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PERMEABILITY OF CEREBRAL BLOOD VESSELS TO PROTEIN MOLECULES IN CONVULSIVE SEIZURES

by

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INTRODUCTION

Histological examination of the brains of experimental animals subjected to convulsive seizures shows an evidence of tissue damage which could be the result, to some extent, of increased vascular permeability. Diapedesis of red cells, edema and nerve cell damage in acute stages, and perivascular hematogenous pigment, nerve cell deficit and gliosis around the blood vessels in chronic conditions are examples of such changes.

More direct evidence was obtained in experiments in which trypan blue was used to test the permeability of the blood-brain barrier (BBB) in various pathological conditions - among others, in the electrically-induced epileptic seizures. Broman, a pioneer in this field of research, has shown that epileptic seizures produce change in the BBB, resulting in a blue stain of the nervous tissue. In his work - and in that of numerous other investigators - substances used to test the permeability of the BBB were of small molecular size. Therefore, it was thought that new information might be obtained by using a substance of large molecular size and it was decided to try radioactive iodinated bovine albumin. It was felt, also, that any light thrown on properties of the BBB in relation to molecules of proteins might be of value for better understanding of such disorders, which may be related to antigen-antibody reaction.

The second problem which I wanted to investigate was the mechanism of cerebral lesions resulting from status epilepticus and leading to a clinical condition known as

infantile hemiplegia. It was known that cortical lesions in this disease are particularly severe in the depth of the sulci. We postulated that lesions resulting from the status epilepticus are due to tissue damage associated with increased vascular permeability. We expected, therefore, to learn more about these problems by studying changes in permeability related to a series of epileptic seizures, which would imitate human status epilepticus.

SURVEY OF THE LITERATURE

The central nervous system is separated from the rest of the body biochemically as well as anatomically. Anatomical insulation is formed by the meninges and neurilemma. The concept of a biochemical mechanism resulted from the observations on the stainability of the CNS by semicolloidal acid dyes. In 1886 Ehrlich (quoted by Bakay) first applied the use of dyes for the study of the central nervous system. He demonstrated that basic dyes such as methylene blue could be used as vital stain to reveal the morphology of nerve cells and their branches. He also observed that, contrary to the behavior of basic dyes, the majority of acid dyes did not appear in the CNS when injected subcutaneously. Ehrlich ascribed the ability of the basic dyes to stain the CNS as due to their lipoidal solubility. The existence of the effective physiological barrier protecting the CNS was first shown experimentally by Lewandowsky in 1900, when he discovered that, although a small amount of sodium ferrocyanide introduced into the CSF would be lethal to a test animal, doses 200 times larger could be well tolerated when injected intravenously.

A classical experiment was carried out by Goldmann in 1913 with the acid semicolloidal dye, trypan blue. Following the intravenous injection of trypan blue solution, the dye colored all the body tissues with the exception of the CNS (Experiment 1). On the other hand, the brain became stained if the dye was injected into the subarachnoid space.

This was followed by severe convulsions and paralysis of the animal (Experiment 2).

Enormous literature on the mechanism which has been called the blood-brain barrier (BBB) has accumulated in the past three decades; however, the exact mode of action of this barrier is not yet satisfactorily elucidated. Spatz (1934) hypothesized that the anatomical localization of the BBB is to be looked for in the endothelium of the CNS capillaries which behaves as a selective membrane. King (1939) proposed a hypothesis of chemical affinity. He found that trypan blue in sufficiently high blood concentration did stain certain areas of the CNS - the choroid plexus, pituitary, tuber cinereum, area postrema, paraphysis and pineal body - which are largely composed of connective tissue and possess the adequate "binding power" comparable to that of other non-nervous organs. King explained that the peripheral nervous system which contains an abundant connective tissue stroma stains much more readily than the CNS. So does inflamed or necrotic tissue, due to the change in the intrinsic factors, i.e. an altered affinity. The stainability of the neonatal brain is indicative of a difference in affinity for trypan blue on the part of juvenile brain tissue.

In 1942 Friedemann arrived at the conclusion that positive charge or absence of electrical charge allows dyes or toxins to pass the cerebral capillaries, which behave as a selective membrane at the normal pH of the blood. The

same would apply to drugs, viruses, and antibodies. Neo-arssphenamine, Bayer 205 and potassium ferrocyanide, which are electro-negative, do not pass the blood-brain barrier while alkaloids, which do act on the CNS, easily traverse the capillary endothelium because of their basic character. Neurotropic viruses (poliomyelitis, rabies, herpes simplex and the neurotropic strain of yellow fever) carry a negative electric charge, and therefore cannot gain entrance into the CNS via the bloodstream. It is a known fact that large doses of tetanus toxin can be tolerated if injected intravenously. According to Friedemann's theory, the hemato-encephalic barrier forms an electro-negative membrane which hinders the passage of substances with a negative electric charge whereas the positive particles can enter the brain tissue with relative ease. This offers an explanation for the greater toxicity of cobra venom and lamb dysentery, which carry a positive charge as compared with the slower action of tetanus, diphtheria, botulinus toxin and staphylococcus toxin which are all negatively charged. As cell membranes in general are permeable to basic and impermeable to acid dyes, Friedemann suggested that the site of the barrier is in the vascular endothelium.

On the other hand, Bakay (1956), using the positively and negatively charged radioactive ions, came to the conclusion that the variation in the diffusion of different substances cannot be explained simply by their electric potential.

The majority of investigators accept the localization of the BBB in the vascular endothelium. Apart from the above-mentioned Spatz, the same opinion is shared by Morgenstern and Birjukoff (1928), Broman (1940), Friedemann (1942) and others.

Other concertions will be mentioned briefly.

An alternative location of the hemato-encephalic barrier was sought by Gaertner (1927), Hauptmann and Gaertner (1933), Richter (1950) in the pia-glia membrane or membrana gliae limitans.

Walter (1934) tried to compromise both theories in ascribing the principal significance to the intimal endothelium of capillaries while the pia-glia membrane would also play a part in the mechanism under discussion.

Bouton (1940), experimenting with dogs, produced the destruction of the intercellular substance by air embolism. Following the intravenous injection of trypan blue, the dye was found to have entered the brain tissue in the same areas where the intercellular substance had disappeared. Bouton concluded that the intercellular ground substance was an essential factor of the blood-brain barrier.

Hess (1955) stressed again the importance of the ground substance in the development of the hemato-encephalic barrier. The ground substance (periodic acid Schiff positive material) appears in guinea-pig embryos at about 45 days gestation and by fifty days it is similar in appearance and quality to that of the adult animal. The brains of newborn

guinea-pigs do not store sodium ferrocyanide nor can they be stained by trypan blue. On the contrary, the brains of neonatal mice, rabbits, cats and dogs do store sodium ferrocyanide and are stained by trypan blue. It is known that, in these animals, the ground substance develops only after birth.

In another article Hess gives experimental evidence in favor of the above theory. Thus, after hot-stab injuries of the brain, the reconstruction of the hemato-encephalic barrier, as tested by trypan blue stainability of brain lesion, conforms to the same schedule as the re-elaboration of the ground substance of the brain tissue.

Summarizing, Hess suggests that the effect of the blood-brain barrier depends on combined properties of: (a) the walls of the brain capillaries (endothelium and/or pia-glial membrane), (b) the ground substance, (c) the cellular elements of the brain.

The BBB shows a delayed permeability to a large number of solutes, including glucose and urea which readily pass cellular membranes in other tissues. Unlike other capillary or cell membranes, the BBB is particularly impermeable to proteins, while in other organs a certain percentage of the plasma proteins may pass the capillary walls. For instance, according to Starling (1909) the blood vessels of liver and of the gastrointestinal tract are also permeable to protein molecules. The antibodies can, apparently, in minimal amounts, gain the entrance into the CSF but only if the titer of the blood is high. Large colloidal particles pass the barrier under pathological conditions, as

for instance the hemolytic amboceptors which pass into the CSF in general paralysis (Weil-Kafka reaction), or isohemaglutinins and ferments in certain infections and intoxications. There is also a slight increase in permeability of the barrier during agonal state.

To summarize, there is a definite evidence of a selective passage of substances from the blood to CNS. The specific permeability properties of the cerebral vessels are called blood-brain barrier (BBB) - King, 1939, Friedemann, 1942, Broman, 1939-1950, Bakay, 1951. The term "barrier" should not be considered in a mechanical sense but rather as a selective inhibition of transfer of certain substances from the blood into the brain.

Disturbances of vascular permeability in general can be produced by a variety of factors of a traumatic, thermic, toxic, embolic, biochemical, and neoplastic nature. This applies equally to the BBB.

Through the work of Broman and Lindberg-Broman (1945) and Broman (1938, 1940, 1944, 1949), it has been shown that the permeability of the cerebral blood vessels is not affected by vasodilatation by means of ultra short waves, by drugs like histamine and acetyl-choline, or by injury of the vasomotor center or of the sinus carotis. The BBB in adults is remarkably resistant to anoxia. As demonstrated by trypan blue experiments, anoxia of several hours' duration has no deleterious effect on the cerebral vascular permeability. Broman (1940) was unable to demonstrate any impairment of permeability in

heparinized small experimental animals until twelve hours after death. He suggested the explanation that the vascular endothelium, being made up of a rather primitive type of cells, could survive several hours in anoxic conditions. In cats the arterial blood supply to the brain could be stopped for one hour without demonstrable disturbance of the vascular permeability. In experiments with more prolonged occlusion, intravascular coagulation made further observation impossible. Similarly a general increase in the intracranial venous pressure has hardly any deleterious effect on the BBB of a normal brain.

Greentoft (1954) studied the problems of the BBB in newborn infants with special reference to asphyxial damage. In his opinion the hemato-encephalic barrier in the foetus can easily be broken down by anoxia. The resistance to anoxia was shown to increase with the growth of the foetus. In the cases of prolonged asphyxia, post-mortem perfusion of the brain with a trypan blue solution stained scattered blue discolored areas, mainly in the central white matter. In the blue-colored areas, the capillaries showed distinctly blue-stained endothelial nuclei and were surrounded by a diffuse halo in which blue-stained glial nuclei could be found. In one case with recurrent convulsions, there were observed short segments of precapillaries or larger vessels with blue-stained nuclei. In other words, in asphyxia of newborn infants, there was a clear relationship between the degeneration of the capillary endothelium (as evidenced by its stainability) and the damge to the BBB.

It is a well-established fact that convulsive seizures are accompanied by serious vasomotor disturbances of the brain circulation. Spielmeyer (1930) has found histological evidence of recurring vasospasm in the brains of epileptic patients. Foster Kennedy, as quoted by Penfield, noted a visible shrinking of the brain in an epileptic attack.

A series of direct observations was made on exposed human brain in this Institute. It was found that certain circulatory changes are associated with all epileptic seizures, whether induced or spontaneous and whether in man or in experimental animals (Penfield, 1955). These are as follows:

(1) Ictal vasodilatation. During the epileptic seizure there occurs an increase in the blood flow through those parts of the gray matter which are involved in the neuronal discharge. This increase in blood flow begins from 4 to 10 seconds after the first convulsive movement and continues for a little time after the seizure is over. A similar and even more marked circulatory increase occurs in caudate, putamen, pallidum and thalamus. The increased flow is due to dilatation of the cerebral vessels by a substance formed in the tissue (such as carbon dioxide or perhaps an unidentified substance) as the result of activity of the discharging ganglion cells. If the attack is

unilateral, the increase occurs only in the contralateral hemisphere. There is little evidence of change in blood flow in the white matter.

Bulging and cyanosis of the brain are occasionally seen but these phenomena are secondary to respiratory embarrassment only. They are a result of the seizure and have nothing to do with its primary mechanism.

(2) Ictal cessation of pulsation. If the seizure is a grand mal in type, visible pulsation in the superficial pial arteries disappears and the hemispheres cease to pulsate. This is caused by opening of the vascular bed which decreases resistance to flow through the tissue. During this time, controlled measurements of the blood flow show it to be increased and the systemic arterial pressure, simultaneously recorded, is decreased as often as it is increased, or, usually, is little altered. Toward the close of the seizure the visible pulsation reappears and becomes more pronounced.

(3) Postictal hyperaemia is an infrequent but significant sequel to seizures. This hyperaemia, which can be called reactive since it is due to antecedent asphyxia, comes on some minutes after the end of the seizures and persists for as long as a half-hour, perhaps longer. It occurs in that part of the brain which has been the chief site of epileptic discharge.

