIG. XXVI.



## THE BRAIN AND MENINGES AT 24 TO 96 HOURS AFTER OPERATION.

Under this heading the pathological changes in the brain and meninges resulting from previous electrical stimulation at operation or their exposure to room air under various conditions are described together. Six cats were used for this study. The general outlines of the experiments are described under technique and the particulars of each are summarized in table I.

In cat P.2117 during electrical stimulation an attempt was made to avoid the larger blood vessels, so that little vasoconstriction of these occurred. In cat P.2124, however, the arterial vessels were systematically stimulated and considerable ischaemia of the exposed cortex produced. This was also true in cat P.2178 where the sigmoid gyrus was stimulated. It should be noted (in table I.) that electrical stimulation was of a greater intensity in cat P.2178 than in the others.

### A. Meningeal Adhesions.

At autopsy it was found that a few fine filamentous adhesions were present between the dura mater and arachnoid in all three cats that had been allowed to survive 72 hours or more. These adhesions were found bilaterally over the area of brain exposed at operation and appeared to be of equal extent whether the brain had been exposed to room air, covered with a petri dish, irrigated with Ringer's solution every fifteen

minutes or stimulated electrically. No adhesions were found in those cats that had been killed 48 hours or less after operation.

There were no other significant pathological findings at autopsy, (or later when the brains were cut) in these animals except in cat P.2178 described below. The wound in each case appeared normal, and there were no abnormal collections of fluid or blood. The temporal muscle had become very slightly adherent to the dura in the cats surviving more than 48 hours. There was no evident oedema of the brain, nor flattening of convolutions at the site of operation. Whether any vascular congestion was present could not be ascertained as each of the brains was injected with formalin before removal. The injections were all satisfactory.

The brain of cat P.2178 showed definite quite marked swelling of the left sigmoid gyrus (gyrus stimulated at operation). This was verified by Dr. Erickson and Dr. Pudenz. Similar swelling had been noted at operation, (see Part I. of the thesis and Fig. XIXb).

B. Cellular reaction in the Meninges.

Histologically the sections stained by Weigert's H.& V.G. method showed the most interesting changes. In each of the six brains, over both hemispheres, at the site of exposure at operation there was a cellular reaction in the meninges with some swelling of both pia mater and arachnoid membranes.

## FIGURE XXVII.

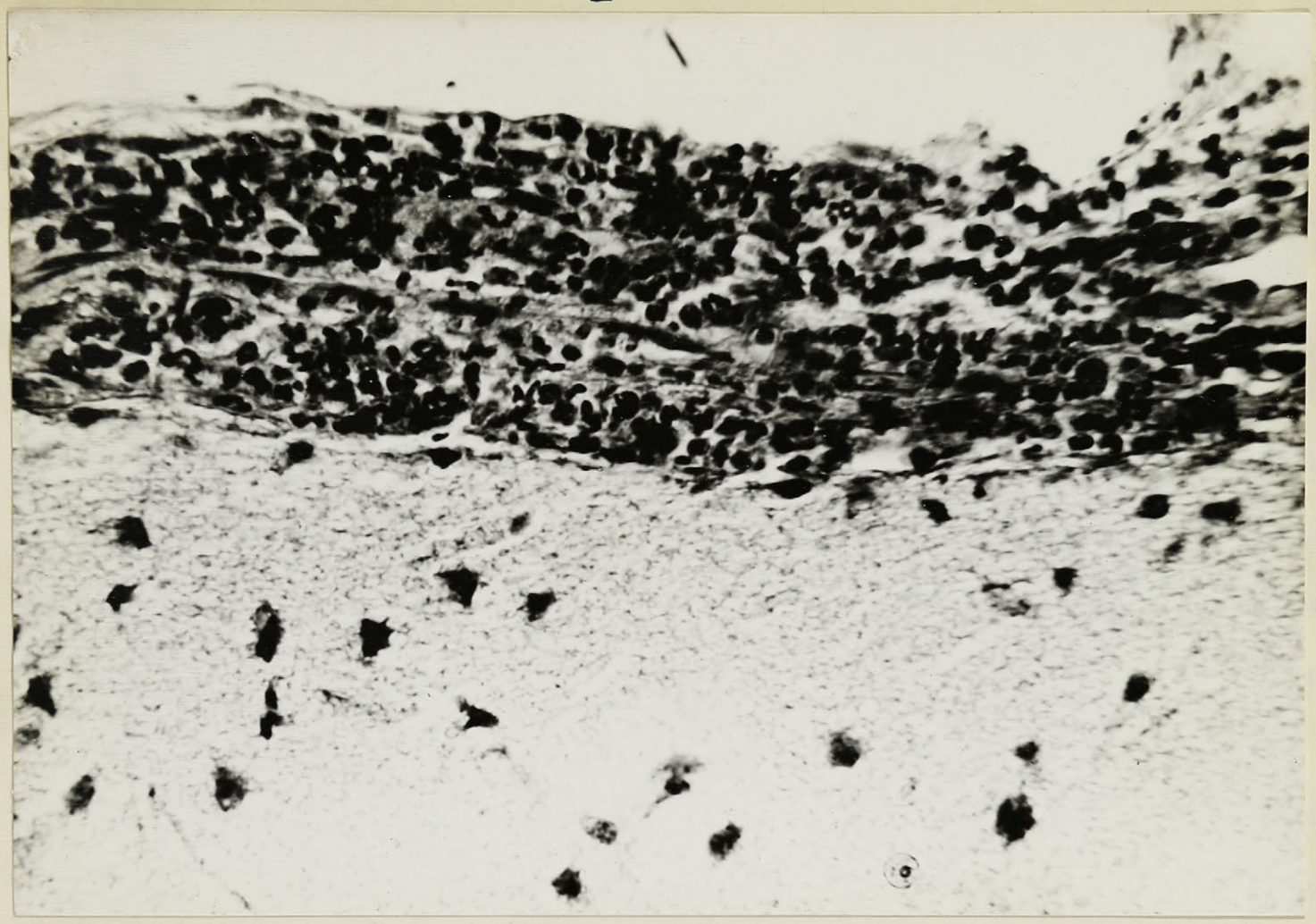
The photomicrographs on the opposite page are of the brain of Cat No.P. 2117, (H.& V.G. stain). Illustration L. is of the pia-arachnoid and molecular layer of the cortex overlying the left suprasylvian gyrus, which was previously stimulated electrically. Note the marked cellular reaction in the meninges.

The illustration marked R. is of the pia-arachnoid overlying the right suprasylvian gyrus. Note that there is less cellular reaction on this side which was not stimulated and covered by a watch glass during the period of the experiment.

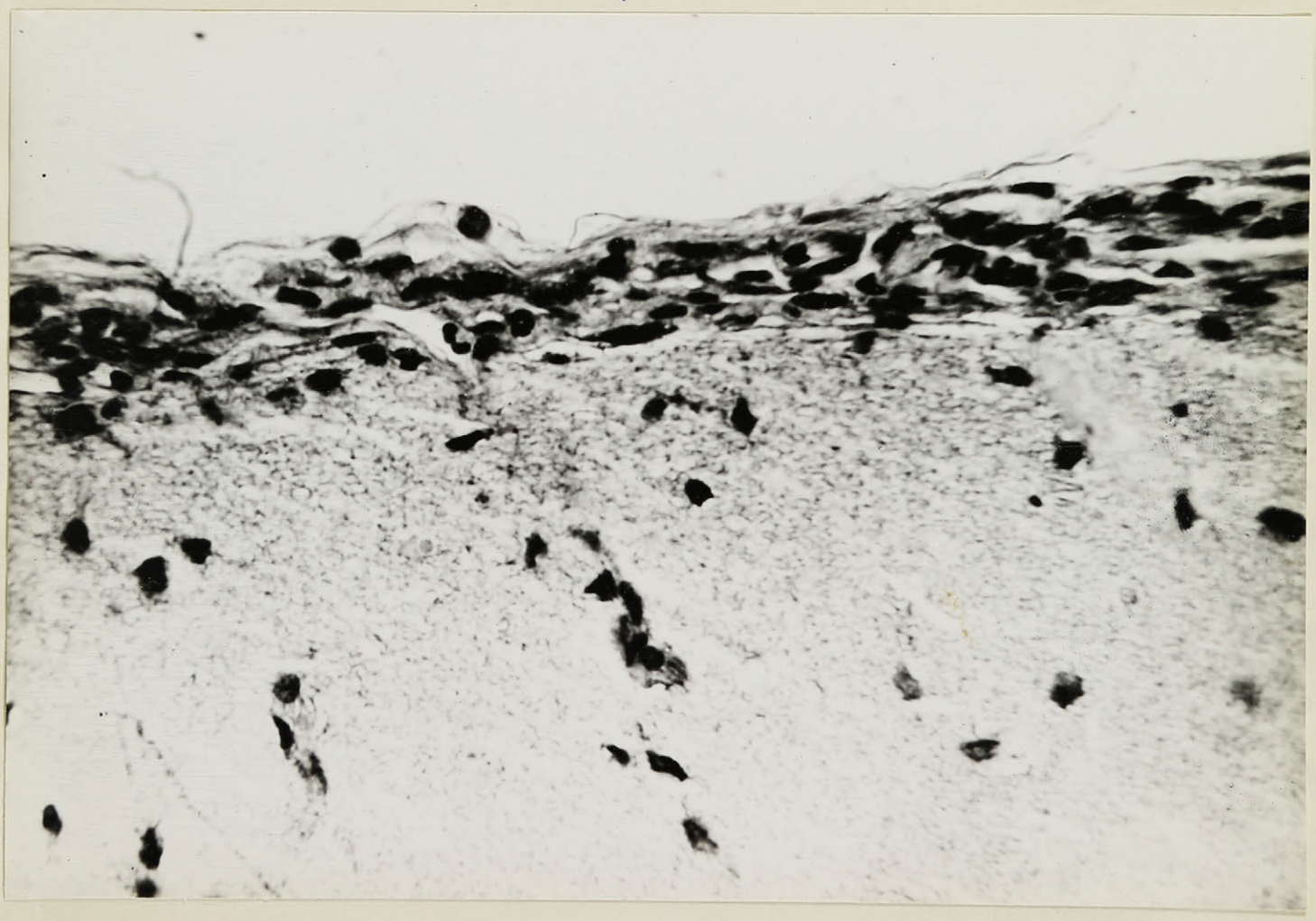
If the suprasylvian gyrus on each side could have been photographed as a whole the difference in the inflamatory reaction in the meninges on the two sides would not have appeared quite so marked.

FIG. XXVII.

L



R



The cellular reaction was strictly localized to the site of operation except for a few cells which had wandered off through the sub-arachnoid space. At 24 to 48 hours polymorphonuclear leucocytes were slightly more numerous than other types but large numbers of small or large monos were present as well as large elongated cells with abundant pale staining cytoplasm and large oval moderately dark staining nuclei. These latter cells appeared to be coming from the pia-arachnoid. The cells penetrated everywhere through the meshwork of the sub-arachnoid space. At 72 hours the polynuclear leucocytes appeared less numerous although the cellular reaction as a whole was a little more marked. At this stage there was more evidence of swelling of the pia-arachnoid and defininte evidence of proliferation of the cells of both membranes.

The cellular reaction and meningeal changes were present in all brains bilaterally but it was a little more marked where electrical stimulation had been used (see Fig. XXVII). It was the same whether the brain had been covered or left exposed to room air and irrigation appeared to make little difference.

The Nissl preparations showed some changes in the nerve cells but these will be described later. In brain number P.2117 it was felt that there were early changes in the microglia in the molecular layer and layer 2. of the cortex but these findings were not conclusive nor were they verified in other experiments. The gold chloride sublimate stain showed no

## TABLE I.

THE MENINGES AT 24 TO 96 HOURS AFTER OPERATION.

| Animal & Number | Operation | Treatment of brain on left. | Treatment of brain on right | Survival period | Findings at autopsy | Histo-pathological findings |
|---|---|---|---|---|---|---|
| P.2115 cat | Decompression bilateral | Exposed Irrigation Q. 15 min. | covered by petri dish Irrigation Q.15 min | 24 hours | no meningeal adhesions | cellular reaction in meninges bilateral |
| P.2117 cat | " | Exposed electrical stimulation for ½ hr. Thyraton at 3. Irrigation Q. 15 min. | Exposed Irrigation Q.15 min | 72 hours | Fine meningeal adhesions bilateral | cellular reaction inmeninges Slightly more on left. |
| P.2124 cat | " | Covered by petri dish Elect.stim. for ½ hr. Thyratron at 3. | Covered by petri dish | 72 hours | " | " |
| P.2132 cat | " | Exposed | " | 96 hours | " | cellular reaction in meninges bilateral low-grade infection on left? |
| P.2178 cat | " | Same as P.2124 but thyratron at 9. | Same as P.2124 | 48 hours | No meningeal adhesions | Same as P.2117 |
| P.2180 cat | " | Same as P.2132 | Same as P.2132 | 48 hours | " | cellular reaction in meninges bilateral Same as P.2115. |

TIME BETWEEN OPENING AND CLOSURE OF DURA IN EACH CASE WAS TWO HOURS.

definite changes in the astrocytes at this early period, although probably with better staining some swelling of the astrocytes could have been demonstrated at this stage as an intermediary step in the reactive gliosis that is described as occurring later.

## THE MENINGES AT 8 TO 105 DAYS AFTER OPERATION.

The findings recorded under this heading were obtained from observations on twelve cats, one dog and one monkey upon which craniotomies had previously been performed. Details of the experiments are recorded in tables II and III.

### Autopsy material.

At autopsy it was found that in all cases where the brain had been left exposed to air for one hour adhesions were present between dura mater and arachnoid membrane. Grossly the adhesions were essentially the same in character and extent whether the brain had been left exposed to room air, covered with a watch glass or irrigated continuously with Ringer's solution during the period that the dura was open. The adhesions were slightly more dense in those cases where electrical stimulation had been used, especially when this had been of a high intensity.

Adhesions between pachy- and lepto-meninges were somewhat less extensive and less firm underlying a bone flap than at the site of a decompression. Also the dura itself was always less

thick in the former than in the latter case. It was only adherent to the temporal muscle through the trephine holes of a bone flap whereas it had become everywhere densely adherent to the temporal muscle in the regions of a decompression. Adhesions were somewhat more dense in animals allowed to survive for long periods than in those killed in eight days.

The adhesions were diffusely spread over the area that had been exposed at operation. They were usually in the form of a meshwork of short dense strands but in places were either plastered densely to the underlying pia-arachnoid or at times were composed of delicate fibre-like strands as long as 2-3 mm. in length. They were usually more tough and extensive along the line of silk sutures in the dura than elsewhere. On stripping the dura from the leptomeninges the surface of the latter usually appeared roughened and felt so to the palpating finger.

In most cases these observations were made after the brain, (with the overlying meninges at the site of operation), had been removed from the skull, (see Figs. XVIII to XXV). The lower edge of the dura, at the open end of the suture line in the dura, was gently elevated so that the underlying adhesions could be seen in part. Further observations were made on each block of tissue after the brain had been cut and the dura was usually stripped from one or more of these in each brain.

No adhesions between dura and arachnoid were found under-

lying a bony decompression, providing the dura had not been opened nor were they present in the case where the unopened dura had been irrigated for two hours with iced Ringer's solution. In those experiments where the dura had been opened and closed again immediately, no adhesions had formed between lepto- and pachy-meninges except at the site of the silk suture line in the dura. Here there were a few adhesions about most sutures, the arachnoid having apparently been injured by the contact of the silk.

Nothing abnormal was noted in any of the brains, (apart from the meninges) either before or after they had been cut except that in some cases there was slight convolutional flattening at the operative site.

Microscopical Examination of the Meninges.

The findings recorded above were corroborated on histological examination of the meninges.

It was found that by eight days after operation the cellular reaction in the meninges described above had almost completely subsided. That is the polymorphonuclear leucocytes and small monocytes had disappeared except for a very occasional one. Scattered large, apparently phagocytic cells (compound granular corpuscles) with abundant vacuolated cytoplasm and small dark staining eccentrically placed nuclei were present however.

By eight days both the pia and arachnoid were quite markedly

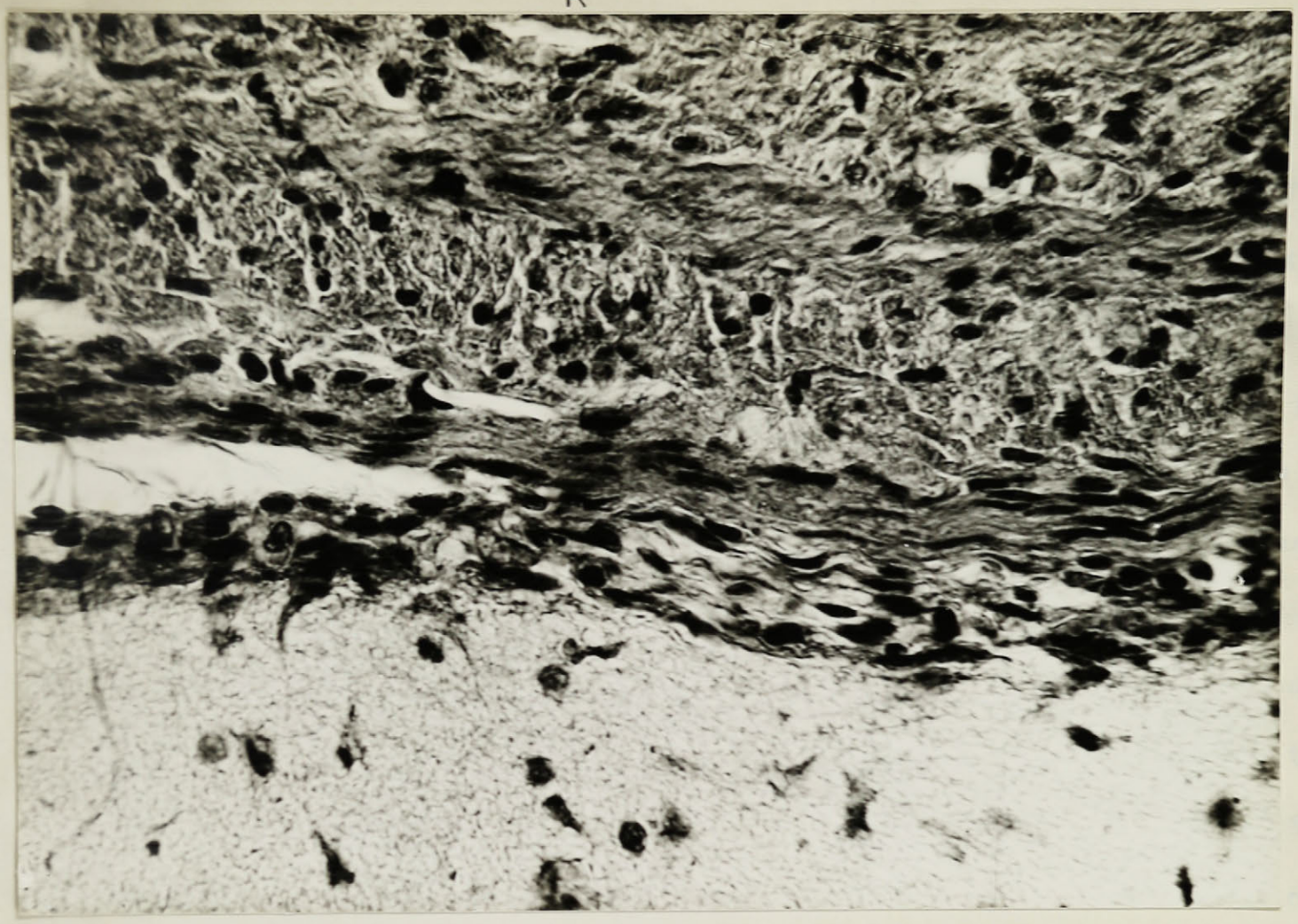
## FIGURE XXVIII.

