Thesis

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THE EFFECTS OF IONISING RADIATION AND CHEMICAL CARCINOGENS ON THE MAMMALIAN CENTRAL NERVOUS SYSTEM.

ABSTRACT

This investigation was aimed at a better understanding of the effects of single doses of ionising radiation on the mammalian brain, with particular reference to delayed white matter necrosis and vascular lesions. The effects of previous local irradiation on cerebral injury and infection with B. pertussis were also investigated.

Hypertension induced by renal damage was shown to lower the threshold dose for the appearance of vascular lesions from 2,000R to 1,00(The latent period was shortened for doses of 2,000 - 3000R. However, with higher doses of 4,000R no shortening of the latent period occurred and animals live for the same length of time as the comparable group of normotensive animals. Theories to explain the phenomenon of vascular irradiation damage were discussed.

Cerebral injury and infection with B. pertussis were used to reveal radiation damage to neuroglial cells. The effect of doses as small as 200R could be demonstrated three days after irradiation.

Cerebral injury and infection in conjunction with previous local irradiation, provided evidence to support the view that brain macrophages are principally of local origin and are only to a limited extent derived from the general circulation.

Evidence is provided to support the general theory that radiation damage is revealed when cells attempt mitosis. In the brain there is an exceptionally slow mitotic replacement of cells and this accounts for the extremely long latent periods observed.

Radiation of the developing brain under different oxygen tensions

demonstrated protection from radiation when animals breathed low tensions of oxygen as compared with high tensions.

Many glial tumours developed following the deep implantation of chemical carcinogens into the cerebral hemispheres so as to involve the subependymal layer. When the carcinogen was placed superficially no glial tumours developed. Tumours appeared earlier in males.

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CHAPTER 1

HISTORICAL INTRODUCTION

A. (i) Irradiation of the central nervous system

Morphological effects of ionising radiation on the mammalian central nervous system (C.N.S.) were first observed by Obersteiner (1904,1905) shortly after the discovery of radium and x-rays. He observed that mice exposed to radium, and dying within 12 hours and 66 days after irradiation, showed haemorrhagic changes in the meninges, cerebral cortex, hippocampus, basal ganglion and spinal cord. Schels (1904) observed similar changes in radium irradiated rabbits.

Since this time, the effects of ionising radiation on the central nervous system have been studied very extensively, spurred on, to some extent, by the early use of x-rays in the treatment of intracranial tumours. The first reported case of the use of x-irradiation for the treatment of an intracranial tumour was by Gramegna (1909). He reported "temporary subjective improvement", after irradiation of the sella by directing x-rays through the mouth. A review of the early literature was given by Warren (1943).

There is general agreement that the central nervous system is relatively resistant to radiation damage. A dose of 500R to the mouse spinal cord will have no effect on the total number of nerve cells, even in old age. (Wright and Jacobs 1966).

In a recent review by Haymaker (1962), it was stated that single doses of x-irradiation up to 1,000R produced no early or late morphological changes in the brain or spinal cord, and animals lived into old age. With doses higher than this, 2,000-4,000R, morphological changes did occur, but only after a latent period of several months (Russell et.al.1949, and Berg and Lindgren 1958). The relationship between the latent period and irradiation dose being exponential (Zeman 1961), although there was much individual variation.

With higher single doses there was evidence of early cell death with the appearance of pyknotic and necrotic nuclei in neurones and interstitial cells, so that after an exposure of 3,000R, early cell death was the predominant feature (Arnold et.al.1954b). Single doses of the order of 10,000R or more, will produce direct cell death to such an extent, that the delayed radiation damage effect can no longer occur, "the cells capable of producing this effect being largely destroyed" (Zeman 1961).

With doses of 50,000R the survival time (for air breathing animals) was cut to between 200-300 minutes (Wright and Shewell 1965).

Of these doses, it is only those up to the intermediate dose ranges, 2,000-4,000R single doses, or their equivalent fractionated doses, that are of practical value. It is these doses which are used in the treatment of intracranial tumours and result in delayed radiation damage of the central nervous system.

(ii) Theories of latent C.N.S. irradiation damage

Although the phenomenon of latent C.N.S. irradiation damage is well documented, both from a study of experimental animals and human material, the process by which it occurs is still not fully understood. The long latent period is particularly interesting because of the apparent lack of demonstrable physiological, biochemical and morphological changes until shortly or immediately before the onset of overt radiation damage.

This has provided an interesting field for speculation, as to the pathogenesis of these latent irradiation lesions. Several theories have been advanced which can perhaps be divided into two broad groups. The first of these is that the irradiation acts primarily on the vascular system and that any necrotic changes are secondary to ischemic anoxia. The other theory suggests that the primary reaction is on the neurones or neuroectodermal cells (glia) or both, and that damage to the vascular system is of minor importance. Since these two theories were suggested, considerable controversy has developed as to which cellular element determines the sensitivity of the nervous system.

The literature in support of these two conflicting views will now be reviewed.

(a) Primary vascular damage.

In a review covering many of the earlier findings in human brains treated by x-rays or radium therapy (for cerebral tumours), Lyman (1933)

expressed the view that the majority of changes observed could be explained by a primary effect on the vascular system and that effects on the nervous tissue were of secondary importance. This statement he supported by observations on four dogs, irradiated with 18-20 erythema doses. In these animals, vascular damage predominates with occasional associated small areas of necrosis, in the cerebral cortex, optic chiasma, hypothalamus and corpus callosum. This is a view supported by Warren (1943), with primary vascular damage resulting in ischemic changes in neural tissue, the degree of damage depending upon the length of latent period. Primary damage to the nervous tissue did occur, but this was not considered to be important.