In occasional instances pial blanching or pial flushing may occur at the end of the seizure. Local postictal spasm can be observed infrequently in pial arteries, especially after major seizures.

There is no evidence of any widespread vasoconstriction during or immediately preceding a seizure. Vascular spasms and anemias may be concerned in the pathological background of epilepsy but, at all events, they play no role in the actual mechanism during a seizure.

(4) Circulation between attacks. In the cortex of patients suffering from cryptogenic epilepsy, as observed on the exposed brain at operation, there seems to be greater variability of blood supply than there is in cases of symptomatic epilepsy. In the former cases there may appear a blanching or flushing of pia and arterial constriction.

In summary, Penfield condluded that a physiological instability of the cerebral blood vessels is common to epileptics of all varieties.

Silfverskiold and Amark (1943) measured the arterial and venous blood pressure during electric shock convulsions in psychotic patients directly. A marked rise was obtained in both the arterial and venous pressure. A mercury pressure as high as 100 mm. was recorded in the cubital vein in several instances. A maximum was reached during the tonic phase. The pressure sank back gradually during the clonic phase. In some cases the venous pressure remained raised, even after the convulsions. Similar values were obtained in the external jugular vein; however, the rise in the pressure was short-lived.

The arterial blood pressure was found in the tonic phase to go up to 250 mm. of Hg. while during the clonic phase there was a fairly rapid drop. The heart sounds disappeared

and then appeared again with gradually increasing strength. The situation resembles that seen during Valsalva's experiment. The minute volume may be decreased by 50% or more. The pulse oscillations are, on the whole, absent during the convulsions and consequently, there is a lessening in the stroke volume. In the authors' opinion the ischemic brain lesions are caused by a general lessening in the blood flow to the brain in conjunction with the convulsion apnoea.

Vascular disturbances in cats' brain following a short series of electrically-induced seizures were studied by Scholz and Jotten (1951). They obtained a patchy type of postparoxysmal anemia of the brain. The animals sacrificed 5 to 10 minutes after the series of seizures showed a poor filling of the large arteries and widespread areas of capillary anemia in which the capillary network was broken up into short segments. This applied particularly to the cerebral cortex, where the anemic areas alternated with areas of relatively better capillary filling indicating some stagnation of blood. This patchy anemic and hyperaemic pattern comparable to the cutis marmorata of the skin showed no definite relationship to the angioarchitectonics of the cortex, although the better-filled portion of the cortex generally corresponded to the 4th cortical layer. It can also be mentioned in this connection that in the cortical lesions of the epileptic brains there is a definite tendency to spare the 4th cortical layer. In addition Scholz's experimental material shows accentuation of the ischaemia of the cortex in the depth of the sulci.

Similar anemic areas were seen frequently in the hippocampus and within the thalamus, as well as in the basal portion of the medulla and in the olives. In the cerebellum there was the same irregular pattern of capillary filling. Finally, a spastic constriction of the arteries, maximally dilated and congested veins and scattered areas of poor capillary filling were observed in the nucleus caudatus.

The first signs of the restitution of the normal vascular conditions were noted 20 minutes after the last convulsive attack. At 40 minutes this tendency was rather far advanced and the vascular filling was found to be fairly uniform.

It is conceivable that some of the vascular disturbances cited above are associated with change in vascular permeability. There is a fair amount of experimental evidence in favor of this assumption.

Most of the experimental work on the effects of the convulsive seizures was aimed at reproducing as closely as possible the conditions to which psychotic patients are subjected during various therapeutic convulsive procedures. The main objective was to investigate the gross and microscopic changes of the CNS. A search was made for hemorrhages, nerve cell and glial changes. Only an indirect conclusion of disturbed vascular permeability was drawn from the presence of diapedesis, edema, perivascular hematogenic pigment, and slight reactive gliosis in the vicinity of blood vessels. (Ferraro and Roizin, 1946-49, Alexander and Loewenbach, Elpers and Hughes, 1942). A complete survey of literature on this subject including

some own work is to be found in a monograph by Hans Hartelins, Copenhagen, 1952. Cerebral ischaemia in connection with the epileptic seizure was suggested as the most probable mechanism causing irreversible nerve cell changes.

Prados (1936) , working with cats, found that convulsions caused by monobromcamphor allowed trypan blue to pass into nervous tissue. The experiment was later criticized by Broman who argued that the dye was too concentrated and crystallized into minute emboli.

Broman's own paper of 1944 deals with disturbances of permeability as the result of hypoglycemic coma, cardiazol and electro-shock therapy. The experiments were conducted on rabbits and the changes of permeability tested with intravenously-injected trypan blue solution. The animals, which were subjected to a series of 2 to 10 electrically-induced convulsions, presented scattered perivascular passages of the dye with a tendency to symmetrical distribution. No exact localization of these lesions was given. Other parallel experiments were carried out, with the substitution of cardiazol seizures or insulin coma for electro-shock. The author concludes that hypoglycemia produces the stronger and electric convulsions the weakest effect. In addition, in the case of hypoglycemic coma, the permeability changes were accompanied by petechial hemorrhages. It was shown that the impairment of the BBB took place during the period of shock treatment, since the animals which received trypan blue after the treatment presented no passage of dye, although the presence of

fresh petechiae indicated recent lesions of the vascular endothelium.

Moore (1953) also attempted to disrupt the BBB by using insulin although his experiments were not uniformly successful. By application of fluorescein or trypan blue as indicators, he found a breakdown of the barrier in only three out of thirty insulin-treated mice and then only in the terminal stage. This had no relationship to the presence or absence of convulsions. However, it was noted that, in the animals which had convulsions, the toxic effects of trypan blue were increased.

MATERIALS AND METHODS

A series of experiments was carried out, using adult cats of an average weight of 1.8 to 2.6 kg. The exact age of the animals was unknown. An attempt was made to eliminate from the experiments all the old-age group or unhealthy-looking animals. Both sexes were used indiscriminately.

The preliminary experiments were conducted under Nembutal anaesthesia 0.05 per kg. weight of the animal. The convulsive seizures were induced in non-anaesthesized animals but otherwise the experiments were conducted in the same way as in the preliminary group.

The permeability changes of cerebral blood vessels were tested with iodinated (I 131) bovine serum albumin suspended in normal saline. This was prepared by Charles and Frosst Company, using a modification of the method published by R. C. Gilmor Jr., M. C. Robins, and A. F. Reid in "Nucleonics" vol. 12, pp. 65-68, February, 1954. With this method not more than 2% of the total radioactivity of the solution may be due to unbound iodine.

The dose per animal was approximately 1 mc. and corresponded to 3-6 cc. of solution, which was given intravenously. Autoradiography was used to study the changes in permeability and at the early stage of our work the procedure was as follows: At the end of the experiment, the animal was bled into

abdominal cavity by cutting the abdominal aorta. Both common carotid arteries were cannulated and the brain was perfused with 100-150 cc. Elliott's A solution at the hydrostatic pressure of 120 cc. water. Subsequently, fixation of the brain was carried out in situ through the same cannulae with 20% formalin and 20% alcohol solution in normal saline. The animals were decapitated, the brain removed and left in the same fixative overnight to complete the fixation. Next morning the brain was sliced in a coronal plane in 0.4 cm. thick blocks and examined grossly. Representative sections 100 microns thick were cut on freezing microtome, mounted on glass slides and left to dry for one or two hours.

Further processing was completed in the darkroom. The slides were put in apposition with one of the following radiosensitive plates or films:

Kodak Medical X-ray film "No Screen", tinted safety base, dupletized.

Kodak industrial X-ray film, type K, tinted safety base, dupletized.

Kodak autoradiographic "A" plates, thickness 25 microns, top-coated.

Kodak autoradiographic "No Screen" plates, top-coated, thickness 25 microns.

Ilford Medium Lantern Slides, thickness of emulsion 50 m.

Kodak N.T.B. nuclear tract plates, 10 m., top-coated.

The time of exposure varied according to the sensitivity of the emulsion. It was found that the "No Screen" or type K emulsion was the most sensitive and required about 72 hours' exposure time. The second in order was Medical "A" type of emulsion with an exposure time of approximately

10 days, while for Ilford Lantern Slides or "Kodak Sheet Film Contrast Process Ortho" it was necessary to prolong the exposure to three weeks. The N.T.B. emulsion was found to be completely unsuitable, since it was the least sensitive and,in order to use it, the exposure time would have had to have been prolonged beyond three weeks; this was unfeasible because of the relatively short half-life of the isotope (8 days). This was rather unfortunate, since N.T.B. plates give the highest degree of resolution and under normal circumstances the background of the image would be entirely free of fogging.

The developing and fixing procedure of the autoradiographs did not differ from that of the ordinary X-ray films.

After the exposure the original microscopic slides were stained with benzidine technique to demonstrate the condition of vascular filling and were then counterstained with a 0.5% solution of basic fuchsin.

The slides and autoradiographs were examined grossly or microscopically, separately or in apposition.

The technique of brain perfusion described above was found to be unsatisfactory. It left a large amount of blood residue in the vessels and capillaries. It appeared that large areas of brain tissue were by-passed by perfusion fluids, probably through the opened side-anastomoses. The evaluation of the experimental results from the autoradiographs at that time was thought to be

difficult, if not impossible. (Compare Figs. 1 and 2).

In order to improve our perfusion techniques, an attempt was made to perfuse the brain through the aorta; however, this failed to bring about any significant change.

Finally, a new approach was made, aiming at the removal of radioactivity from the circulation of the animal by a hemodilution technique. A modified exchange transfusion technique, which is applied clinically in cases of Rh incompatibility in newborn infants, was used. At the termination of the experiment, the abdominal cavity of the animal was opened and the inferior vena cava cannulated at the level just distal to the entrance of the renal arteries. The abdominal aorta was clamped at approximately the same level. One of the plasma substitutes, dextran or subtosan, was injected through the cannula into venous circulation at a rate of 100 drops per minute. At the same time the right external jugular vein was cut, its proximal end was clamped and the distal stump was left open to let the animal bleed. In this way the amount of blood lost was immediately replaced by the plasma substitute through the vena cava. By application of this technique, it was possible to attain a high degree of hemodilution, depending on the condition of the animals. In most cases, at the end of this procedure, the heart of the living animal would pump a blood-tinged plasma substitute while all the mucosal membranes and internal organs became deadly pale. The average amount of plasma substitute used for one animal was 150 to 250 cc. and the time required was about 30 minutes. At the end of that time, when the heart stopped beating, the perfusion

and fixation of the brain was carried out in the manner previously described.

This method brought about a considerable improvement in the results. The autoradiographs obtained showed hardly any residue of radioactivity within the cerebral blood vessels. Some blackening of the emulsion remained only at the level of choroid plexuses and the segments of leptomeninges. (Fig. 3).

RESULTS

A. Experiment No. 1, Local Application of Heat.

(a) Technique.

A male cat (P56-117) weighing 1.8 kg. was anaesthesized with 0.8 cc. of 5% Nembutal solution. The skin covering the skull of the animal was incised in the coronal direction and retracted on the right side. The right masseter muscles were also separated from the bony surface and thus the bone all over the right cerebral hemisphere was exposed. When all the bleeding points were controlled, 3 cc. of iodinated I 131 bovine serum albumin corresponding to 1 mc. of radioactivity were injected intravenously. Thirty minutes later, a brass cylinder, pre-heated over a gas burner, was applied to the skull for one minute.

The replacement transfusion with plasma substitute and the perfusion and fixation of the brain were performed according to the procedure described in the previous chapter.

Gross examination of the brain revealed several small groups of petechial hemorrhages on the surface of the right hemisphere. The corresponding areas were also slightly softer to palpation as compared with the left cerebral hemisphere, which was pale and fairly firm.

After the fixation, sectioning and mounting of the slides, autoradiographs were obtained after 3 days' exposure to "No Screen" films and plates.

(b) Results.

The autoradiograph (Figs. 4,5) shows an increased uptake of radioactivity by the leptomeninges of the right (heated) hemisphere. In the areas in which the effect of thermal injury was particularly severe, as evidenced by the presence of petechial hemorrhages in the gross specimen, the radiosensitive emulsion showed a diffuse blackening. The injured areas expanded into the depth of the brain tissue in a wedge-shaped manner for a distance of 1 cm., with the base of this wedge directed toward the surface of the brain.

Microscopic examination of the corresponding slides stained with benzidine technique and counterstained with a weak solution of basic fuchsin showed coagulation necrosis of the superficial cortex, as evidenced by increased stainability and loosening of the tissue, while the capillaries were dilated and contained laked blood. The deeper layers of the cortex and the subjacent white matter remaining in the region corresponding to the diffuse blackening in the autoradiograph showed no blood content in the capillaries. The blood had been successfully removed by perfusion (Fig. 6).