The two photomicrographs in Fig. XXVIII. show the adhesions overlying the suprasylvian gyrus of each hemisphere of cat No.P.2144, (H.& V.G. stain).

The upper illustration is from the right hemisphere and the lower from the left. Note that the molecular layer of the cortex fills the lower portion of each photomicrograph. The thickened pia overlies this and is densely adherent in places to the arachnoid, which in turn is adherent to the dura throughout. The cells and nuclei of the arachnoid, for the most part, are more elongated than the rounder nuclei and cells of the pia. This will be seen to be true also in Fig. XXIX. There is slightly more thickening of the pia-arachnoid in the upper illustration (right side of brain - exposed to air) than in the lower (left side of brain - irrigated continuously with Ringer's solution).

FIG. XXVIII.

R



L

thickened and adhesions were present between lepto- and pachymeninges in all cases where the brain had been left exposed for two hours, regardless of whether it had been left exposed to room air, irrigated continually or covered with a petri dish. In cat P.2155 there was slightly more thickening of the pia-arachnoid and the adhesions were somewhat more extensive on the side left exposed to air than on the side irrigated, (see Fig. XXVIII). However in cat P.2168 where an identical experiment was carried out, the meninges appeared about the same on the two sides. The thickening of the pia-arachnoid and the adhesions were more marked on the side where strong electrical stimulation had been used, (see Fig. XXIX), than where none had been employed. The duration of survival of the animal seemed to make little difference in the thickness of the leptomeninges as they showed about as much proliferation at eight days as at the end of three months. They presented less reaction under a bone flap than under a decompression and this was also true of the dura.

The leptomeninges appeared essentially normal in those cats where the dura was opened and closed again immediately, (except along the silk suture line in the dura where there were adhesions and thickening), where the dura was irrigated with iced Ringer's solution or where the dura was not opened under a decompression.

The thickening found in the leptomeninges was due to pro-

liferation on the part of the cells of these membranes. (This is well illustrated in Figs. XXVIII and XXIX). The cells of both pia and arachnoid membranes were increased in number and had lined themselves in layers parallel to the original membranes. The cells of the arachnoid showed a tendency to become more elongated than normal and the arachnoid had become easily distinguishable from the pia. By eight days and thereafter the arachnoid was several, (or more), cell layers in thickness. The cells had darkly staining elongated nuclei in which the chromatin was quite evenly distributed. The cytoplasm was drawn out in elongated tails parallel to the surface of the brain. The pia reacted in a similar manner but the cells of this membrane had larger, rounder dark staining nuclei and more oval cell outlines, (see Figs. XXVIII and XXIX).

In many places the sub-arachnoid space did not appear obliterated but the arachnoid was firmly adherent to the under surface of the dura, being drawn away with this membrane during fixation or handling of the tissue. In these cases the arachnoid appeared to be a layer of cells lining the inner surface of the dura. However, in many places it could be seen that this "lining membrane" was continuous with the arachnoid, (see Fig. XXVIII).

In other places the arachnoid was densely adherent to both dura and pia, the cells of the arachnoid being diffusely intermingled with those of the pia on the one side and the

## FIGURE XXIX.

The photomicrographs in Fig. XXIX. are of the adhesions overlying the right and left suprasylvian gyri of cat No.P.2184. (H.& V.G. stain).

Note that the pia-arachnoid is more adherent to the dura over the right hemisphere (illustration A), than over the left (illustration B). One delicate adhesion between arachnoid and dura is shown in illustration B. The meninges have been torn away from the molecular layer of the cortex in handling and fixation.

The right suprasylvian gyrus of this cat was stimulated electrically. The left was not.

R

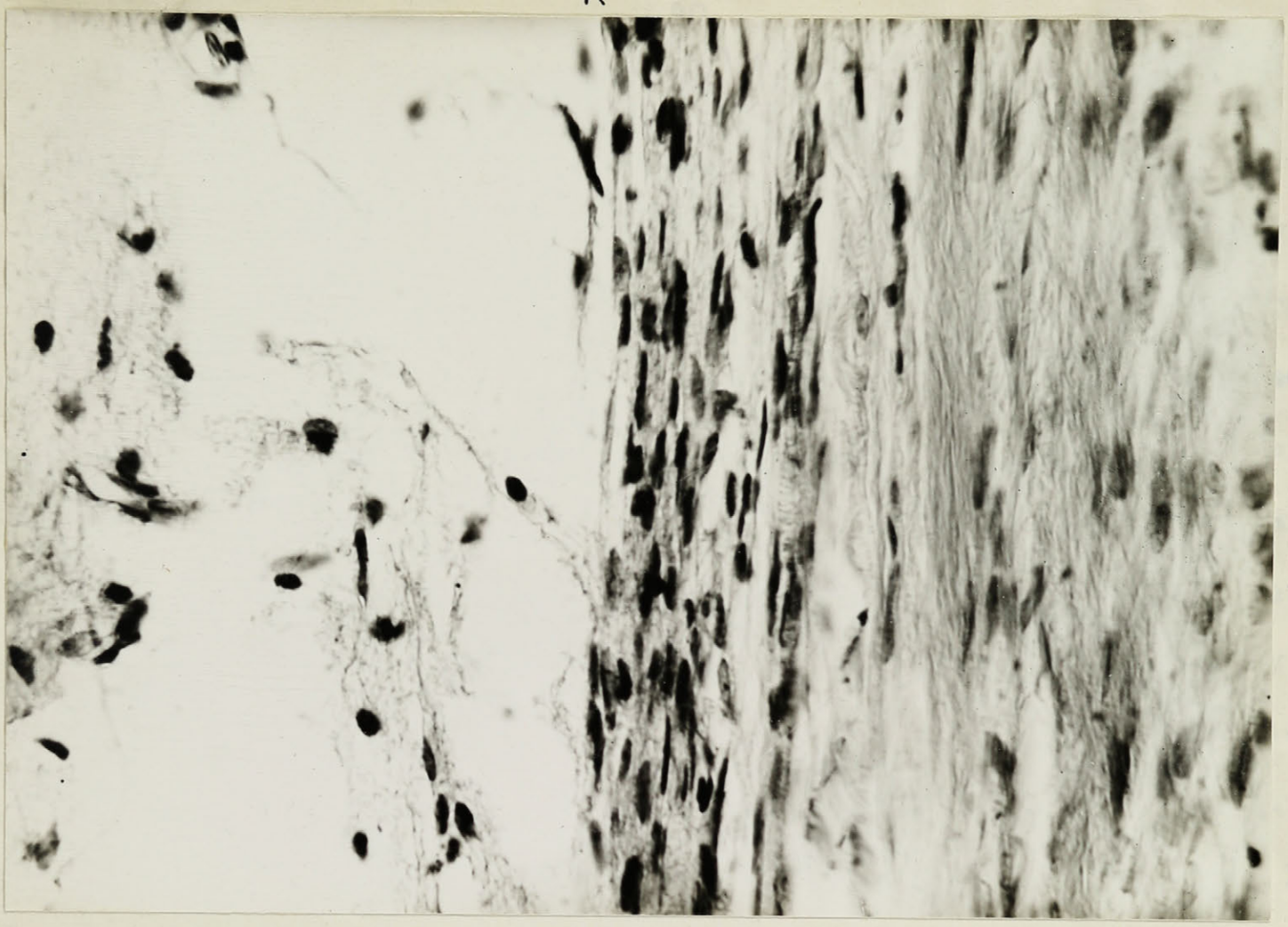
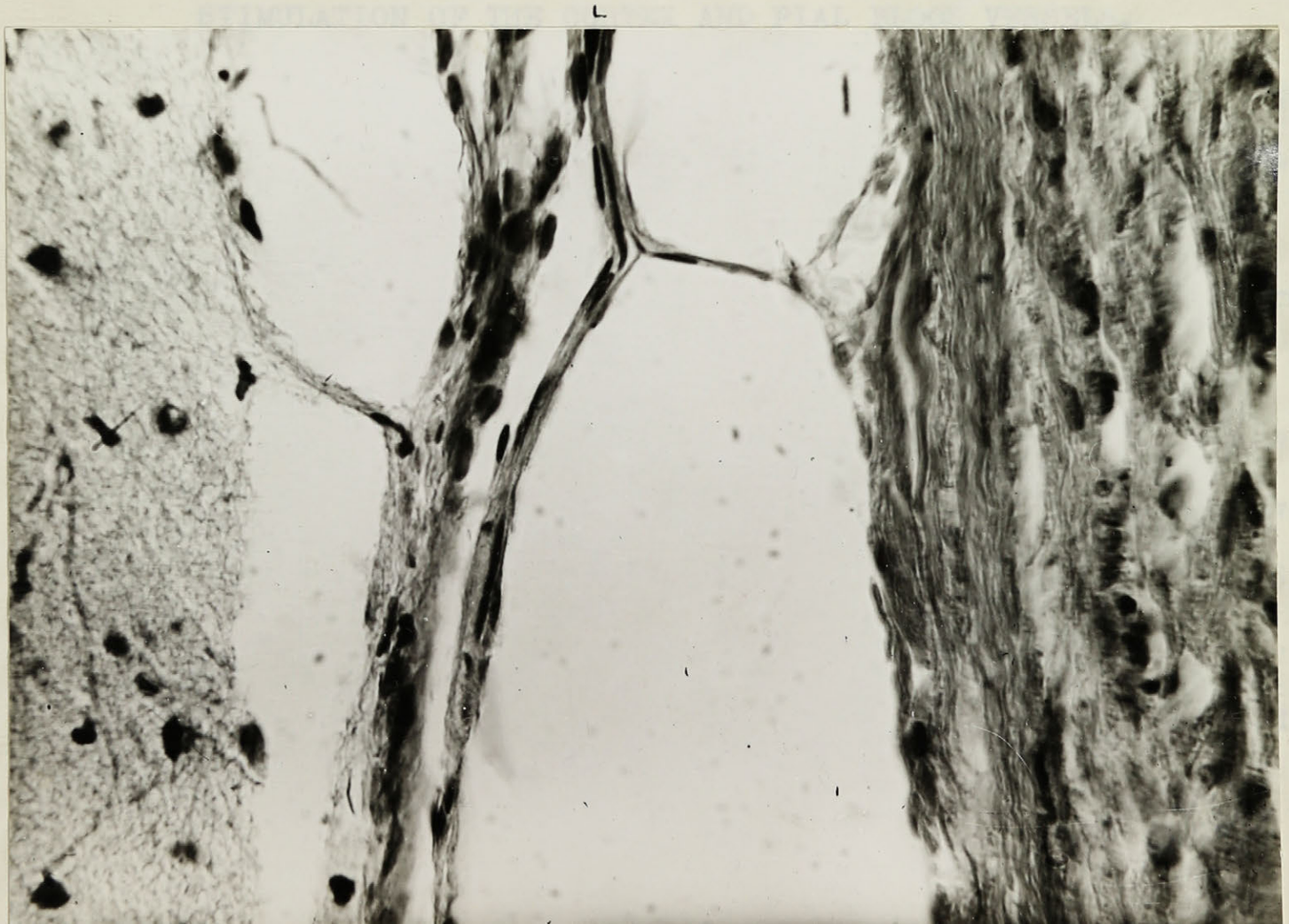


FIG. XXIX

L



dura on the other, (see Fig. XXIX.A.).

In those cases where the adhesions were less dense, delicate thread-like strands of arachnoid, at times only one cell layer in thickness, joined the arachnoid to the dura. These adhesions became most evident where the dura had become separated away from the arachnoid during handling of the sections. Such an adhesion is well shown in Fig. XXIX.B.

The firmness of many of the adhesions described above was shown by the fact that when the dura was teased away from the brain (when mounting the paraffin sections) it, at times, drew with it not only the leptomeninges but also a portion of the underlying molecular layer of the cortex. (See Fig. XXIX.).

CEREBRAL CICATRIX RESULTING FROM ELECTRICAL OR MECHANICAL STIMULATION OF THE CORTEX AND PIAL BLOOD VESSELS.

In Part I. of the thesis it was shown that focal ischaemia could be produced in the cerebral cortex if the pial blood vessels were stimulated electrically or mechanically. A series of experiments were therefore carried out to determine what type of histological lesion, if any, might occur in the brain following such electrical or mechanical stimulation.

In this particular study the brains of six animals were used (4 cats, 1 dog and 1 monkey; nos. P.2004, 2006, 2135, 2137, 2151 and 2184). It should be noted, however, that before undertaking this experiment a large number of animals had

been experimented on in a similar manner so that the technique had become fairly well perfected. The brains of a number of these other animals used in the earlier experiments were stained with Weigert's H.& V.G. method and Nissl's (thionin) method and the hisological changes noted. Some refernece will be made to these findings below and although not directly included in the thesis have been of help in arriving at the final conclusions.

For particulars of experiments see technique and tables II & III.

Histopathology.

Cat P.2004. Left cerebral hemisphere following electrical stimulation of the cortex and pial blood vessels. In this experiment stimulation at operation was applied directly to the larger pial vessels so that considerable obvious ischaemia of the cortex occurred.

The sections stained by Cajal's gold chloride sublimate method (modified) were the most interesting, (see Fig. XXX). In these sections was found a triangular area of gliosis in the grey matter of the suprasylvian gyrus and a smaller similar area, which was more patchy in the ectosylvian gyrus, (as illustrated in the drawing accompanying Fig. XXX). The triangle was formed with its base at the pia and its apex at the white matter. (It should be noted that in the majority of the experiments described in Part II. of the thesis the suprasylvian

FIGURE XXX.

The two photomicrographs on the opposite page are of the left suprasylvian gyrus of cat No.P.2004, which had been stimulated electrically. The upper illustration A. is a high power magnification of the area shown in the right upper hand corner of illustration B., indicated by the arrows.

In illustration B., the pia is shown at the right lower corner. Radiating in from the pia two large perforating vessels are present. An increase in number and hypertrophy of astrocytes is present between these two vessels. This area of gliosis fades away into a zone of normal cortex in the lower portion of the illustration. Between the vessels it extends through all the layers of the cortex to the white matter. Note that there are fewer hypertrophied astrocytes subpially and in the molecular layer than deeper in the cortex. (G.C.S. stain)

Diagram of areas of gliosis in Cat No.P.2004.