The vascular hypothesis was further amplified by Schels in 1934 and reaffirmed later (Schels et.al.1962), that vascular damage increased vascular permeability (see also Clemente and Holst 1954) producing oedema, with eventual neuronal and myelin degeneration. These changes were, to a large extent, selective, producing white matter necrosis of the rabbit's spinal cord, with doses ranging from 3,000 - 11,000R x-rays. This is a feature demonstrated by Berg and Lingren (1958), who were also of the opinion that the primary damage was of vascular origin. Irradiation of rabbit brain with doses of 2,100 - 3,000R, showed that cystic necrosis of white matter increased with irradiation dose (2,500 - 3,000R), while small foci of damage predominated with the lower dose (2,100R).

With doses of 1,500 - 2,850R the predominance of vascular lesions was stressed (Marberg et.al.1945, Russell et.al.1949).

The latter also expressed this view after the examination of four human brains (Pennybacker and Russell 1948), although they did mention evidence of glial reaction in one case; "Case 4 showed loss of pyramidal cells from the cerebral cortex with associated proliferation of large abnormal astrocytes".

Supporting the view of the predominant role of vascular damage, is the observation that radiation induced pathogenic processes may become progressive and spread into non-irradiated parts of the brain (Russell et.al.1949), although Berg and Lindgren (1958) claim that x-ray lesions are strictly limited to the irradiation field. The rich capillary bed of the hypothalamus, it was postulated, (Haymaker et.al.1962) was responsible for the high radiosensitivity of this region.

The recent findings of Asscher and Ansen (1962), that hypertension apparently facilitates the development of vascular lesions in the irradiated (1,500 - 3,000R) rat spinal cord adds weight to the hypothesis of primary vascular damage.

The findings of Crane and Dutta (1963), enable us to explain the phenomenon of vascular irradiation damage in more usual radiobiological terms. They demonstrated, using H^3 thymidine cell labelling, that the smooth muscle cells of arteries and arterioles and the endothelial cells of capillaries, veins and arterioles have a slow mitotic replacement in adult life. The additional finding, that replacement is increased by hypertension, adds great importance to the findings of Asscher and Anson. This will be discussed later

in the light of our own findings.

(b) Primary damage to neural tissues.

Theories covered by this title can include damage to either neurones, supporting glial cells, or a combination of the two cellular types.

Several of the earlier workers reported that there was always a predominance of delayed radiation necrosis in white matter (Davidoff et.al.1938, Wachowski et.al.1945, Crompton et.al.1961), which they considered to be a direct effect on neurones and glia, "both showing retrograde changes". Fibrinoid thickening of vessel walls was observed (Crompton et.al.1961), but it was not considered that these produced the large necrotic areas, which were not, it was said, typical of ischemic necrosis. A similar view was held by O'Connel and Brunschwig (1937), who observed vascular changes in only one out of five patients given radiation treatment for intracranial gliomas. In this case the degeneration of neurones and hypertrophy of astrocytes and oligodendrocytes was most evident. The slight thickening of vascular walls did not appear to produce changes in the parenchyma.

Changes were observed in glial cells 6 to 24 hours after irradiation (Hicks and Montgomery 1952, Brownson et.al.1963). Oligodendrocytes were considered to be the most sensitive cell type and "necrotic cells" particularly noticeable in the corpus callosum after 1,200R (Hicks and Montgomery 1952). Cells of the subependymal plate showed necrotic changes with 200R. It is difficult to draw any firm conclusions as to the role of these changes in delayed radiation necrosis, although the view of direct damage to the oligodendrocyte-

myelin complex was given by Lampert et.al.(1959). He observed, in a study of the brain of a woman, dying 3 months after irradiation (5,750R at 288R/day), pronounced demyelination in the irradiated area. These irradiation areas were devoid of oligodendrocytes. Astrocyte alterations were observed, with bizzare mitotic figures and many nuclei in hypertrophied cells.

The extensive studies of Arnold and Bailey (Arnold et.al.1954a, 1954b, 1954c.) on the irradiation of primate brains, all support the direct action of radiation on neural parenchyma. The white matter, it was reaffirmed, was the most radiosensitive. Abnormal gliosis was noted around necrotic areas. Slight vascular lesions, it was again believed, were not of importance in the pathogenesis of the radiation lesions. This view was held by Hassler (1966a, 1966b) who demonstrated that some vascular changes occurred after direct parenchymal damage.

For the spinal cord of rats, Innes and Carsten (1961a, 1961b, 1962) also reported selective destruction of white matter with a single dose of 3,500R; there was no apparent associated blood vessel damage. The extent of damage was dependant on the duration of the neurological symptoms. No "gitter" cell formation was observed around the necrotic areas, so it was postulated that they were destroyed at an equal rate to that of white matter.

The detailed effects of x-irradiation on glial cells were reported by Arnold and Bailey (1954d). With doses of 7,000R, acute necrosis resulted in the irradiation field. In this necrotic region it was noted that few, if any, microglia appear; astrocytes showed some

proliferation at first but later degenerate. Lower doses of 3,000 - 5,000R still inhibit gitter cell formation, although astrocytes appear to be a little more resistant.

Following single doses of 2,370 and 4,740R, Hassler (1966c) showed that the normal H^3 thymidine uptake of the brain was quickly reduced. This was most marked in the subependymal plate where, following 2,370R, a reduction of 80% in the number of labelled cells was observed in the first 24 hours. A reduction of 85% in the same time interval was observed following the higher dose. Reduction in the labelling index of other cell types was also observed at later intervals of time, after irradiation.

In other investigations using H^3 thymidine cell labelling, Zeman (1963) demonstrated a marked glial disturbance in irradiated rat spinal cord (3,500R), prior to the development of paraplegia and delayed radiation necrosis. It was first noted that a marked increase in cell numbers occurred in white matter prior to the onset of paraplegia and that thereafter, the cell numbers rapidly decreased. This increase in cell numbers was a result of local mitotic activity, as was shown by the high cell labelling index found in white matter three hours after an injection of H^3 thymidine. "The majority of these cells were non-viable and died a rapid post mitotic death". The stimulus for this high mitotic activity in the interstitial cells of the white matter was unknown, although it was postulated, that it resulted from an initial reproductive cell death, occurring at the termination of these cells' natural life span.