(c) Discussion.

The severe effect of thermic injury was applied in this preliminary experiment in order to determine the suitability of this method for possible application in subsequent experiments.

The review of literature reveals that in experimental animals the heating of the local region of the brain by diathermy produces a regional disorder of the vascular permeability (Schmid, 1931). This result has been verified by Broman, Radner, and Swanberg, 1949, who demonstrated that initially such heating causes local pathological changes in the permeability of all the vessels in the damaged area, some of them also showing diapedesis of blood cells. Later, however, the central part of the damaged area becomes the site of necrosis with total stasis while the disturbance of vascular permeability in the peripheral zone lasts for a week.

In our experiment we are dealing with a severe damage of the BBB produced by heat effect. Although the microscopic examination revealed necrosis and a marked dilatation and congestion of the capillaries in the superficial cortex, the pattern of the autoradiograph leaves little doubt that the homogenous intense darkening of the emulsion at the level of the lesion is produced by a diffuse inbibition of the tissue by blood plasma. In this experiment the BBB was rendered permeable to large molecules of serum albumin in an area significantly larger than that showing evidence of damage in an ordinary histological stain.

B. Experiment No. 2, Air Embolism.

(a) Technique.

Two cats (P55-403, P56-78) weighing 2.o and 2.6 kg. respectively were anaesthetized in the usual way and injected with 3 cc. of iodinated (I 131) bovine serum albumin corresponding to 1 mc. Half an hour later the right common carotid artery was exposed and injected with 0.3 cc. of air 3 times at 30-minute intervals. Half an hour after the last air injection, the exchange transfusion with Dextran was begun. The latter procedure lasted 25 minutes and required 220 cc. of plasma substitute. The perfusion and fixation of the brain was completed as outlined under the description of the method.

Gross examination of both specimens failed to reveal any significant abnormalities. A special search was made for areas of congestion or hemorrhages which were both absent. There were also no areas of softening of the brain tissue. The right and left hemispheres were pale and of the same moderately firm consistency.

Autoradiographs were taken by three days' exposure of 100 m. thick sections to "No Screen" radiographic plate of 25 m. thickness, top-coated.

(b) Results.

Examination of the autoradiographs revealed a complete absence of radioactivity in the left cerebral hemisphere. The right hemisphere, which had been pre-treated with air emboli, presented a peculiar autoradiographic pattern which, when compared with that of a non-perfused or poorly-perfused brain, showed an essential difference. (See Figs. 7,8,9,11).

The differences are obvious as far as the number, size, shape, distribution and intensity of the component images are concerned.

The autoradiographic pattern of the embolized hemisphere is made up of individual component parts which are smaller in number but larger in size as compared with those of a non-perfused brain. Their shape is round or oval; the central core shows an increased darkening of the radiosensitive emulsion, as compared with the peripheral portion, which gradually fades out into the surrounding tissue. Occasionally two or more lesions coalesce. There are also a few lesions presenting a roughly wedge-shaped outline, reminiscent of those produced by thermic injury. The embolic lesions are mostly located in the cortical gray matter.

The pattern of the non-perfused or poorly-perfused brain is entirely different. It is composed of a large number of minute, small or medium-sized circles localized mostly in the meninges and in the gray matter. There are also thin, long streaks which are found in the white matter. No tendency was noted to produce large confluent conglomerates of these autoradiographic images; they are all distinctly separate and the individual images possess the same intensity throughout their surface. Their limits are rather sharp.

When these autoradiographs are correlated with the original microscopical slides, one comes to the conclusion that the embolic lesions correspond mostly to the precapillary arteries which are devoid of any blood content and are found in the center of

the autoradiographic dark regions. The diameter of the latter exceeds many times (10 to 15) the diameter of the blood vessel. On the contrary, all the blood vessels of the non-perfused brain are invariably filled with blood, and the autoradiographic image exactly overlies the blood vessel and is of the same or, at most, three times the diameter of the vessel.

Finally, not all the blood vessels in the embolized hemisphere produce the radioautographic picture. In fact, there are a large number of precapillary vessels which fail to produce any radiographic image.

All these findings are summarized in a tabular form, page 27a.

(c) Discussion.

In this experiment we are dealing with a phenomenon other than an insufficient perfusion or postembolic congestion of the cerebral vessels. The contralateral hemisphere serves as a control of the efficiency of the perfusion method. The benzidine stain of the microscopic slides confirms the absence of any blood residue in the blood vessels. In Broman's experiments the postembolic congestion was found to be short-lived and to disappear quickly after the air emboli were removed from the vascular lumina by the circulating stream of blood. In our experiments the only interpretation which can be offered is that of an increased vascular permeability to serum albumin produced by air embolism.

According to Broman (1940) the air embolism produces a disturbance in the cerebrovascular permeability

AIR EMBOLISM VS. UNSATISFACTORY PERFUSION IN AUTORADIOGRAPHS.

	Number of component images	Size	Shape	Distribution	Intensity	Relation vessel/ image
Air embolism	Moderate	large	rounded, oval, occas. wedge-shaped, (confluent)	one hemisphere, mainly cortex	decreases towards periphery	Vessel in the center of the image. Diameter of vessel a fraction of the diameter of the image. (1/15-1/10)
Poor perfusion	Large	small to moderate	punctate or round in the cortex, streaked in white matter, never coalesce.	both hemispheres, cortex and white matter	uniform, sharp limits	the image of vessels of the same diameter or at most 3 times the diameter of the vessel.

immediately after the passage of the emboli. This was explained as the effect of the interrupted contact between the wall of the vessel and the blood plasma. In his experiments the lesions caused by air emboli surround the precapillary arteries like a sleeve and are found more frequently in the cerebral cortex than in the white substance. These lesions are produced in the segment of the arterioles situated proximately to the embolus. The latter usually lodges at the point of bifurcation of the artery. Broman was unable to demonstrate any definite passage of colored plasma into the perivascular brain tissue as a result of a single dose of air embolism. In our experiment, there is an undisputable permeation of serum albumin around the embolized blood vessels following a repeated embolization. As noted above, not all the precapillary arteries produce an autoradiographic image. It has to be assumed that only those blood vessels which are the site of emboli for a sufficiently long period of time (10 minutes, according to Broman) will present evidence of impaired vascular permeability.

C. Experiment No. 3, Convulsive Seizures.

Having obtained the results of the preliminary experiments described above, we embarked on the series of experiments which were originally planned, i.e. testing of the cerebrovascular permeability to large molecules in relation to convulsive seizures.

Particular attention was paid to the selection of young, healthy-looking animals for these experiments. Their respective weights were between the limits of 1.8 and 2.6 kg.

(a) Technique.

The shocks were given with a Model "N Electro Shock Therapy" machine designed to operate on 60-cycle alternating current. The electrodes were made of brass; they were round and measured 1.5 cm. in diameter. In every case they were applied symmetrically bitemporally in front of each ear. The hair in this area was shaved off and an EEG jelly was rubbed in. During the seizures the head of the animal was protected from possible injury resulting from clonic contractions. The quantity of current was kept as low as possible. It was found that for the majority of animals, 80 volts over 0.3 seconds was sufficient to produce grand mal type of seizure. The latter was characterized by a short latency period and the tonic, clonic and tonic phases. Occasionally only abortive seizures were obtained. These were characterized by the absence of tonic phase and of the postconvulsive coma stage. Following the typical grand mal seizure, the animals remained for a variable period of time in the state of coma

until they recovered sufficiently to exhibit spontaneous movements, usually standing up or starting to react to sensory stimuli. After the coma stage the animals remained apparently amnestic with a blunted sensorium and poorly coordinated movements, more evident in the hind legs than in the forelimbs. Occasional tachypnoea was observed and, as a rule, other phenomena of autonomic origin supervened as an increased salivation, urination, and defecation. Only in the case when abortive seizures did occur, the animals exhibited fear of the electrodes; otherwise, no reaction was noticeable.

An attempt was made to keep the animals in the stage of artificial status epilepticus for a period of 6 hours. Convulsive seizures were repeated every five minutes or ten shocks were applied per hour, thus giving a total number of 60-70 treatments. There is a remarkable ability of these animals to withstand a large number of convulsive seizures. Only two animals of the total number of twelve died, after having been given approximately 20 electroshocks.

In a group of 12 animals, 10 experimental animals and two controls, the injection of radioactive bovine serum albumin was given intravenously before the onset of treatment. The usual amount injected was 1 mc. of radioactivity in 3-6 cc. At the termination of the last convulsive attack, the animals were allowed to rest for a period of time of about 30 minutes to 1 hour. Two control animals received the same dose of radioactive serum albumin, but were not subjected to convulsive seizures. The exchange

transfusion, the perfusion and fixation of the brain were performed in the same way as in the preliminary experiments.

(b) Results.

Of the group of 12 treated animals, two died, as mentioned above, after having received 18 and 20 convulsive seizures, respectively. They were eliminated from the subsequent examinations, following a short autopsy which, however, failed to reveal any obvious cause of death.

Macroscopic examination.

The brains of the remaining ten animals were examined grossly. In four animals the dorsal surface of the brain showed a very faint, pinkish discoloration presenting an irregular, mottled pattern. Both marginal gyri showed this peculiar discoloration in all four cases. All the brains were otherwise apparently well-perfused and fixed, possessing a fairly firm consistency. Slicing of the brains in a coronal plane in 0.4 cm. thick blocks revealed no petechial or diffuse hemorrhages. In several cases, the basal ganglia also were found to show pale pinkish discoloration.

Representative blocks of tissue were cut serially on a freezing microtome, 100 m. thick. About 20 autoradiographs from each case were obtained with the usual contact technique, using Industrial K films, Medical "No Screen" films, "No Screen" autoradiographic plates and Ilford lantern slides. After the exposure and development of the autoradiographs, the original slides were stained by Alexander's benzidine and counterstained with 0.5% basic fuchsin.

Examination of the autoradiographs shows an increased uptake of radioactivity by all gray matter, including the cerebral and cerebellar cortex, the basal ganglia, thalamus and hypothalamus. The most conspicuous change is seen in the thalamus. In all cases the autoradiographic pattern is approximately the same. The darkening of photographic emulsion is diffuse in character and, as compared with the original microscopic slides, shows no relationship to the individual blood vessels. It can be definitely ruled out that this image is produced by the residue of non-perfused blood in the vascular network. The improved perfusion technique leaves hardly any blood in the vessels as evidenced by benzidine stains. Otherwise it was shown above that the blood-filled vessels never produce a diffuse confluent autoradiographic picture such as that seen in this series of experiments.

The distribution of regions which concentrate a large amount of labeled serum albumin is constant and most striking (Figs. 16, 17, 19, 21, 23). The strongest concentration is in the thalamus and hypothalamus, which take up the labeled serum albumin in a diffuse manner, although in individual cases slight variation in distribution and intensity may be present. The most constantly and severely affected are: anterior thalamic nuclei, dorsomedial nucleus, ventralis posterior and pulvinar. A high uptake of radioactivity is also found in the zona incerta and particularly in the tuber

cinereum. The amount of tagged proteins in the lateral geniculate body varies in wide limits and is, on the whole, less than in the thalamic nuclei. The cerebellar cortex is also involved as a rule; however, no definite pattern of distribution could be observed. The cerebellar vermis takes up a slightly larger amount of radioactivity than the other groups of folia (Fig. 15). A rather faint but definite darkening of photographic emulsion is seen at the level of the cerebral cortex and basal ganglia. In several instances an impression was gained of a slight accentuation of the darkening in the depth of the cerebral sulci (Figs. 13, 14).

(c) Discussion.

A considerable amount of evidence has accumulated pointing to the disturbances of the BBB as a precipitant factor in epileptic convulsions. Numerous preconvulsant conditions such as dehydration, alkalosis, anoxemia, and inflammatory processes known to favor the occurrence of seizures may alter the properties of the barrier and create the intracellular conditions leading into a convulsive state. Spiegel and Spiegel-Adolf (1936), using an indirect method of electrical conductivity, have demonstrated that conditions which predispose to the production of convulsive seizures are associated with an increase in the permeability of the cortical tissue. There are recorded in the literature occasional cases of angioneurotic edema with typical manifestations of disturbed vascular permeability associated with signs of increased intracranial pressure, convulsive seizures, transitory hemiplegias, aphasias, and other

neurological symptoms which could be explained as the result of pathological changes in the BBB. Stern and Lockschina (1928) reported alterations of the hemato-encephalic barrier in an anaphylactic shock with a resulting permeation of the brain tissue by certain colloidal substances.

