A NEW METHOD OF STUDY OF THE BRAIN CAPILLARIES AND ITS APPLICATION TO THE REGIONAL LOCALISATION OF MENTAL DISORDER

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The natural state of the capillaries in the cadaver or experimental animal cannot be demonstrated by the usual methods of injection of coloured substances, since it is quite impossible to avoid or make allowances for artefacts consequent upon the necessary pressure employed. The vessels may be either unduly distended giving a wrong appearance of congestion, or owing to a minute particle or clot remain empty thus simulating ischaemia.

After some years of experimenting, a method of staining the contents of the blood vessels applicable to the demonstration of capillaries in histological brain section has been developed, which enables us to see exactly what is required, namely, states of anaemia or congestion in various regions of the brain. The field opened by this method is a large one, not only in mental research, but also in its applications in physiology, anatomy, and pharmacology. The research has its limitations, since death itself intervenes between the physiologically active condition and post-mortem examination; and since those pathological processes leading up to the death of the patient must necessarily affect brain tissue, it is not easy to judge the value of abnormalities even when found. It is pointed out, however, that such apparent difficulties may, when investigated by the following method, yield information and understanding invaluable to medical science. The finding of local irregularities of the vascular supply as compared with the remarkable evenness of the normal is a valid reason for attaching importance to such conditions; and associated tissue changes of cerebral softening, residua of blood pigment, etc., are useful in estimating the probable duration of existence of the lesion. The method may be used for comparing the vascularities of all the body viscera under normal and pathological conditions.

The method consists of the histological application of a blood stain. The stable blue-black pigment, resulting from the reaction between the haemoglobin of the corpuscles and the benzidine-nitroprusside mixture under the influence of a weak oxidising agent, is quite insoluble in organic fluids, and thus sections may be cleared in alcohol and xylol prior to mounting in balsam or oil. The vessels can be most conveniently studied in thick frozen sections—250 μ—using a binocular microscope of the Greenough pattern with objectives of 48 mm. Thinner sections stain equally well and the times of staining are les-

sened, but the stereoscopic effect seen in the thicker sections is of superior value in the study of brain tissue; highly vascular tissues from other viscera require much thinner sections for convenient study (e.g. 50 μ for kidney tissue). The quantities of the reagents have been chosen carefully, over one thousand experiments with varying quantities, etc., having been first carried out so as to allow a moderate amount of latitude in excess or deficit, thus reducing the personal factor to a minimum.

The initial preparation of the material is of greatest importance. Handling of the tissues must be done with extreme care to avoid pressure effects or redistribution of the blood whilst it is yet fluid within the vessels. Fortunately, owing to the minute size of the capillaries, applied mechanical forces must be quite considerable to disturb the relationships. Animal brains can be fixed *in situ* with the top of the cranial vault and a thin slice of the cerebrum removed, to allow penetration of the fixative. In the human cadaver the skull is best cut as usual with a horizontal saw-cut 1 in. above the auditory meatus and 1 in. above the eyebrow, then before disturbing the vault, cutting through all tissues and brain with a fine hack-saw blade. The part of the brain thus removed with the vault can be fixed *in situ*, and the remainder of the brain also fixed *in situ* by attaching a rubber band, i.e. a 4 in. section of a 6 in. motor-car inner tube—to form a basin which can be filled with fixative.

Fixation in hot solutions or alcohol produces a remarkable contraction of the membranes of the brain so that the blood may be squeezed out of the inner unfixed regions. The mechanical pressure thus effected is enormous, so that artificial cooling during fixation is an advantage.

The only satisfactory fixative is formalin hypertonic saline (approx. 10 per cent. of formalin and 2 per cent. salt). Fixation for short periods in Muller, or bichromate and formalin made hypertonic with salt, has given satisfactory results, but not preferable to the above. Many other fixatives have been tried all of which have proved unsatisfactory, especially those containing acid or alcohol. Salt solutions or sodium sulphite have no deleterious effect, but sulphates must be carefully washed away since benzidine forms an insoluble sulphate.

METHOD AND REAGENTS REQUIRED

The brain should be fixed *in situ* for 24 hours if practicable, then cut into slices of not more than 1 cm. thick and again placed in the fixative.

(1) Fix slices in formol hypertonic saline. 2 days.

(2) Wash well in water. Several hours.

(3) Soak slices in "gum phenol" for 2 days, and cut sections at 250 μ with freezing microtome.

(4) Wash sections in running water for 2 hours to remove gum and formalin.

(5) Place sections in shaking machine at 37° C. with constant gentle shaking for 1 hour in the sodium nitroprusside benzidine mixture.

(6) Rinse quickly in water.

(7) Place in weak hydrogen peroxide at 37° C. with constant gentle shaking for 1 hour.

(8) Wash in water. Dehydrate in 90 per cent. and absolute alcohol. Clear in xylol and mount in canada balsam.

A suitable apparatus for gentle shaking is the electric revolving table, as used for shop-window display, set at an angle of about 12° with the horizontal, and revolving 1½ times a minute; a table 16 in. diameter will accommodate a dozen Petri dishes in which the sections can be washed and stained. The whole apparatus is placed in a dark box or cupboard with a red electric lamp or hot water bottle to keep the temperature 35–40° C.

Formalin hypertonic saline

Formalin, commercial, 40 per cent.	100 c.c.
Salt	20 gm.
Water	1000 c.c.

Gum phenol solution

Gum arabic, coarsely powdered	500 gm.
Water	2000 c.c.
Phenol	20 gm.

Warm to dissolve; filter through wool and then by pressure through a Seitz bacterial filter.

Sodium nitroprusside benzidine mixture

Sodium nitroprusside	0·1 gm.
0·5 per cent. benzidine in 2 per cent. acetic acid	25·0 c.c.
Distilled water	75·0 c.c.

Dissolve the sodium nitroprusside in 20 c.c. water and add the benzidine. Make up to the full amount and filter.

Solutions must be prepared immediately before use; they must not be used after 24 hours, and exposure to strong light must be avoided.

Hydrogen peroxide solution

Hydrogen peroxide (20 vol.)	2 c.c.
Distilled water	400 c.c.