The literature cited provides very convincing evidence in support of both the above-mentioned theories, and so the controversy that one is correct and the other not, is perhaps without foundation. It may be better to consider an interplay between the two opposing theories, the predominance of one or the other, depending on the effective dose of irradiation. This may then explain why workers using doses of the order of 2,000 - 3,000R (Russell et.al.1949, Berg and Lindgren 1958) have supported the view of primary vascular damage, while others using higher doses, 3,500R (Zeman 1963, Innes and Carsten 1961) have been in favour of a direct effect on neurones or supporting glia.

The brain and spinal cord is a complex tissue with numerous cellular elements and it might be wrong to consider that a single mechanism acts over such a wide range of doses.

The present study is intended to test the radiosensitivity of certain cellular elements in the brain, with the hope that it will provide a better understanding of the importance of each in the phenomenon of latent cerebral irradiation damage.

B. <u>The subependymal plate</u>

In the early development of the central nervous system the neural tube consists of an inner layer of closely packed mitotically active nuclei (the ependymal layer), and a anuclear peripheral region the marginal layer (Kershman 1938). No cellular differentiation was observed in the ependymal layer, which is of ectodermal origin, and from which originate the precursors of both neurones and glia. These

cells migrate peripherally to form a layer between the ependymal and marginal layers known as the mantle layer.

At a later stage in the development of the cerebrum the ependymal layer differentiates into two layers. One is a dense layer of neuronal and glial precursors, the sub-ependymal layer, and the other is a single layer of cubical cells, the ependyma, which lines the central canal. Mitotic activity continues in the subependymal layer and cells migrate out to produce a wider mantle layer.

After birth, the subependymal layer of rats and mice is still present and mitotically active (Altman 1966, Smart 1961). It is a prominant feature in new born mice, completely surrounding the anterior horn of the lateral ventricles and extending forward as a cord of cells into the olfactory lobes (Smart 1961). Where the layer passes close to the corpus callosum, "streams of cells" extend out between the fibres. Posteriorly to the foramen of Monro only remnants of the layer remain on the lateral side of the lateral ventricles.

As the animal grows the subependymal layer becomes reduced in thickness. In a extensive cell labelling study carried out by Altman (1966, 1966a), it was shown that cell migration from the subependymal layer played an important part in the postnatal neurogenesis of the rat. The information provided by Altman's investigation is too extensive to be mentioned comprehensively here so that only a few of the most important points relating to the migration of cells from the subependymal plate will be quoted. Anteriorly, they migrate out to form the internal granular layer of the olfactory bulbs, while

more posteriorly cells can be traced migrating along the fimbria of the hippocampus to the dentate gyrus. Here they differentiate to form new granular cells. The migration of cells from the subependymal layer along the white matter tracks of the corpus callosum was demonstrated by Smart(1961).

In the adult mouse the subependymal layer is much reduced. It consists of a narrow layer of cells immediately under the ependyma and limited to the lateral wall of the lateral ventricles. Rostrally the layer extends to the tip of the ventricles where the cells become more numerous, a few extending onto the median wall and out into the central core of the olfactory lobes. Caudally the layer thins out and disappears. The layer is widest towards the roof of the lateral ventricles where columns of cells extend out between the corpus callosum and caudate nucleus rostrally, and between the hippocampal cortex and corpus callosum caudally, besides extending between the fibres of the corpus callosum (Smart 1961).

The layer still shows mitotic activity in the adult rat and mouse (Allen 1912, Bryans 1959, Smart 1961) even in animals over one year old (Bryans). Although the subsequent fate of these newly formed cells is not definitely known it has been postulated that they migrate out into the brain substance and thus represent a continuous source of glia in the adult brain (Bryans 1959, Globus and Kuhlenbeck 1944).

An investigation of mitotic activity in other parts of the adult brain was carried out by Hommes and Leblond (1967). They indicated that a slow mitotic replacement of cells does occur in the brain in the

region of the optic chiasma where it is maximal in the corpus callosum and minimal in the cerebral cortex.

Thus, two possible interstitial cell replacement mechanisms exist in the brain, one a migratory one originating from a stem cell population in the subependymal layer, and the other, a local one. This slow mitotic turnover of cells is a factor that should be considered in any study of delayed cerebral irradiation damage.

C. Wound healing in the central nervous system.

One of the first studies of the origin of cellular types participating in the response to cerebral injury was carried out by Hortega and Penfield (1927). Using selective staining methods for astrocytes, oligodendrocytes and microglia it was shown that only microglia and astrocytes reacted to cerebral injury, while oligodendrocytes and neurones appeared to play no part in the reaction.

Microglia play a primary role in the phagocytosis of the products of destruction. They loose their spider-like form as early as the first day after injury, taking the amoeboid form of compound granular corpuscles which accumulate about the wound edge. Mitotic activity is observed in these granular cells, the cells remaining until the products of destruction are removed. Hypertrophied astrocytes with shortened processes are observed close to the margin of the wound one day after injury. Granules accumulate in the cytoplasm and the nuclei become larger and less dense. By the third and fourth day astrocytes are found with several nuclei, nuclear divisions were said to have taken place by

amitosis as no mitotic figures could be detected in this cell type. Later the astrocytes become fibrous and produce the radially arranged scar tissue found around the stab wound.

This view was generally accepted with a few reservations. One of these was the consideration of a larger stab wound, where connective tissue originating from and connected with the meninges, fills the centre of the wound. The glial reaction occurs around this (Penfield 1924).