Before discussing the results of our experiments, it would be best first to list the pathological changes of the central nervous system which are thought to be sequelae of convulsive changes. It must be mentioned, however, that it is not always possible to distinguish the postconvulsive lesions from those which are the cause of epilepsy.

Cerebral and cerebellar cortex.

Spielmeyer (1933) reported histological evidence of spasm of small cerebral vessels, both in the case of symptomatic epilepsy and idiopathic or cryptogenic epilepsy. He discovered small perivascular areas of destruction of neurons in the hippocampus and in the cerebellum. The vessels were normal in appearance and Spielmeyer concluded that the perivascular injury could only have been produced by spastic vasoconstriction during the seizures.

The hippocampus is the most frequent area of the brain to be affected during convulsive seizures of various etiology. Lesions of the hippocampus are found in approximately 50% of epileptic brains. The most sensitive - h_1 or Sommer's sector - shows necrosis with neuronophagia and an increase in rod cells in acute cases and in chronic conditions a loss of neurons and gliosis.

However, the lesions of Ammon's horn are of a nonspecific character and are found in a variety of morbid conditions, such as cerebral trauma, poisoning with or without convulsions, arteriosclerosis and infections.

Hartelius (1952) had the impression that irreversible lesions of nerve cells following electrically-induced seizures in cats were possibly somewhat more common in Ammon's horn than in the cerebral cortex; however, he admitted that phenomena suspect of neuromophagia were also seen in the control animals.

The second most common pathological change found in the brain in epileptics is atrophy of the cerebellar folia which varies within wide limits and occasionally involves the whole cerebellar hemisphere. There is a marked tendency towards accentuation of the cortical atrophy in the depth of the sulci. This, according to Scholz, is the result of compression of the pial blood vessels and is caused by congestion and increased intracranial pressure during the seizures.

Scholz listed three types of lesion found in the cerebral cortex: 1. Disseminated necrosis or disappearance of nerve cells in small circumscribed areas, corresponding to the distribution of blood supply by pial arteries. 2. Laminar lesions. The 4th cortical layer is usually spared. Necrosis or gliosis of the cortex tends to be accentuated at the bottom of the sulci. 3. Shrinkage of the whole gyri, "ulegyria". The exact mechanism of this lesion is unknown. It cannot be explained by passive

vasomotor disturbances alone.

Finally, in rare cases, atrophy may involve the entire cerebral hemisphere.

Thalamus. Following convulsive seizures there may be found disseminated necrosis of nerve cells, especially in the lateral nuclei group (ventral and dorsolateral nuclei). The boundaries of the individual nuclei are not respected. The anterior nuclear group and the pulvinar are only rarely involved. Occasionally, secondary myelinated glial scars are seen, producing a picture comparable to the so-called "status marmoratus" of the striatum.

Corpus striatum. Involvement of this structure is a rather rare occurrence. The acute stage is characterized by ischemic ganglion cell changes, mostly of the small cells, neuronophagia and rod cell proliferation. The acute lesion terminates in a reparative gliosis, and occasionally status marmoratus may be present. The latter is discernible even with the naked eye on the gross specimen.

As far as the specificity of all the changes described above is concerned, the problem is difficult to solve. The lesions of the hippocampus, as mentioned above, are apparently of a nonspecific character. It seems that most of the lesions considered previously to be the result of epileptic attacks are actually an evidence of brain damage which occurred before the onset of seizures.

Our experiments indicate that a series of electrically-induced epileptic seizures is able to produce a marked concentration of labeled serum albumin in the thalamus,

tuber cinereum, and to a lesser degree in the cerebral and cerebellar cortex and in the basal ganglia. The most likely explanation of this autoradiographic effect is that the labeled protein molecules actually pass the capillary wall and infiltrate the brain tissue. In favor of this speaks the diffuse character of the autoradiographic picture and the apparent lack of relation to the larger blood vessels. However, with our present technique of contact autoradiography and its poor resolution we have to admit that definite proof of this assumption is lacking.

There is another possible interpretation, that the proteins only infiltrate the endothelium of the capillaries. We expect that the final answer will be forthcoming from our work which is underway and in which thin paraffin sections are directly coated with radiosensitive emulsion, thus providing better resolution.

As far as the peculiar distribution of the albumin concentration is concerned - predilection of the hypothalamus and the thalamus - no satisfactory explanation can be offered. Jung (1950) measured the production of potentials in various subcortical centers during electrically-induced seizures and found that during the grand mal type of seizure, the discharges were recorded from all the regions of the thalamus and from the cerebral cortex. However, in abortive seizures numerous potentials are generated in the medial, dorsolateral and anterior nuclear groups of the thalamus, while the electrical activity of the cerebral cortex is minimal. In the case of atypical seizures which can be considered as intermediate between the abortive and grand mal ones, long-lasting discharges

are produced in subcortical centers only, primarily in the thalamus, subthalamic area and in the hypothalamus. Simultaneously, the cerebellum takes part in the production of electric potentials.

The increased metabolic rate of the gray matter during an epileptic seizure may have some relation to the mechanism of the production of the pathological changes. It is probable that a relative hypoxemia, caused by a marked increase in oxygen requirements and only a moderate elevation of blood supply, can lead to irreparable tissue damage (Penfield, Jung). Our experiments suggest an impaired permeability of the BBB in the thalamus, hypothalamus and in the cerebellum to be a constant phenomenon accompanying electrically-induced convulsive seizures in the cat. It may be hypothesized that these changes arise in those areas of the brain which, according to Jung, are most active. In fact, a variable percentage of convulsive seizures induced in our animals can be classified as abortive or atypical and they may have produced increased electrical activity in the thalamus and subthalamic regions. On the other hand, we were unable to demonstrate any changes in the hippocampus, which is one of the most frequently affected regions of the human cerebral cortex in epileptic brains. It is possible that the species differences may be responsible for this discrepancy.

D. Experiment No. 4, Influence of the Variation of the Number of Seizures and of Time of Injection.

(a) Technique.

A group of six animals was used in this experiment. The average weight of the cats used was 2 kg. and they appeared to be in a good condition. They were subdivided into three subgroups of two animals each.

The usual dose of iodinated serum albumin (1 mc.) was injected intravenously into the first two animals and then both of them were subjected to a series of ten convulsive seizures at intervals of five minutes. One of the animals was transfused and sacrificed immediately , while the other was killed after five hours.

The second subgroup of animals was given 30 electrically-induced convulsive seizures under the same experimental conditions. Again, one of the cats was sacrificed immediately after the last attack and the other was left alive for three hours.

The last group of two animals received a series of 60 seizures, comparable to that applied in the previous experiment except for the fact that one of the cats was injected with radioactive albumin before and the other after the seizures. The latter survived for three hours after the last convulsive attack.

(b) Results.

The autoradiographs of the brains of all six animals were correlated and studied. The same autoradiographic pattern, but of varying intensity, was found in all cases except that which was obtained from the animal

injected with radioactive substance after the seizures. In this last case, only the tuber cinereum produced an autoradiographic image (P56-163, Fig. 30).

The first subgroup of animals killed after ten seizures showed evidence of a very slight tracer concentration, although the familiar convulsive pattern of the autoradiographs was readily recognizable. No difference was noticeable between the animal sacrificed immediately (P56-181, Fig.25) and that killed after a 5-hour period of survival (P56-180, Fig.26).

In the second subgroup, the animal killed soon after the termination of the series of 30 seizures (P56-182, Fig.27) produced a strongly positive radioautograph which was only slightly lighter than that of a 60-seizures animal. It was much darker than that of another 30-seizures animal (P56-193, Fig.28), although the latter survived for an extra 3 hours after the last convulsive attack.

(c) Discussion.

The experiments described above indicated that the intensity of the uptake of radioactivity is proportional to the number of convulsive seizures given. No tracer concentration takes place if the animal is injected after the seizures, nor does a change in the density of autoradiograph occur if the animal is left alive for several hours after the seizures. It would appear that the concentration of tagged albumin takes place only at the very moment of the convulsive attack and that this process is reversible.

If our interpretation of increased vascular permeability is correct, it would be in keeping with the observations of Broman who found that the staining of nervous tissue develops during the convulsions only and that the vascular damage responsible for this effect is repaired almost immediately after cessation of the seizures.

E. Experiment No. 5, Venous Congestion.

The effect of increased venous congestion on the BBB was tested in two animals, one of which had received an additional intravenous injection of epinephrine in order to raise the systemic blood pressure.

(a) Technique.

A male cat (P56-149) weighing 1.8 kg. was injected with the usual dose of radioactive serum albumin and the experiment was carried out half an hour later under Nembutal anaesthesia. The jugular veins were exposed and both internal ones were ligated permanently. The external jugular veins were clamped for one-minute periods of time at intervals of two minutes. This procedure was repeated 60 times, leaving a half-hour rest-period in the middle of the experiment. At the end, the animal was subjected to the usual blood replacement and perfusion procedure.

In another cat (P56-195) weighing 2.3 kg., all the four jugular veins were clamped for a 30-minute period of time. In the initial ten minutes of the experiment, the animal received a slow intravenous injection of 1 cc. of 1:1000 solution of epinephrine diluted in 5 cc. of saline.

(b) Results.

Gross examination of the brains of both animals showed a very faint pale-pinkish discoloration of the dorsolateral surface of the cerebral hemispheres, producing an irregular, patchy appearance. The latter closely resembled the discoloration of some of the brains of the animals subjected to convulsive seizures. However, the autoradiographs obtained from this material were disappointing.

There was a negligible concentration of the radioiodine in the gray matter as a whole, showing no gradation in the intensity. In particular, the thalamic area revealed no increased uptake of the isotope.

(c) Discussion.

This experiment as well as the subsequent one, No. 6, was aimed at reproducing a convulsive autoradiographic result by other means than convulsive seizures. From the review of the literature, it appears possible that the impairment of the BBB could be the result of disturbance of cerebral circulation associated with the relative hypoxemia, increased intracranial blood pressure, possibly brain edema, or maybe the combination of some of these factors.

As demonstrated by the work of Broman previously quoted, venous congestion and anoxemia are not the factors responsible for the disturbances of the hematoencephalic barrier. The results of our experiment on the limited number of animals substantiate Broman's findings, particularly in relation to large protein molecules. These results were not influenced by additional elevation of the systemic blood pressure produced by epinephrine injection.

F. Experiment No. 6, Brain Edema.

(a) Technique.

Artificial brain edema was produced in an animal which had been previously injected with labeled serum albumin. The cat was anaesthesized with 0.05 g. Nembutal. The skin overlying the skull was reflected and a large bony portion of the skull was removed, exposing nearly the whole dorsolateral surface of the right hemisphere. The dura was left intact. After the bleeding was controlled, radioactive substance was injected intravenously and half an hour later, 250 cc. of 0.1% solution of glucose were injected over a period of time of 1½ hours at a rate of about 40 drops per minute. In this way, a marked edema and a moderate degree of herniation of the brain was produced. Replacement transfusion, perfusion and fixation of the brain were carried out in the usual manner. The perfusion process was rather difficult and required a higher hydrostatic pressure. The same observation was made by several investigators concerning the perfusion of edematous brains.

(b) Results.

Gross examination of the brain showed no significant abnormalities. Both hemispheres were of approximately the same size. There were no grossly discernible lesions. The autoradiographs revealed a slight increase of isotope concentration in the meninges of the right hemisphere. There were also several punctate lesions in the same hemisphere (P56-161, Fig. 31). These measured 1-3 mm. in diameter and were found to be produced by minute hemorrhages or microscopical areas of necrosis in the

cerebral cortex and in the subjacent white matter.

(c) Discussion.

In this case, experimental brain edema failed to reproduce anything which would resemble the convulsive autoradiographic pattern. No increased iodine concentration was obtained in the thalamus or in the cerebral cortex. Similarly, in Broman's experiments, an injection of distilled water, given either intravenously or into the carotid artery produced cerebral edema without disturbing the vascular permeability. Several small disseminated lesions can be explained as having been produced by herniation of the brain or by operative trauma. Broman listed three causes of disturbed vascular permeability in brain hernia.

1. rupture of small vessels.
2. necrotic processes.
3. raised venous pressure.

Rupture of the vessels takes place several millimeters below the surface of the cortex and is caused by deformation of the tissue.