The cleared section should be transparent and practically colourless except for the vessels, which should show as well-defined jet black threads. The experimenter is advised to stain sections of normal brain (e.g. cat) with each section required for study, as a control of the process, until confidence has been attained. Control animals must be perfectly healthy, as even minor ill health is accompanied by local abnormalities of the cerebral blood supply. The permanence of the stain about equals that of other aniline dyestuffs when suitably mounted in acid-free balsam. Some sections over six months old show the vessels equal to the original staining.

Figs. 1 and 2 indicate the usual architecture of the vessels in the cerebral and cerebellar cortex respectively, both show some slight but definite congestion, and minute microscopic haemorrhages can be seen in areas near the

centre of the second figure. *Unevenness* is the foremost abnormality seen in brains from mental patients and occurs both in relation to the size and shape of individual vessels and to the local cortical or subcortical blood supply, these are well shown in fig. 3. *Congestion* is often seen in local cortical areas as in fig. 4, or it may be confined to the deeper layers as in fig. 5. (Loss of brain cortex may imitate congestion by the apparent increase in vessels consequent upon collapse of the brain parenchyma, this is illustrated by fig. 6.) Congestion occurs also in the neighbourhood of focal haemorrhages in the white matter as in fig. 7, and adjacent to microscopic areas of meningitis, it is of course synonymous with encephalitis. *Ischaemia* is found: (*a*) as circumscribed loss of a single vessel of the cortex (fig. 8), or of the white matter (fig. 9); (*b*) as widespread areas, diffuse or involving well demarcated focal lesions of the white matter (fig. 10), indicating the earliest stage of cerebral softening; (*c*) it also frequently involves limited areas, or the separate layers of the cortex, most usually the outer third as in fig. 11. *Microscopic capillary haemorrhages* varying from pin-point to pin-head in size are quite commonly found either single or multiple, their shape being grapelike or plane depending upon the disposition of the neighbouring nerve fibres. Fig. 12 shows haemorrhages near the lateral ventricle which are distinguishable from very recent haemorrhages by their diffuse outline; other abnormalities seen are, (*a*) thrombosis of vessels, (*b*) vessels bristling with diapedesis of corpuscles, (*c*) empty vessels, (*d*) perivascular cuffing, spaces and debris, (*e*) scars of vessels and brain membrane, (*f*) loss of brain parenchyma, (*g*) new vessels in granulation tissue, and (*h*) the method also indicates minute cerebrospinal fluid cysts occasionally found in the outer layers of the cortex.

APPLICATION TO THE STUDY OF MENTAL DISORDER

Although over a thousand sections of brains of mental patients have been examined, the work must be considered as still in its preliminary stage. From the anatomical point of view, however, interesting results have already been obtained. Although it is stated that the cerebrum is one of the most vascular of organs (14) we have found that the vascularity of the brain is far less than for instance that of the kidney (about one-sixteenth), which is surprising in view of the vascularity of the brain membranes but understandable since a large proportion of the brain substance consists of organic soluble "electric insulating" material. The preponderance of vascular abnormalities is in the cerebral cortex; often the outer half of the cortex of the whole brain has deficient blood supply amounting in many cases to almost complete anaemia (see fig. 11). In many cases it is the subcortical part of the brain which is almost devoid of normal staining haemoglobin, merely the shell of the cortex showing the vessel stain, i.e. early stage of general softening of the brain. These and the smaller ischaemic areas indicate neighbouring brain paralysis (15); congestion may be associated with increased irritability of brain tissue. Loss of brain substance follows continued ischaemia which becomes evident as a reduction of thickness

of the cortex (fig. 6), or as changes in pattern of the architecture; owing to the plastic nature of brain substance the volume of any given focal lesion becomes greatly reduced in course of time. Quite commonly we have found softenings of the brain (fig. 10), unsuspected during life, which vary in size from microscopic areas to an inch or more in length. These occur in any part of the white matter of the brain and its stem. Capillary haemorrhages are commonly seen, occurring in great numbers in the basal ganglia of the few cases we have examined which during life showed choreaiform symptoms. Abnormalities of the tissues adjacent to the lateral and third ventricles are not uncommonly present (see fig. 12), and often thick-walled tortuous vessels in the basal ganglia are the seat of pathological changes sometimes accompanied by masses of yellow brown granules.

Further statements with regard to findings might be misleading in view of the hitherto insufficient control of manipulation prior to fixation, which has of necessity occurred in the removal and sectioning of certain of the brains during routine post-mortems.

DISCUSSION OF THE REGIONAL LOCALISATION OF MENTAL DISORDER

In assisting others wishing to make a further study of the problem the following may be of practical interest:

(1) Gosline(1) has conveniently summarised previous work upon the localisation of the pathological changes in dementia praecox; his summary is given verbatim:

> Only two writers have attempted any definite correlations between disease of cell layers and dementia praecox, or disease of brain areas and dementia praecox. Cotton, as admitted by Alzheimer, was the first to correlate disease of the second and third cortical layers with dementia praecox, mentioning at the same time that the fat occurs over the entire cortex. Southard was the first to claim a relation of disease of the post rolandic areas and dementia praecox of the katatonic form, and between disease of the frontal areas and the paranoid form. Later, Southard and his co-workers have shown some connection between lesions of the cingular gyrus and katatonia, gliosis of the thalamus and dementia praecox, and cerebellar hemiatrophy and katatonia. Other writers have laid emphasis in their cases on a predominance of the pathological findings in one layer or another, or in one brain area as against another, but no one has made any special claims for these observations before the writers mentioned above. Thus Alzheimer mentioned changes in the deeper layers at first, later pointing out that they occurred in the more superficial layers to a greater extent. Kleppel and L'Hermitte found the changes more in the zones of association, in the larger pyramids of the third layer of cells and in the fusiform cells of the sixth layer of Hammarberg. Dunton, in a case of katatonic dementia praecox, found chromatolysis most marked in the fifth layer of Hammarberg. Lubouchine mentions the molecular layer and the layer of the small pyramids; Lannois and Paviot mention the purkinje cells of the cerebellum; Kollac mentions increase of the glia in the superficial layers and in the white matter; Elmiger mentions the free edge as being most affected; Orton finds the amoeboid changes more in the deeper layers and in the white matter; Sioli finds more change in the upper layer; Mott in the infra granule

layers. As for the fibres: Cramer mentions thinning of the interradiary and tangential systems; Weber, of the supraradiary network; Elisath Hall, Meynert's layers. All have support in the shape of corroborative work by other investigators. Dunton and Maschtschenko claim that the frontal lobes are more affected; Zalplachita, the frontal and central; Orton, that the temporal and occipital are less affected than other parts.