These observations have been confirmed by numerous workers (Linell 1929, Lewis and Swank 1953, Klatzo et.al.1958); all reaffirming the view that the microglia are phagocytic after cerebral injury, and that this reaction is associated with a more general glial reaction in the formation of scar tissue.

The glial reaction in the hypoglossal nucleus, following nerve crush has been reported by Sjostrand (1966). Using an H^3 thymidine cell labelling technique he showed that in response to nerve crush, glial cells synthesise DNA and divide by mitosis, and not as reported by Hortega, by amitosis. Oligodendrocytes, pericytes and endothelial cells were also shown to react during nerve regeneration, along with astrocytes and microglia. The mitotic activity of microglia was much higher than for any other of the proliferating cell types reacting in response to injury. All the above observations support the view that the majority of cells reacting in response to neural injury are of local origin. This is not a view supported by the findings of Konigsmark and Sidman (1963), who claimed that the majority of brain

macrophages were of haematogenous origin and not locally derived as previously believed. Their technique was to label a high proportion of circulating leucocytes, prior to injury, with H^3 thymidine. Autoradiographic studies after injury enabled them to determine the proportion of leucocytes participating in the lesion. From these they were able to conclude "less than 50% of the macrophages appearing in the first two days after wounding and less than 30% of the ones arriving thereafter, arise endogenously. All the others arise from circulating mononuclear cells, probably monocytes. These leucocytes assume other forms in the wound area, continue to divide, and may transform into brain macrophages".

The above findings are not in agreement with the preliminary report of Hopewell and Wright (1967, and reported more fully in Chap.3). This demonstrated that the cellular reaction to injury could be reduced by previous local irradiation. With a prior irradiation dose of 4,000R the cellular reaction was reduced to half by the seventh day after injury. This result suggests that the majority of brain macrophages are not of haemotogenous origin but are derived from local cells. This view is supported by the findings of Cavanagh (1968), who using the same technique of previous local irradiation was able to report a reduction in the cellular response to injury of peripheral nerve. In this case, a previous dose of 2,000R produced a 42.5% reduction in the cellular reaction by the 14th day after nerve crush. Similar conclusions were drawn to these of Hopewell and Wright, that the majority of cells were of local origin and not derived from circulating mononuclear cells.

Possible explanations of the findings of Konigsmark and Sidman will be discussed later (Chapter 8).

D. Intracerebral infection with B. pertussis.

Bordetella pertussis lives on the ciliated lining of the respiratory tract and is the causative organism of whooping cough in man.

The mouse was first used in the potency testing of B. pertussis vaccines in 1937 when mice were infected by the intanasal route (Burnett and Timinus, 1937). Ten years later, Kendric (1947) described the intracerebral route of challenge. This was shown to correlate better with the field trials carried out on the potency of pertussis vaccines than mice challenged by the intranasal route (Standfast 1958). The similarities between the two sites of infection being the ciliated epithelium of the respiratory tract and ciliated ependymal cells which line the ventricles of the brain. Berenbaum (1960) and Iida (1963) have reported seeing the bacteria on the ciliated lining of the ependyma.

Different strains of B. pertussis have been isolated and these have shown to be of varying virulence for mice following intracerebral infection. These strains can be basically classified into two groups; those of high virulence for mice and those of low virulence.

The virulent strains eg., strain 18/323 have been investigated by several workers (Delby and Stanfast 1961, Blyth 1955, and Berenbaum et.al.1960). These workers have shown that after an initial challenge-dose of organisms there is a rapid initial fall in the bacterial count

in the brain by as much as 90%. This level was then maintained for a short time being followed by a rapid multiplication of the bacteria to give a viable count after 24 hours approximately the same as that of the initial inoculum. Bacterial counts then increase by ten fold daily and all mice die when the bacterial count reaches 10^7 to 10^8 viable organisms.

Until recently low-virulence strains have received little attention and only a few brief reports existed in the literature. Blyth(1955) using strain H154, injected 2 x 10^3 viable organisms; these multiplied slowly reaching a maximum count on the fifth day, then bacterial counts decreased and all brains were sterile by the 21st day. Brown (1958) with strain 1011 injected 10^3 organisms; these multiplied rapidly for the first 3 - 4 days and then rapidly disappeared from the brain.

However, in the last few years these low virulence strains have been more extensively studied (Adams 1968). He has shown that when approximately 10^4 organisms of a low virulence strain (B.1772) of B. pertussis are injected intracerebrally, there is a 90% loss of organisms over the first hour (cf. high virulence strains). They then multiply for 24 - 48 hours to reach a peak of approximately the initial inoculum size, before rapidly disappearing from the brain, which was nearly sterile by the fifth day.

He also found that if the inoculum was raised to between 10^5 to 10^6 organisms the bacterial counts remained at a high level for five

days before they disappeared from the brain. All mice survived.

Prior to our investigation and other studies by Adams several suggestions have been put forward to explain the difference in response between the two types of strains. Local antibody production was postulated by Anderson (1957) and phagocytosis by Brown (1958). The participation of blood leucocytes, commonly observed after the third day of infection with the high virulence strains was suggested by Berenbraum (1960). This was further supported by the findings of Iida et.al.(1963) who, after the induction of leucopenia by subcutaneous injection of cortisone, was able to detect a considerable reduction in the LD_{50} of their low virulence strain (Tohama I) from 2×10^6 to 2×10^3 viable bacteria.

The hypothesis that blood leucocytes are responsible for the irradiacation of B. pertussis intra-cerebral infection was the starting point of our investigation.

E. The oxygen effect.

The effect of variation in oxygen tension on radiosensitivity is well documented for cells in tissue culture and in some intact mammalian organs. Using ascites tumour cells Gray et.al.(1953) was able to show a marked variation in irradiation response with different oxygen tensions. Cells irradiated in air were more sensitive to irradiation than cells irradiated in nitrogen by a factor of 3.1.