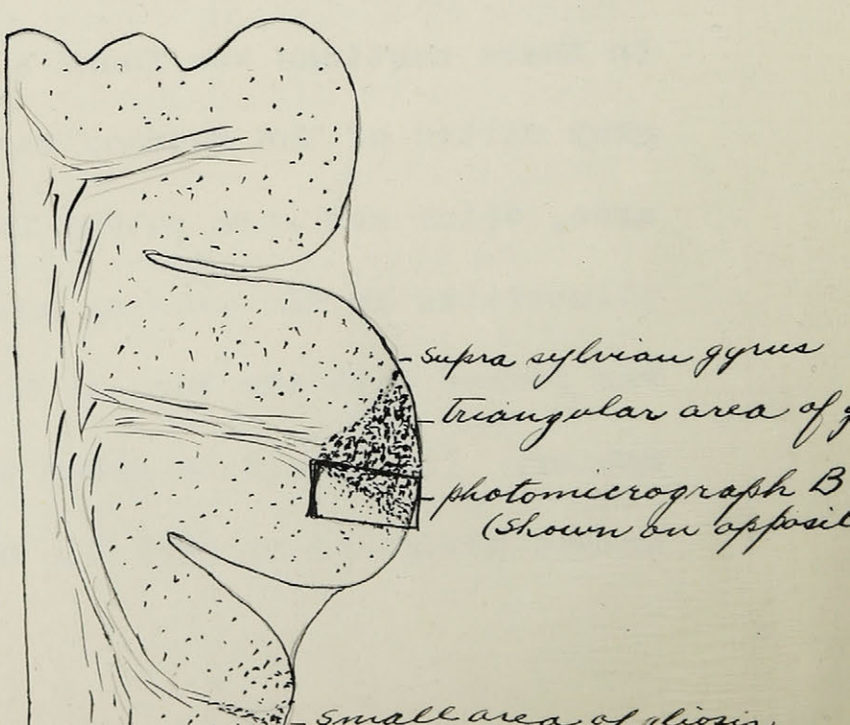
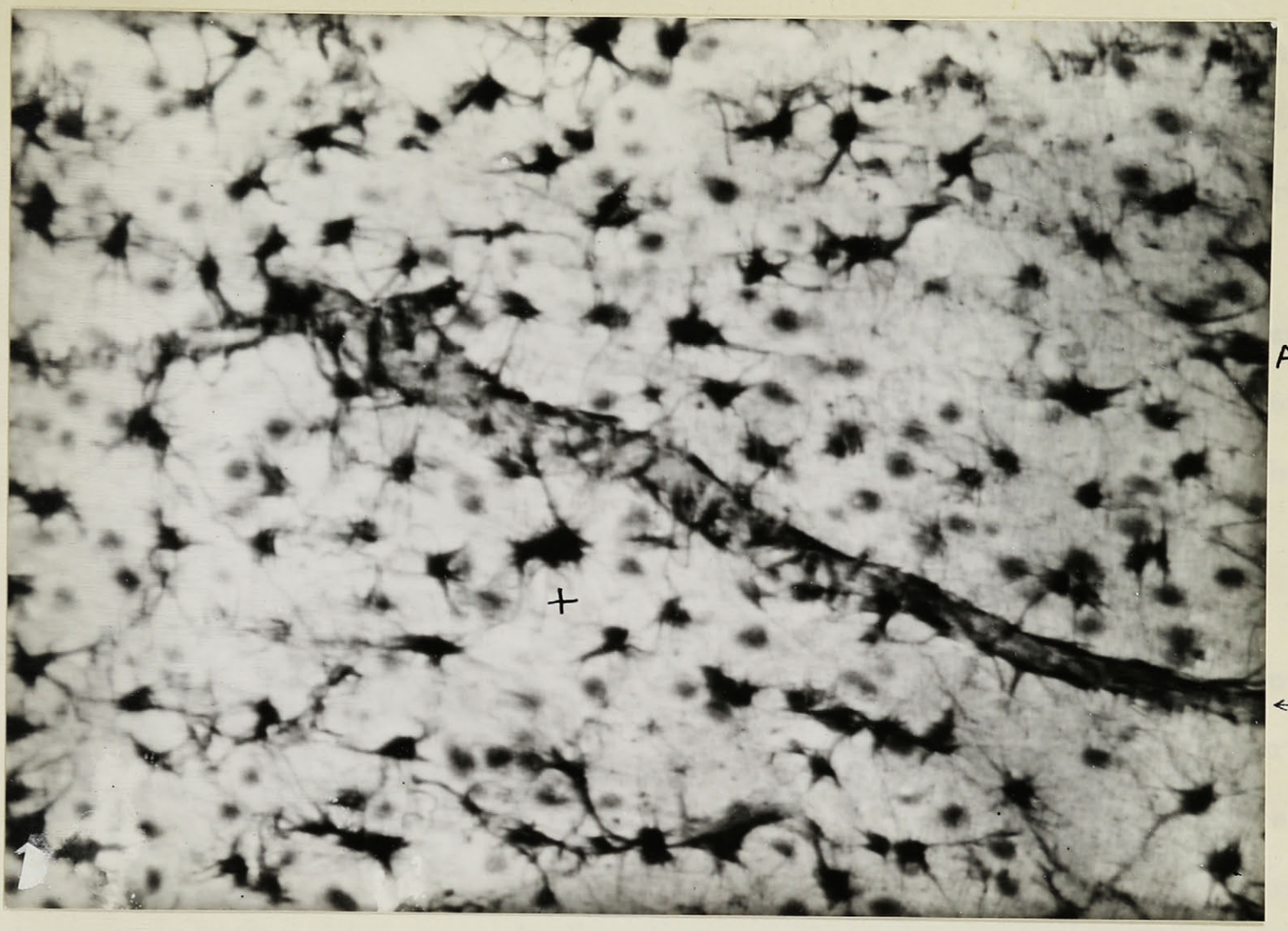
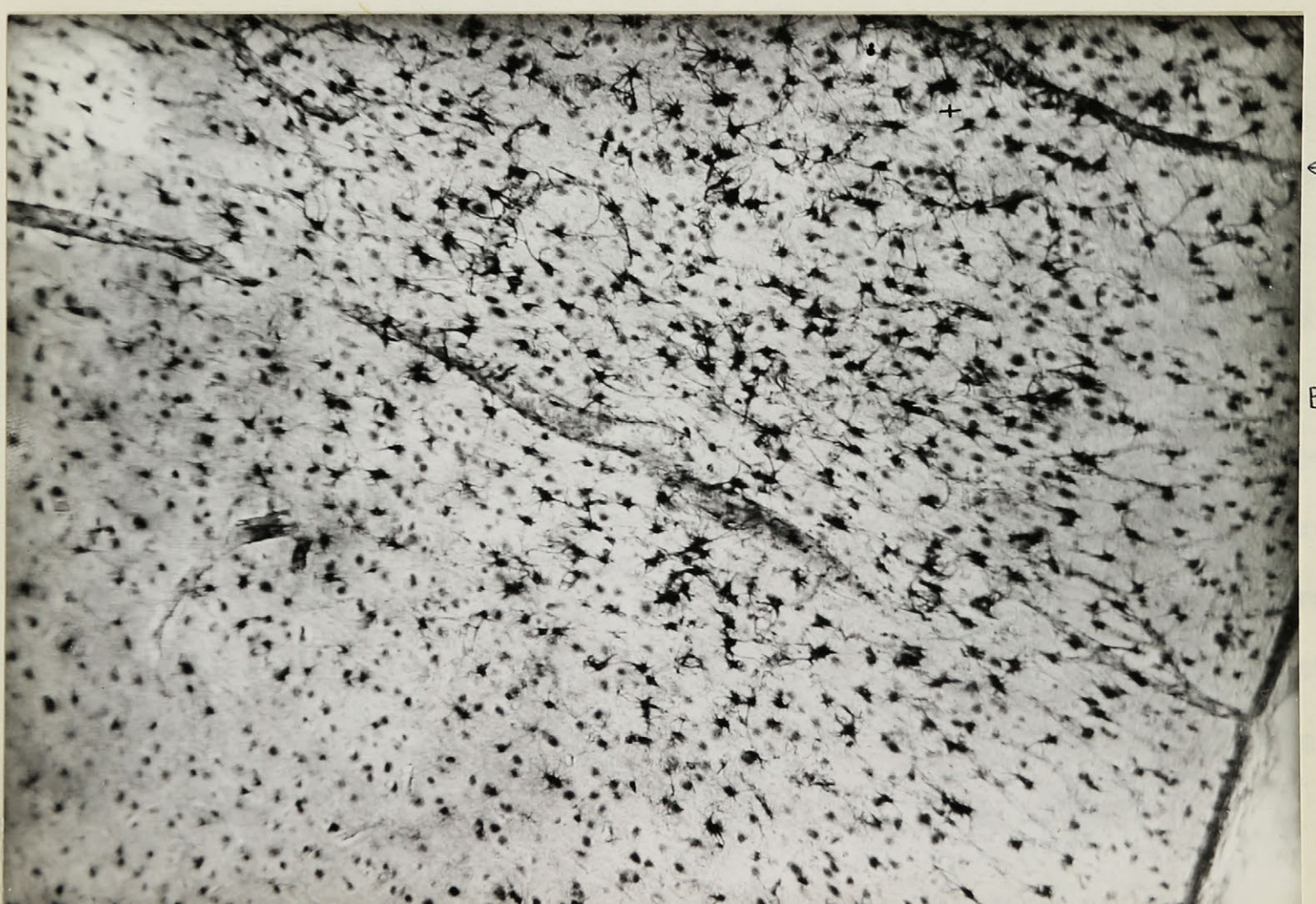


FIG. XXX.



A



B

gyrus was the one most stimulated or exposed at operation and this was true in this case. The stimulation was restricted to this gyrus and small/portion of the neighbouring lateral and ectosylvian gyri.)

The triangular area of gliosis mentioned was composed of hypertrophied astrocytes, most of which had laid down fibres and become changed from the usual protoplasmic to the fibrous form. There was very evident thickening of the vascular foot processes of the astrocytes and an increase in number of these. There was also a definite increase in the number of astrocytes over the normal in this area. This could easily be ascertained by observing the number in the triangular area as compared to that in the adjacent normal grey matter. Also in the zone of gliosis was abundant evidence of direct cellular division of astrocytes.

The triangular patch of gliosis extended from the pia to the white matter and was of about equal extent in all layers of the cortex. There was however surprisingly little reaction of the subpial astrocytes or of those in the molecular layer of the cortex.

There was a definite tendency for the astrocytic hypertrophy to be greater in the perivascular zones. The sides of the triangle were beautifully defined by the large perforating pial and intracerebral vessels, the triangular shape of the gliosis in fact being apparently determined by the anatomical

arrangement of the vessels, (see diagram of Fig. XXX).

The small area of patchy gliosis in the ectosylvian gyrus was identical to that described above but was not as extensive and hence did not form such a complete triangle.

The sections stained with Mallory's P.T.A. and Rio-Hortega's S.C.A. methods verified the findings already reported but added little, nor did they demonstrate the lesion so clearly.

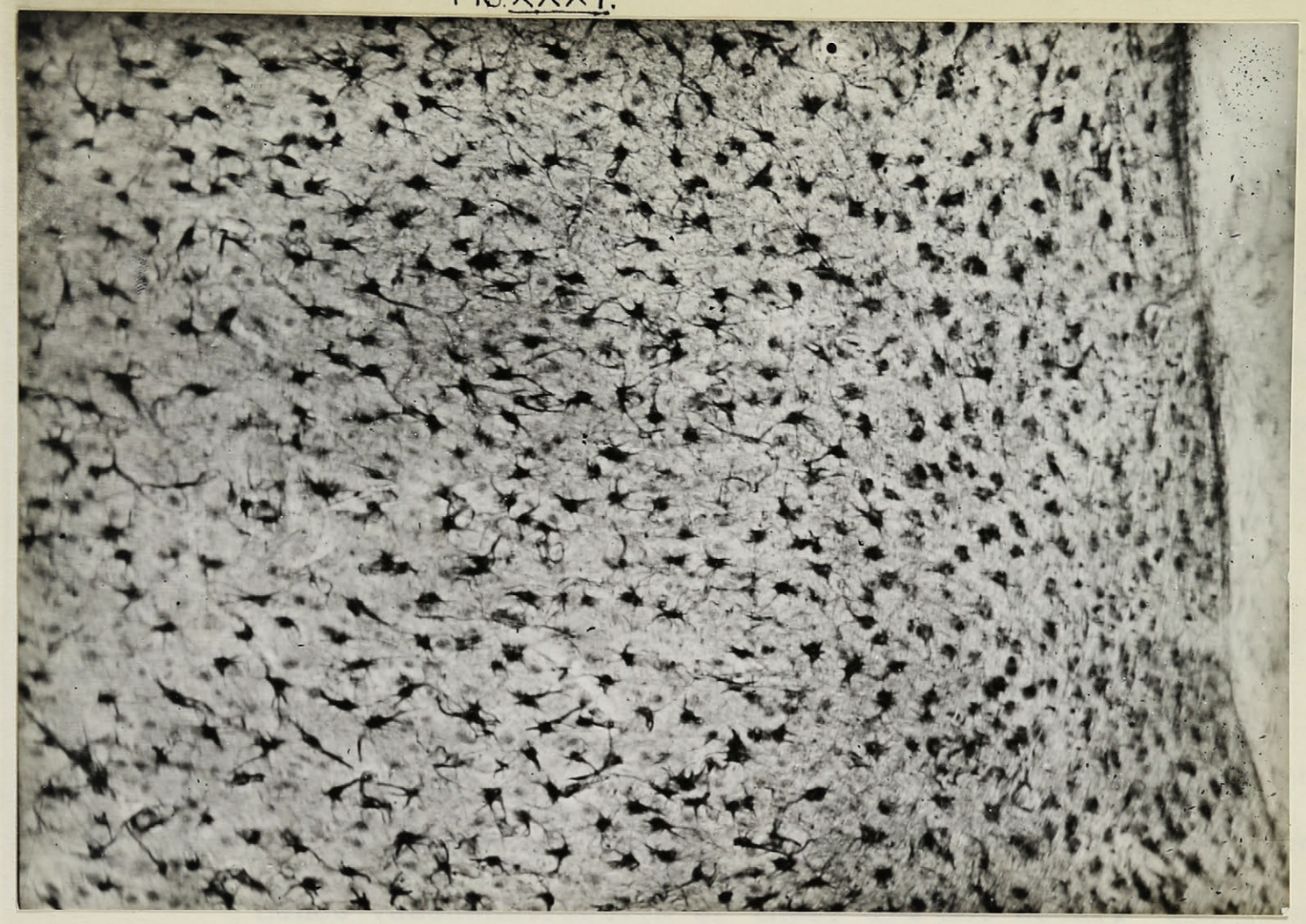
The changes in the meninges have been described from the H.& V.G. stains and nothing else abnormal was noted in these sections. There was no evidence of haemorrhage, petchial or otherwise, nor of vascular thrombosis in any of the sections.

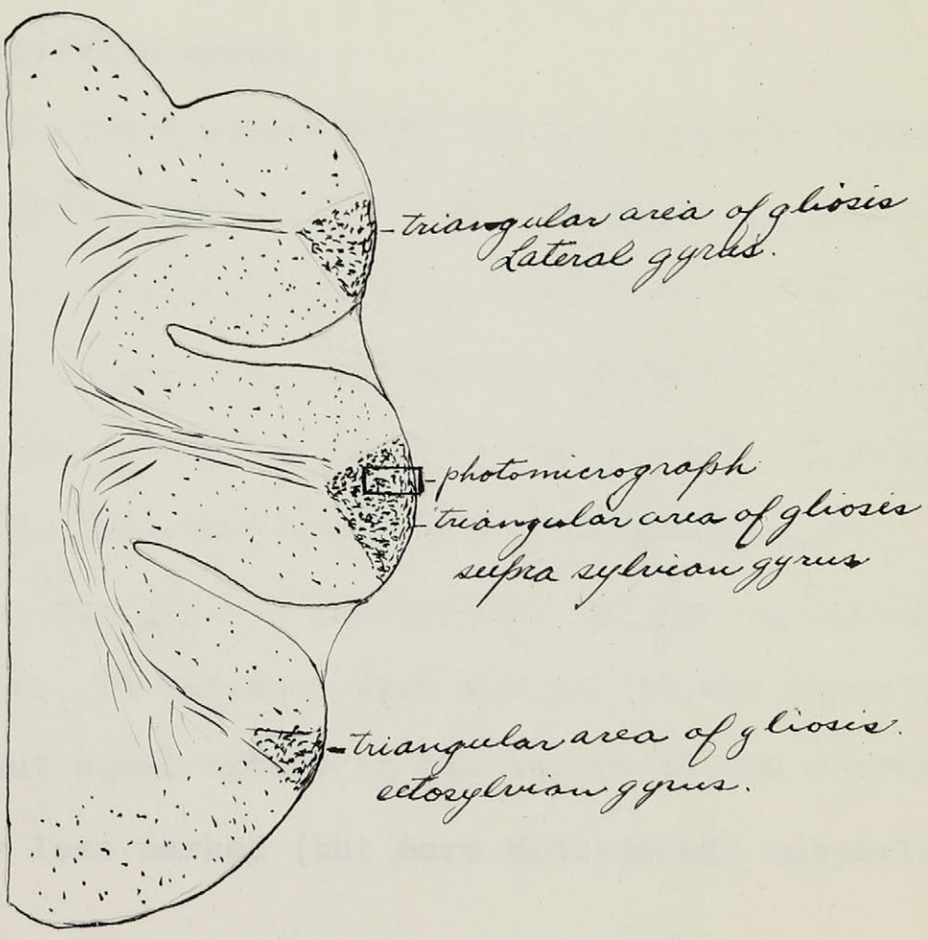
What was felt to be an ischaemic change in some of the nerve cells in the first two layers of the cortex of the suprasylvian gyrus were seen in the Nissl's (thionin) preparations. This change was characterized by shrunken elongated pyknotic cells in which the nucleus could no longer be discernable in the dark cytoplasm. Also by the fact that the cytoplasm of many of the cells in this gyrus (as compared with the neighbouring gyri or the same one in the opposite hemisphere) were much more pale staining than usual, the chromatin being finely dispersed or almost absent, the processes being frequently invisible, and the nucleus small, dark staining and frequently eccentrically placed. Scattered cells, especially the larger ganglion cells, showed aggregations of the Nissl substance into clumps at the periphery of the cell and an eccentrically placed nucleus.

FIGURE XXXI.

The photomicrograph on the opposite page was taken from the left suprasylvian gyrus of cat No.P.2006. The pial vessels over the surface of this gyrus were constricted by mechanical stimulation. Note that there is a marked gliosis (hypertrophy and increase in number of astrocytes), extending from the pia to the white matter. The subpial astrocytes are arranged parallel to the surface of the gyrus and some show clasmatodendrosis. This change in the subpial astrocytes is believed to be due to trauma from mechanical stimulation. The gliosis in the deeper layers of the cortex, which is in the form of a clearly demarcated triangle as shown in the diagram is apparently due to ischaemia following vasoconstriction of the pial vessels. (G.C.S. stain, modified).

FIG. XXXI.




triangular area of gliosis
Lateral gyrus.
photomicrograph
triangular area of gliosis
supra sylvian gyrus
triangular area of gliosis.
ectosylvian gyrus.

Still other cells were vacuolated and there was some increase in perineuronal satellitosis of oligodendroglia.

These changes in the nerve cells were not marked by any means, however, and all the changes described were rarely found in the same section. It is felt however that the changes in nerve cells were definite and this is easily understandable since the gyrus was made ischaemic for nearly half an hour.

Cat. P.2151. Left cerebral hemisphere after electrical stimulation of the cortex.

It will be noted in table II . that in this experiment the larger vessels were avoided during electrical stimulation and hence vasoconstriction of these did not occur. The resultant ischaemia in the cortex was therefore less than that in Cat P.2004 described immediately above. It was largely restricted to the suprasylvian gyrus.