G. Experiment No. 7, Protective Action by Trypan Red.

(a) Technique.

Two cats, weighing 2.8 and 2.4 kg. respectively, received a series of preliminary injections of trypan red according to the technique of Aird (1948). The dye was injected intraperitoneally in a 1% solution in a dose of 10 mg. per kilogram of body weight per injection. Four injections were given over a period of seven days. A pink discoloration of the skin and mucous membrane was noted, which increased in intensity with each subsequent injection. It was noted that the animals had lost their appetite and, at the end of the treatment, presented definite toxic symptoms, such as sluggishness, ataxic gait and disturbance of body equilibrium. They had lost 0.5 and 0.25 kg. of weight, respectively.

Both animals were injected with iodinated serum albumin (1 mc. each) and were subjected to an electrically-induced convulsive seizure every five minutes. It was noted that the cats were more refractile to the convulsive treatment and required a voltage of 100-115 volts as compared to an average of 80-90 volts for other animals. One of the cats, which had appeared to be in a relatively good condition at the beginning of the experiment died after having received 12 convulsive shocks. Another one withstood all the series of 60 shocks, was transfused with Dextran and its brain was perfused successfully.

(b) Results.

At autopsy both animals appeared to be dehydrated. The animal which died showed no gross pathology. All the

internal organs were pinkish-discolored. The brain was grossly normal, apart from pink discoloration of the dura and plexuses.

The animal which received the full series of convulsive treatment revealed, apart from the pink discoloration of the skin and other organs, a moderate degree of symmetrical hydrocephalus. Autoradiographs of the brain showed a relatively slight uptake of radioactivity of the known convulsive pattern. The intensity of the radiograph corresponded approximately to that of the 10-seizures animals of Experiment No. 4 (See Fig. 32).

(c) Discussion.

Cobb and his associates (1937) demonstrated that brilliant vital red could be used in both experimental animals and humans to protect against the convulsant agent, triphenylphosphite. Aird (1939) used the same dye and succeeded in protecting dogs against convulsive doses of cocaine. The same author (1948) applied trypan red to patients with amyotrophic lateral sclerosis and claimed to have obtained some improvement of the neurological condition of these patients. An intravenous injection of trypan red, when given to experimental animals before the trauma, also prevented some of the ill effects of cerebral concussion. Consequently further studies were undertaken on the application of trypan red to electroshock therapy. Aird expressed the opinion that trypan red might counteract both the increase in permeability of the BBB and the cerebral dysrhythmia associated with electroshock in animals.

In our experiment, which could not be repeated on a larger number of animals because of lack of time, there is some suggestion of protective action of trypan red against the development of our convulsive autoradiographic effect. However, trypan red, at least in the dosage recommended by Aird, was found to be toxic to the animals. This problem deserves further investigation.

H. Experiment No. 8 - Control Experiment with Unbound Iodine.

(a) Technique

Two cats of approximately the same weight were each injected with 40 microcuries of standard oral solution of potassium iodide (I 131). Thirty minutes later, one of the animals was subjected to electrically-induced convulsions and received 60 seizures in six hours. Following this procedure, both animals were anaesthesized and their brains were perfused in the usual manner.

Sections of the brain and of the thyroid gland were cut and autoradiographs were taken as in the previous experiments.

(b) Results.

No concentration of radioactivity was discovered in the brain of either the convulsed or the control animal. There was instead a high uptake of iodine in the thyroid gland in both instances.

(c) Discussion

This experiment was carried out in order to exclude the effect of the unbound iodine which is a contaminant in the solution of iodinated serum albumin used in the previous series of experiments. The method of preparation of the iodinated serum albumin allows up to 2% of radioactivity to be due to unbound iodine, that is, 20 microcuries per one millicurie of radioactivity (See page 17). The amount of forty microcuries injected in the animals in this experiment is double the quantity which could be introduced into the blood circulation of our experimental animals with each

injection of 1 millicurie of iodinated serum albumin. The result of this experiment rules out the possibility that the unbound iodine was responsible for the radioautographic pattern of convulsed animals. The unbound iodine seems to be picked up selectively by the thyroid gland.

CONCLUSION

Autoradiographs of the brains of control animals showed the BBB to be impermeable to large protein molecules under normal circumstances. Preliminary experiments using heat injury revealed diffusion of plasma into the brain substance beyond the limits of histologically demonstrable tissue damage. Air embolism produced an impairment of vascular permeability at the level of the precapillaries.

Larger series of electrically-induced seizures imitating status epilepticus produced a peculiar autoradiographic pattern which was rather diffuse in character and showed no definite relation to the individual blood vessels. Lack of this relation and the absence of blood in the lumina of the blood vessels including the capillaries would rule out the possibility that this autoradiographic effect is produced by an unsatisfactory perfusion technique. There are two other possible interpretations for the results obtained:

The labeled protein molecules might infiltrate only the endothelium of the capillaries without actually passing the blood vessel wall. However, this mechanism would be less likely to produce a diffuse autoradiographic image of such a high degree of concentration.

In our opinion the most plausible interpretation is that of an increased vascular permeability at the level of the capillaries. In favor of this speaks a diffuse darkening of the photographic emulsion as compared with the pattern of a control non-perfused brain in which the blood vessels stand

out fairly distinctly against a very faint background. However, a definite proof for our hypothesis of increased permeability is lacking. We expect to obtain a solution of this problem by coating radiographic technique in which very thin 7-10 micron sections are coated by a layer of radio-sensitive emulsion. This, we hope, would secure a satisfactory degree of resolution and might furnish the final answer.

The most conspicuous concentration of tagged albumin took place at the level of the thalamus and hypothalamus. The tuber cinereum, dorsomedial and lateral nuclear groups of the thalamus were those which suffered most frequently. Of the other regions of the brain, those which deserve to be mentioned are the zona incerta, cerebellar vermis, and other groups of cerebellar folia. Lateral geniculate bodies concentrated the isotope with a variable intensity. A slight concentration of the radioactive iodine was also present in the cerebral cortex and basal ganglia. There was a certain accentuation of this change in the depth of the sulci but this has been found to be of a relatively slight degree. A satisfactory explanation for the reason why these particular areas undergo this peculiar change is lacking. It can only be conjectured that these centers are the seat of increased metabolism and are active participants in the production of high electric potentials, especially during abortive and atypical seizures. An increased rate of metabolism and energy production would result in an abnormal concentration of the products of metabolism. In fact Spiegel Adolf (1945), using spectrometric methods, demonstrated the

breakdown of nuclear substances during the convulsive seizures. Richter and Dawson suggested that in electroshock the ammonia may accumulate in metabolically active regions and the release of ammonia might induce the seizure. Similarly, the concentration of guanidine was reported to be high in essential epilepsy and to rise sharply at the time of seizures. These or other normal metabolites in abnormal concentration (e.g. lactic acid) or perhaps also foreign to the brain substances might create favorable physico-chemical conditions for the production of permeability change. The disturbance of cerebro-vascular circulation, stasis, and relative hypoxemia may also play some part in this mechanism in the highly vascularized areas.

The mechanism responsible for our convulsive auto-radiographic effect develop in close relation to the seizures and it ceases to operate immediately after the termination of the attack.

It became apparent that the intensity of the tracer concentration was proportional to the number of convulsive attacks.

Hartelius found that no generalized chromophobia (tigrolysis) could be observed in the nerve cells of the animals subjected to convulsions with a survival time of at least 24 hours. These changes reached a maximum after 48 to 96 hours. He concluded that the cellular changes do not appear to be immediate sequelae of the convulsions but secondary ones. In our work we succeeded in demonstrating the existence of some of the earliest processes connected with convulsive trauma long before any evidence of histo-
logical

abnormality can be detected.

The attempts to reproduce a convulsive autoradiographic effect by other means, such as long-lasting venous congestion with the simultaneous increase of systemic blood pressure or by brain edema were unsuccessful. It would give some support to the hypothesis that certain intermediate factors (metabolic?) play a decisive role.

Aird's technique of protection of the BBB by premedication with trypan red was applied to a limited number of experimental animals. No definite conclusion can be drawn as far as the concentration of albumen molecules in the pretreated brain is concerned. However, some suggestion of the protective action of trypan red in relation to convulsions was obtained which would warrant further investigation along these lines.

SUMMARY

Permeability changes of the blood-brain barrier to large protein molecules were investigated in cats in relation to a large series of electrically-induced seizures imitating status epilepticus in man.

Iodinated (I 131) bovine serum albumin was injected intravenously before or after the seizures. A method of replacement transfusion with plasma substitute was used as an adjunct to the conventional brain perfusion technique.

Contact radiography secured a fair degree of resolution of the precapillary blood vessels; however, for the capillary network it was found to be unsatisfactory.

A series of preliminary experiments was carried out, using the effect of heat injury and air embolism. The latter was found to break the blood-brain barrier at the level of the precapillaries.

Convulsive seizures produced a peculiar autoradiographic pattern in which the highest concentration of the tagged albumen was found in the thalamus and in the hypothalamus. The intensity of the autoradiograph increased in proportion to the number of seizures given. If the labeled serum albumen was injected after the seizures, no uptake of radioactivity took place with the exception of the tuber cinereum. It would appear that the concentration of serum albumin in the thalamus and in other locations takes place during convulsions only and that this process is reversible. It is postulated that the concentration of labeled serum albumen in the diencephalon, in the cerebellar vermis and

in the depth of the cerebral sulci is the result of impaired permeability of the cerebral capillaries to protein molecules during artificial status epilepticus.

An attempt to reproduce the convulsive autoradiographic pattern by passive venous congestion, increased systemic blood pressure, and cerebral edema was unsuccessful.

The possibility of participation of the unbound iodine in the production of the convulsive autoradiographic effect was experimentally excluded.

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Fig. 1 Autoradiograph of a non-perfused brain (positive print).

Fig. 2 Autoradiograph of a perfused brain. Note abundant residue of blood in the vessels (positive print).

Fig. 3. Autoradiograph of a perfused brain following replacement transfusion with Dextran. A small amount of radioactivity is noted in the choroid plexus only (positive print).

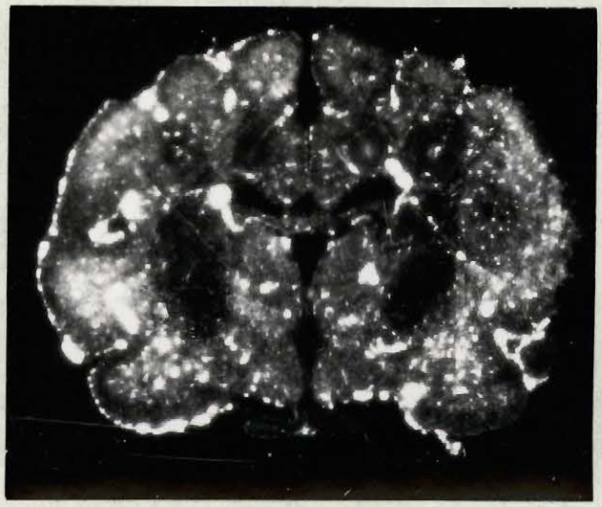

Fig. I.

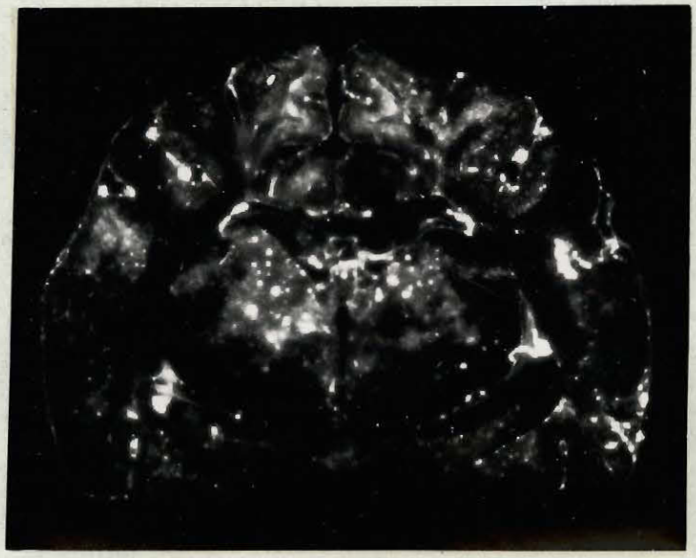

Fig. 2.

Fig. 3.

Fig. 4. Effect of heat injury. Note heavy tracer concentration in two wedge-shaped areas and some uptake of radioactivity in the meninges (positive print).

