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EXPERIMENTAL RADIO-NECROSIS OF THE BRAIN IN RABBITS

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Wide acceptance of the statement that the central nervous system is relatively insensitive to *x* rays has undoubtedly encouraged the use of excessive doses for the treatment of lesions in and about this region. A series of human cases has been reported (Pennybacker and Russell, 1948) in which massive focal necrosis and gliosis of the brain followed at some period after therapeutic irradiation. The latent period varied from nine months to five years. In two of these (Cases 1 and 2) the changes occurred in areas that were presumed, on reasonable grounds, to have been previously normal; in the remainder the picture was complicated by a pre-existing or co-existing tumour. Nevertheless the general pattern of the tissue alterations was consistent throughout the series, and agreed with descriptions already published by others.

We concluded, from the histological appearances in the human material, that the degeneration, necrosis, and hæmorrhage were brought about by profound circulatory disturbances resulting from the vascular changes also demonstrated. These included fibrinoid necrosis of the walls, thrombosis, and pronounced collagenous thickening of the perforating vessels. The wide-spread distribution of extra-vascular fibrin filaments throughout the affected areas of the cerebrum, and independent of hæmorrhage, pointed to an increased permeability of the vessels as a part of the process. And in Case 1 the presence of granules of thorotrast in and outside the walls of vessels in the affected area provided additional evidence of this.

But any estimate of the precise sequence of events in such a fully developed lesion must be largely guesswork unless this can be studied experimentally Hence an attempt has been made, in the work to be described, not only to reproduce the lesions seen in the human subject but also to study their evolution and, in particular, the part played by structural alterations in the blood vessels. These experiments were briefly mentioned, in an incomplete stage of the work, in an earlier communication (Russell, 1946).

Experimental

Monkeys and dogs not being available for our purposes we used rabbits. Our first experiments, using a single trial surface dose of 2,850 r, proved successful in the induction of lesions, accompanied by neurological disturbances, after a latent period of about a hundred days. We then examined the brains of similarly treated animals at different periods before the time at which symptoms were anticipated in order to demonstrate earlier stages. In other experiments we reduced the *x*-ray dosage to find the level at which histological changes would fail to appear, and to examine the relation between the dose given and the latent period.

Technique.—Unanæsthetized young adult rabbits of no special breed received a single dose of *x* rays directed to the left side of the skull, the scalp being unshaved. The radiation was generated at 200K (Villard Circuit) filtered by 0·5 mm. Cu. and 1·0 mm. Al to give a half-value layer of 0·95 mm. Cu. Field size 3×4 cm.; surface dose-rate 154·5 r per minute. Focus—skin distance 25·5 cm. With the conditions the approximate depth doses are: 1 cm. depth, 85 per cent.; 2 cm. depth, 70 per cent.; 3 cm. depth, 57 per cent. Group I (13 animals) received 2,850 r; Group II (5 animals) 2,440 r; Group III (2 animals) 2,030 r; Group IV (2 animals) 1,625 r. The dose stated is that at the surface in each case, delivered as one exposure.

Under these conditions a rectangular patch of epilation appeared in the scalp, in animals surviving one month or longer, which was bounded medially by a sagittal line corresponding either to the centre of the skull or a few mm. to the right of it; anteriorly it generally fell just behind the palpebral fissure; laterally it overlapped the temporal muscles and passed backwards to skirt the base of the left ear and usually extended over the back of the skull covering the left side of the cerebellum. In animals of Groups I and II the skin at late stages lost its suppleness and became dry and scaly. In those of Group I which survived longest the centre of the affected skin sometimes became ulcerated and scabbed.

The animals were killed by bleeding them from the carotid arteries when deeply anæsthetized with chloroform. The brain was then removed, fixed in 10 per cent. formaldehyde, and divided coronally, when hardened, with slices about 2 mm. thick. The cerebellum was obliquely divided on each side so that the paraflocculus remained attached (Fig. 1). Most of the blocks were embedded in paraffin wax, but some were cut on the freezing microtome and stained by various silver techniques, and for fat. Paraffin sections were stained with Ehrlich's hæmatoxylin and eosin, Weigert's iron hæmatoxylin and van Gieson's mixture, Weigert's fuchselin for elastic fibres and neutral red, and phosphotungstic-acid hæmatoxylin.

Group I

2850 r: Thirteen Animals

Apart from one rabbit, which died of intercurrent disease on the twenty-first day after irradiation, all remained in normal health until sacrificed or, if allowed to survive, until about the hundredth day. On the ninety-ninth, hundred and third, and hundred and fourth days respectively three of the animals became irritable, lost interest in their food, and developed neurological signs including transitory attacks of head-retraction accompanied by salivation, circling movements which were usually towards the right, and phases of nystagmus. Two of these animals were killed, at forty-eight hours and fourteen days respectively, after the development of symptoms; the third was allowed to survive for a further six weeks. By this time it appeared blind and unable to feed itself and was therefore killed. These three rabbits, which will be referred to as "post-symptomatic" examples, all showed extensive irradiation lesions of the brain.