Marburg (2) has described cortical changes in manic depressive insanity. Benvenuti (3) has described the cerebellum as the psychical component of the brain. Other examples could be multiplied indefinitely.

(2) Mental functions are absolutely dependent upon the integrity of the supply of blood to the brain. According to Trotter (15) practically all symptoms of intracranial lesions can be explained in terms of circulatory disturbance. Simple physiological anaemia of the brain as with high altitudes or diminution of air pressure in a Haldane steel chamber is accompanied not only by disturbed consciousness and a marked diminution of acuity of vision and hearing, but also by emotional instability (7). About 15 min. is the limit of cerebral anaemia in animals which is followed by recovery; even then, convulsions, paralyses, dementia or death may follow at a later period.

(3) The brain does *not* "act as a whole" in mental processes for the simple reason that much of the brain mass has definitely known unconscious function (vital and righting reflexes, etc.). Owing to the extreme specialisation of brain tissue it would entail a prodigious waste of energy if all irrelevant neurones acted with every mental process.

(4) Mental disorder is of the nature of a *functional disturbance of physiologically intact mechanisms* within the brain since remissions may occur during which the individual is sane. Even mute and apathetic cases of dementia praecox of many years' duration have shown a short temporary resuscitation of their memory and powers of rational conversation by means of the methods of cerebral stimulation devised by Loevenhart (4). McCutcheon's case (5) maintained a mute vegetative existence for 16 years, and "couldn't have been more like a cabbage." He then had a fatal attack of pneumonia, coincident with which his mental clarity returned, and he asked for his friends, with whom he conversed in a perfectly rational and lucid manner.

(5) One must assume that by far the greater part of the cortex is only occasionally active; otherwise concentration and attention would not be possible. The mechanisms for these processes possibly consist in an active restriction of blood supply to unwanted cortical areas (anaemia = brain paralysis), and an increased blood supply and/or congestion (which causes heightened irritability of the neurones) to parts of the brain where active function is required. Thus mental processes are to some extent subordinate to sympathetic control. Dissolution of the integrity of the sympathetic mechanisms would, according to its extent, cause irrational thought.

(6) Intellectual and memory processes are dependent upon the development and activity of the cerebral cortex. The cerebral cortex is the most probable localisation of subconscious memory and of the higher reflexes of instinctive behaviour. There must be considered the possibility that the

cerebellum plays a large part in the mental processes of judgment, such being a higher function of the valuation of difficulties in reaching and procuring food, for which the most important association is the spatial relationship of the individual—a function of the 8th nerve by otolithic elaboration.

(7) From theoretical considerations, intelligence and memory defect are in the author's opinion *not* themselves really characteristic of certifiable mental disorder. To forget past experiences is a normal physiological process; conversely it is possible to demonstrate in a large proportion of mental hospital patients a degree of intelligence and memory which is in marked contrast to an irrational behaviour consequent upon their emotional state. Even in cases of confusional insanity or dementia, where the intellectual and memory defects constitute the main clinical picture, there is an accompanying underlying emotional perversion, which is of more importance from the research point of view and which distinguishes these cases from simple non-certifiable toxic confusion or senile childishness and forgetfulness. Although dementia frequently follows insanity this may be due to the same pathological processes as affect subcortical tissue, and thus there is at present no justification for the localisation in the cerebral cortex of anatomical changes upon which mental disorder solely depends. Mental disorder is not the simple converse of mental order, and active insanity is not a simple atavistic reversion to the behaviour of a *normal* animal. The following symptoms are more pertinent of insanity as such.

(8) Cases of certifiable mental disorder include the happiest as well as the most miserable of mankind, every sensory stimulus may cause pleasure, one inmate described the asylum as the most beautiful hotel imaginable. In other cases the mental anguish may be so intense that they prefer death to their intolerable existence, and often exhibit remarkable ingenuity and intelligence in their methods of attempts at suicide. Pathological irritation of the thalamus in such cases is to be inferred from our knowledge of this region of the brain as the "pleasure-pain" centre.

(9) Almost constantly associated with mental disease are states of fear, varying from mild unfounded apprehension to appalling terror; these often constitute the motive for conduct involving injury to themselves and others. The closely allied symptom of rage in animals has been shown by Bard and Cannon(6) to have its origin in the subthalamic region.

(10) Sufferers from mental disorder may be in a condition of such intense and prolonged shock that additional peripheral shock is scarcely perceived. Under such circumstances a patient may pull out an eye or avulse a testis with little if any shock reaction.

The usual response to systemic infection may be absent, there being for example no pain or cough in an attack of pneumonia. There can be little doubt but that the "head ganglion of the sympathetic" in the hypothalamic area is functionally disturbed in such cases, and perhaps most cases of active insanity.

(11) The association of mental disorder with metabolic and sexual dis-

turbances is well recognised. These disturbances may be correlated with pituitary hypothalamic dysfunction (see author's publications (8,9,10)).

(12) A frequent symptom in mental disorder, which can be distinguished from fear, is that of evil foreboding of the future with or without a feeling of unreality and occasionally a conviction of being about to die (also a common symptom in rabies), the whole suggesting vaso-vagal attacks. Dramatic cures of insanity are usually associated with violent disturbance of the "vital" centres by which the patient is brought nigh unto death; such are the massive asphyxia by CO_2 (Loevenhart); massive doses of hypnotic drugs; some fatal infections; removal of the colon (Cotton); and some attempts at suicide which are almost successful.

(13) The removal of the right hemisphere of the brain by Dandy (11) even effected a cure of incipient mental disorder. The patient, a clergyman, had of course a left-sided paralysis, but nevertheless retained as far as could be ascertained full possession of his mental capacity; he was able subsequently for two years to conduct services, etc. (There has not yet been a case of subtotal resection of the left hemisphere; but such would cause greater loss of mental faculty, language and intelligence being to some extent synonymous.) A decorticate dog may exhibit signs of mental defect but does not necessarily show the behaviour typical of madness.

In contrast to the above, abnormal behaviour and symptoms of mental disorder are commonly found in subcortical affections such as chorea, disseminated sclerosis and post-lethargic encephalitis. Russell Brain (12) quotes a case of mental disorder "like a decorticate cat"; and Devine (13) shows that dementia praecox is essentially not so much an intellectual as a vital centre failure.