Fig. 5. One of the lesions of Fig. 4 under higher magnification. (negative print).

Fig. 6. Original histological section showing area corresponding to the previous autoradiograph. Note small area of coagulation necrosis in the superficial cortex.

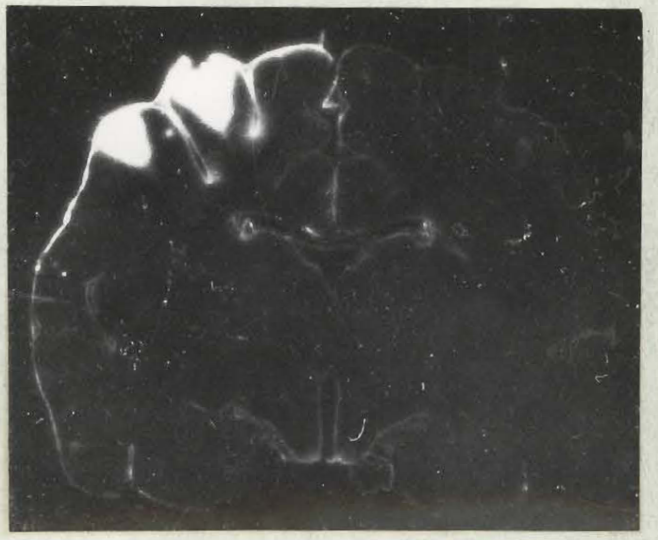

Fig. 4.

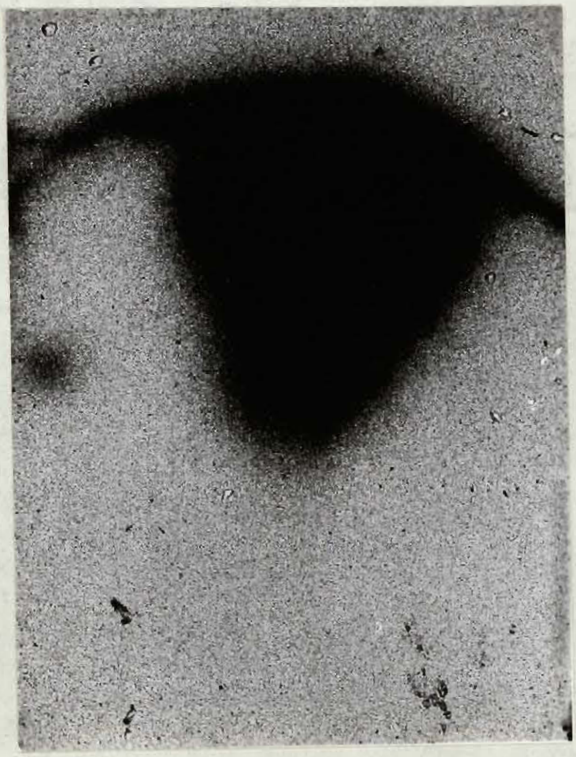

Fig. 5.

Fig. 6.

Fig. 7. Air embolism. The distribution of lesions is confined to the embolized hemisphere. (Positive print).

Fig. 8. Same as Fig. 7. Two gyri under higher magnification (Negative print).

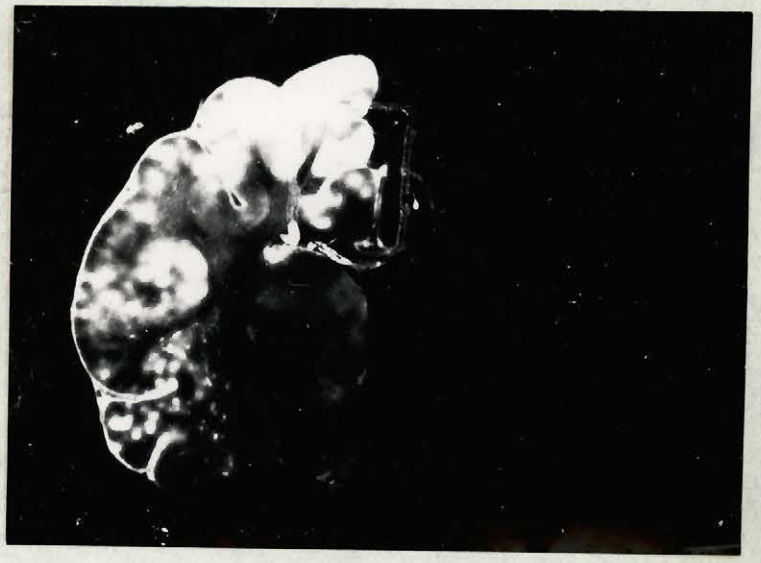

Fig. 7.

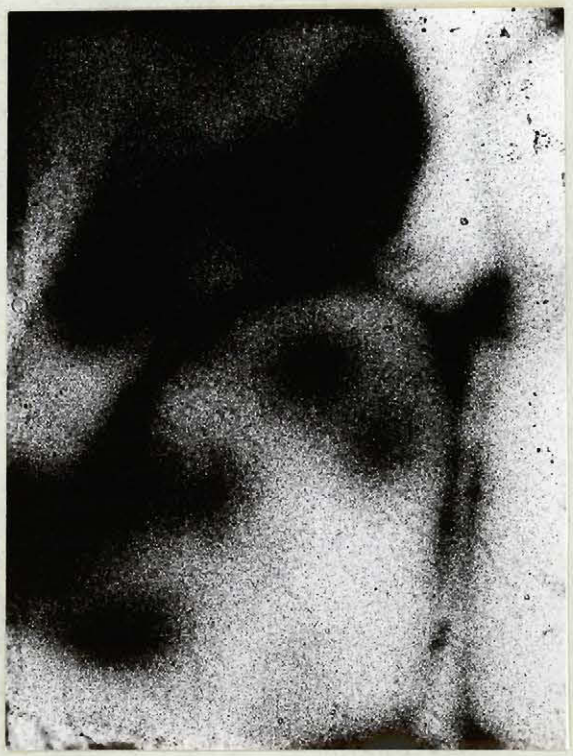

Fig. 8.

Fig. 9. Embolized gyrus. Note peripheral distribution of lesions (negative print).

Fig. 10. Original histological section of which autoradiograph for Fig. 9 was obtained. Note the absence of blood in the vessels.

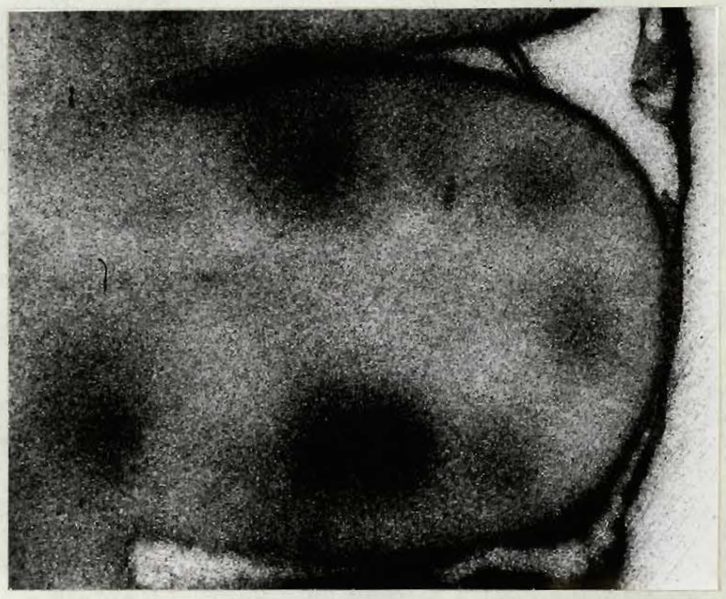

Fig. 9.

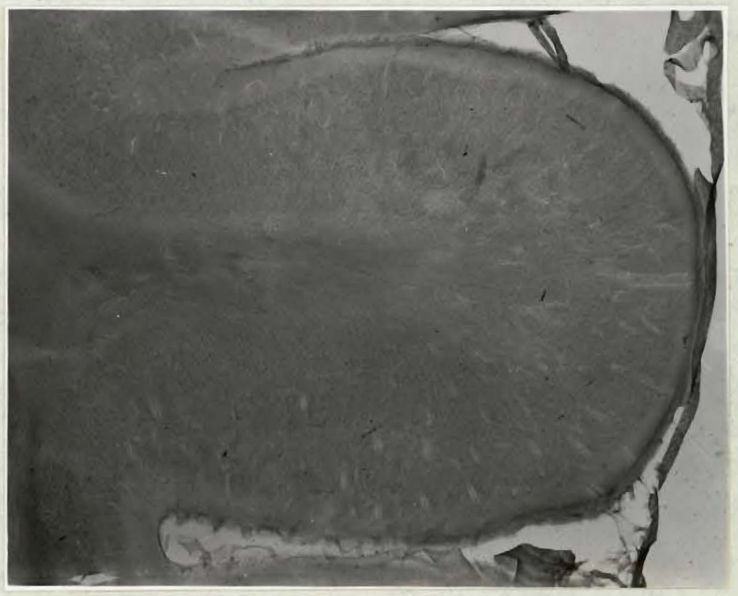

Fig. IO.

Figs. 11 and 12. Autoradiograph and histological section of a non-perfused brain. Note the blood vessels filled with blood. Histological section - benzidine stain and basic fuchsin counterstain.

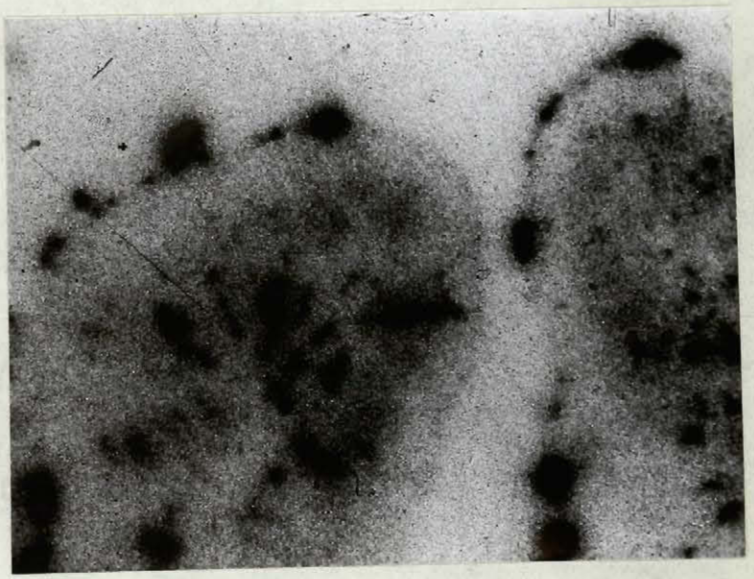

Fig. II.

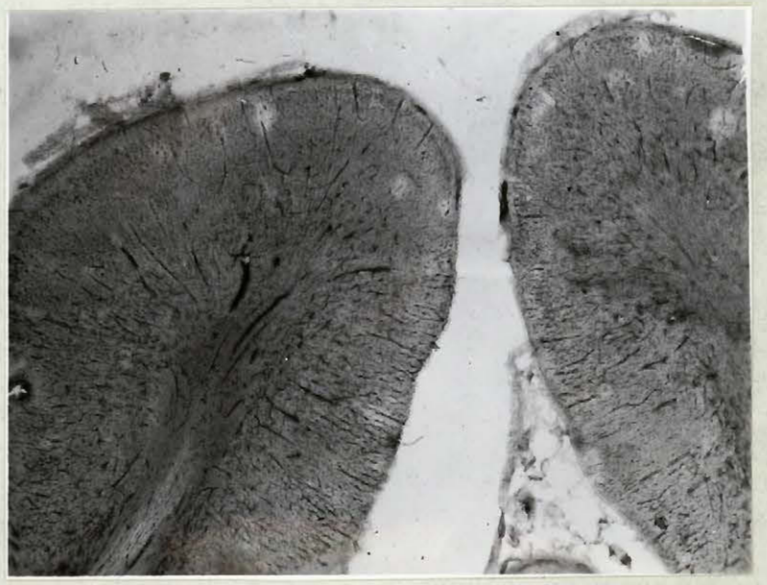

Fig. I2.

Figs. 13, 14. Convulsive seizures. Positive prints obtained from autoradiographs showing some concentration of radio isotope in the gray matter of the cortex and basal ganglia. Note increased radioactivity in the depth of the sulci.