The remaining nine animals of the series were killed before the development of symptoms at seven, twenty-six, fifty-seven, seventy-three, seventy-six, eighty-two, eighty-five (two animals), and ninety days after irradiation, with the object of demonstrating earlier lesions. The search was successful in three; at eighty-two, eight-five (one animal) and ninety days. These will be referred to as "pre-symptomatic" examples. The remainder showed no histological changes in the brain. With the object of demonstrating possible changes in capillary permeability five of the rabbits were given an intraperitoneal injection of trypan blue (20 ml. of a 1 per cent. solution per kg. of body weight) twenty-four hours before death. These were killed at seven, twenty-six, fifty-seven, eighty-five, and one hundred and forty-three days after irradiation.

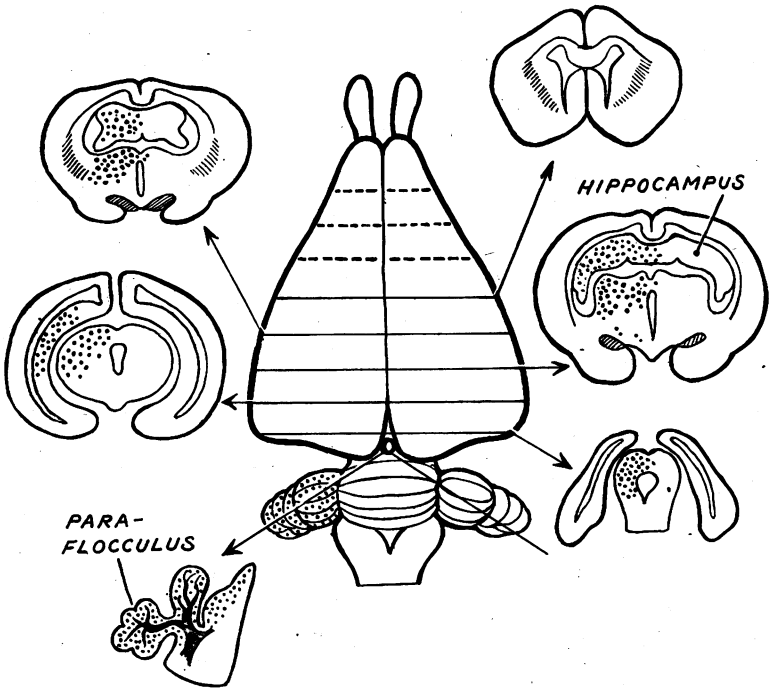


FIG. 1.—Diagram of rabbit's brain. Straight lines represent planes of section; arrows directed to show pattern of structures on corresponding cut surfaces. Dotted areas on latter represent sites of observed lesions.

Pathological Changes in Group I

Macroscopic. — Alterations in the skin of the irradiated area have already been noted. In none of the rabbits examined before eighty-two days were any further changes found. After this period dissection revealed fibrosis of the subcutaneous tissues; the scalp and temporal muscles were often abnormally adherent to the skull. The skull itself showed no change beyond pallor in the irradiated area when viewed by transmitted light. The meninges were invariably unaltered. The brain, too, showed as a rule no superficial changes beyond diffuse orange or café-au-lait pigmentation of the left side of the cerebellum (three animals; Fig. 2) accompanied, in one, by a few petechial hæmorrhages in the same areas. Occasionally the hind-end of the left cerebrum

appeared slightly fuller than on the right.

On section no alterations were found before the eighty-second day after irradiation. In the rabbit killed at this stage a limited area, about 4 mm. in diameter, of the left lobe of the cerebellum showed slight diffuse yellowish discoloration of the cortex, with a few punctate hæmorrhages. The paraflocculus was not affected. At eighty-five days (two rabbits) and ninety days no changes were visible to the naked eye. In the *post-symptomatic examples* there were ill-defined grey or yellowish-grey patches of discoloration in deep-seated parts of the hind end of the left cerebrum, the left basal ganglia, and left dorsal quadrant of the mid-brain (Fig. 1). The cerebral areas affected were the dorsal part of the left hippocampus, the left fimbria, and the adjacent parts of the basal ganglia. The folia of the left lobe of the cerebellum were shrunken and yellowish in those animals which showed superficial discoloration ; in one of these the paraflocculus was similarly involved, and in another there were a few petechiæ in the adjacent left side of the medulla oblongata. It is particularly noteworthy that the cerebral cortex in the irradiated area was unaltered except in the animal, killed at 143 days, which received trypan blue. This, the most advanced of the series, showed a diffuse patch of blue coloration over the lateral border of the hemisphere and similar staining of the necrotic foci in the deeper tissues.