As in all these experiments the pathological lesion was best demonstrated with Cajal's gold chloride sublimate method (modified). In the sections stained by this method there was found an area of gliosis, irregularly triangular in shape in the grey matter, (with its base at the pia and its apex at the white matter), over the tip of the suprasylvian gyrus, (see Fig. XXXII). The gliosis was similar in type to that described in cat P.2004. It extended from the pia to the white matter being of about equal extent in all layers of the cortex. It was slightly less marked (but more widespread) subpially and

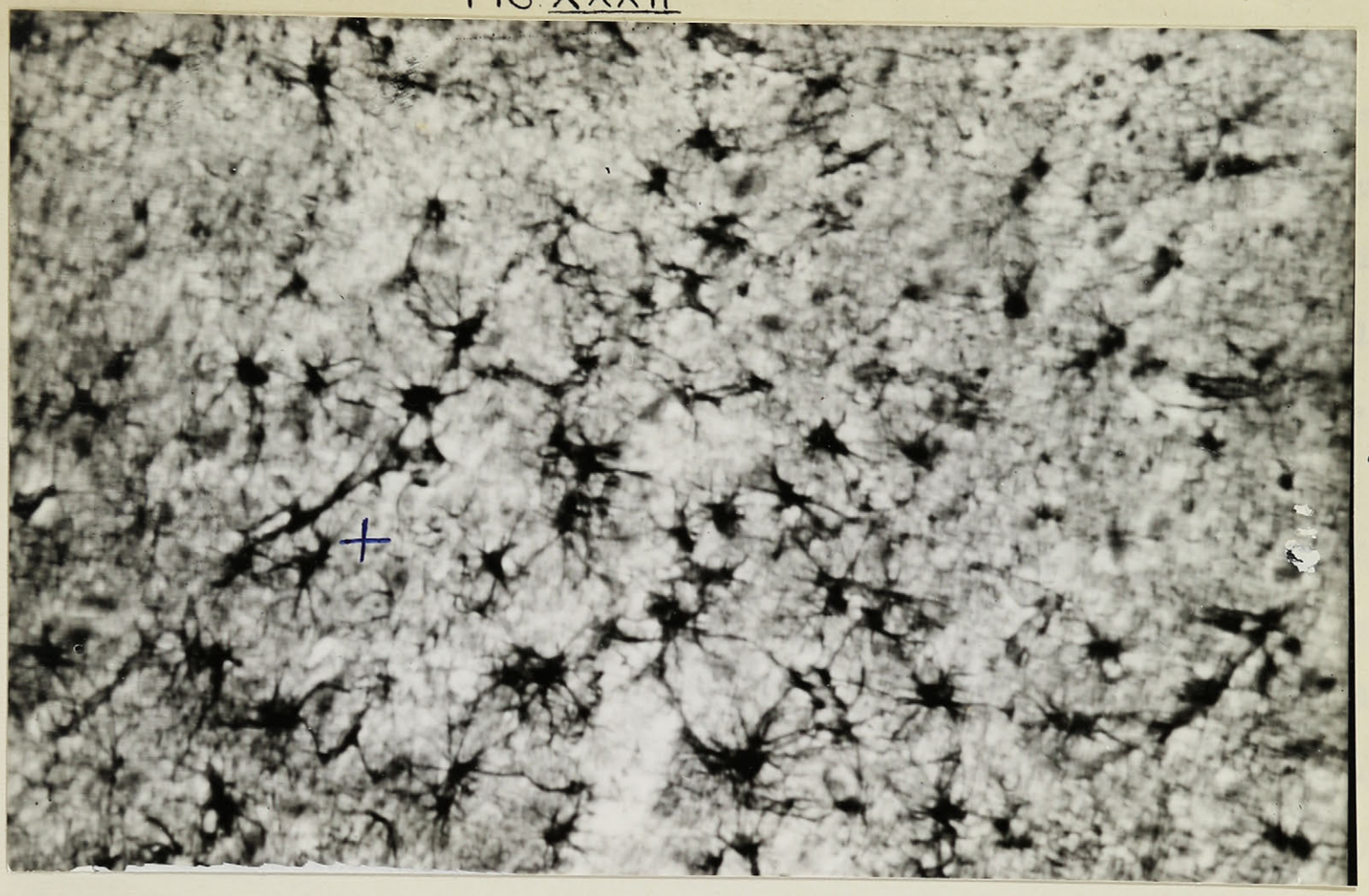
## FIGURE XXXII.

The two photomicrographs on the opposite page are of the left suprasylvian gyrus of cat No.P.2151. This gyrus had been stimulated electrically.

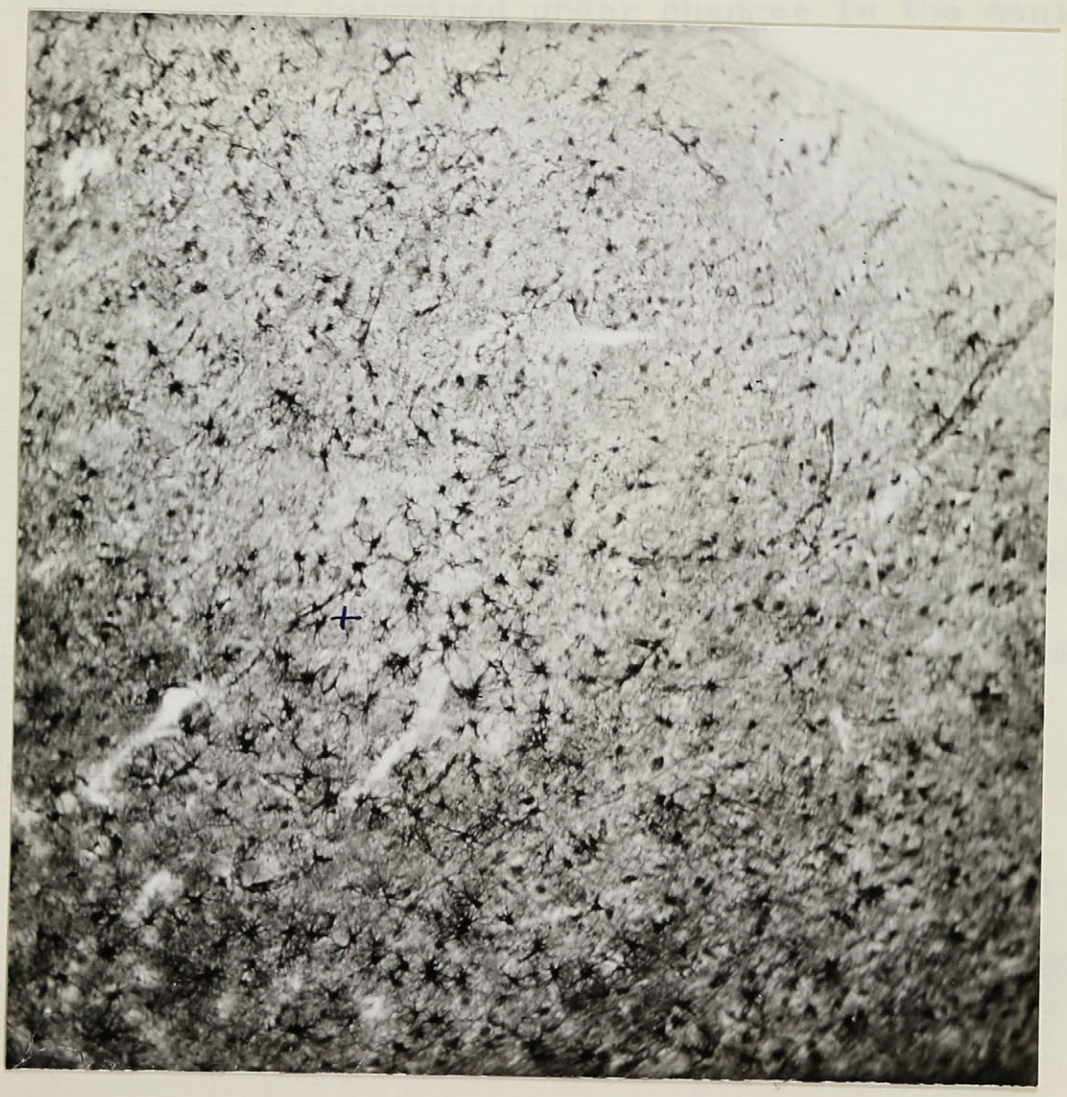
Illustration A. is a high magnification of the area marked + in illustration B. and shows well that the protoplasmic astrocytes of the grey matter have not only increased in number but have been transformed into hypertrophied fibrous forms. Evidence of direct division of astrocytes is seen in several places.

Illustration B. shows a patch of gliosis deep in the grey matter in the region marked + and can be followed out toward the surface of the pia at the left upper corner of the photomicrograph. A few hypertrophied astrocytes are seen along the perforating vessel from the pia in the right upper portion of the illustration. Examination of this and other sections from the same gyrus showed that the gliosis, although patchy was in the form of a triangle. (G.C.S. stain).

FIG. XXXII



A



B

in the molecular layer than deeper. The gliosis in this case, however, was more patchy than in Cat. P.2004 and did not form a complete triangle. However, on examining a number of sections it was evident that its anatomical distribution, although less extensive and patchy, was essentially the same as in cat P.2004. Its limits were demarcated by perforating vessels from the pia which, as we know, form a triangle. In places it appeared to be perivascular and near the sides of the triangle was quite marked to the medial side of a perforating vessel and faded away to normal on the other side of this vessel (where collateral circulation was apparently sufficient to prevent ischaemia).

The sections stained with Weigert's H.& V.G. method have been partly described under changes in the meninges. Nothing else of importance was noted in these sections. There was no evidence of hemorrhage or thrombosis in the brain. No definite changes in the nerve cells were noted in the Nissl (thionin) preparations.

Histological examination of the brain exposed through a right sided craniotomy in this cat revealed nothing abnormal in the cortex. More will be said concerning this finding when the histological changes in the brain following exposure to air are described.

Cat P.2184. Right cerebral hemisphere.

In this experiment the right suprasylvian gyrus was stimulated for 20 minutes with the thyratron at 9. The intensity

FIGURE XXXIII.

Cat No.P.2184 - Sections stained-Cajal's G.C.S. method (modified).

Illustration A. is taken from the right suprassylvian gyrus and B. from the same gyrus of the left hemisphere. The pial vessels of the gyrus shown in A. were constricted electrically for 20 minutes (thyratron-fairly high intensity). The gyrus shown in illustration B. was merely exposed (see particulars of experiment in text).

Note that in photomicrograph A. there is an evenly distributed, marked increase in number and hypertrophy of the astrocytes of the grey matter. The area in which this astrocytic change was present formed a clearly demarcated triangle as shown in the diagram below. There is marked thickening of the pia in this case and sections stained by other methods showed severe changes in nerve cells and a complete disorganization of the normal cytoarchitecture in the triangular area shown in the diagram.

Photomicrograph B. shows an evenly distributed, definite hypertrophy and increase in number of the astrocytes in the grey matter. Although this change is not nearly so marked as in A, its distribution is also triangular as shown in the accompanying diagram.

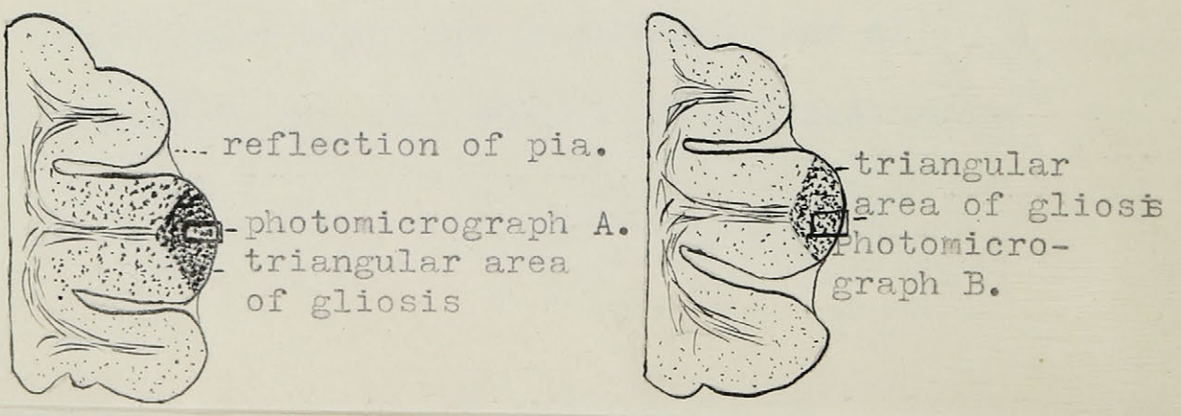
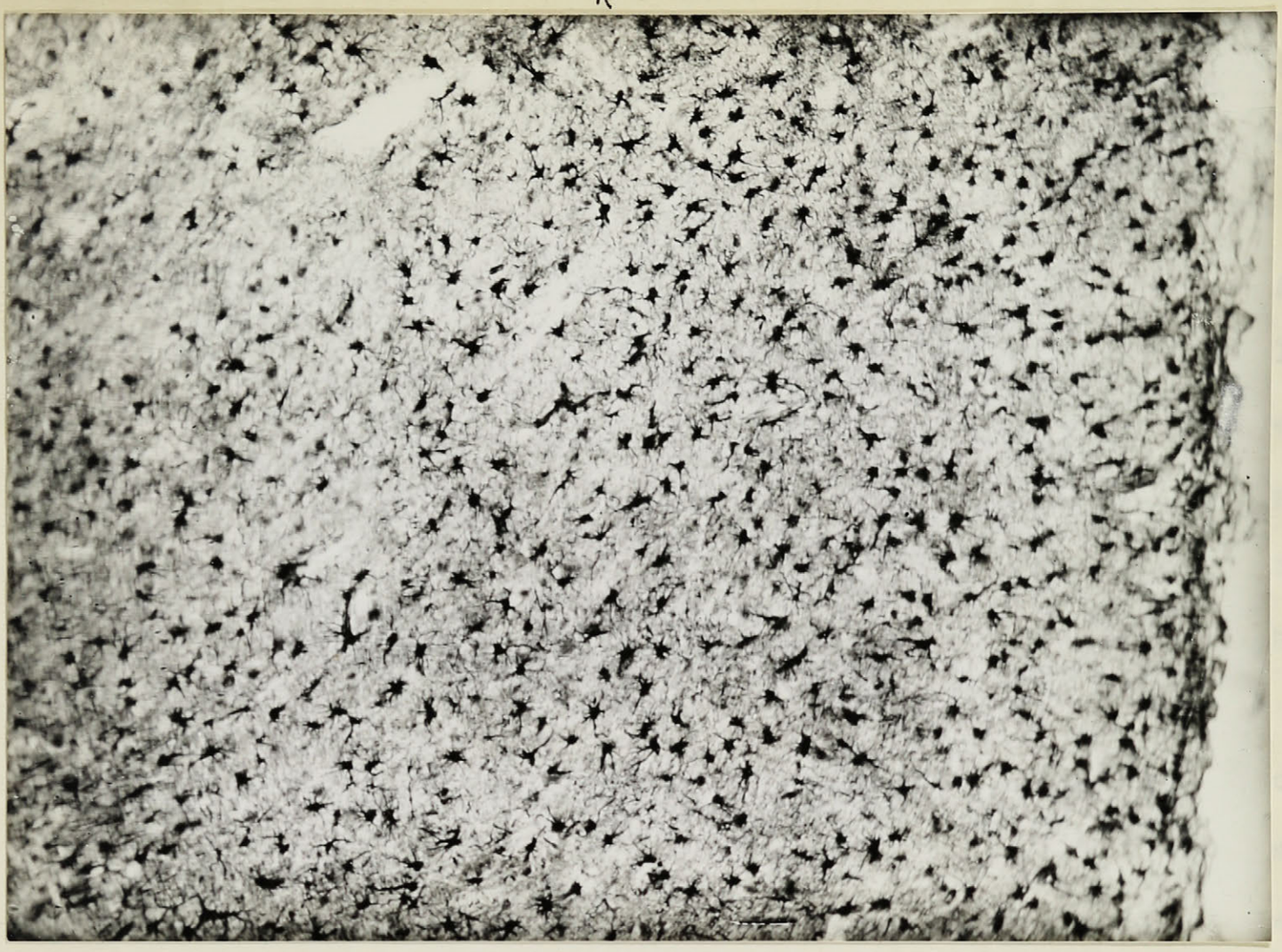


FIG. XXXIII

R



A

L

B

of the electrical stimulation was therefore greater than in any of the other experiments in this series and the resultant ischaemia of the cortex was marked.

Eleetrical stimulation, of the right suprasylvian gyrus, was applied by touching the electrodes to the cortex (along the vessels) for periods of 2 seconds with intervals of 2 seconds between stimulations. As a control the suprasylvian gyrus of the opposite hemisphere was stimulated with electrodes in an identical manner but in this case the electric current had been turned off.

The histological sections of the right hemisphere of this cat showed very marked pathological changes in the suprasylvian gyrus, (see Fig. XXXIII). The changes were of more intense degree than in any of the other cats and this is easily explainable on the basis of the intensity of the stimulation and degree of ischaemia.

The sections stained with Cajal's gold chloride sublimate method (modified) showed a triangle of very marked gliosis in the tip of the suprasylvian gyrus. The triangle involved the whole tip of the gyrus from the region where the pia is reflected to the neighbouring gyri, (as shown in the diagram of Fig. XXXIII). Throughout the whole triangle there was an evenly distributed increase in number and hypertrophy of astrocytes. The astrocytes were fibrous in type and had plumper cell bodies and shorter thicker processes than in sections from other

experiments in this series. It is interesting that there was also hypertrophy of astrocytes throughout this whole gyrus, along its lateral borders in the grey matter as well as over the tip. The triangular area of marked gliosis over the tip of the gyrus, however, was sharply demarcated from the lesser gliosis along the sides of the gyrus.

The hypertrophy of astrocytes throughout the whole grey matter of this suprasylvian gyrus as well as over the tip seems easily explained by the fact that vasoconstriction and hence ischaemia occurred through the gyrus as a whole, because of the intensity of stimulation. The perforating vessels over the exposed surface of the gyrus, however, were more constricted than elsewhere and this explains the more marked hypertrophy of astrocytes in the triangle of cortex which they supply.

The gliosis mentioned was entirely restricted to the suprasylvian gyrus, the astrocytes in the neighbouring gyri (in the same section) appearing normal and protoplasmic in form - except for a few hypertrophied ones in the borders of the gyri immediately adjacent to the suprasylvian. Due to spread of the electric current a slight effect on the immediately adjacent tissues is to be expected.

The sections stained with Nissl's (thionin) method show very marked changes in the nerve cells and a destruction of the normal cytoarchitecture. The cortex was reduced to about two-thirds of its normal width. Many nerve cells had obviously

disappeared, leaving, in some areas, clear spaces behind. Some nerve cells were shrunken, dark staining and homogeneous in appearance, the nucleus not being distinguishable from the dark cytoplasm. Other cells were pale staining, vacuolated and with small dark staining eccentrically placed nuclei. The picture as a whole was one of destruction and was sufficiently marked to be very evident in the H.& V.G. sections. Although there was some rearrangement of the vessel pattern in the cortex there was no evidence of hemorrhage or thrombosis. The sections stained with the P.T.A. and S.C.A. methods bore out what has already been described.

Cat P.2006. Left cerebral hemisphere.

The experiment on this cat was different from those three described immediately above in that the pial vessels were constricted by mechanical rather than by electrical stimulation. This was done by stroking the vessels from side to side as described in Part I. of the thesis. They were kept constricted in this manner over portions of the lateral, suprasylvian and ectosylvian gyri during half an hour.

Sections stained by the G.C.S. method showed three triangles of gliosis, one in each of the gyri stimulated. The triangles in the lateral and in the suprasylvian gyri were more complete than in the ectosylvian where the gliosis was patchy. The gliosis was the same as that described in the preceeding experiments except that in addition there was a layer of

hypertrophied subpial astrocytes (in each triangle) arranged parallel to the surface of the gyrus, (see Fig. XXXI). Evidence of clasmatodendrosis of astrocytes was also present in this layer.