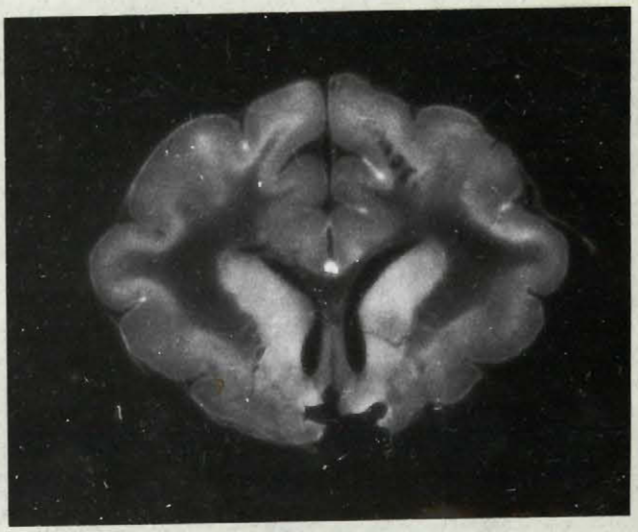

Fig. I3.

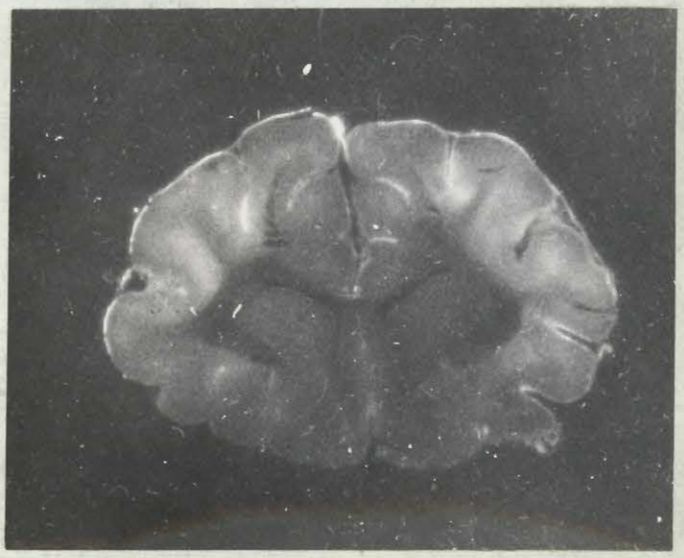

Fig. I4.

Fig. 15. Convulsive seizures. Autoradiograph of cerebellum. Increased tracer concentration in the vermis (positive print).

Fig. 16. Convulsive seizures. Autoradiograph of brain. Note high uptake of radioactivity in the thalamus and geniculate bodies.

Fig. I5.

Fig. I6.

Figs. 17 and 18. Convulsive seizures. Autoradiograph (negative print) and the corresponding histological section stained with basic fuchsin. Note the distribution of radioactivity in relation to the individual nuclear groups.

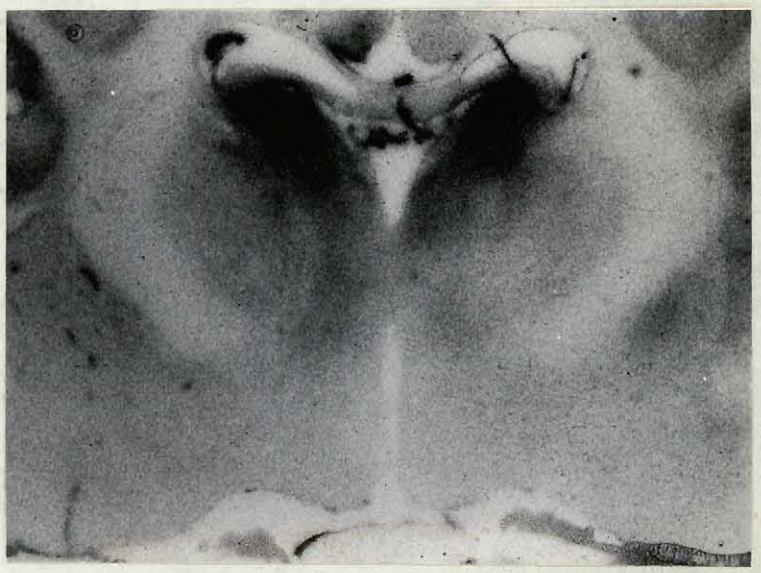

Fig. I7.

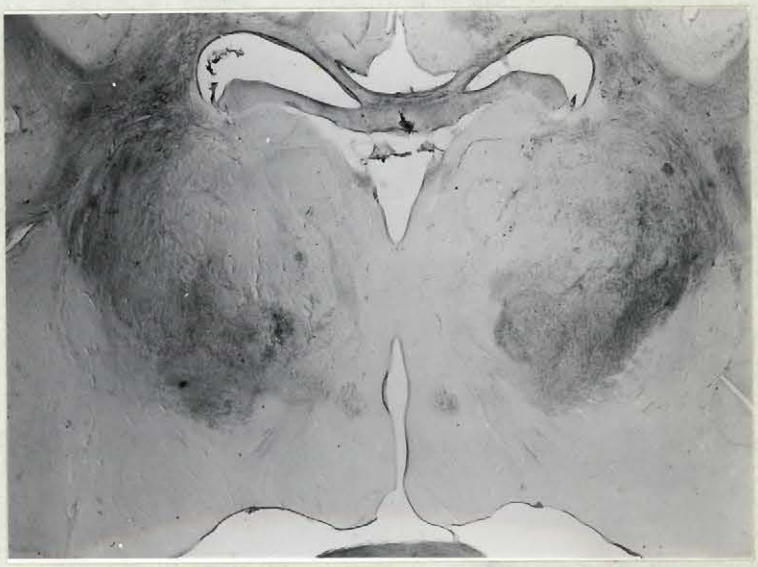

Fig. I8.

Figs. 19 and 20. Convulsive seizures. Diencephalon in autoradiograph and the corresponding histological section. Note the distribution of the radioactivity in the individual nuclear groups.

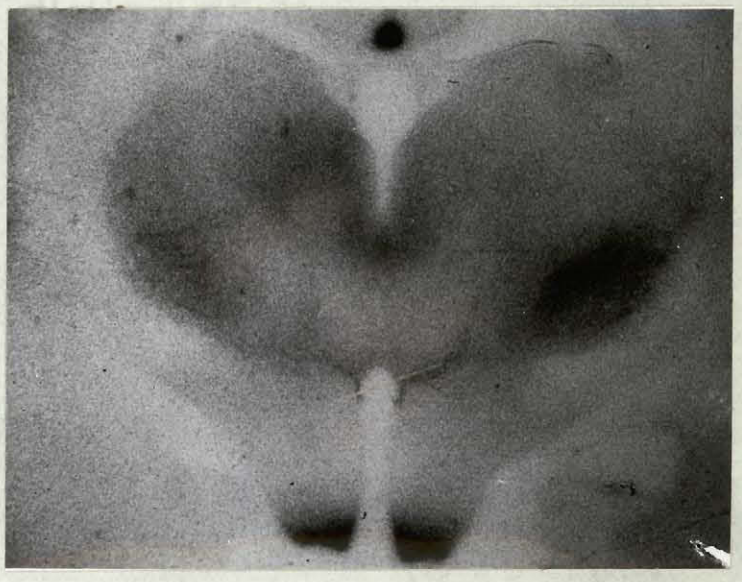

Fig. 19.

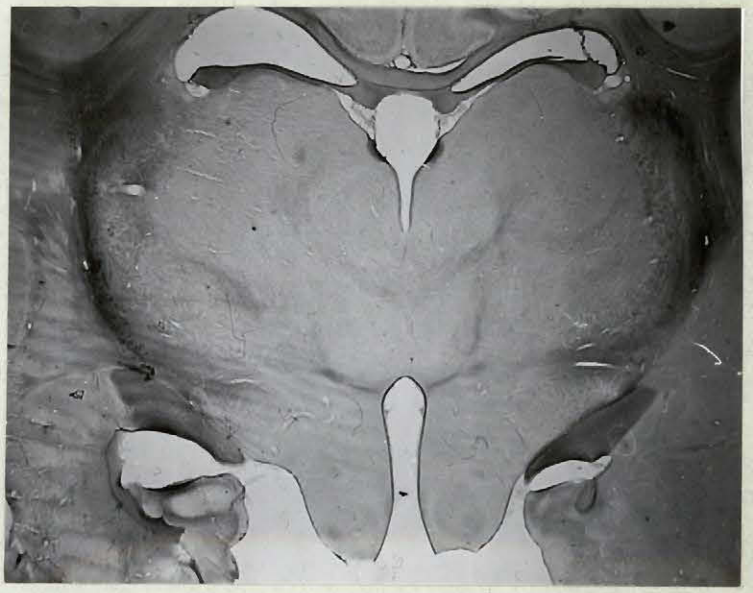

Fig. 20.

Figs. 21 and 22. Convulsive seizures. Thalamus. Autoradiograph and original histological section.

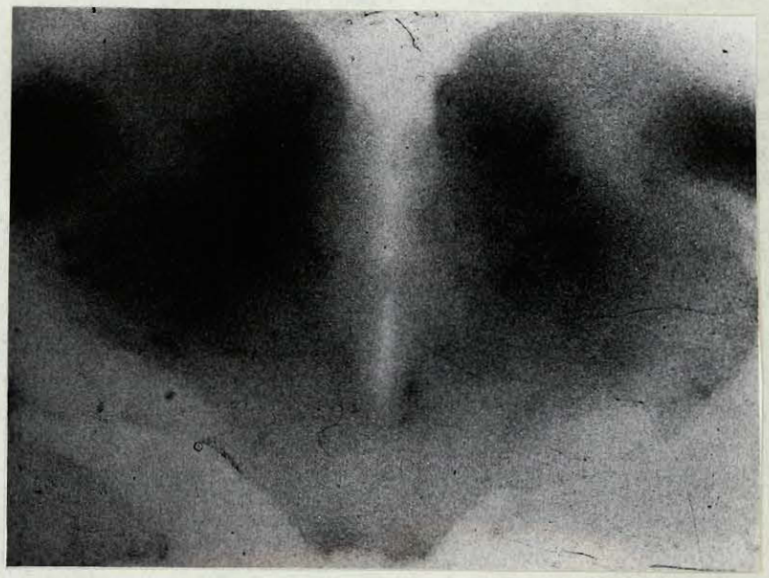

Fig.2I.

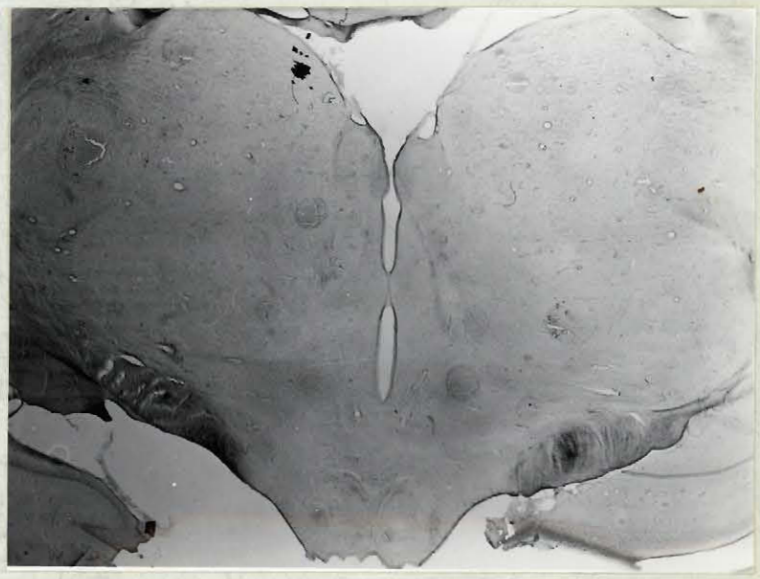

Fig. 22.

Figs. 23 and 24. Convulsive seizures. Autoradiograph (negative print) and corresponding area in the histological section.