Microscopic Examination.—No changes were identified in rabbits killed before the eighty-second day after irradiation. Early changes were found in the pre-symptomatic group and progressively later stages in the three post-symptomatic examples. No changes, however, were found in one of the two animals killed at eighty-five days.

The *earliest lesions* found, at eighty-two, eighty-five, and ninety days, consisted of minute ball- and ring-hæmorrhages, too small to be seen with the naked eye, which lay in the dorsal part of the left hippocampus, the adjacent lateral border of the basal ganglia, and the left dorso-lateral quadrant of the mid-brain (Fig. 1). The same areas, in the rabbit at eighty-two days, contained a few foci of anæmic necrosis of similar size. Both the hæmorrhagic and anæmic foci were generally near a capillary but sometimes surrounded an arteriole. The basement-membranes of these capillaries appeared rather blurred and stained feebly with the usual aniline dyes ; the endothelial cells were often swollen but were free from any demonstrable nuclear degeneration or fatty change ; the lumina contained discrete red corpuscles. Occasionally spheroidal particles, from about 4 to 8 μ, staining like fibrin with phospho-tungstic-acid hæmatoxylin, appeared in the tissues near the walls of these vessels. In silver preparations amœboid forms of microglia had collected in these perivascular foci and some cells had ingested red corpuscles, but as yet contained no Sudanophil droplets. There was no gliosis. Bielschowsky preparations showed well-preserved neurones in all areas, and even in the neighbourhood of the perivascular lesions. Axis-cylinders were fragmented in the centre of some foci, but in places traversed them intact.

Changes were present in the cerebellum in two of these animals, but appeared more recent in the rabbit killed at ninety days than at eighty-two days. In the former, a few cortical hæmorrhages occupied the layer of Purkinje cells and there was considerable focal degeneration of these cells. No vascular changes were observed. At eighty-two days, however, there were many more hæmorrhages, affecting all the cortical layers, distorting their pattern and accompanied by a good deal of œdema. There was a patchy loss of the Purkinje cells, especially at the summits of the folia, and associated here with gliosis resulting from multiplication of the Bergmann cells (Fig. 3). The perforating blood vessels were rather tortuous and, in silver preparations, showed collagenous thickening. Foci of microglial reaction were present in all layers, including the sub-cortical white matter, the appearances being similar to those already described in the cerebrum. In Bielschowsky preparations no notable changes were found ; the axis-cylinders were often tortuous but were intact except in areas of tissue destruction. A few " torpedoes " were however found attached to Purkinje cells.

The three *post-symptomatic examples* showed progression of the damage both in respect of the actual size of the areas involved and also in the presence of old as well as recent lesions. While the distribution of the lesions retained the same character as in those already described (Fig. 1) there appeared a tendency for the necroses and hæmorrhages to spread both ventrally and towards the right cerebrum, but in the cerebellum they did not cross the mid-line. In these cerebral extensions homologous structures were usually involved. Thus in the rabbit that survived to 143 days there were recent hæmorrhages and necroses even at the frontal poles, occupying the central white and adjacent grey matter ; the left pole was more affected than the right. Patches of necrosis also appeared in the optic tracts on either side of the tuber, thus accounting for the blindness (Fig. 4). Here again the left side was more affected than the right. In this animal the cerebellum escaped damage ; correspondingly it was noted that the area of epilation was more anterior than usual, its

anterior border being at the level of the palpebral fissure. In the animal killed at 118 days the cerebellar cortex of the left lobe showed advanced gliosis (Fig. 5), with destruction of many neurones and considerable collagenous thickening of the perforating blood vessels (Fig. 6). But progression of the lesion in the cerebellum here was also demonstrated by fresh hæmorrhages and necroses (Fig. 7).

The oldest lesions in the cerebrum of these three rabbits were confined to the sites where they were first identified in the pre-symptomatic group (Fig. 8). In and about these there was dense gliosis, the astrocytes being exceptionally large, with bulky cytoplasm and stout fibrillated processes (Figs. 9, 10). Iron-pigment within microglial cells indicated the sites of earlier hæmorrhages. Many fat-granule cells were found in the borders of the necrotic areas. Fresh necroses and hæmorrhages were, however, mixed with the older lesions in all areas where the latter were found (Fig. 11). In these three animals further pathological developments were found in the perforating vessels associated with the lesions already described. Thus in occasional arterioles and capillaries the walls had undergone fibrinoid necrosis (Figs. 12, 13), and some of these were occluded by thrombus. In the longest surviving rabbit a group of vessels in the left hippocampal gyrus showed great dilatation with thickening of the walls by œdema, fibrin, and reduplication of collagen fibres (Fig. 14). In this animal alone were a few hæmorrhages found in the subcortical white matter of the posterior third of the left cerebrum. In the area of cortex that was vitally stained with trypan blue there was an amœboid change in the microglial cells, but no further histological alterations were demonstrated. It is remarkable that, in the group as a whole, the cerebral cortex and meninges remained unchanged.