Noel and Wright (1968) demonstrated a dose modifying factor of 2.69 for growing mouse tail bones irradiated in nitrogen as compared with oxygen at one atmosphere. The sensitising effects of higher oxygen tensions have been successfully demonstrated for growing mouse tail bones with 3 atmospheres of oxygen (Howard - Flanders and Wright 1957). The question of the oxygen effect and its potential applications to radiotherapy have been reviewed Wright (1966) and in Modern Trends in Radiotherapy I. (1967).

However, little information appears to be available on the oxygen effect as applied to the irradiation of the central nervous system. Wright and Shewell (1965) were able to report a protective effect for nitrogen breathing animals, when brains were irradiated with high doses of irradiation (50 - 400 Krads). They were not however, able to demonstrate sensitisation with 100% oxygen, in fact slight protection was indicated. With lower doses, 4,700 - 21,380R, a variable protection was obtained with anoxia for brain irradiation (Sharples and Jacobs 1966). This, it was assumed, was due to variation in tissue damage resulting from anoxia. For the spinal cord, Zeman (1966) demonstrated a slight enhancement of irradiation damage with 95% oxygen (5% carbon dioxide) for grey matter, but not for white matter. A factor of 1.15 was quoted, as compared with animals breathing air. This field is clearly in need of further investigation.

F. <u>The production of experimental cerebral tumours</u>

The incidence of tumours of meningeal and neuroglial origin in the central nervous system accounts for only 2 - 5% of malignant

diseases in man (Willis 1967). The incidence of spontaneous central nervous system tumours in rodents, in particular the rat, is a little hard to determine, as few comprehensive investigations have been carried out.

The studies that have been undertaken however, have been very unproductive regarding the discovery of spontaneous tumours in the central nervous system in rodents. In wild rats, McCoy (1909) found 103 tumours in 100,000 animals and Woolley and Wherry (1911) only 22 tumours in 23,000 rats, but none of these were primary tumours of the central nervous system. Curtis et.al. (1931) on the other hand, on examination of 31,868 rats found one C.N.S. tumour, a ganglioneuroma of the optic nerve.

A similar pattern is reported in mice. Steward (1950) reported only two meningeal tumours in 70,000 mice examined.

Thus we can conclude that the incidence of spontaneous tumours of the central nervous system are very low, and can probably be ignored as a factor in the study of the effects of carcinogens on the central nervous system.

The effect of implants of chemical carcinogens into the brain of rodents has met with mixed and often contradictory results. Some workers have reported that cerebral tumours are not produced while others have produced a high proportion of tumours, both of the meningeal and neuroglial origin.

The mouse has been most extensively used in the study of the effects of chemical carcinogens on the brain, and as will be shown later, has until now been a more successful animal to use than the rat. The results however have still been very variable and so in the previous work that is quoted I shall try and explain the various results on the basis of the different techniques used.

The first significant success was obtained by Seligman and Shear (1939) who implanted pellets of 20 methylcholanthrene deeply into the brain of 20 male C_3H mice. This resulted in the induction of 13 tumours, 11 of these being of glial origin. Shortly after this, Peers (1940) and Zimmerman (1941) produced similar results with the same carcinogen, again in pellet form, using albino and C_3H mice respectively. Zimmerman produced 26 tumours (15 gliomas) in 57 experimental animals, the pellet was quoted as being 3mm. deep within the brain. Peers produced 32 tumours in 87 animals of which 15 were neuroglial in origin. In the latter experiment it was stated that the pellet was placed flush with the surface of the brain. The pellets were cylindrical and on average 2mm. in length; then by nature of their implantation method (trocar and plunger) the pellets would in a number of cases, penetrate into the brain at least 2mm.

This early success with methylcholanthrene in mice has been confirmed in more recent years by a number of workers (Table 1).

TABLE 1

Intracerebral tumours in mice produced with methylcholanthrene

Worker	No. of mice	No. of tumours	% tumours
Sato (1963)	135	49	38
Haelst (1963)	77	23	30
Kawai (1963)	242	112	46
Nishikawa (1963)	528	45	9
Ishida (1964)	107	28	26

These results thus confirm that methylcholanthrene implanted into the brains of mice will produce neoplasms of both glial and mesodermal origin. However the results are variable and the percentage of tumours varied from 65% (Seligman and Shear) to only 9 % by Nishikawa. A possible explanation of this is in the strain of mice used, and in support of this, both Nishikawa and Kawai claimed a higher incidence in coloured mice than in albino strains. Other factors derived from these results are that sarcomas tend to develop earlier than gliomas, an interval of 50 days is quoted by Peers, and 85 days according to Haelst. The importance of sex is very difficult to evaluate, for Sato showed a higher incidence in males (41%) than females (32.7%); while Peers claimed the reverse with a ratio of 6:2 females to males for gliomas, although the sex of seven other mice with gliomas was unknown. In the two results with the highest

incidence of tumours, 65% (Seligman and Shear) and 52% (Zimmerman) only male mice were used.

Another carcinogen to be used is 3.4 Benzpyrene, although it has only been used in mice by Zimmerman (1955). He implanted 1mm. cubes of carcinogen into the right cerebral hemisphere of 51 mice. 47 of these survived into "tumour-bearing age" and 28 developed tumours. Of these, 14 were glial in nature and 13 classified as sarcomas with one mixed glio-sarcoma. Again, as with the work using methylcolanthrene, sarcomas tend to develop earlier, 245 days as against 276 days for gliomas. The tumours were equally distributed between the sexes.