The reader will be able to associate the above clinical symptoms with the disordered functions of the basal ganglia, the thalamus and the hypothalamus respectively. Which of these localities is the most important and the part played by the cerebellum requires further research by the method suggested; but enough has been said to indicate that, even though our present findings consist of a wealth of vascular abnormalities in the cerebral cortex, it is probable that less recognised subcortical affections are of primary importance in the causation of those clinical symptoms associated with certifiable mental disorder. The cortical lesions present may be a source of irritation of the subcortical centres just as lower brain-stem affections (especially of the fifth nerve) can be.

In the study of insanity, medical science is not concerned with the existence or function of a vital principle or "soul" using a material brain for its expression; its main concern is with unhealthy mechanisms of the nervous system which lead to disordered conduct. The customary idea of a soul (presumed rudimentary or absent in animals) which controls human conduct has wrongly limited our conception of insanity to human beings, with the result that mental research has been almost exclusively directed to histological changes in the highly developed human cerebral cortex. There is therefore urgent need of

research into the existence and causes of functional derangements of subcortical and vital centres in cases of active mental disease by methods not altogether dependent upon the personal factor. If we could establish by histological methods, such as put forward in this paper, that pathological changes capable of disturbing the function of the nerve cells or tracts occurred in certain subcortical areas in cases of active mental disorder, it would materially assist our conception of insanity as part of general medicine, and enable clinical pictures and medico-legal interpretations as to sanity to be more scientifically controlled than at present.

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EXPLANATION OF PLATES I–VI

(All untouched photographs; magnification × 15.)

PLATE I

Fig. 1. Normal architecture of capillaries of cerebral cortex. There is some congestion. Parietal region of a man aged 53.

Fig. 2. Normal architecture of the cerebellar capillaries. There is some congestion, and minute haemorrhages are to be seen at the surface near the centre of the figure. Female aged 28.

PLATE II

Fig. 3. Irregularity of vessels and blood supply of local areas of cortex. Frontal region of an idiot aged 26.

Fig. 4. Marked congestion of the capillaries of the cortex of the frontal region. Female aged 31, case of dementia praecox.