No layer of astrocytes similar to this was found in any of the experiments except where mechanical stimulation had been applied with some force to the surface of the brain. It is felt that these astrocytes were the result of the mechanical trauma. The triangular distribution of the underlying gliosis, however, it is believed was the result of ischaemia from vasoconstriction of pial vessels.

Sections were also studied that had been stained with the H.& V.G., P.T.A., S.C.A. and Nissl's thionin methods. Nothing else of significance was found except some suggestive changes in the nerve cells, (in the same areas as the gliosis), similar to those described in cat P.2004.

Dog. P. 2135. Right cerebral hemisphere.

In this animal a large bilateral craniotomy was performed and the pial vessels stimulated electrically over a large area of cortex (thyratron at 3) for half an hour. Stimulations were for periods of 3 seconds at 3 second intervals. There was good constriction of pial arterial vessels but the vessels were not as sensitive as in the cat and the resultant ischaemia was less marked. The stimulation in this case was spread over a large area and not confined to one gyrus.

Histologically the lesions found in this brain were not as striking as in those found in cats. Only a few small areas of patchy perivascular gliosis was to be seen and one definite triangle of gliosis. This triangular area of gliosis was best seen in the G.C.S. sections and was similar to those lesions described above. This lesion however was in the cortex directly underlying an area of considerable thickening in the meninges and it is felt that the changes in the meninges with pressure upon pial vessels might have been the cause of the gliosis rather than the ischaemia at operation caused by electrical stimulation. There were no other abnormal findings of significance in the sections stained by the H.& V.G., S.C.A., P.T.A., or Nissl's (thionin) methods. No thromboses of vessels or hemorrhages were noted.

Monkey P.2137.

A large left osteoplastic flap was turned and the cortex of the left hemisphere stimulated with the thyratron at 3 for 20 minutes and at 8 for 10 minutes. The stimulations were applied for 3 seconds at 3 second intervals and ranged over the whole exposed cortex. Although the pial vessels were stimulated directly only a few of these showed slight constrictions, one vessel only constricted over a small area almost to obliteration. The lack of reaction to electrical stimulation of these vessels is quite different from the prompt constrictor responses obtained in cats - as described in Part I. of the thesis.

FIGURE XXXIV.

Photomicrograph of a patch of hypertrophied, fibrous astrocytes in the grey matter of monkey No.P. 2137 whose cortex had been stimulated electrically. It was in layer V. of the cortex. Similar patches were found in all layers of the grey matter and appeared to be perivascular in distribution. See text and tables of experimental data for further particulars. (G.C.S. stain.).

FIG. XXXIV.

The pathological changes found in this brain were not marked but quite definite. Patches of perivascular gliosis were found in the grey matter over the tips of the gyri in the area of brain stimulated electrically. These patches were found in all layers of the cortex from the pia to the white matter and were of about equal extent in all layers. A typical patch is shown in Fig. XXXIV. These patches of gliosis were made up of hypertrophied astrocytes which had become definitely fibrous in form.

Although no definite complete triangular areas of gliosis were found it seems more than likely that the perivascular distribution of the lesions and the fact that they were present equally in all layers of the cortex can be explained on a vascular (ischaemic) basis rather than on a traumatic one. The picture in this brain seems to be only an incomplete counterpart of the typical triangular lesions described in cats. The reason for this seems most likely to be that in cats the ischaemia produced by electrical stimulation was more complete. Ischaemia resulting from other causes at operation will be discussed in detail later and these may play some part in all these lesions.

Note. All the brains described in the above section were also studied after being stained by Rio-Hortega's silver carbonate method for oligodendroglia and by Penfield's second modification of this method for oligodendroglia and microglia. The staining with the former method was kindly performed by Dr. McCarter. A good proportion of the stains were satisfactory. No changes

TABLE OF EXPERIMENTAL DATA, NO. II.

| Animal & Number | Operation | Treatment of brain on left | Treatment of brain on right | Survival period in days | Findings at autopsy | Cortex Histo-pathologic findings. |
|---|---|---|---|---|---|---|
| CAT P.2004 | Left decomp-ression | Exposed under glass 1 hour. Elect. stim. ½ hr. Constriction of pial vessels Thyratron at 5. | No craniotomy | 39 | Quite dense meningeal adhesions on left. | Triangular areas of gliosis in grey matter left hemisphere |
| cat P.2006 | " | Exposed under glass 1 hour. Mechanical stim. Constriction of pial vessels ½ hr. | " | 38 | " | " |
| dog P.2135 | Bilateral decomp-ression | Exposed under glass 1 hour & to room air 1 hour. | Exposed under glass 1hr. & to room air 1 hr. Elect. stim. Constriction pial vessels Thyratron 3. | 105 | Dense meningeal adhesions bilateral convolutional flattening | Patchy areas of gliosis in right hemisphere |
| monkey | Left osteo-plastic craniot-omy. | Exposed under glass 40 mins. Elect. stim. Thyratron at 8. for 10 mins & at 3 for 20 mins. | No craniot-omy. | 27 | Quite firm meningeal adhesions on left. | Patchy areas of gliosis in left hemisphere |
| cat P.2144 | Bilateral decomp-ression | Exposure Cont. irrig. Ringer's soln. 2 hours. | Exposure to room air for 2 hours | 8 | Quite dense meningeal adhesions bilateral | Triangular areas of gliosis both hemispheres |
| cat P.2151 | " | Exposure under glass 45 mins. Elect. stim. ½ hr. Thyratron 5. Vessels not constricted. | Exposure under glass 45 mins. | 63 | " | Patchy triangular areas of gliosis left hemisphere |
| cat P.2155 | " | Exposure Cont. irrig. Ringer's soln. 2 hours | Exposure under glass 2 hours. | 18 | " | Triangular areas of gliosis both hemispheres |

in the oligodendroglia were noted in any of the brains. Changes in microglia were found only in Cat P.2184. In this brain a considerable number of early amoeboid forms of microglia were present throughout the areas involved in the gliosis and a few compound granular corpuscles were to be seen subpially in the suprasylvian gyrus. In all the other brains the microglia appeared normal but unfortunately it stained poorly over the tips of the gyri in the area of the lesions as compared with the grey matter elsewhere.

## CEREBRAL CICATRIX FOLLOWING EXPOSURE OF THE BRAIN AT OPERATION

Eight cats were used in this study. Details of the experiments are given below, in table III., and in the description of technique.

Triangular areas of gliosis were found in the grey matter of a number of these cats whose brains had been merely exposed to room air under various conditions for two hours. These areas of gliosis were similar to the ones found in cats whose pial blood vessels had been constricted electrically or mechanically and it is felt that they are likewise probably due to ischaemia although the mechanism of production of the ischaemia was different.

The pathological findings in the sections stained by Cajal's G.C.S. method(modified) will be described separately for each cat. The sections stained by other methods will be

TABLE OF EXPERIMENTAL DATA, NO. III.

| Animal & Number | Operation | Treatment of brain on left | Treatment of brain on right | Survival period in days | Findings at | Cortex Histo-pathological findings |
|---|---|---|---|---|---|---|
| cat P.2168 | Bilateral decomp-ression. | Exposure under glass 2 hours. | Exposure Continuous irrig. Ringer soln. 2 hrs. | 65 | Quite dense meningeal adhesions bilateral | Triangular areas of gliosis both hemispheres |
| cat P.2182 | " | Dura exposed 15 mins. not opened | Dura opened & closed again immediately | 55 | No adhesions on left Adhesions about silk sutures on right. | No pathology in cortex. |
| cat P.2184 | " | Exposure under glass 1 hour. Electrodes touched to brain as on right No elect. stim. | Exposure under glass 1 hr. Elect. stim. Thyratron 9. Constriction of pial vessels 20 min | 15 | Quite dense meningeal adhesions bilateral | Triangular areas of gliosis both hemispheres More marked on right. |
| cat P.2186 | osteoplastic craniotomy right. Decompress-ion left. | Exposure under glass 2 hours. | Exposure under glass 2 hours. | 16 | " | Triangular areas of gliosis both hemispheres |
| cat P.2189 | Bilateral osteoplastic craniotomy | Exposure to room air 2 hours. | " | 18 | " | No definite pathology Poor stains |
| cat P.2194 | " | Exposure of dura Irrig. iced Ringer's soln. 2 hours. | Exposure of kept warm - 2 hours. | 9 | No adhesions | No pathology |
| cat P.2214 | " | Dura opened & closed again immediately | Exposure to room air 5 hours. | 9 | Quite dense adhesions on right Adhesions about dural sutures on left. Extra-dural haematoma on left | Triangular areas of gliosis both hemispheres |

described collectively.

Cat P. 2144. Right hemisphere - The suprasylvian and a portion of the lateral and ectosylvian gyri were exposed to room air for 2 hours.

The extent of the craniotomy and brain exposure was the same in all of the experiments to follow except where stated.

In the sections stained by the Cajal G.C.S. method (modified) there was gliosis throughout the whole of a triangular area in the suprasylvian gyrus as shown in Figs XXXV and XXXVI and in the diagram of Fig. XXXV. Another smaller triangular area of gliosis was present in the grey matter of the ectosylvian gyrus. Immediately overlying each of these areas of gliosis, the dura was adherent to the pia-arachnoid and there appeared to be slight flattening of the gyri in this region.

The gliosis consisted of a quite marked hypertrophy and increase in number of the astrocytes throughout the whole triangular zone from pia to white matter. The base of the triangle in the suprasylvian gyrus was at the pia and extended across the whole exposed surface of the gyrus from the point, on each side of the gyrus, where the pia was reflected to the neighbouring gyri, (as in the diagram of Fig. XXXV.) The apex of the triangle was at the white matter. The area of gliosis in the ectosylvian gyrus was similar in distribution but its base was narrower covering only the central portion of the gyrus. In the triangular zones mentioned the astrocytes had laid down

fibres and become transformed from protoplasmic into fibrous forms. There was abundant evidence of direct cellular division of astrocytes. The increase in number and hypertrophy of the vascular foot plates of the astrocytes along the larger vessels was very evident. The hypertrophied astrocytes were quite evenly distributed throughout both triangular zones mentioned, and there was a quite sharp demarcation between abnormal fibrous and normal protoplasmic astrocytes at the sides of the triangle. Over a considerable area, however, the astrocytes were less numerous in the molecular layer of the cortex than in the deeper layers. However in some places in the molecular layer, especially where the dura was most adherent to the pia-arachnoid, there was hypertrophy and increase in number of the astrocytes in the molecular layer and some of these showed clasmatodendrosis. (Sections stained by other methods than the G.C.S. will be described below).

Cat P. 2144. Left cerebral hemisphere - which was irrigated continuously for two hours with Ringer's solution at body temperature, i.e. during the entire period that the dura was open. The exposure was of the same extent as on the right side.

The sections stained by the G.C.S. method showed two triangular areas of gliosis almost identical to those described in the right hemisphere of the same cat - one large triangle in the suprasylvian and one smaller one in the ectosylvian gyrus. The hypertrophy and increase in number of the astrocytes in these

FIGURE XXXV.

Two photomicrographs from the right suprasylvian gyrus of cat No.P. 2155, photographed from the same microscopic section. They are shown in an attempt to illustrate the triangular nature of the patches of gliosis mentioned in the other photomicrographs and in the text.

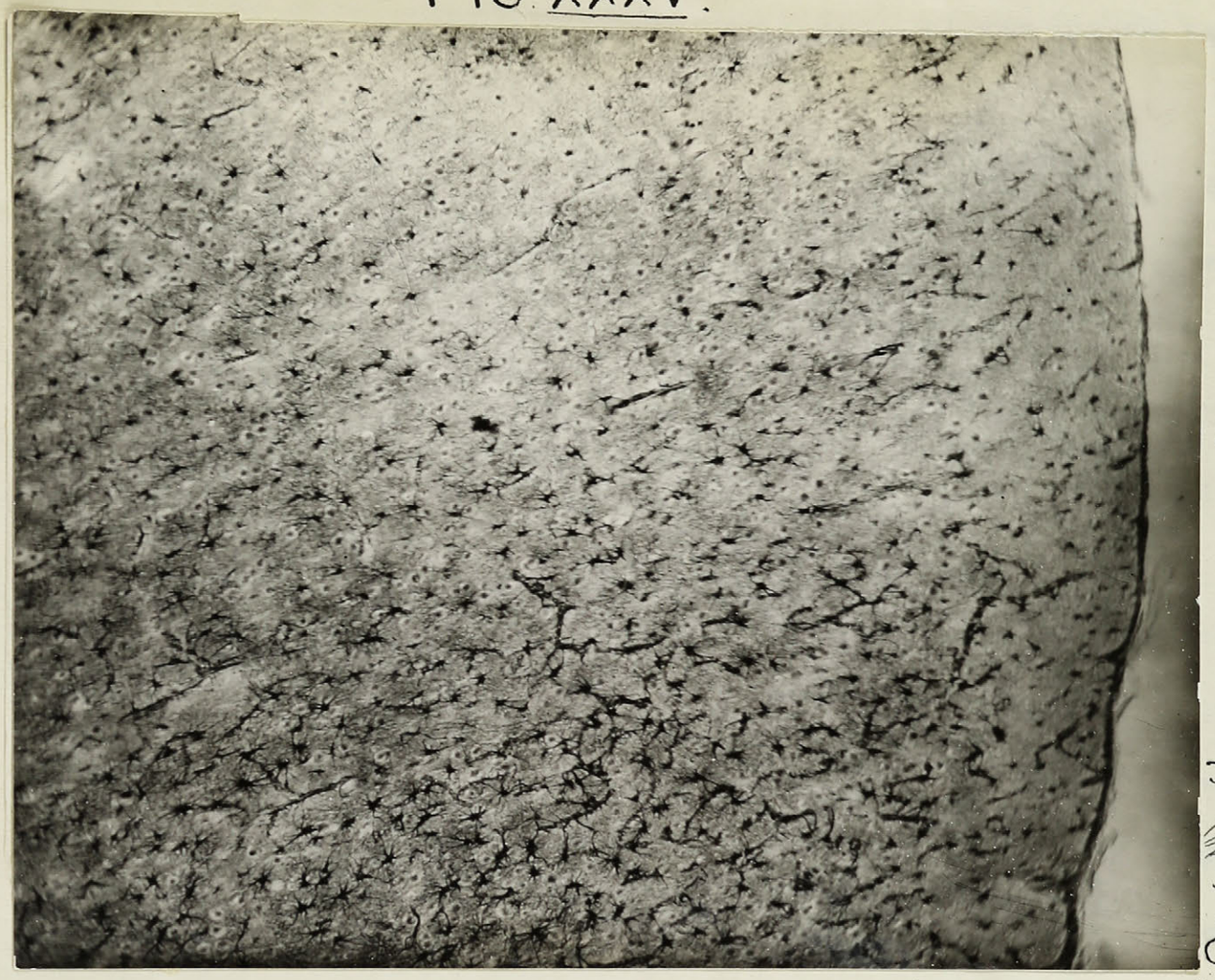
In the lower portion of photomicrograph A. and in the upper portion of B. is a zone in the grey matter in which the astrocytes are hypertrophied, (transformed into fibrous astrocytes) and increased in number. This zone extends from the pia to the white matter. Its lateral border in each case is quite sharp and slants in the same direction as the perforating vessels from the pia. In photomicrograph B. the lateral border of the gliosis stops at and is demarcated by the large vessel perforating the cortex from the pia (and marked with an arrow). On the opposite side of this vessel (i.e. in the lower portion of photomicrograph B.) the astrocytes are normal in size and number - and are protoplasmic. Normal astrocytes are also present in the upper half of A. Note that there is little reaction of the subpial astrocytes. The areas from which the photomicrographs were taken are shown in the diagram.

DIAGRAM OF AREAS OF GLIOSIS IN BRAIN P.2155 -R.

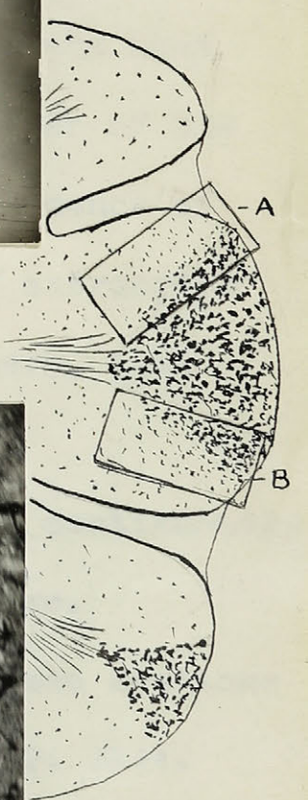
These are shown on the opposite page.