Group II

Five rabbits each received 2,440 r, the physical conditions being the same as before. Two developed intercurrent disease and were killed, at eighty-nine and 194 days respectively, after irradiation. They showed no cerebral changes due to the experiment. Two others spontaneously developed neurological symptoms of the kind observed in Group I at 239 and 240 days respectively, and were then killed. The fifth had several episodes of irritability, with indifference to food, at about 240 days; after this it recovered and appeared normal. It seemed well when killed at 355 days. These three rabbits all showed advanced changes, similar in distribution and character to those noted in the post-symptomatic members of Group I. In one animal, however (239 days), the cortex of the left cerebral convexity, at the coronal level of the tuber cinereum, showed recent hæmorrhages in the deeper laminæ. The cortex as a whole appeared slightly atrophied in this region. It is perhaps significant that this rabbit became suddenly ill, with irritability and retraction of the head upwards and to the left, and it flopped repeatedly on to its left side. There was a rapid flickering of the right eye, the left eye being almost closed and immobile. There was rapid, panting respiration and excessive salivation. This rabbit was killed two hours after the development of symptoms.

In comparison with Group I the vascular changes were more pronounced; not only was fibrinoid necrosis more conspicuous but there was, in addition, a good deal of hyaline collagenous thickening in occasional foci. It is noteworthy that, in all three animals, fresh hæmorrhages and anæmic necroses were conspicuous in addition to older lesions.

Group III

Two rabbits each received 2,030 r by the same technique. They were kept for fifteen and twenty-five months respectively and remained normal. Their brains showed no abnormality.

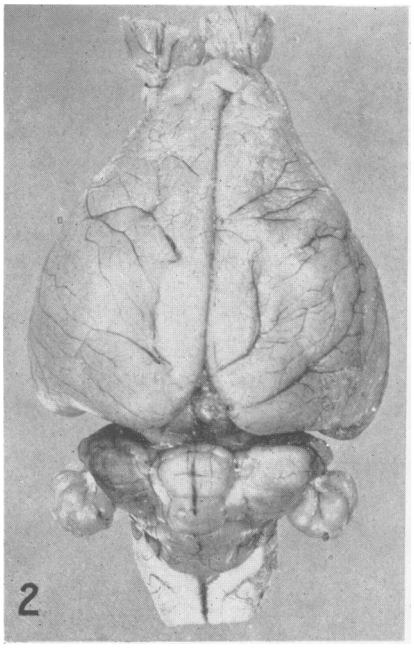
Group IV

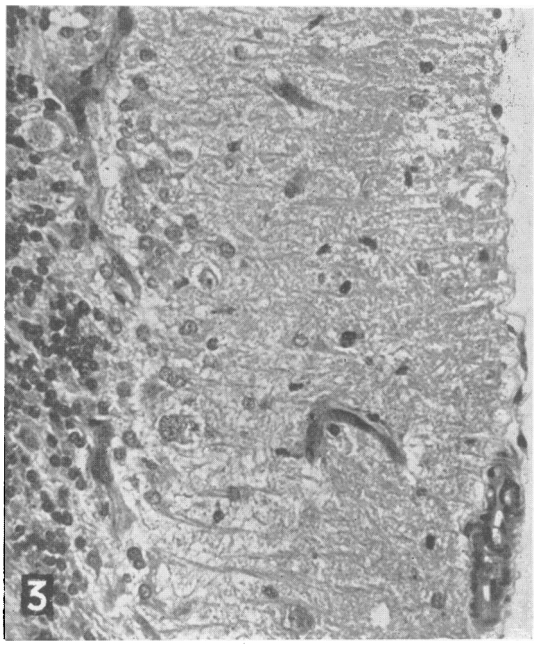
Two rabbits each received 1,625 r and were kept for fifteen and twenty-four months respectively. They also remained healthy and their brains were normal on microscopical examination.

Discussion

Comparison of the histological effects produced in these rabbits with those recorded in human material (see literature in Pennybacker and Russell, 1948) shows a close correspondence in the range and character of the delayed lesions that arise in the brain after *x* irradiation. It is important to emphasize the existence of this latent period, during which the subject may enjoy normal health and vigour, because certain more diffuse, mainly neuronal, changes have been described as the *immediate* effect of *x* irradiation in experimental animals. We are not concerned with these here.