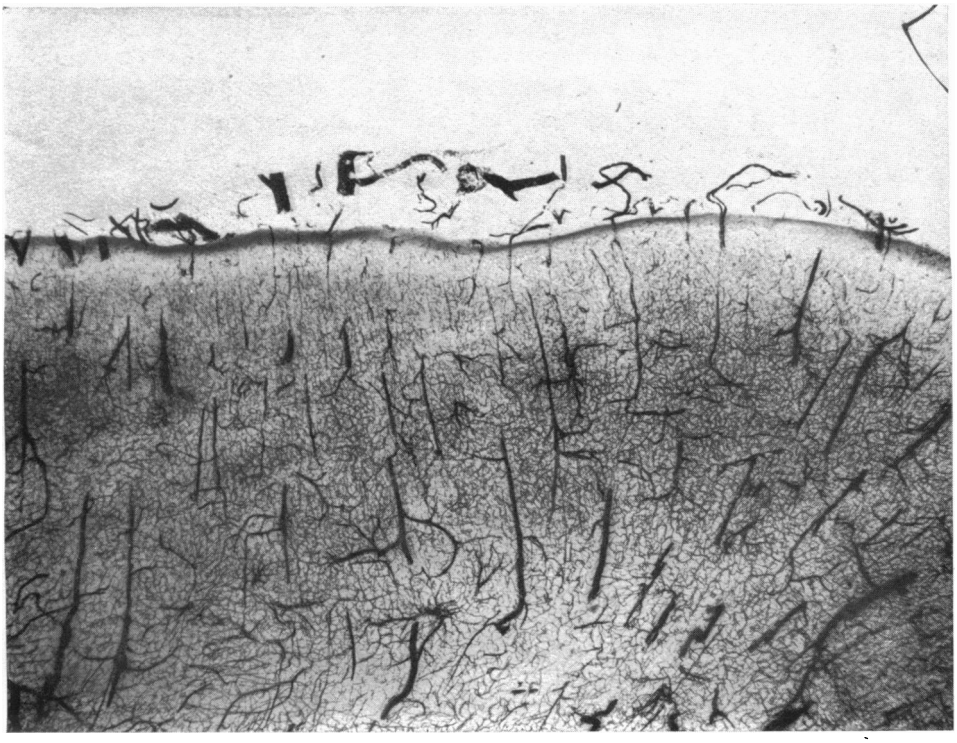


Fig. 1

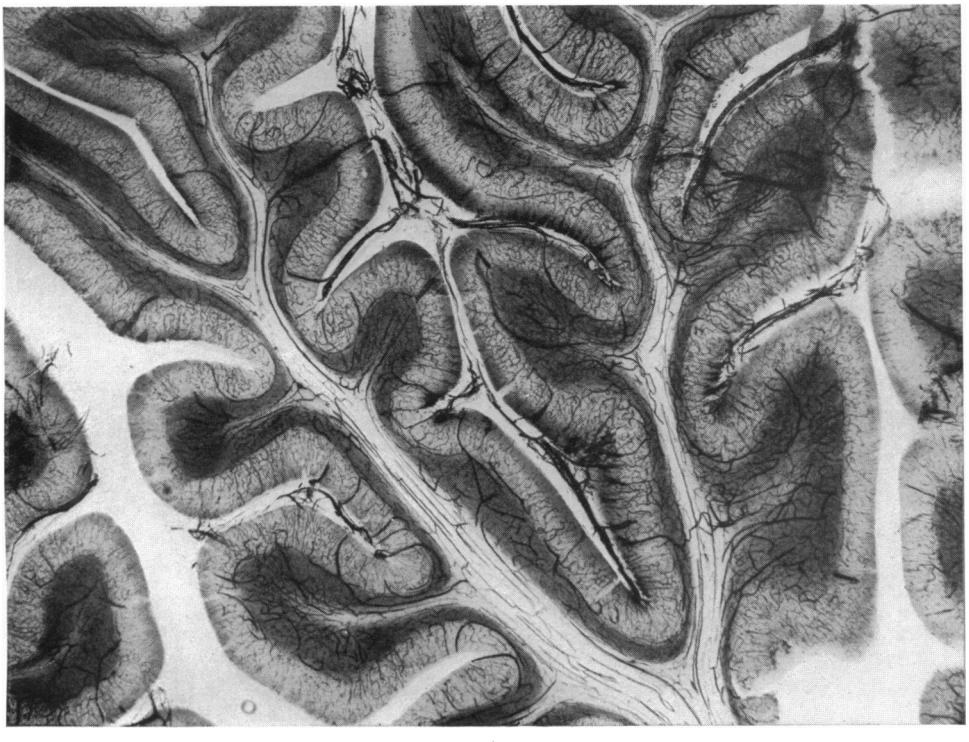
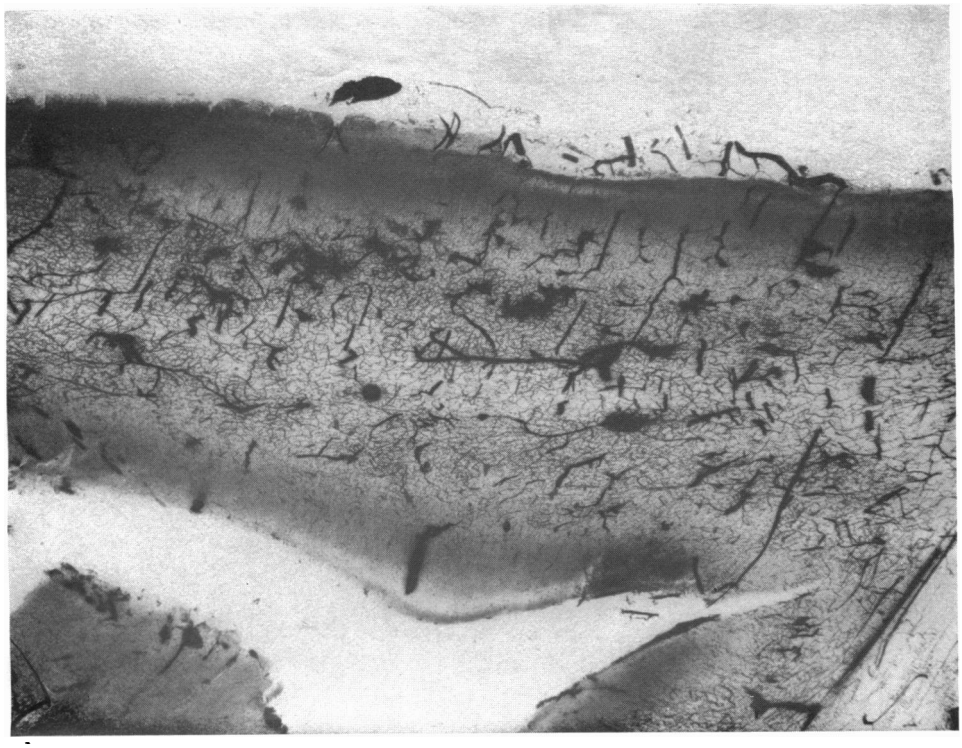


Fig. 2



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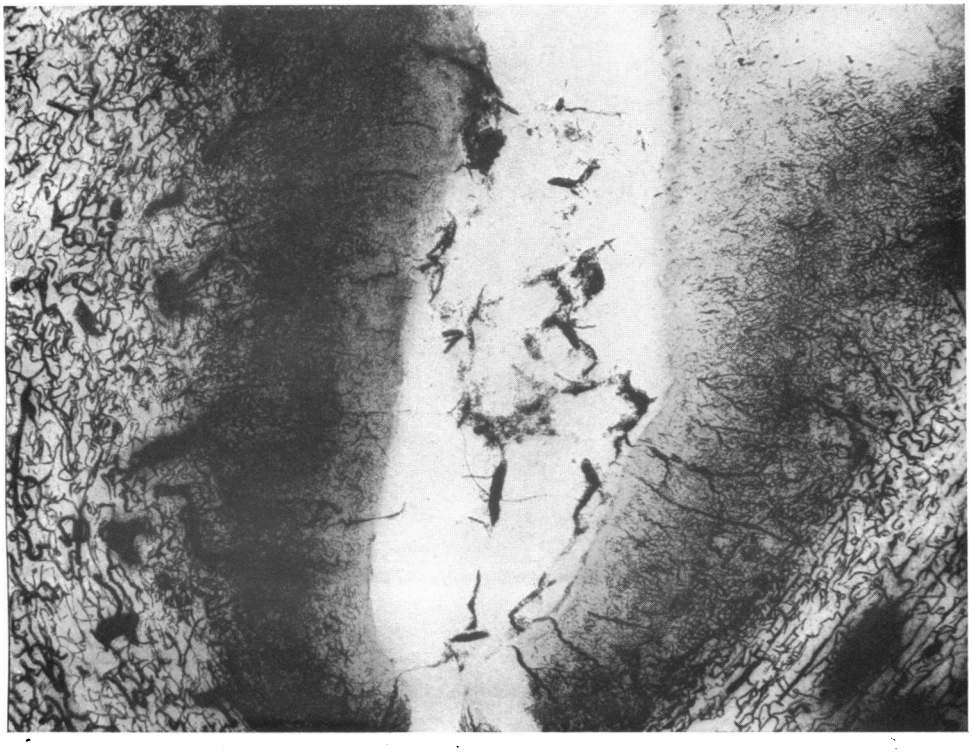
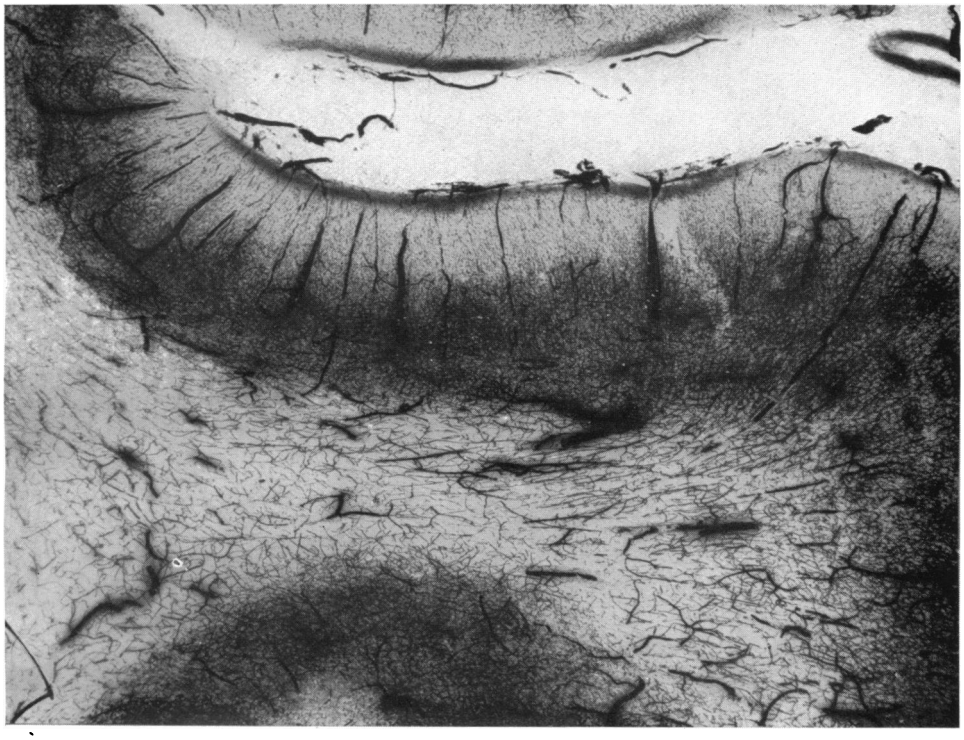