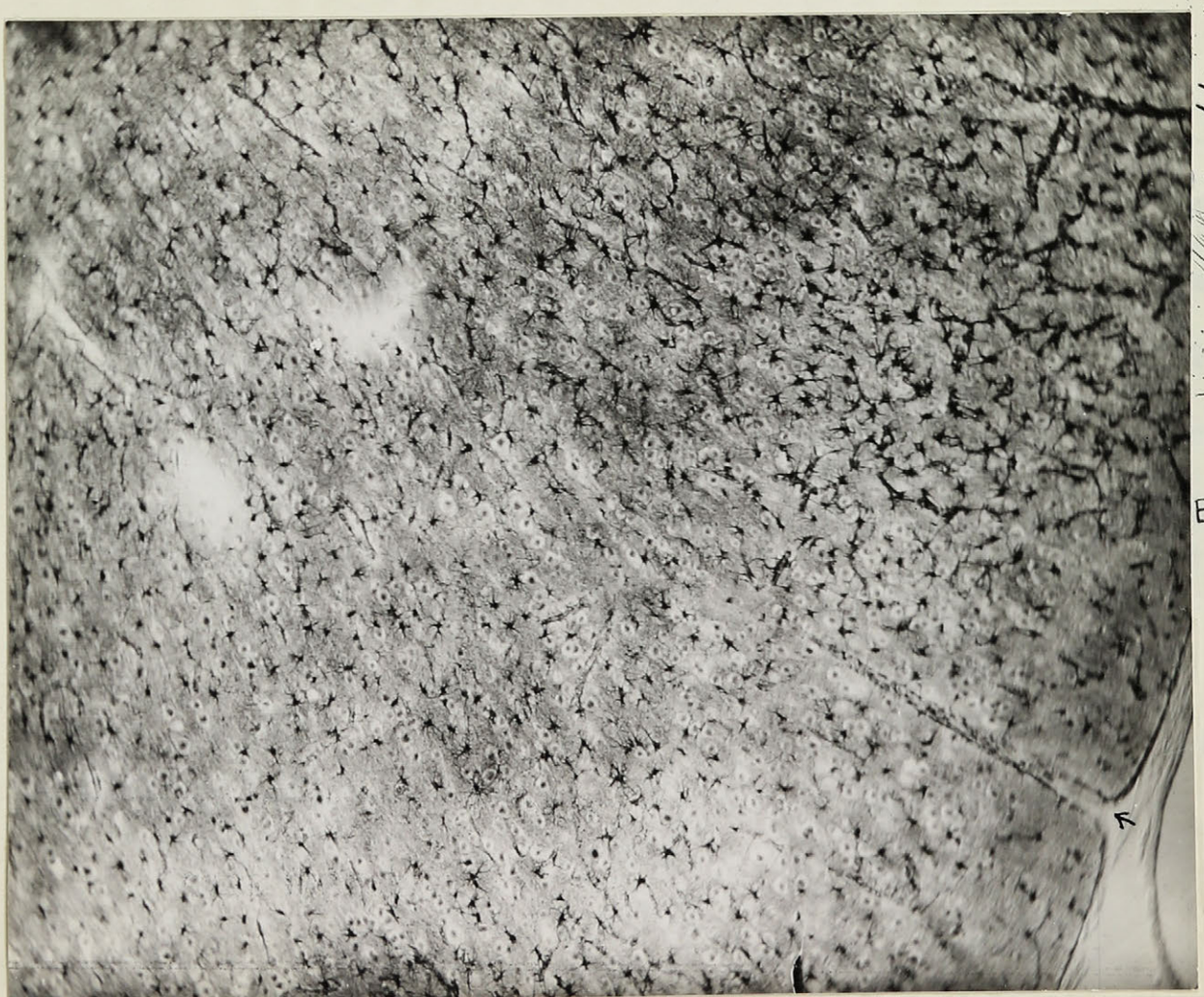
The enclosed areas marked A. and B. in the diagram represent the areas shown in the photomicrographs A. & B.

FIG. XXXV.



A



B

zones was somewhat less, however, than in the right hemisphere of this cat.

Cat P. 2155. Right hemisphere - the brain was exposed to air for two hours but covered with a watch glass immediately on opening the dura.

G. C. S. section: In the grey matter of the suprasylvian gyrus was present a beautifully demarcated, large, triangular area of gliosis similar to that described in cat P.2144. The astrocytic hypertrophy was perhaps even slightly more marked in these sections than in those taken from the right cerebral hemisphere of cat P.2144. The reaction of the astrocytes was less pronounced subpially and throughout the molecular layer of the cortex than in the deeper layers and there was no evidence of clasmatodendrosis in this case. The hypertrophy of astrocytes was slightly greater in layers 2 and 3 than elsewhere, (see Fig. XXXVII, XXXVIII and XXXIX).

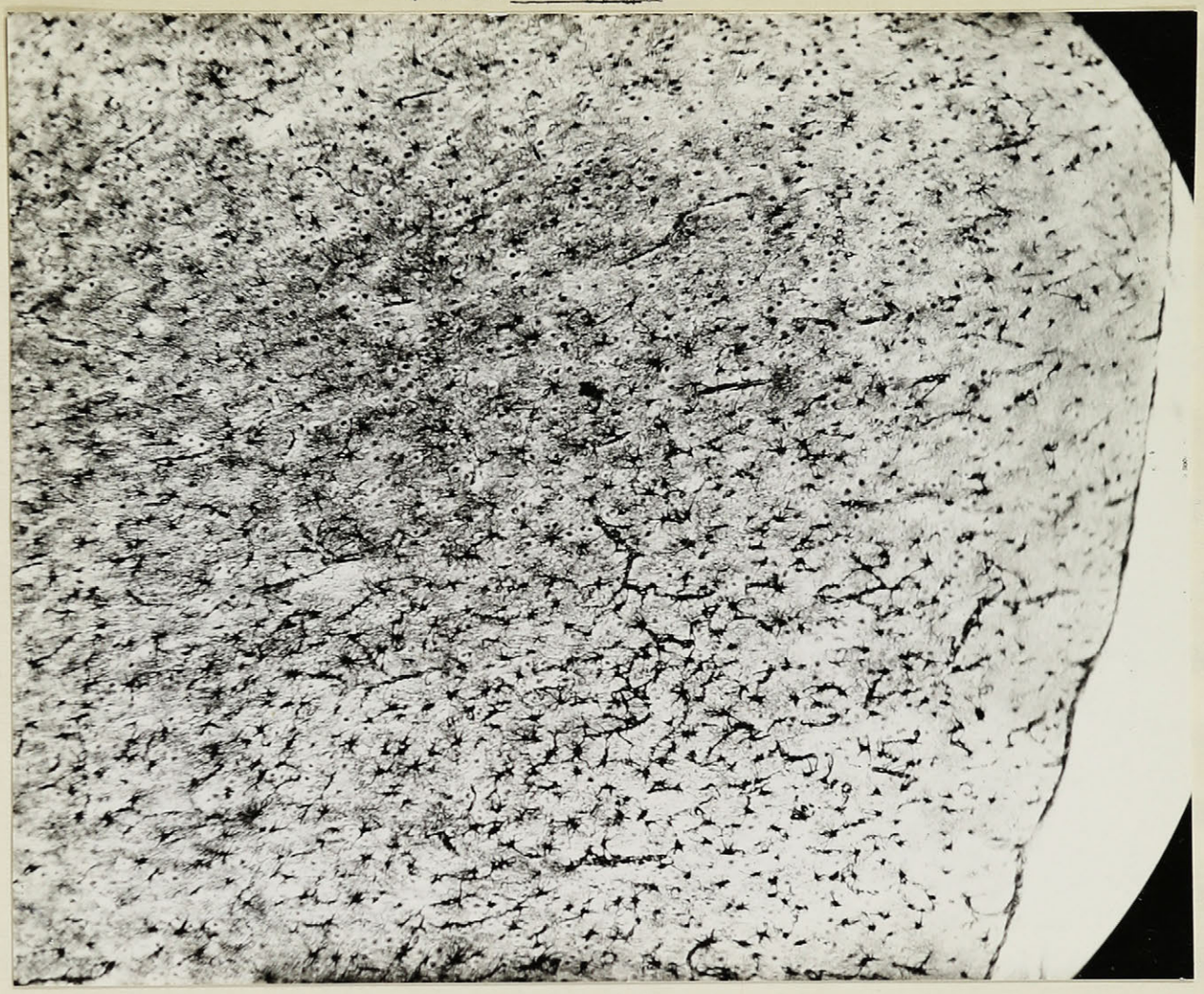
Cat. P. 2155. Left hemisphere - this was irrigated continuously for 2 hours with Ringer's solution at body temperature.

G.C.S. sections : Similar changes were present in these sections as found in the right hemisphere of cat P. 2155 and P. 2144. A large triangular area of gliosis was present in the suprasylvian gyrus and also a smaller less complete triangular zone of astrocytic hypertrophy in the ectosylvian gyrus. The hypertrophy and increase in number of astrocytes was less marked in these sections than in those from the right hemisphere of cat P. 2155, being about the same as in the left hemisphere of

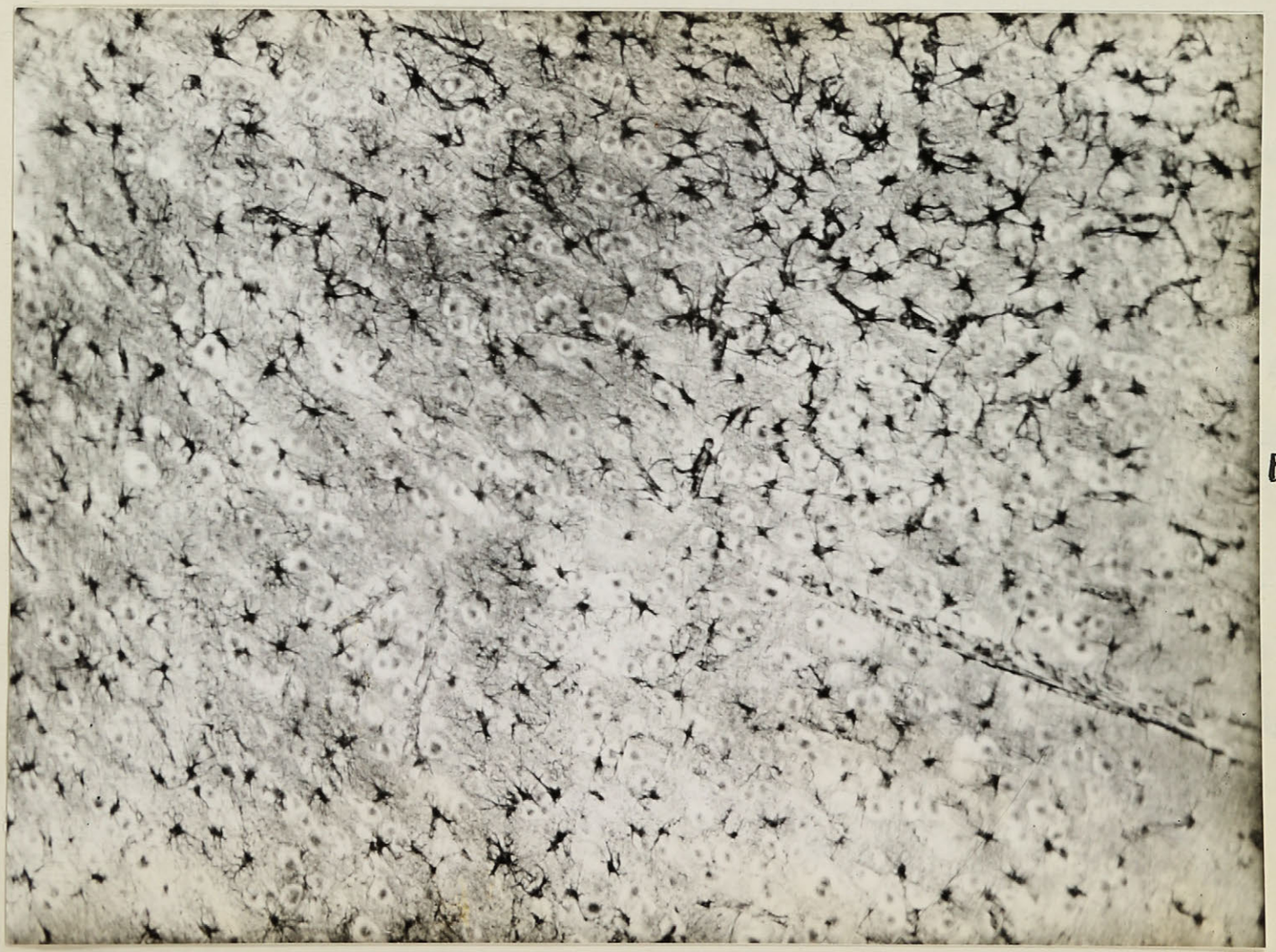
## FIGURE XXXVI.

These photomicrographs are the same as those shown in Fig. XXXV. but are of different magnifications. Photomicrograph A. illustrates how the gliosis (in lower half of picture) extends from the pia to the white matter. It is however a little more marked in layers 2 and 3 than elsewhere. Photomicrograph B. shows how sharply the area of hypertrophied astrocytes is demarcated from normal astrocytes by a perforating vessel from the pia mater, (vessel marked with an arrow). (G.C.S. stain).

FIG. XXXVI.



A



B

cat P. 2144.

Cat P. 2168. Right hemisphere - the brain was irrigated continuously for 2 hours with Ringer's solution at body temperature.

Left hemisphere - this was exposed to air for 2 hours, but covered with a watch glass.

G.C.S. sections: Sections from each hemisphere showed a large triangular area of gliosis in the suprasylvian gyrus similar to that described in cats P.2144 and P. 2155. However, despite the fact that the experiments were more or less identical in cats P. 2155 and P. 2168 (except for the duration of the survival periods), the gliosis was considerably more marked in cat P. 2155 than in cat P. 2168. In the latter case the gliosis was of similar anatomical distribution but the hypertrophy of the individual astrocytes was less and they were not so increased in number.

Cat. P. 2186. In this cat a bone flap was turned on the right and a decompression performed over the left hemisphere. Both wounds were covered with a large petri dish immediately on opening the dura. The dura was left open for two hours.

G.C.S. sections: A large triangular area of gliosis was found to be present in both suprasylvian gyri identical in anatomical distribution to those described in the other cats above. The hypertrophy of astrocytes was only moderate, however, but definite. Evidence of direct division of astrocytes was more plentiful in these sections than in any of the others despite

the fact that the total increase in number of astrocytes was not as great as in some of the other sections. It is interesting that the gliosis was somewhat more pronounced on the side of the bone flap than on that of the decompression where it was somewhat patchy in distribution. In both suprasylvian gyri the hypertrophied astrocytes were more abundant in layers 2 and 3 of the cortex (although they extended to the white matter), with only slight astrocytic reaction in the molecular and subpial zones.

Cat P. 2189. Both cerebral hemispheres.

G.C.S. sections: No gliosis or other pathology was found in either cerebral hemisphere of this brain. This is hard to explain as the experiment was the same as in some of the cats described above where areas of marked gliosis were present.

There are perhaps two reasons for these findings. One is that the craniotomies in this case (bone flaps) were smaller than in any other cat and secondly that the stains were poor and might not have demonstrated the hypertrophied astrocytes.

Cat P.2214. A portion of the right cerebral hemisphere was exposed to air for 5 hours. On the left side the dura was opened and then closed again immediately. At autopsy a large extradural haematoma was found on the left side and about half a c.c. of thick mucous-like material was present extradurally. The frontal sinus on the left side had been opened at operation accidentally. It was swabbed out with mercurochrome and the

sinus plugged with bone wax.

G.C.S. sections: A triangular zone of gliosis was found in the suprasylvian and ectosylvian gyri of each hemisphere. The gliosis was quite marked and similar in nature and anatomical distribution to that described in cat P.2144.

It is interesting that there was gliosis in the left cerebral hemisphere, on which side the dura was closed again immediately after it had been opened. It is believed that the gliosis in this hemisphere may be explained by the presence of the extradural haematoma and the collection of the mucous-like material found in the wound at autopsy. More will be said in the final discussion concerning this finding.

Cat. P.2194. A bilateral decompression was performed on this cat. Immediately on exposing the dura (which was not opened) on the right side it was covered by a petri dish surrounded by gauze moistened with Ringer's solution. An electric light bulb was then placed above the watch glass. Heat from the lamp was prevented from reaching the wound on the opposite side by draping a towel over the lamp. In this fashion the dura and brain on the right side were kept warm for 2 hours. On the left side the dura (which was not opened) was irrigated continuously for 2 hours with iced Ringer's solution.

G.C.S. sections: No pathological changes were found in this brain

Cat. P. 2182. A bilateral decompression was performed on this cat. On the right side the dura was opened and closed again immediately. On the left side nothing was done to the dura.

The wound was closed bilaterally immediately after the above procedure on the right side had been completed.

G.C.S. sections: Nothing abnormal was found in the sections from either hemisphere - except in the meninges as described above.

Cat. P. 2184. The histological findings in the right hemisphere of this cat following electrical stimulation have already been recorded.

The treatment of the brain exposed through a left decompression was described above. The duration of the exposure was for one hour.

G.C.S. sections from left cerebral hemisphere.

A large triangular area showing moderate gliosis was present in the suprasylvian gyrus. The pathological picture both in its nature and anatomical distribution was about the same as that described in the left suprasylvian gyrus of cat P. 2144 or P. 2155. There was less hypertrophy of astrocytes than occurred in the right cerebral hemisphere of cats P.2144 and P.2155. The duration of the exposure was not as long as in these cats and this might have played a part.

Cat. P. 2151. The histological findings in the left cerebral hemisphere of this cat have already been described above.

It should be noted that the duration of the exposure of the brain in this case was only forty-five minutes as compared to two hours in many of the other cats.

G.C.S. sections: Nothing of pathological significance was noted in the right cerebral hemisphere of this brain - except in the meninges as described before.

The pathological findings in the sections of the above brains, (of animals P.2144, 2151, 2155, 2168, 2182, 2184, 2186, 2189, 2194 and 2214), stained by S.C.A., P.T.A., H.& V.G., Nissl's (thionin) methods, as well as by Loyez's method for myelin sheaths, Bodian's method for axis cylinders, Rio-Hortega's S.C. method for oligodendroglia and Penfield's second modification of the latter method for oligodendroglia and microglia, may be summarized together.

With these methods the findings recorded above were verified. No definite changes in nerve cells, axis cylinders, or myelin sheaths were found. There was no evidence in any of the sections of thrombosis of vessels or hemorrhage in the cortex. No changes were seen in the oligodendroglia. No definite alterations in the microglia were observed but in some areas where the gliosis was quite marked early changes may have been present.

FIGURE XXXVII.

The two photomicrographs are of the right supra-sylvian gyrus, (which had been exposed to air) in cat No.P.2144. A sharply demarcated triangular area of gliosis was present in this gyrus as shown in the diagram below. Photomicrograph A., shows how the astrocytes are hypertrophied and increased in number in the grey matter. Note that there is little reaction of the subpial astrocytes and of those in the outer molecular layer of the cortex, shown to the right side of the picture. This was not true over the whole surface of the gyrus, however.

Photomicrograph B. shows the demarcation between hypertrophied and normal astrocytes at the side of the triangle - as illustrated in the diagram below. (G.C.S. stain).

DIAGRAM OF LESIONS IN RIGHT HEMISPHERE OF CAT NO.P.2144

Areas A. and B. represent photomicrographs A. and B.