Delayed necroses of the brain, associated with degenerative and obliterative changes in the blood vessels, have been produced by *x* rays in the dog by Lyman and others (1933). Of the four dogs used, one survived for six months and showed lesions of this kind; in the other three, which were killed at seven days, five weeks, and six weeks, there was no obvious change in the first and "acute meningo-encephalitis" was described in the other two. Davidoff and others (1938) produced a variety of local effects in the brain in a series of





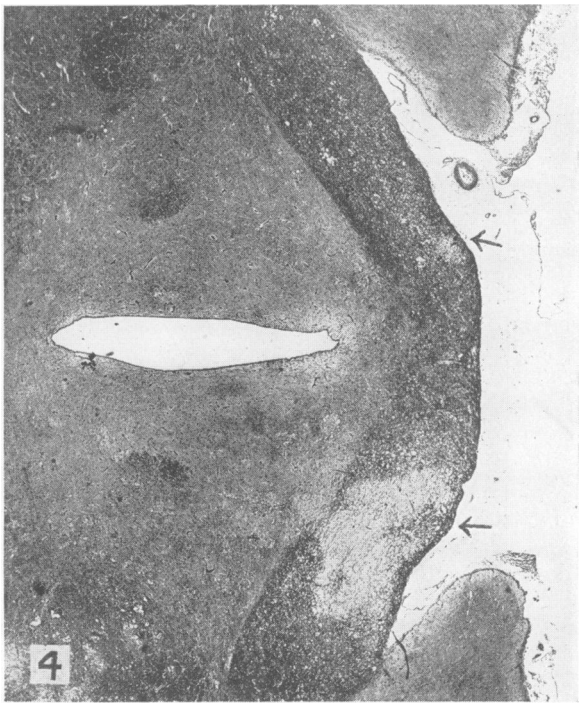
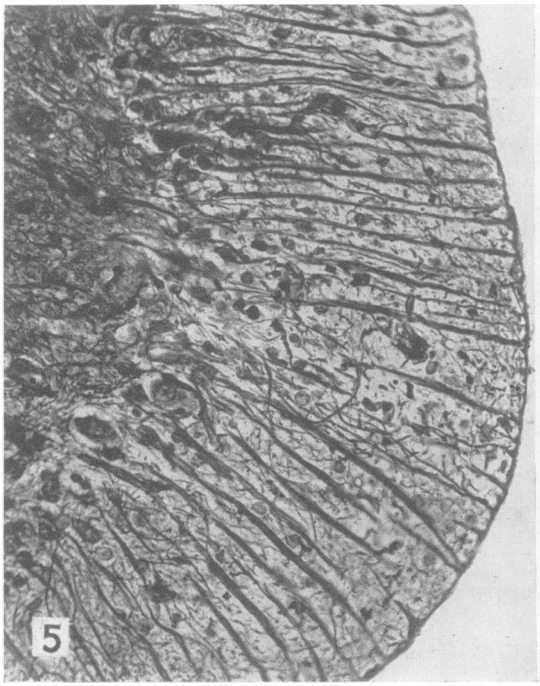
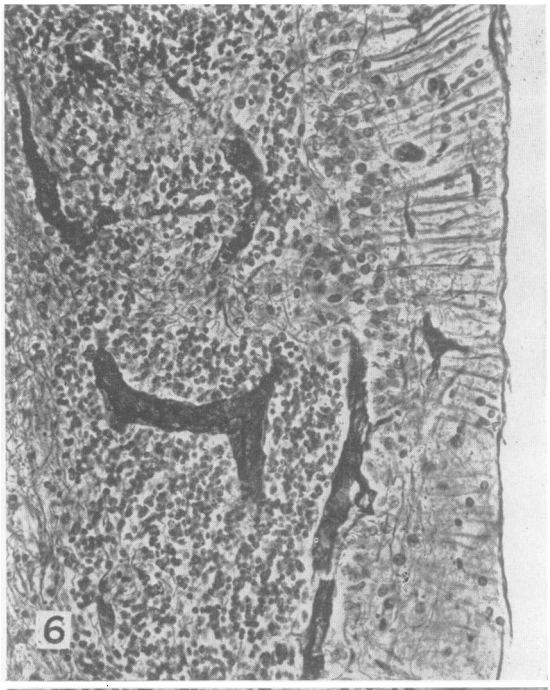


FIG. 2.—Group I; 101 days. Brain showing pigmentation of left lobe of cerebellum.

FIG. 3.—Group I; eighty-two days. Cerebellar cortex showing loss of Purkinje cells and early multiplication of Bergmann cells. Hæmatoxylin and eosin, ×240.

FIG. 4.—Group I; 143 days. Arrows indicate pale areas of necrosis in optic tracts. Phosphotungstic-acid hæmatoxylin, ×12.