Fig. 4



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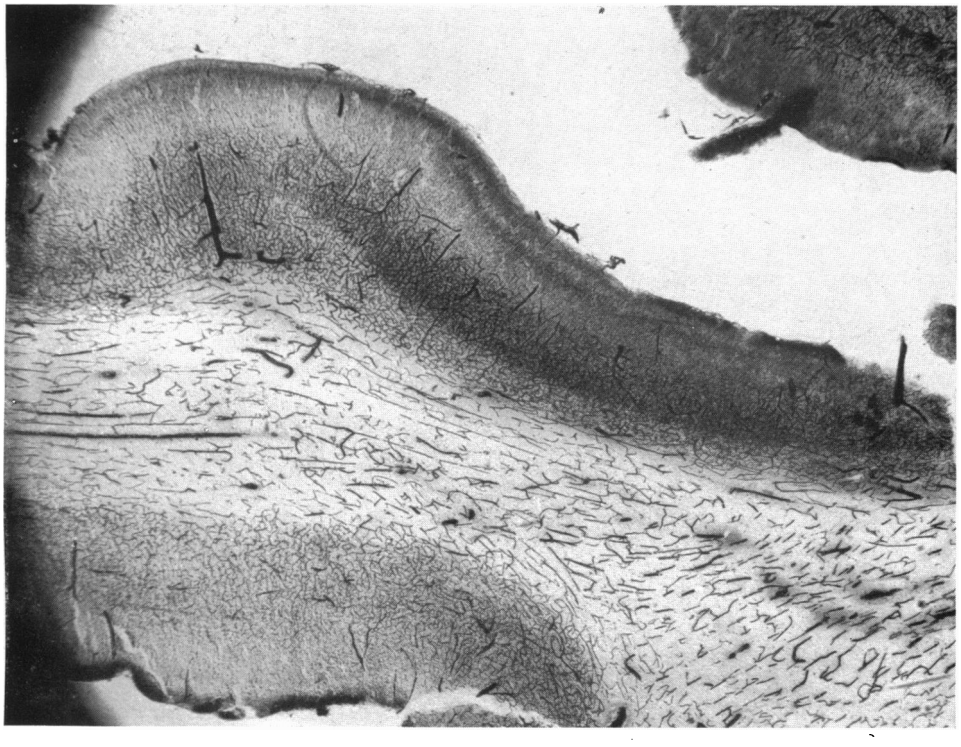

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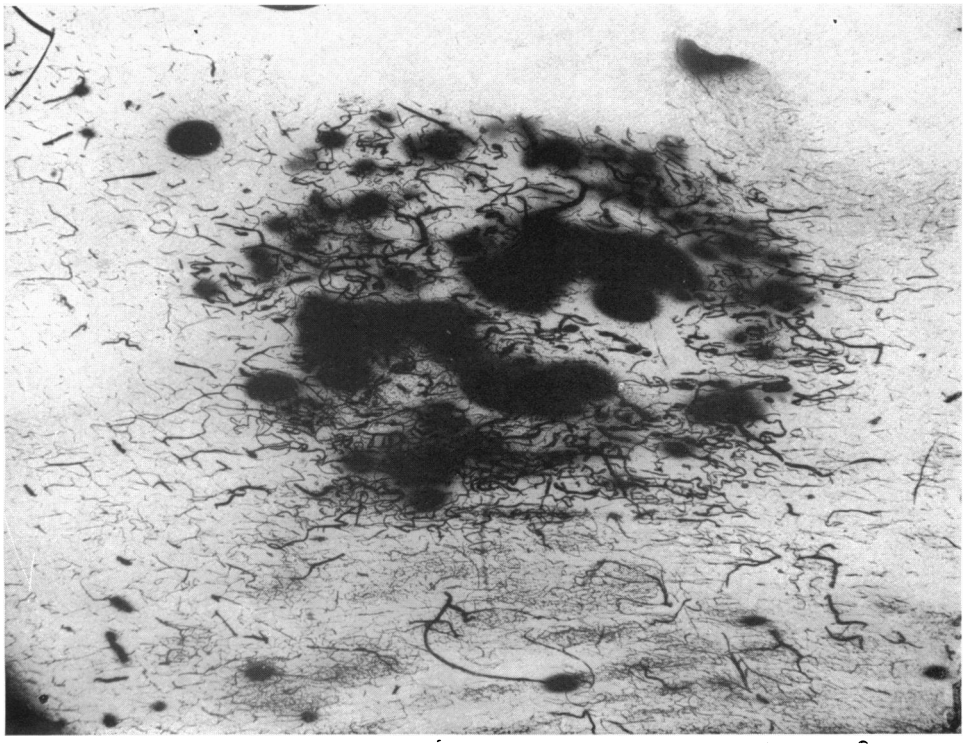