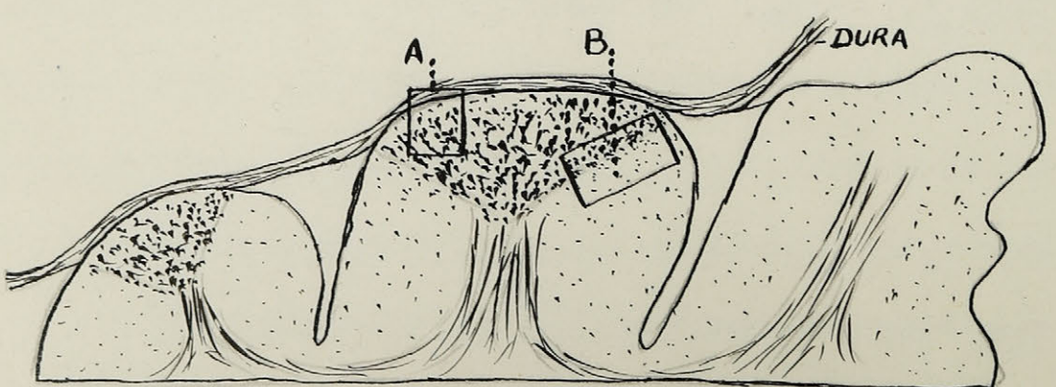
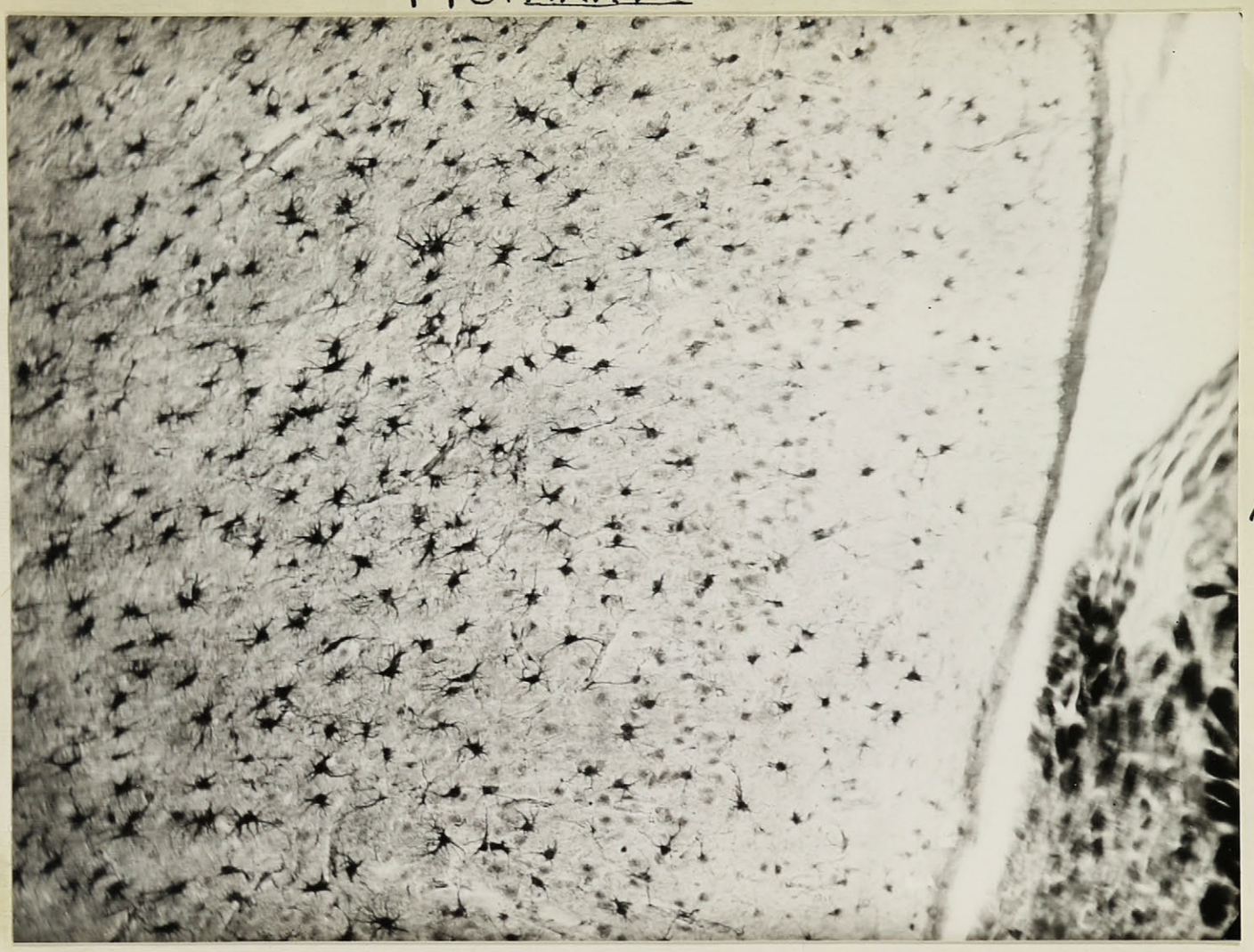
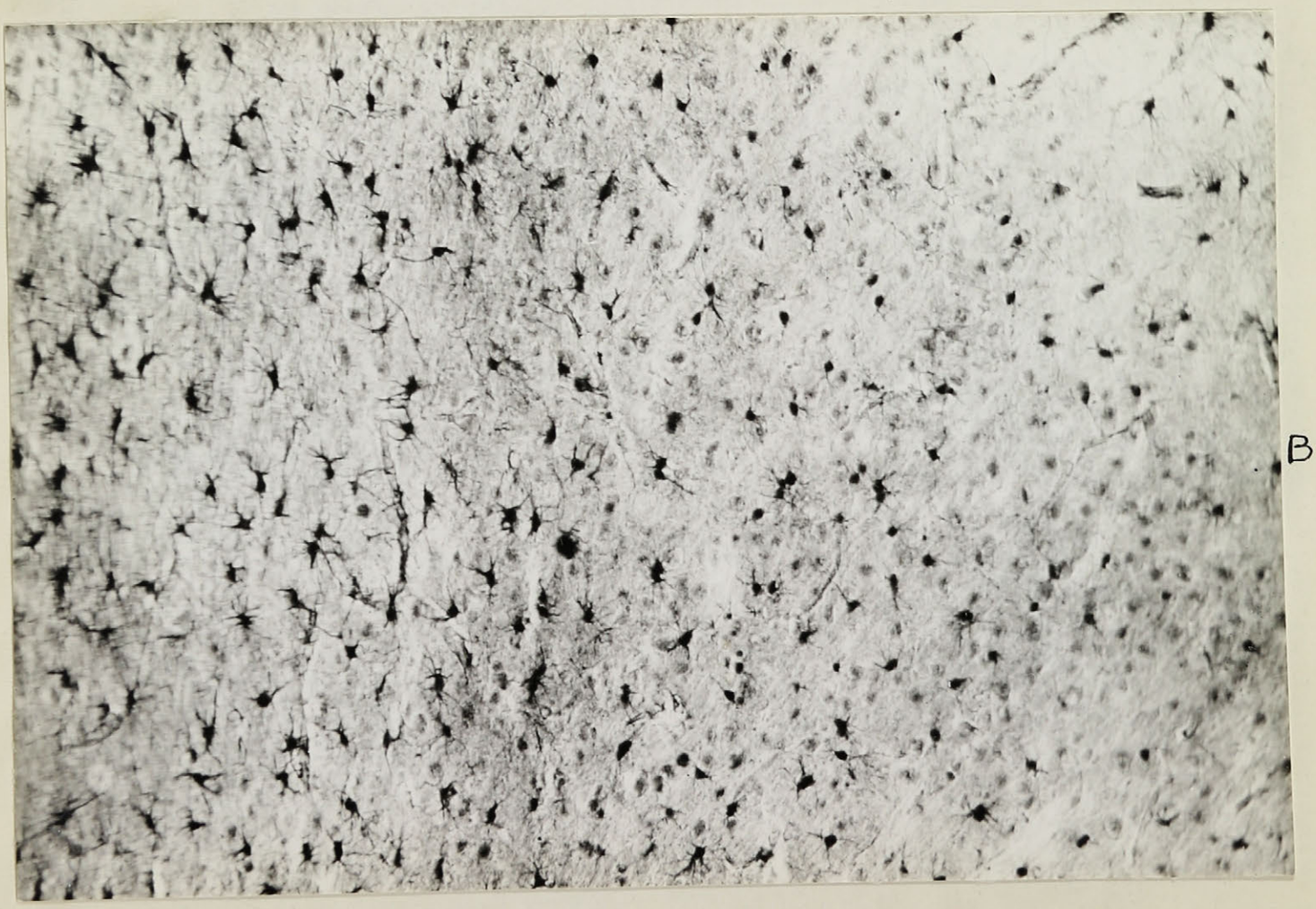


FIG. XXXVII.



A

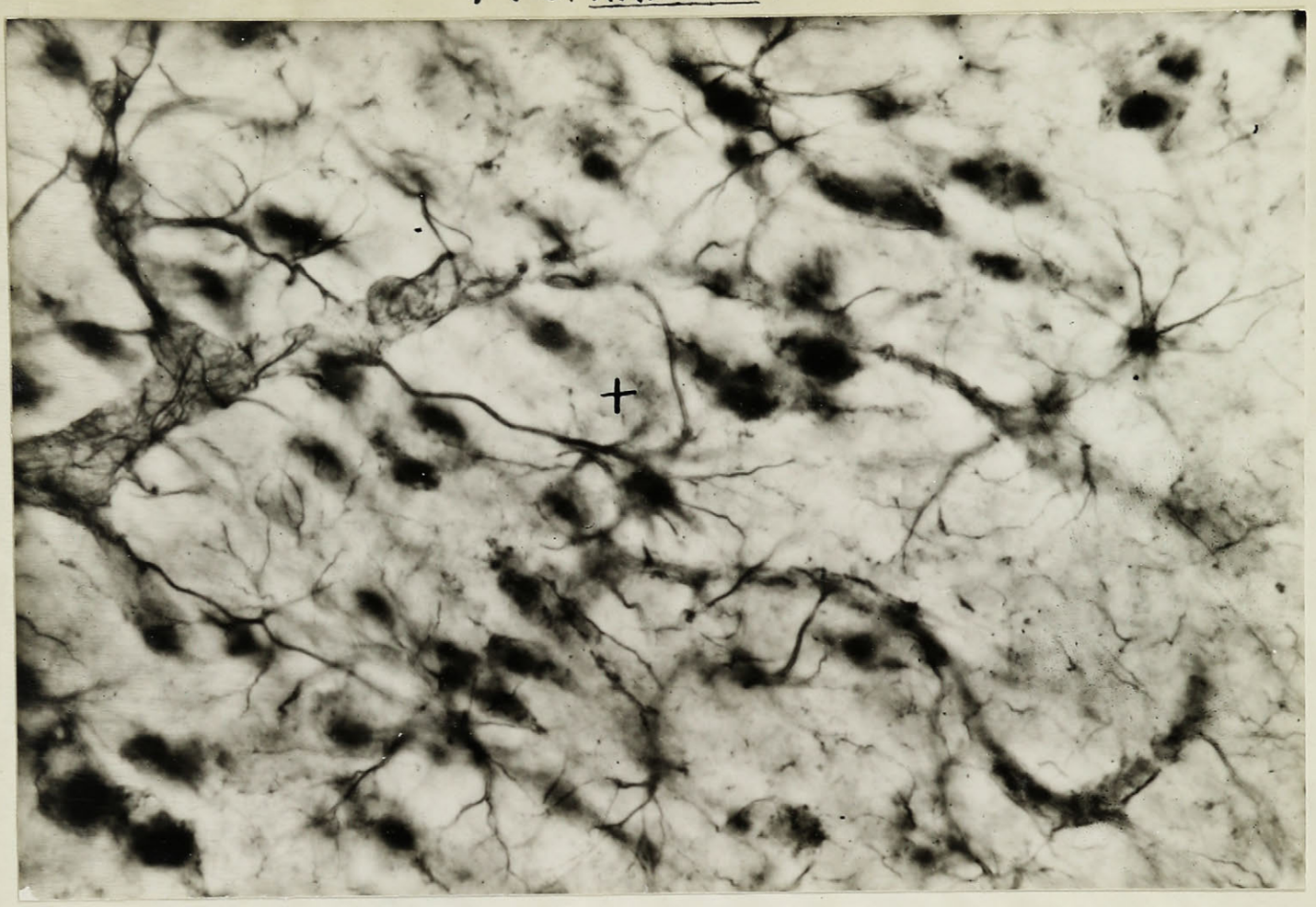


B

## FIGURE XXXVIII.

High power magnification of astrocytes in the grey matter of the same gyrus shown in Fig. XXXVII. Note that the astrocytes are hypertrophied and have been transformed from the protoplasmic form (which are normally present in the grey matter), into fibrous astrocytes. Note also the hypertophy of the foot plates. (S.C.A. stain).

FIG. XXXVIII.



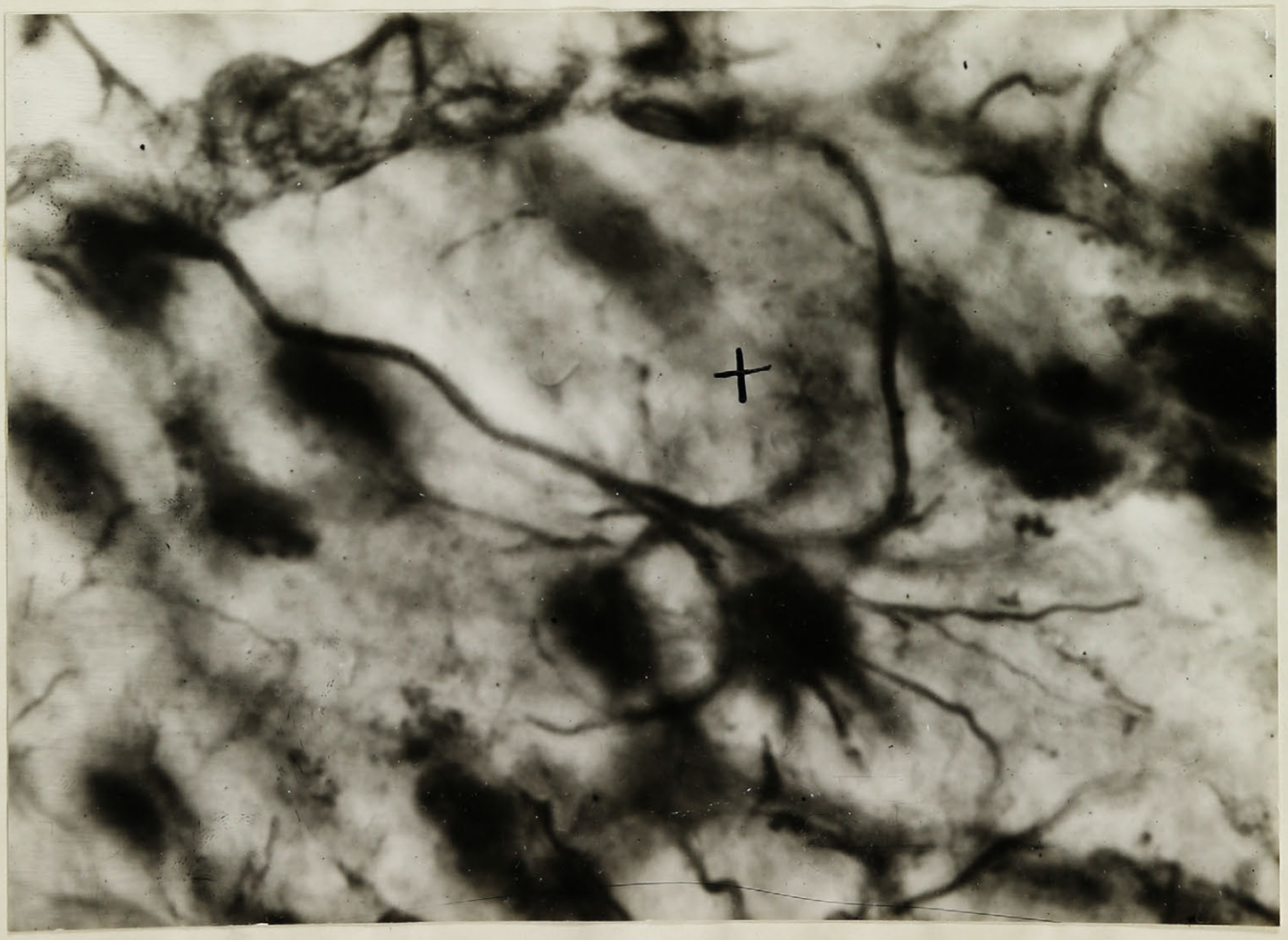
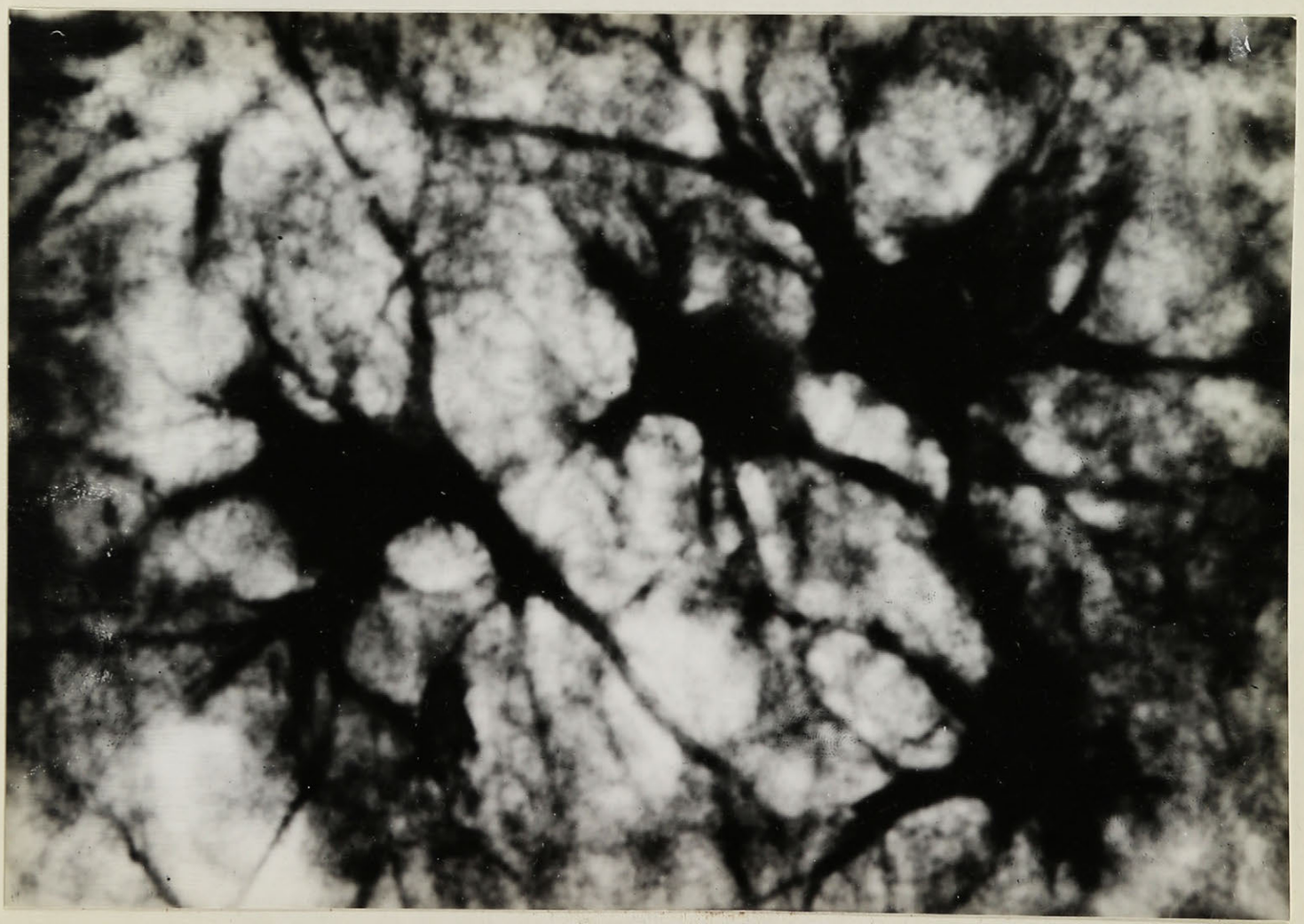


FIGURE XXXIX.