The other carcinogen frequently used in cerebral carcinogenesis is 1.2.5.6. dibenzanthreeene. This was first used by Peers (1939) who implanted 5% dibenzanthreeene in cholesterol pellets superficially into the brains of mice, so that the pellets were flush with the surface of the brain. The result of this experiment was a single tumour, a meningioma, over an observation period of two years. Four years later Arnold and Zimmerman (1943) published a more positive result using this carcinogen with 11 tumours in 21 animals. Zimmerman's explanation of this was twofold; pure carcinogen as opposed to 5% in cholesterol and secondly the strain of mouse used was C_3H as opposed to the albinos used by Peers.

Early attempts to produce neoplasms in rats were not very encouraging. In two reports by Oberling (1936), who placed crystals of benzpyrene subdurally into albino rats. This produced no gliomas,

only three pituitary adenomas, and 12 sarcomas from 10 and 40 animals respectively. Weil (1938) also failed to produce any gliomas in 6 rats which were injected with a mixture of dibenzanthracene and lard. A single carcinoma developed, around which there was a definite glial granuloma.

About the same period, Sweet and Bailey (1941) using 20 methylcholanthrene produced 5 tumours in 42 Wistar rats. The crystals of carcinogen were implanted in the cerebrum and cerebellum, and the tumour types produced were three spindle-celled sarcomas, a fibroblastoma of the cerebellum and a lymphosarcoma associated with a glioblastoma.

Two other workers, Zylbersgac and Scherer (reported by Seligman and Shear 1939), produced completely negative results, the latter using 1% benzpyrene in lard in 70 rats, and the other using pure carcinogen injected subdurally into 35 animals.

More recent work, notably by Japanese workers, has produced gliomas in rats but the incidence obtained was still not as high as induced in mice. Kawai (1963) at the Gumman School of Medicine has made the most extensive study, using 20 methylcolanthrene implanted into the subcortex, cerebrum and cerebellum of 212 rats of two strains (Donryu, 158 and Wistar, 54). Tumours only (21) developed in the Donryu strain the results for Wistars being completely negative. Of the 21 tumours, 13 were gliomas, 7 mesodermal and one was a mixed gliosarcoma. This is a result which would indicate that strain was a factor in the induction of cerebral tumours. A year later

Ishida (1964) with the same carcinogen produced only a single menigioma in 30 animals of the Donryu strain.

The work of Delorme at the Chester Beatty Institute (personal communication) was again negative, only producing a single tumour, a meningioma, from 120 Wistar rats, given an intracerebral implant of 3 - 4 Benzpyrene. In his experiments the pellets were carefully placed 1 - 2mm. below the surface of the brain (fig.1) so as to lie half-way between the meninges and the ependyma of the lateral ventricles but involving neither. Only a single tumour occurred where a pellet had worked its way to the surface.

In the above review of some of the literature on the effects of chemical carcinogens in the brain a rather confused picture between comparative success (Zimmerman 1941,1943) and total failure (Delorme) is presented. Despite this I feel several conclusions can be drawn:-

1. The importance of strain - This is a point raised by many of the researchers using mice in their experiments, and in an extensive study Kawai noted a variation of between 60.6% and 25% tumours in different mouse strains. A similar view was put forward by Zimmerman (1944) after his very extensive studies on cerebral carcinogenesis.

The same thing could be stated using rats for Wistars have given near negative results. The Donryu strain on the other hand,

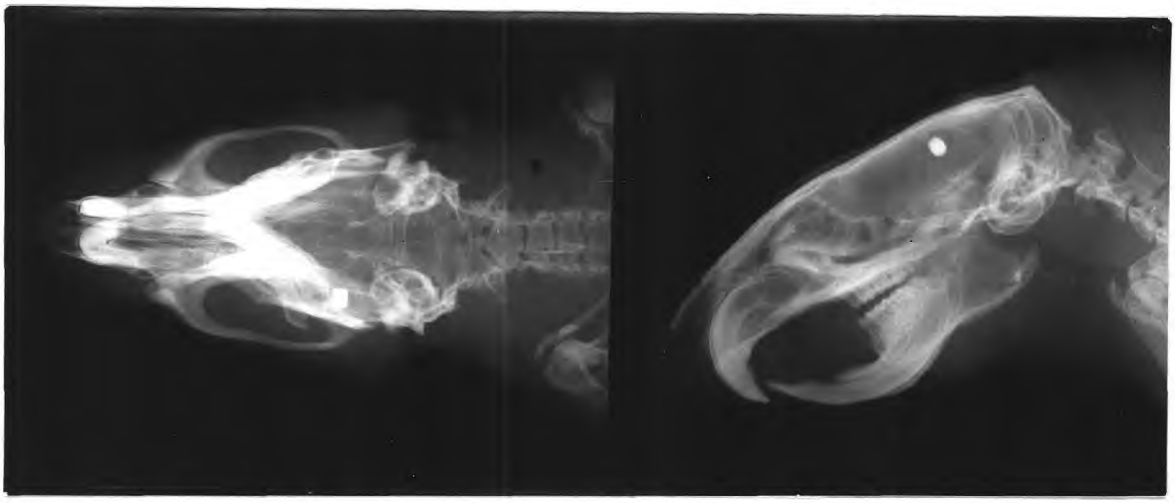


Figure 1. Dorsal and lateral radiographs of a rat skull to show the position of carcinogenic implant (Delorme).

60 male and 60 female six week old Wistar rats were implanted with 2-3mg. pellets of benzpyrene. The depth to which the pellet was inserted was controlled by using a trocar and stylet (Arnold and Sons, No.3) fitted with a guard set at 1.5mm. The pellet was designed to lie midway between the surface of the brain and the lateral ventricles, the position being checked at the time of operation by radiography. The carcinogenic pellet being made radio-opaque by the addition of iron powder.

used by Kawai, produced 21 tumours although this result was not confirmed by Ishida whose results were very comparable with those for Wistars. It would appear that mice on the whole are more sensitive to intracerebral implants of carcinogens than rats.