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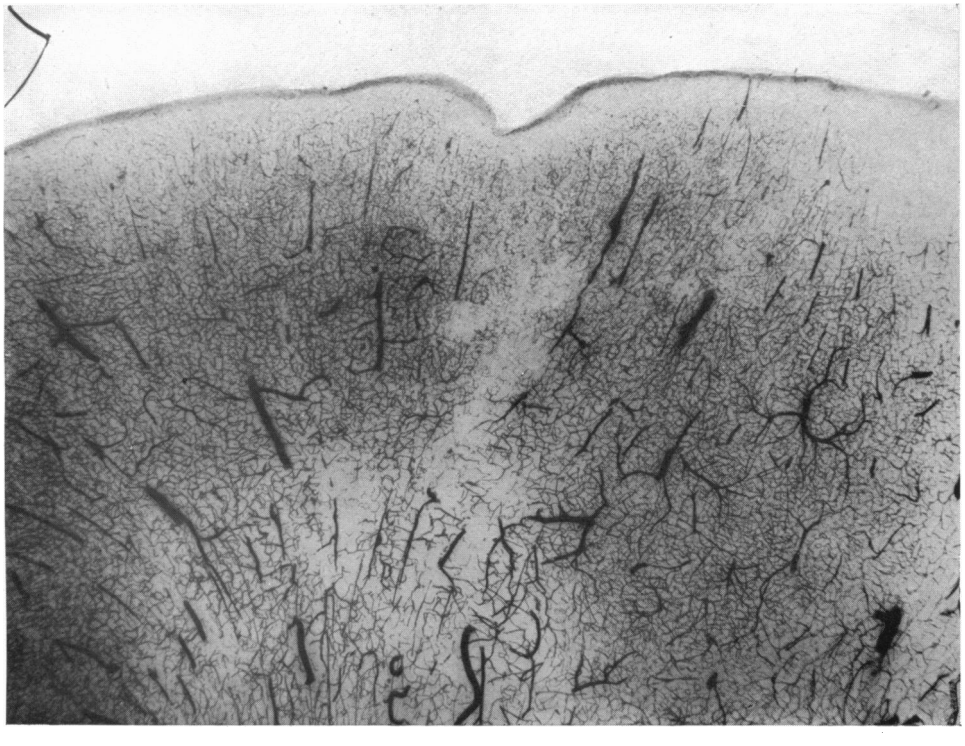

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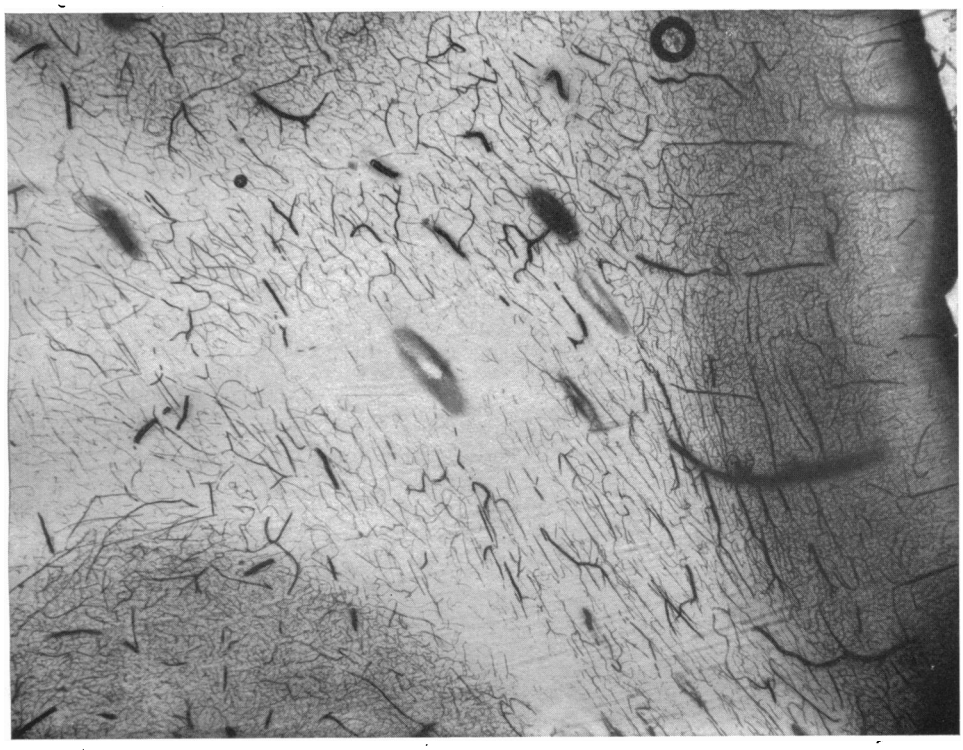