High power photomicrographs of astrocytes in a triangular area of gliosis in the left suprasylvian gyrus of cat No.P. 2144, (whose brain had been exposed on the left and irrigated continuously with Ringer's solution at body temperature). Note that the astrocytes are hypertrophied. They are distinctly fibrous in type. (G.C.S. stain).

FIG. XXXIX.

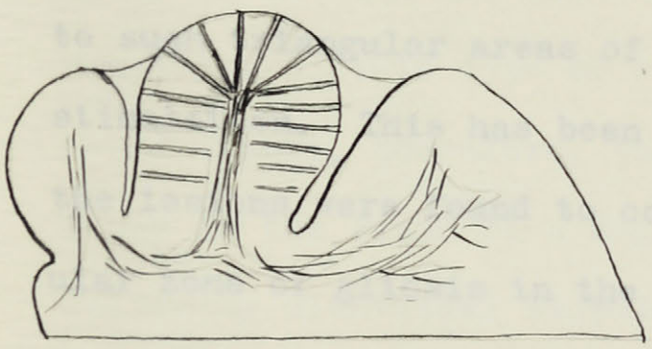


## DISCUSSION AND SUMMARY.

In Part II of the thesis are described experiments which were carried out on eighteen cats, one dog and one monkey. The brains of these animals were exposed under sterile technique at operation and either stimulated electrically or mechanically, or simply left in contact with air under different conditions. After varying periods of survival the animals were killed and their brains and meninges examined grossly and histologically. Definite pathological lesions were found in the brains and meninges of these animals and have been described in the preceeding pages. The controlled method of experimentation made it possible to come to some conclusions concerning the nature and mechanism of production of these lesions, especially those occuring in the cortex. A discussion of these conclusions follows.

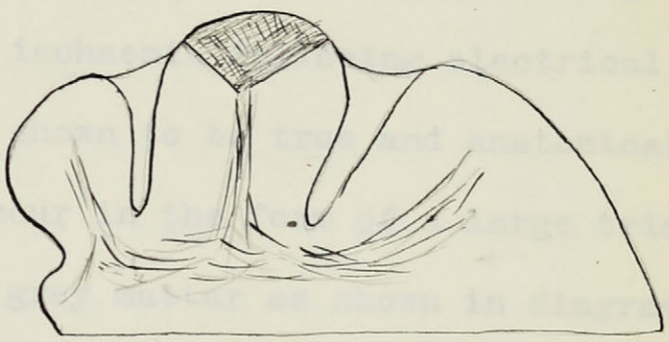
It was shown in Part I. of the thesis that areas of focal ischaemia could be produced in the brain (of cats) by electrical or mechanical stimulation of the pial (and possibly intracerebral) arterial vessels. This ischaemia was brought about by vasoconstriction of these vessels and was frequently in the form of a triangular patch in the cortex, (see Fig XIII). It is felt that the triangular shape of the ischaemic patches is undoubtedly explained by the anatomical distribution of the perforating arterial vessels from the pia in each gyrus. The

two diagrams below illustrate this adequately.



A

Showing perforating pial vessels and blood supply of gyrus.



B

Showing triangular area of ischaemia.

For instance if the vessels over the surface of the central gyrus in diagram A. are stimulated electrically they will be constricted and the greater part of the blood supply to the triangle shown in diagram B. will be cut off. If only a few vessels are completely constricted over the surface of a gyrus then the ischaemia will be in the form of a smaller triangle corresponding to the distribution of these vessels. At times, of course, the ischaemia will be patchy in nature, first because the constrictions may be patchy and also because of the uneveness of the supply of collateral blood vessels. Patchy

ischaemia of this type would be expected to occur where stimulation was carried out so as to avoid applying the electrodes to the larger vessels. Even with this precaution, however, constriction of the smaller vessels would of course result.

Knowing that ischaemia occurs in this manner one might expect to find pathological lesions in the cortex corresponding to such triangular areas of ischaemia following electrical stimulation. This has been shown to be true and anatomically the lesions were found to occur in the form of a large triangular zone of gliosis in the grey matter as shown in diagram B., or in the form of a smaller triangle or they were perivascular and patchy. The lesions have been mainly evidenced by a hypertrophy and increase in number of astrocytes, but in the more severe ones changes in nerve cells and even complete disorganization and destruction of the normal ctyoarchitecture in the triangular area has been found. It has also been demonstrated that the lesion can be varied from one of patchy nature to a small or large triangular area of marked pathological change by varying the intensity and duration of the electrical stimulation, i.e. the degree and duration of the ischaemia, (compare Figs. XXX, XXXII and XXXIII). No definite evidence has been discovered that electricity in itself (without coincident ischaemia) produces any histological change in the cells of the central nervous system. A discussion of these findings as regards their application to the electrical exploration of the

human cortex has been given in the introduction to Part II. of this thesis.

It should be recalled here that, as shown in the first part of the thesis, pial blood vessels in dogs do not constrict quite as readily on electrical or mechanical stimulation as do those in cats and that the monkey's pial vessels are less sensitive to this type of stimulation than the dog's. One would expect, therefore, that less histological change would be found in the brain of a dog or a monkey, ( and probably in man), following electrical stimulation of the cortex, than in that of a cat. This has proved true in the experiments described in Part II. of the thesis; for in dog P.2135 and monkey P. 2137 the lesions were scattered, patchy, perivascular and did not make up complete triangular zones as described in the cat.

In those brains, which had been simply exposed to air, under various conditions, at operation for two hours, triangular areas of gliosis were found in the surface grey matter. These areas of gliosis were similar in nature and anatomical distribution to the lesions described as resulting from focal cerebral ischaemia produced by electrical or mechanical stimulation of pial vessels. They were, however, less patchy and although in some cases the astrocytic hypertrophy was only slight, the area over which it occurred was triangular in shape. The lesions were present only in the surface grey matter of those gyri exposed and were more marked and consistently triangular

in distribution in the suprasylvian gyrus which received the maximum exposure. The base of the triangle was at the pia and the apex at the white matter. They were found in brains which had been protected from dry air by a watch glass surrounded with moistened gauze, or continuously irrigated with Ringer's solution during the period of exposure. The gliosis was possibly a little less marked in the latter than in the other experiments but a larger series would be necessary to be sure of this point. Adhesions between lepto- and pachy-meninges overlay the lesion in each instance (regardless of whether the brain had been covered or irrigated continuously)., and in some cases the gliosis in the grey matter was present only where adhesions were present and visa versa.

It is believed that these triangular patches of gliosis were the result of a relative ischaemia of the grey matter. Their anatomical distribution, which corresponds to the distribution of the perforating vessels (as described in the first part of the discussion) strongly suggests that the lesions were vascular in origin. The similarity between these lesions and those produced by vasoconstriction of pial vessels upholds this point and their perivascular arrangement is also evidence in this direction. It should be stated that no evidence of hemorrhage or thrombosis of vessels was found in the cortex.

It seems very unlikely that the lesions could have been traumatic in origin as the increase in number and hypertrophy

of astrocytes was evenly distributed throughout the triangular zones, from the pia to the white matter. If they were traumatic one would expect the gliosis to have been more marked near the surface of the brain (subpially and in the molecular layer) where the maximum degree of trauma would have been delivered. This however was not true and in fact in many of the lesions there was less gliosis in the subpial and molecular zones than in the deeper layers of the grey matter. Also, the brains of these animals were handled with extreme gentleness at operation and care was taken not to touch the arachnoid.

In determining the cause of this gliosis one must consider the factors that might have played a part at the time of operation. First there is the possibility that vasoconstriction of the pial or intracerebral vessels could have occured due to the trauma of operation. Although there is frequently some vasoconstriction of pial vessels observable (due to trauma) on first opening the dura, this is transitory and not always present. Another possibility is that stasis of blood flow during exposure might play a part. It has been shown that pial vessels dilate and that there is a slowing of circulation when the brain is left exposed to room air for more than an hour. However this dilatation and slowing of circulation in pial vessels does not occur if the brain is protected from dry air with a watch glass surrounded by gauze moistened with Ringer's

solution, - yet essentially the same type of patholgical lesion (consisting of a triangular zone of gliosis) has been found in brains treated in either manner. It has also been shown that temperature is not the important factor in the cause of these lesions; for brains irrigated with iced Ringer's solution for two hours showed no evidence of the typical lesions described, (the dura over these brains was not opened but the temperature in the region of the pia must have reached a low level). Also, brains irrigated with Ringer's solution at body temperature for a two hour period, while the dura was open, showed the same type of lesion as those brains left exposed to dry air.

There seems to be no doubt that the lesions described (in brains following simple exposure at operation), were vascular in origin and in all probability were due to relative ischaemia in the grey matter. This ischaemia might have been brought about in one of three ways, or by a combination of these, (other causes mentioned above having been eliminated); (a). The inflamatory reaction in the meninges, (evidenced by a cellular exudate and oedema of the pia-arachnoid), shown to be present in all these brains during the first eight days after operation, might have compressed the pial vessels thus causing a slowing of the circulation. (b). The adhesions that develop following exposure of the brain at operation and bind the arachnoid to the pia could likewise compress the pial vessels and diminish the

blood supply to the triangular zone as shown in diagram B., page 112. (c). Finally the thickening of the lepto- and pachymeninges and of the temporal muscle and other soft tissues, coincident with some oedema of the brain, which occur after operation, would exert considerable pressure on the brain exposed at the operation site and perhaps compress the pial vessels. This would of course be more likely to occur at the site of a decompression than under a bone flap. In the experiments, although triangular areas of gliosis were found underlying bone flaps, the increase in number and hypertrophy of astrocytes was less than found in most of the lesions at the site of a decompression. However this finding of a difference in the degree of gliosis following the two types of operation is based on too small a series of animals to be certain that it always occurs.

The belief that the triangular zones of gliosis, found in brains previously exposed to air under various conditions at operation, are due to relative ischaemia of the grey matter is supported by Evans and McEachern's (and others) hypothesis that a diminished blood supply will cause glial proliferation.

A point that deserves attention here, before the final stage of this discussion is reached, is the possibility that some of the lesions described as being due to electrical constriction of pial vessels might be caused by the same factors responsible for the lesions found in brains merely exposed

at operation. The lesions in these two series of experiments were essentially the same and in both the brain was exposed to air. The answer is that the lesions found in brains following electrical stimulation were probably in part due to factors resulting from merely exposing the brain. However it was shown that the severity of the lesion could be graded by the intensity and duration of the electrical stimulation, i.e. the degree and duration of the ischaemia. Also in brain P.2151 where the exposure of a portion of each hemisphere was for less than one hour, a typical area of gliosis was found in the hemisphere stimulated electrically and none was present in the opposite non-stimulated one.

The lesions described in this thesis whether due to ischaemia produced by electrical or mechanical stimulation of pial vessels, or resulting from merely exposing the brain to air under different conditions, closely resemble certain of the epileptogenic lesions of the brain, (brain scars-microgyri), described by Penfield and Humphreys in 1939. Their cause, as in certain lesions found by Penfield and Humphreys would seem to have been due to relative ischaemia of the grey matter. The lesions reported in this thesis, however, represent the initial insult whereas those comprising an epileptogenic focus show evidence of progressive destruction of cerebral tissue which Penfield and Humphreys point out might be due to repeated

episodes of ischaemia from vasospasm.

If the cause of the lesions found in brains following their exposure to air at operation are due to ischaemia produced in the manner described above then one should look for similar lesions in the cortex underlying areas of meningitis and probably beneath tumors and subdural haematomas. In other words lesions like those described in this thesis should be looked for in human brains where there has been an inflamatory reaction in the meninges (as occurs after operation), and where there are adhesions, thickening in the meninges or other lesions that might cause compression of the pial blood vessels.

## FINAL SUMMARY AND CONCLUSIONS.

### PART I.

1. The effect of mechanical and electrical stimulation on the pial vessels of thirty cats has been studied.

2. The pial vessels in animals contract vigourously on mechanical or electrical stimulation.

3. The dog's pial vessels are not as sensitive to this type of stimulation as the cat's and the monkey's are less so than the dog's.

4. Such evidence as there is, suggests that these spasms are not dependent upon a neurovascular mechanism. They resemble closely the local spasms in peripheral vessels described by Thomas Lewis and others as being muscular and not neuro-muscular. They are localized to the site of stimulation and are not propagated as in the neurovascular reactions described by Chase and Ricker in the mesenteric vessels of rabbits.

5. In the experiments mechanical stretch of the vessel wall appeared to be an adequate stimulus to produce vasoconstriction. It is therefore suggested that the tone of a pial vessel, at least in the cat, might be partly maintained by a purely local mechanism, i.e. intra arterial pressure (or stretch). This hypothesis might in part explain the fact that pial vessels constrict with a rise in blood pressure and dilate with a fall.

6. Diminished blood flow in the cortex has been demonstrated as a result of these vasoconstrictions by thermo electric blood flow recording. Areas of focal cerebral ischaemia have been shown to be present, following constriction of pial vessels by electrical or mechanical stimulation, by intravital staining. It has been demonstrated that these areas of focal ischaemia are frequently in the form of a triangle corresponding to the anatomical distribution of the cortical vessels.

7. No clinical evidence was found that temporary focal cerebral ischaemia from vasospasm in the temporal, parietal or occipital cortex of animals recovered from ether gave rise to any epileptiform neuronal discharge. That subclinical neuronal discharges occurred as the result of ischaemia could not be ruled out with the methods employed.

8. The fact that electrical stimulation causes focal cerebral ischaemia through spasm of vessels should be taken into consideration in certain physiological problems where the effect of electrical stimulation on nerve cells is being studied.

9. A theoretical discussion of the findings as regards their application to human vessels is presented, especially in relation to their possible significance in electrical stimulation of the brain at operation, in electric shock, and in head trauma.

## FINAL SUMMARY AND CONCLUSIONS.

### PART II.

10. An attempt has been made to determine if the brain or meninges are injured by exposure at operation and whether electrical stimulation (from a thyratron) has any harmful effect upon them. Experiments were performed on eighteen cats, one dog and one monkey.

11. Adhesions between lepto- and pachy-meninges occur in those animals whose brains are exposed at operation for more than one hour, regardless of whether the brain is protected with a glass covering or irrigated continuously with Ringer's solution at body temperature. Adhesions appear as early as the third day postoperatively.

12. The adhesions are not due to drying or changes in temperature of the tissues although these factors may influence the extent of the adhesions.

13. Adhesions do not occur under a dura that is opened and closed again immediately - except in the region of the silk sutures in the dura.

14. A cellular, inflamatory reaction occurs in the lepto-meninges if the brain is exposed to room air for two hours. Irrigation of the brain or protecting it from dry air with a covering does not prevent this reaction. Electrical stimulation increases it. The inflamatory cells largely

disappear from the lepto-meninges by eight days after operation.

15. If the pial vessels in a cat's brain are constricted electrically or mechanically, patches of underlying cortex are rendered ischaemic. These patches are frequently triangular in shape corresponding to the anatomical arrangement of the perforating vessels, (as shown in Part I. of the thesis). Following this type of stimulation, triangular areas of gliosis occur in cat's brains, which correspond exactly in anatomical distribution to the ischaemic patches mentioned.

16. Such areas of gliosis can be varied from ones of patchy nature to small or large triangular areas of marked pathological change, (in nerve cells as well as in neuroglia) by varying the intensity and duration of the electrical stimulation, i.e. the degree and duration of the cortical ischaemia. The gliosis is therefore due, for the most part at least, to ischaemia of the grey matter, (otherwise it would not be triangular in distribution).

17. No definite evidence has been discovered in these particular experiments that electricity in itself, (without coincident ischaemia) produces any histological change in the cells of the central nervous system.

18. Areas of gliosis occur in the brains of dogs and monkeys after electrical stimulation but tend to be more

scattered, patchy, perivascular and do not make up such complete triangular zones as those described in cats. The explanation for this appears to be that the pial vessels in cats constrict more readily on electrical stimulation than do those in dogs and monkeys and the resultant cortical ischaemia would therefore be less in these animals.

19. Triangular areas of gliosis occur in cat brains that have been exposed to air for two hours. The lesions are not prevented by protecting the brain from dry air with a glass covering, or by irrigating it continuously with Ringer's solution at body temperature.

20. These triangular areas of gliosis are similar in nature and anatomical distribution to the lesions described as resulting from focal cerebral ischaemia produced by electrical or mechanical stimulation of pial blood vessels. It is believed that they also result from a relative ischaemia of the grey matter.

21. It appears that the lesions described might be caused by compression of the pial blood vessels. The inflamatory exudate and adhesions that were shown to follow operative exposure of the brain could possibly do this.

22. It is probable that similar lesions to those found in animals might occur in humans where the pial blood flow is interfered with. This might be the case in the brain under, (a) an inflamatory reaction in the meninges, (b) meningeal

adhesions, (c) an expanding extracerebral mass.

23 The similarity between certain epileptogenic lesions in human brains and those produced experimentally in animals (in the present investigation), is pointed out. Relative ischaemia would appear to have been the cause of a number of the initial lesions in these brains, as well as in animals, and a mechanism is suggested whereby this might be brought about in some cases.

24. The possibility that similar lesions, (to those described in animals), might follow craniotomy on humans and especially in those cases where electrical exploration of the cortex was carried out, is discussed.

25. It is shown experimentally, (as suggested by Evans and McEachern and others), that a relative ischaemia of the brain will produce a reactive hypertrophy and increase in number of the astrocytes in the affected area.

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