2. The question of carcinogen type may also be important as methylcolanthrene is relatively successful in the production of tumours while benzpyrene, apart from the work of Zimmerman in mice, has met with little success. The question of pure carcinogen against a low percentage in cholesterol or lard was raised by Zimmerman. It has however been stated by several workers that only minute amounts of carcinogen are required (Leiter and Shear, 1943; Byan and Shimkin 1943). The latter stated that 100% Subcutaneous tumours were produced in C_3H mice by 0.5mgs. of benzpyrene and 0.125mgs. of methylcholanthrene.

3. The question of site is another factor which may be of greater importance. Zimmerman (1955), one of the most successful workers in this field always placed his pellets deeply into the brain, or to quote his own words "An effort was made in these experiments to implant the carcinogen rather deeply within the brain tissue, and in four instances where this resulted in the deposition of the pellet within the ventricular system, ependymoblastomas developed". This was true of the work of Seligman and Shear who also placed their pellets in a deep position and produced a high incidence of tumours. Peers, on the other hand, in his work with dibenzanthrecene, placed the

carcinogen flush with the surface of the brain and in doing so produced only a single tumour, a meningioma. Other experiments carried out on the mouse are a little difficult to evaluate as the workers only state that the carcinogens were implanted into the brain. The exact positioning of pellets in the mouse brain may be difficult owing to its small size.

In the rat, its larger size makes exact positioning of the pellet more feasible. Several workers, implanting pellets subdurally, (Oberling, Weil, Zilberzac and Shear) have produced no glial tumours, only a few sarcomas.

The findings of Delorme are even more interesting, because with the pellet in this mid position he did not involve the meninges, as did the previous workers, nor did he involve the lateral ventricles, as did Zimmerman with mice. No tumours resulted from this implant.

Basing our work on the findings of Delorme it was decided to place our carcinogens in the two positions that he did not use, namely, superficial and deep to involve either the meninges or the lateral ventricles of the brain.

CHAPTER II

THE INFLUENCE OF HYPERTENSION ON LATENT BRAIN IRRADIATION DAMAGE

The cerebral hemisphere of young adult rats of the Wistar strain aged 3 - 4 months and weighing approximately 200 grams, were irradiated under chloral anaesthesia with doses of 1,000, 2,000, 3,000 and 4,000R, given laterally through the side of the head to most of the cerebral hemispheres. Groups of six animals were irradiated at each dose and a further six unirradiated animals were used as controls. The experiment was duplicated using hypertensive animals. Hypertension was obtained by a modification of the Koletsky kidney infarct method (1955).

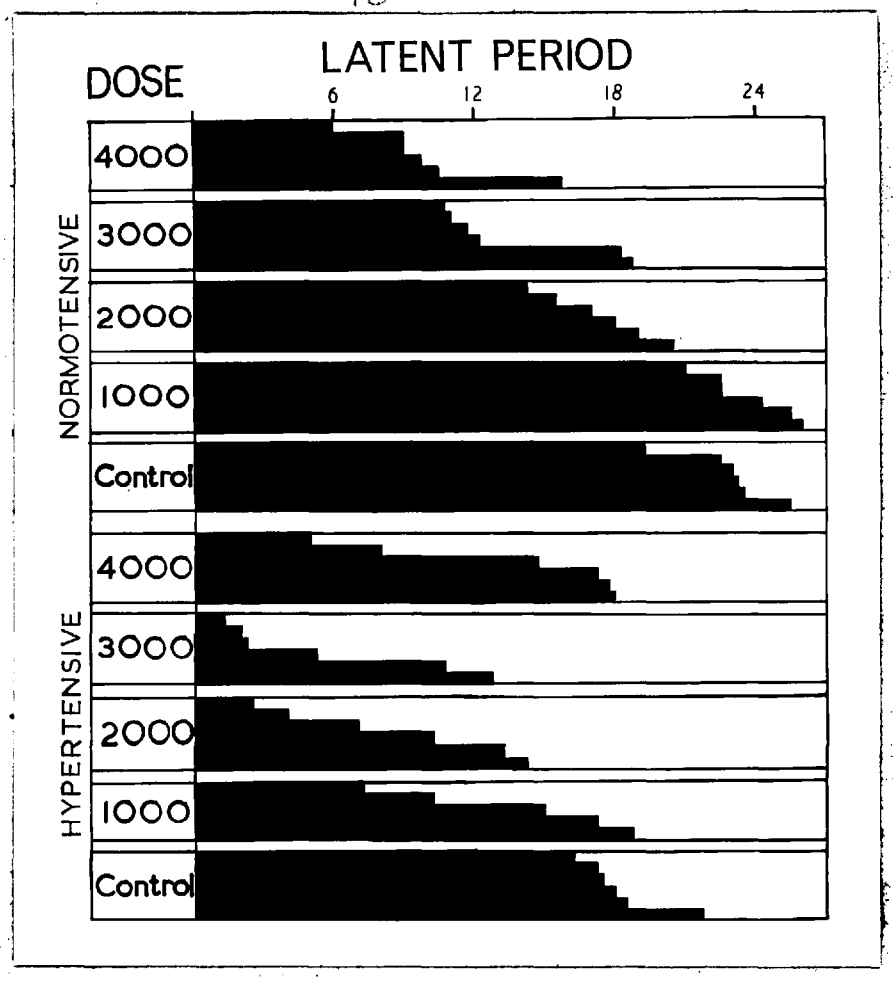
After irradiation the animals were returned to the animal room where they were fed on Dixons 41B rat cake and water ad. lib. The animals were inspected daily and weighed at regular intervals.