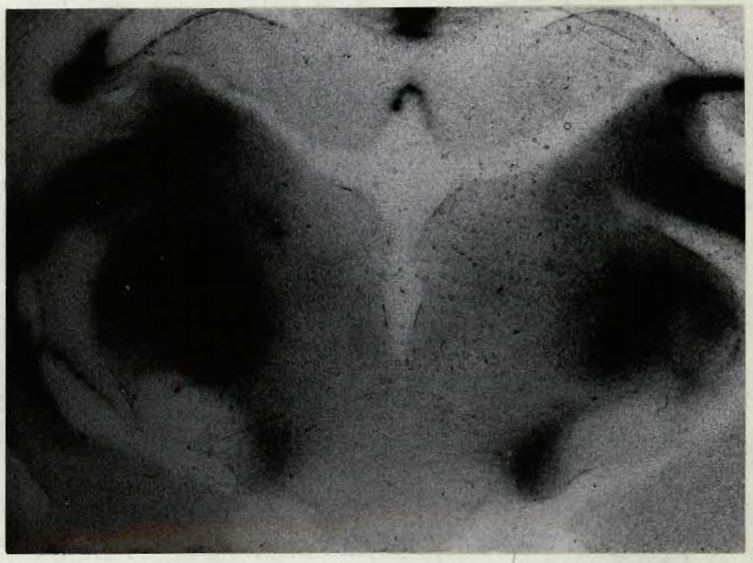

Fig. 23.

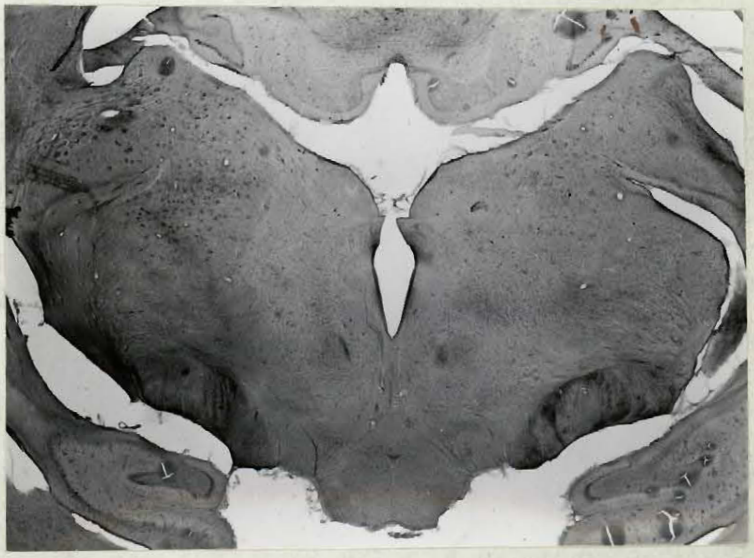

Fig. 24.

Fig. 25. Autoradiograph of brain of a cat which was subjected to 10 convulsive seizures and operated on immediately.

Fig. 26. Autoradiograph of brain of a cat which was subjected to 10 convulsive seizures and survived 5 hours.

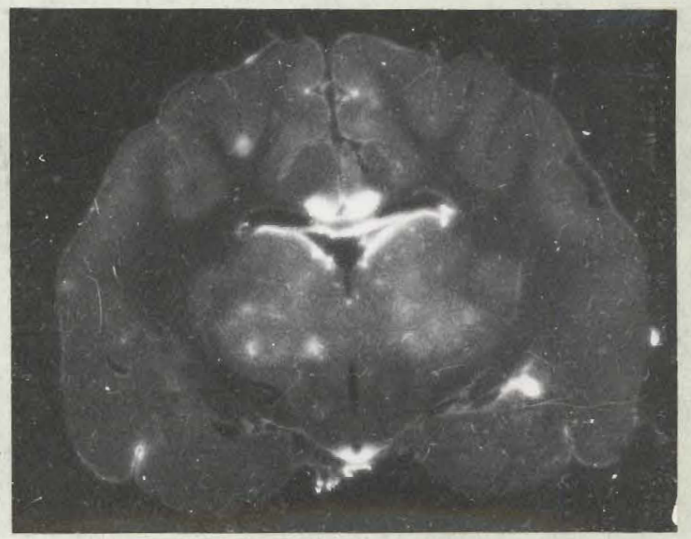

Fig. 25.

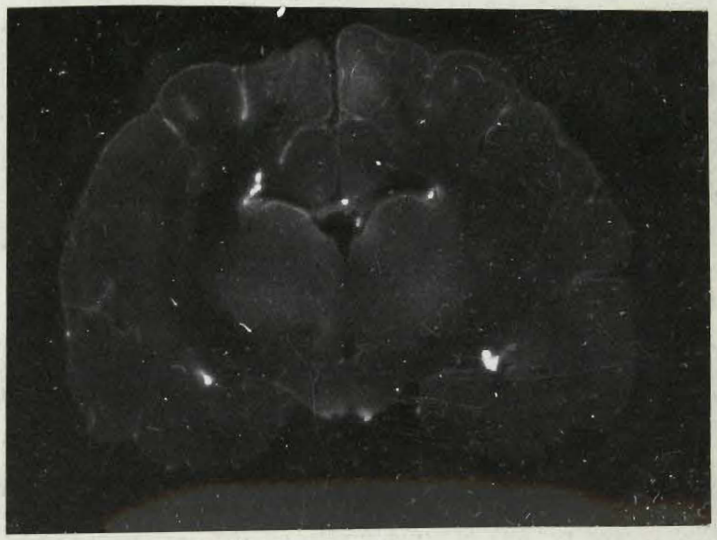

Fig. 26.

Fig. 27. Autoradiograph of a brain of 30-seizures animal sacrificed immediately.

Fig. 28. Another 30-seizures animal which survived for 3 hours.

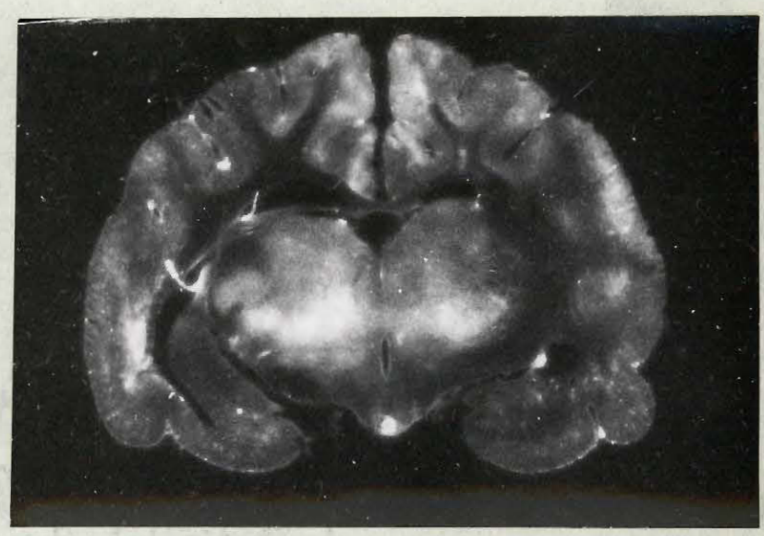

Fig. 27.

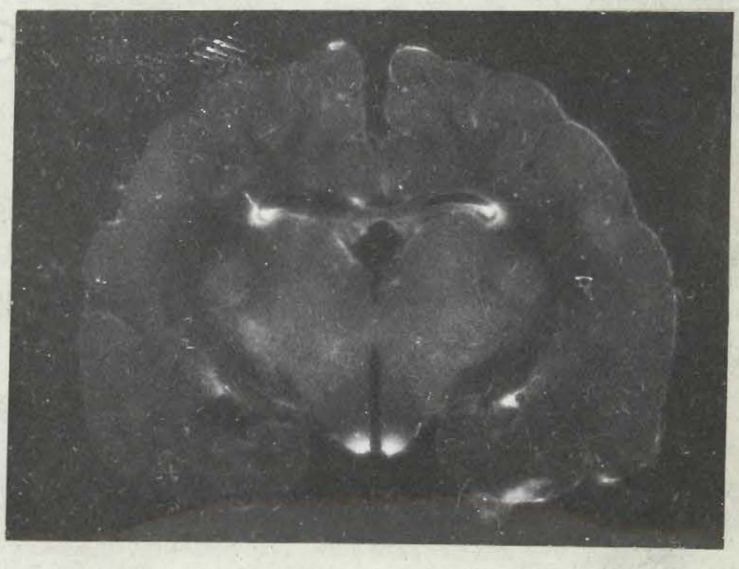

Fig. 28.

Fig. 29. Autoradiograph of brain of a 60-seizures animal injected with radioactive serum albumin prior to the treatment.

Fig. 30. Sixty-seizures animal which received radioactive serum albumin immediately following the last convulsive attack. Note concentration of radioactivity in the hypothalamus and pituitary only.

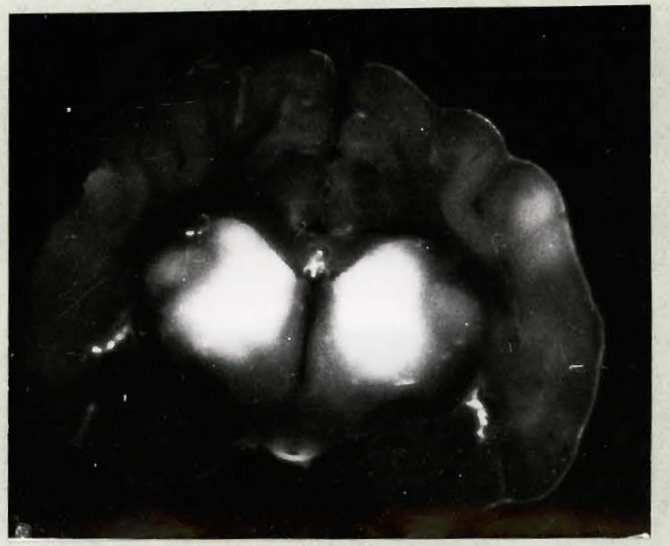

Fig. 29.

Fig. 30.

Fig. 31. Brain edema. Petechial hemorrhages in the cerebral cortex and subjacent white matter produced some uptake of radioactivity. There is also some tracer concentration in the meninges.

Fig. 32. Protective action of trypan red. The animal received 60 convulsive seizures.

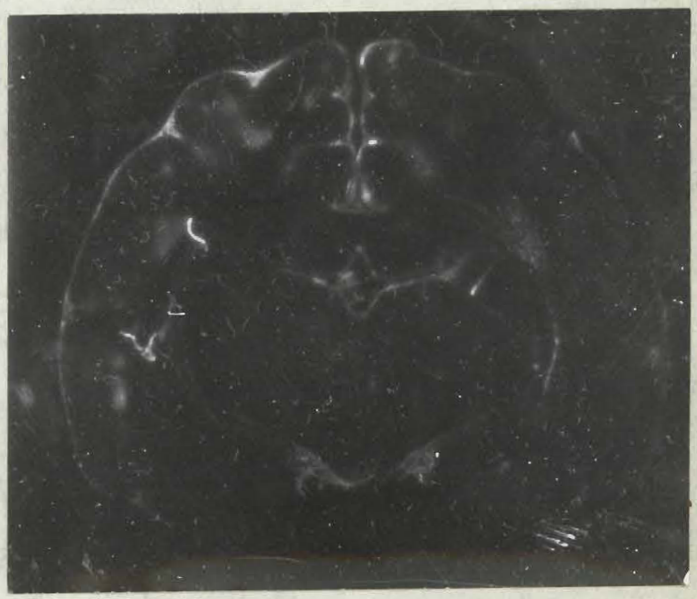

Fig. 31.

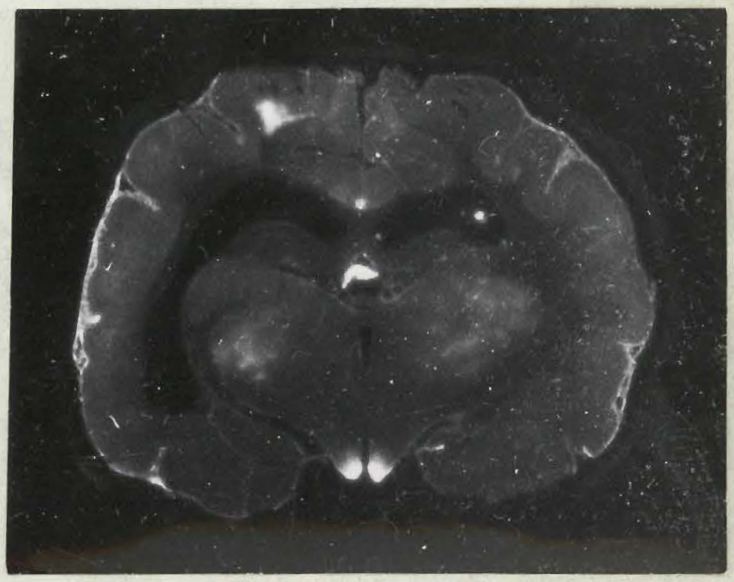

Fig. 32.