Fig. 9

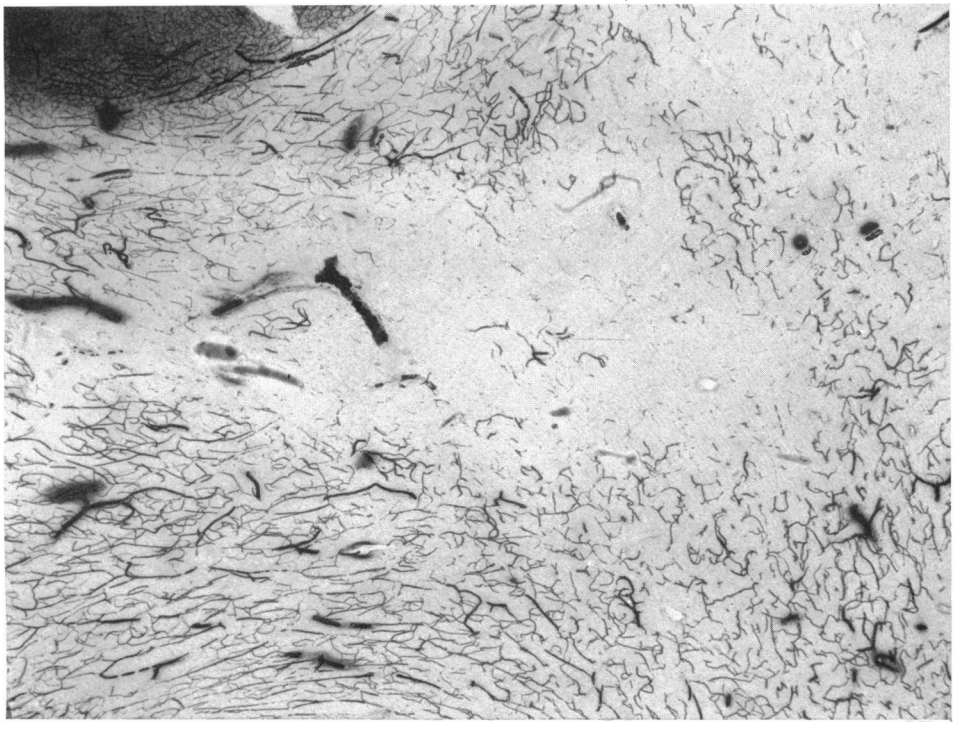

Fig. 10

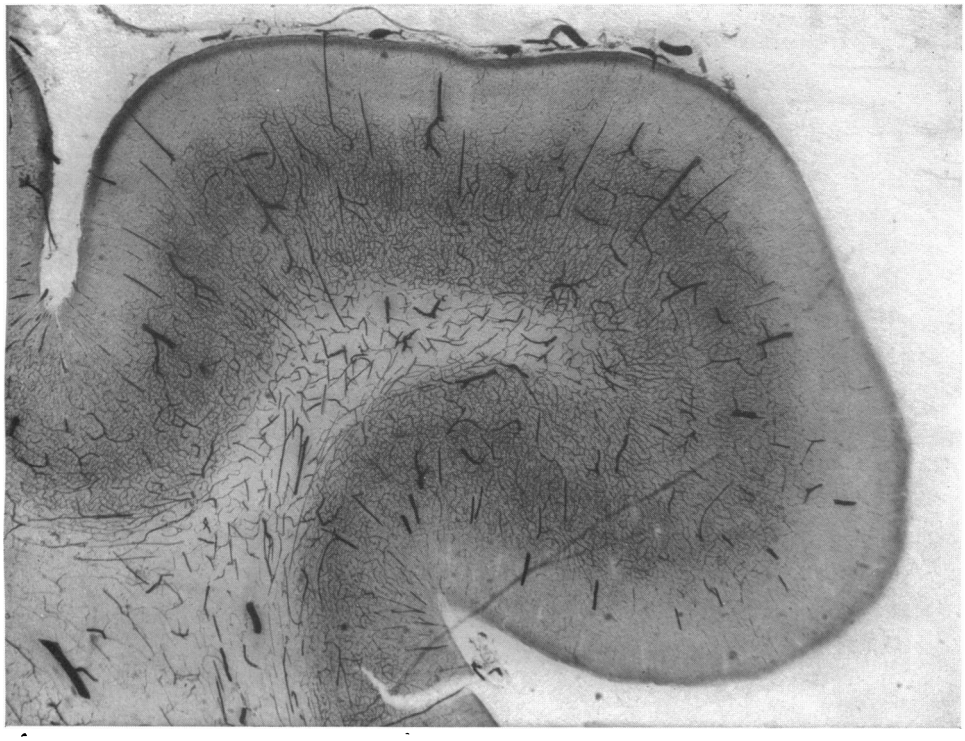

Fig. 11

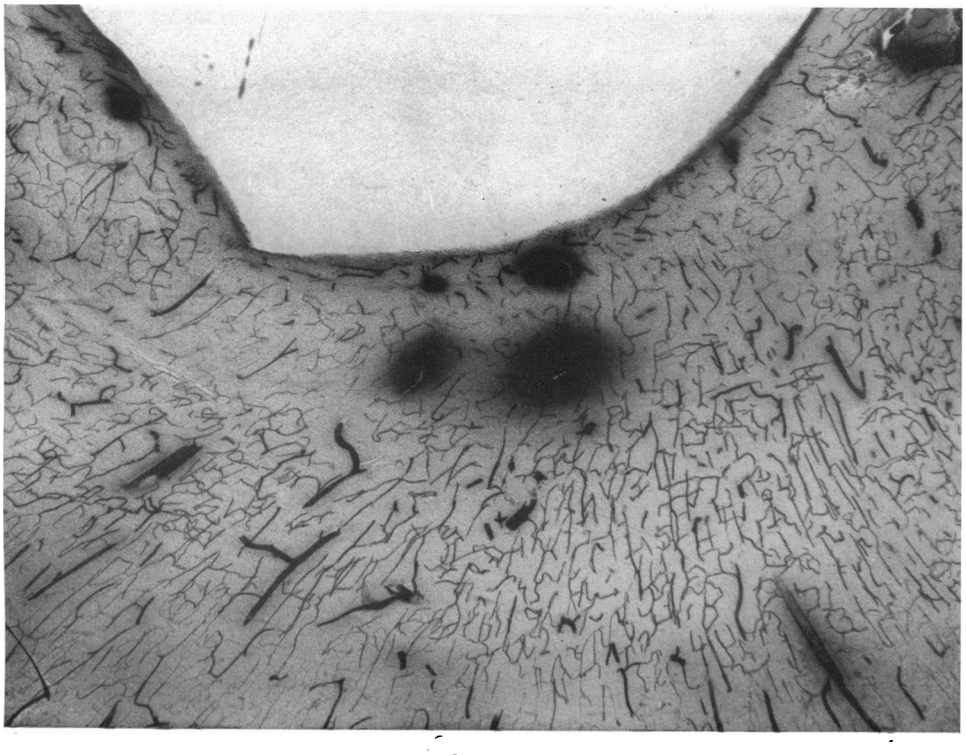

Fig. 12

PLATE III

Fig. 5. Congestion of capillaries limited to the deeper layers of the cortex. Island of Reil, man aged 71, acute confusion.

Fig. 6. Spurious appearance of congestion due to approximation of capillaries following loss of cortical brain substance. Island of Reil, man aged 19, recent mania.

PLATE IV

Fig. 7. Area of congestion near a small haemorrhage in the white matter. Man aged 67, arteriopathic dementia.

Fig. 8. Anaemic focal area extending from surface throughout cortex. This is seen from inspection to be due to loss of a single vessel. Parietal region of a man aged 53, insanity with epilepsy.

PLATE V

Fig. 9. Loss of vessel in white matter with consequent adjacent area of ischaemia.

Fig. 10. Small focal area of ischaemia in the white matter—the earliest stage of cerebral softening. Parietal region, man aged 19, recent mania.

PLATE VI

Fig. 11. Almost complete absence of blood supply of the outer third of the cortex with normal even regular blood supply in the deeper layers. Occipital region of a man aged 19, recent mania.

Fig. 12. Resolving haemorrhages near the lateral ventricle. (Recent minute haemorrhages have a sharp outline and are without surrounding haziness or granules.) Man aged 53, insanity with epilepsy.