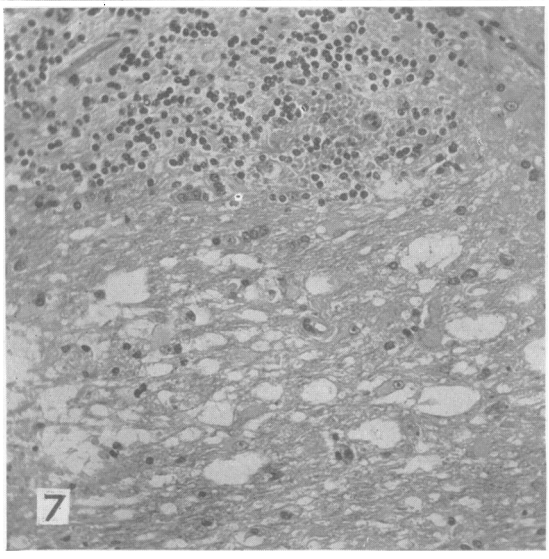


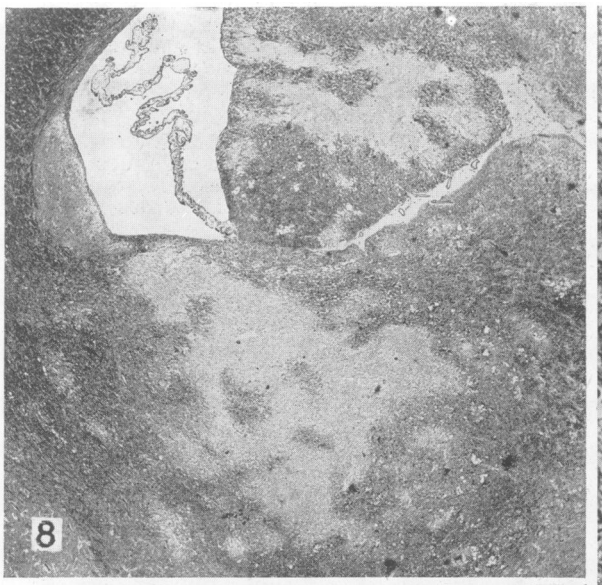
FIG. 5.—Group I; 118 days. Cerebellar cortex to show radial gliosis. Silver carbonate, ×190.

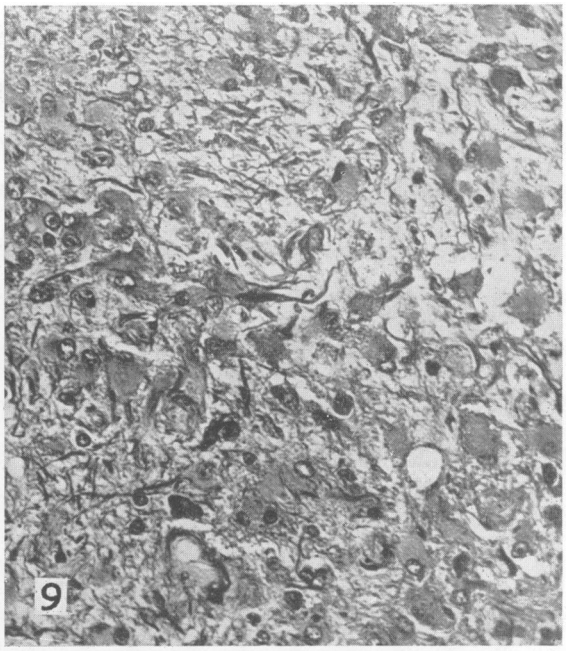
FIG. 6.—As in Fig. 5. Destruction of cortical pattern and collagenous thickening of blood vessels in another area. Silver carbonate for reticulin, ×160.

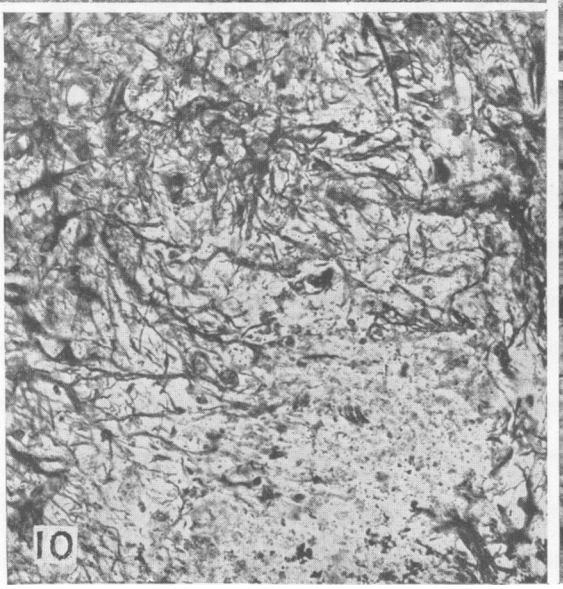
FIG. 7.—As in Figs. 5 and 6. Deeper area showing a fresh hæmorrhage in border of granule-cell layer and vacuolar necrosis in adjacent white matter. Hæmatoxylin and eosin, ×160.

rhesus monkeys. They varied their single dose of *x* rays from 5,000 to 1,000 r and obtained, with doses of from 2,000 to 3,000 r, a hemiparesis that developed after several months due to softening in the irradiated hemisphere. Although they observed occasional hyaline changes and collagenous thickening of related blood vessels, they inclined to the view that the primary effect of the *x* rays was upon the parenchyma of the nervous system.

From our own observations, both on human material and in these rabbits, it appears that vascular damage plays an important, if not determining, part in the production of the lesions though it may not be responsible for the complete histological picture. Thus the earliest change recognized, in rabbits killed shortly before neurological symptoms were anticipated, consisted of minute perivascular hæmorrhages and anæmic necroses. Gliosis quickly







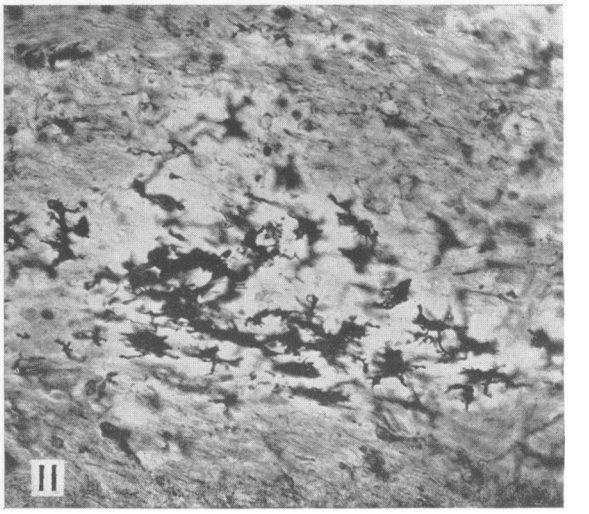


FIG. 8.—Group I ; 143 days. Pale areas of necrosis in left basal ganglia and fimbria (above). Phospho-tungstic-acid hæmatoxylin, × 15.

FIG. 9.—As in Fig. 8, to show gliosis at border of necrotic area, × 270.

FIG. 10.—As in Fig. 9, to show glial fibres. Weil-Davenport, × 190.

FIG. 11.—As in Fig. 10, showing adjacent early necrosis with active microglial reaction, × 230.

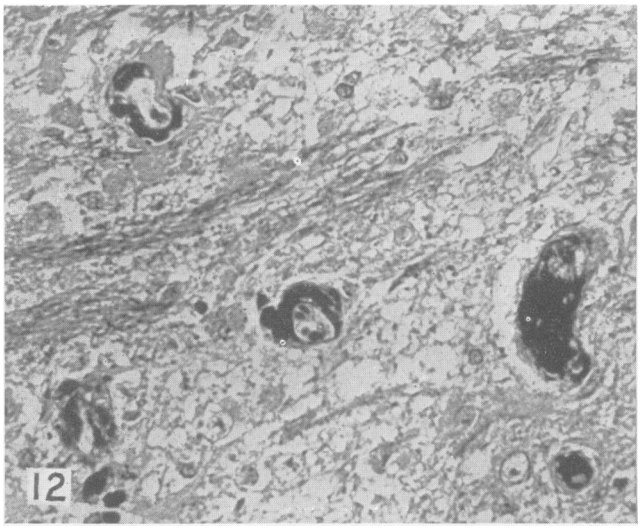
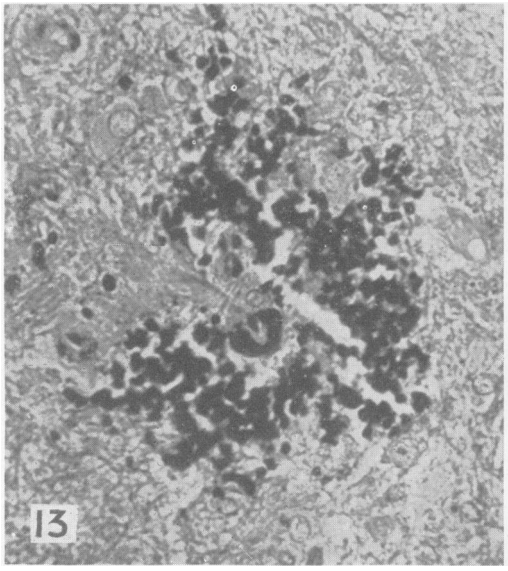
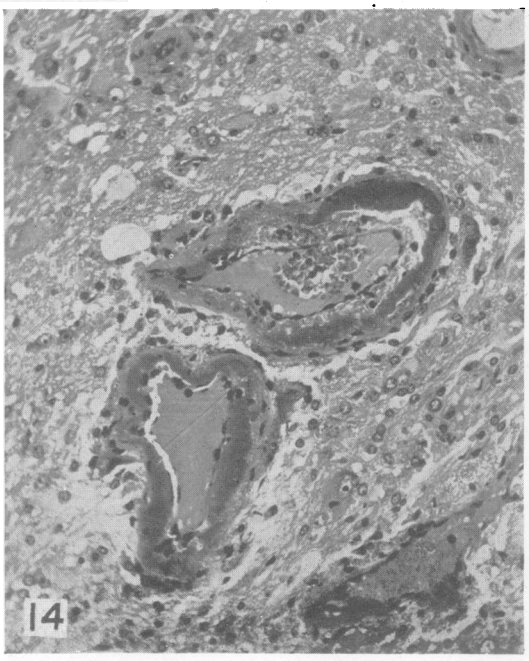


FIG. 12.—Group I ; 101 days. Vessels in left basal ganglia showing fibrinoid necrosis of walls and (right) thrombosis. Phosphotungstic-acid hæmatoxylin, ×360.

FIG. 13.—Group I ; 118 days. Recent hæmorrhage in mid-brain about vessel showing fibrinoid necrosis. Phosphotungstic-acid hæmatoxylin, ×400.

FIG. 14.—Group I : 143 days. Vessels in left hippocampus showing expansion of walls by fibrinoid material, œdema and collagenous thickening. Hæmatoxylin and eosin, ×190.





followed. At the earliest stage no alteration could be demonstrated in the walls of these vessels beyond swelling of the endothelial cells and poor definition of the basement membranes of the capillaries. In rabbits killed two or three weeks later the vessels occasionally showed a frank fibrinoid necrosis sometimes accompanied by thrombosis (Figs. 12, 13). Collagenous thickening was demonstrated in addition (Fig. 14).

Irradiation necrosis, when once initiated, appears to progress with remarkable rapidity. In our animals it began in the irradiated area and later extended more widely beyond the immediate field. This was demonstrated in the rabbit that survived six weeks after the onset of symptoms. The distribution of the initial lesions was remarkably constant and, at first sight, curious in that the deeper parts of the left cerebrum were affected whereas in the cerebellum the cortex appeared to bear the main brunt. Possibly the greater thickness

of the skull over the hind-brain may have been a factor in this, although even an additional thickness of 2 mm. would not have absorbed more than 8 per cent. of the radiation. The immunity of the meninges and of the cerebral cortex was remarkable. In only two rabbits were lesions demonstrated in the cortex, and they were confined to the deeper strata.

Though the number of animals irradiated was small there was a definite indication, comparing Groups I and II, that reduction of the dose of *x* rays (from 2,850 r to 2,440 r) more than doubled the latent period. For this reason it was considered advisable to maintain the animals of Groups III and IV for a proportionately longer period (up to two years). From the absence of any demonstrable lesions in the latter groups it seems likely that the minimal single surface dose of *x* radiation of the quality used that will evoke delayed radio-necrosis in the rabbit's brain lies somewhere between 2,440 and 2,030 r.

The pathogenesis of this delayed radio-necrosis is difficult to conceive. Close microscopic examination of the tissues during the latent period has yielded no clue. And when the earliest foci of hæmorrhage and necrosis are detected it is difficult to interpret their pathogenesis in the absence of any fatty or fibrinoid changes in the walls of the associated blood vessels. Fibrinoid necrosis soon appears, but it has not, in these animals, preceded the parenchymal changes. It may, however, be supposed that some alteration in the permeability of the vessel walls, unaccompanied at first by any obvious structural changes, initiates the pathological processes. The presence of small spheroidal particles, with the staining qualities of fibrin, that are found about some of the vessels in the earliest stages would support this hypothesis. Scholz and Hsü state that there was some indication, in two of their schizophrenic patients, that an increase of permeability took place at the end of four and a half to six and a half weeks respectively after irradiation. But they do not give the evidence for this statement. The widespread distribution of fibrin and the presence of extravascular granules of thorotrast (Case 1) in our human examples (Pennybacker and Russell) indicate an increased permeability when the lesions are advanced. Similarly a local increase of permeability was demonstrated in the most advanced of our series in Group I, when trypan blue was injected before death (see above p.189). But a similar test performed in the pre-symptomatic stage yielded negative results at seven, twenty-seven, fifty-seven, and eighty-five days. It is of interest that, in the last of these, early lesions were identified with the microscope. There is therefore no evidence from our experiments that an increase in vascular permeability precedes the development of parenchymal lesions.

It cannot of course be assumed that the evocative dose of *x* rays for the rabbit's brain will be followed by similar changes in other species. One of us (K.T.) subjected a series of mice to the dose received by the rabbits of Group I: neither histological effects nor neurological symptoms were noted in survivors up to twenty weeks.

Conclusions

A single *x*-ray dose of 2,850 r, under the physical conditions that have been defined, will produce delayed irradiation-necrosis in the rabbit's brain after a latent period of approximately one hundred days.

The latent period is lengthened by reducing the dose to 2,440 r.

The initial histological changes consist of minute foci of hæmorrhage and necrosis which are intimately related to the perforating vessels and, in particular, capillaries. Increase of capillary permeability accompanied the established lesion but was not demonstrated at earlier stages.

The lesions, when once initiated, appear to extend rapidly, and to progress to non-irradiated parts of the brain. They are characterized, in later stages, by pronounced gliosis, and by progressive degeneration and sclerosis of the adjacent vessels.

The neurones are only focally and secondarily affected.

The meninges and their blood vessels are unaffected, and the superficial parts of the cerebrum escape, although the cerebellar cortex may be extensively damaged.

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