A. The influence on survival

Deaths occurred 1½ to 25 months after irradiation, the latter time being approximately the normal life span of the normotensive non-irradiated control animals. The survival time of all animals in this experiment are given in the form of a histogram (Table 2) and the results are summarised in Table 3 and fig. 2. In the graph, the mean survival times after irradiation (for practical purposes the

Table 2. Histogram giving the latent period (months) between irradiation and death for normotensive and hypertensive irradiated animals.

Figure 2. Mean latent period between irradiation and death for normotensive and hypertensive animals, against dose of X-rays.


LATENT PERIOD
DOSE
6
12
18
24
NORMOTENSIVE
4000
3000
2000
1000
Control
HYPERTENSIVE
4000
3000
2000
1000
Control

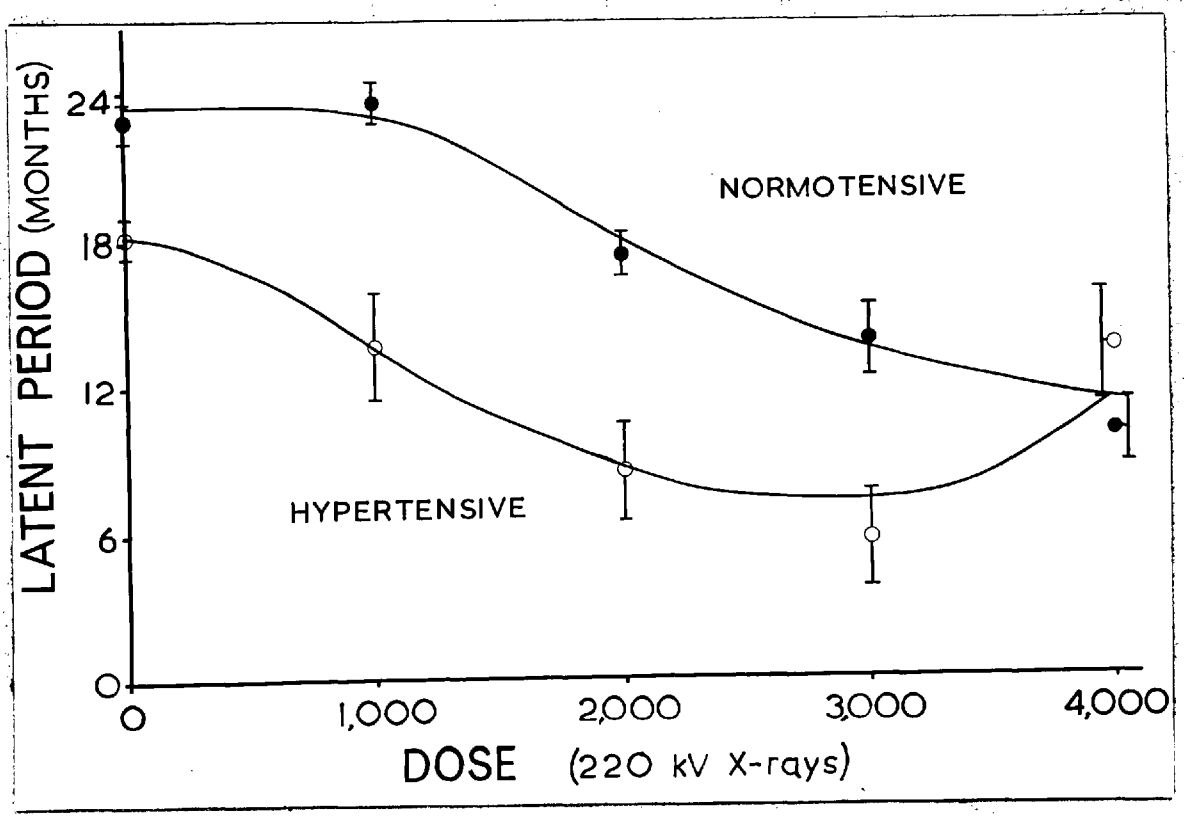

LATENT PERIOD (MONTHS)
24
18
12
6
0
NORMOTENSIVE
HYPERTENSIVE
0
1,000
2,000
3,000
4,000
DOSE (220 kV X-rays)

TABLE 3

Mean survival time between irradiation and death

Dose (R)	Normotensive			Hypertensive		
	Male	Female	Mean	Male	Female	Mean
0	23.25 (3)	22.5 (3)	23.0	18.25 (5)	18.0 (1)	18.25
1000	23.25 (2)	24.0 (4)	23.5	17.0 (3)	9.0 (2)	13.75
2000	-	17.5 (6)	17.5	7.5 (5)	14.25 (1)	8.5
3000	18.25 (1)	13.0 (5)	13.75	6.5 (5)	2.0 (1)	5.75
4000	6.0 (1)	11.0 (5)	10.0	11.25 (4)	18.0 (2)	13.5

( ) number of animals used.

same as the latent period) with their associated standard errors are plotted against the irradiation dose. This demonstrates the effect of renal hypertension in shortening the latent period between irradiation and death, in the 1,000 to 3,000R dose range. It also shows that this effect is lost with a higher dose of 4,000R because both the normotensive and hypertensive animals die after a similar latent period. Hypertension it would appear, has lost its potentiating effect.

B. Weight changes and Neurological signs

1. Normotensive animals - In all the irradiated groups, animals lost weight in the week following cerebral irradiation. This was most marked in the 4,000R group where a mean weight loss of 4% was recorded in the first week. By the second week, in the 1,000 - 3,000R groups the slight weight loss had been regained and the animals had gained weight slightly. In the 4,000R group the 4% loss was maintained and a mean weight gain was not observed until the fifth week after irradiation. (fig.3).

Further weight increases were maintained until between the second and third month, when the majority of the irradiated animals lost weight quite suddenly. Up to 17 grams (9% of maximum) was lost in one animal in the 4,000R group.

In the growth curves for female rats (fig.3), which predominate in all of the Normotensive irradiation groups (Table 3), a 3%

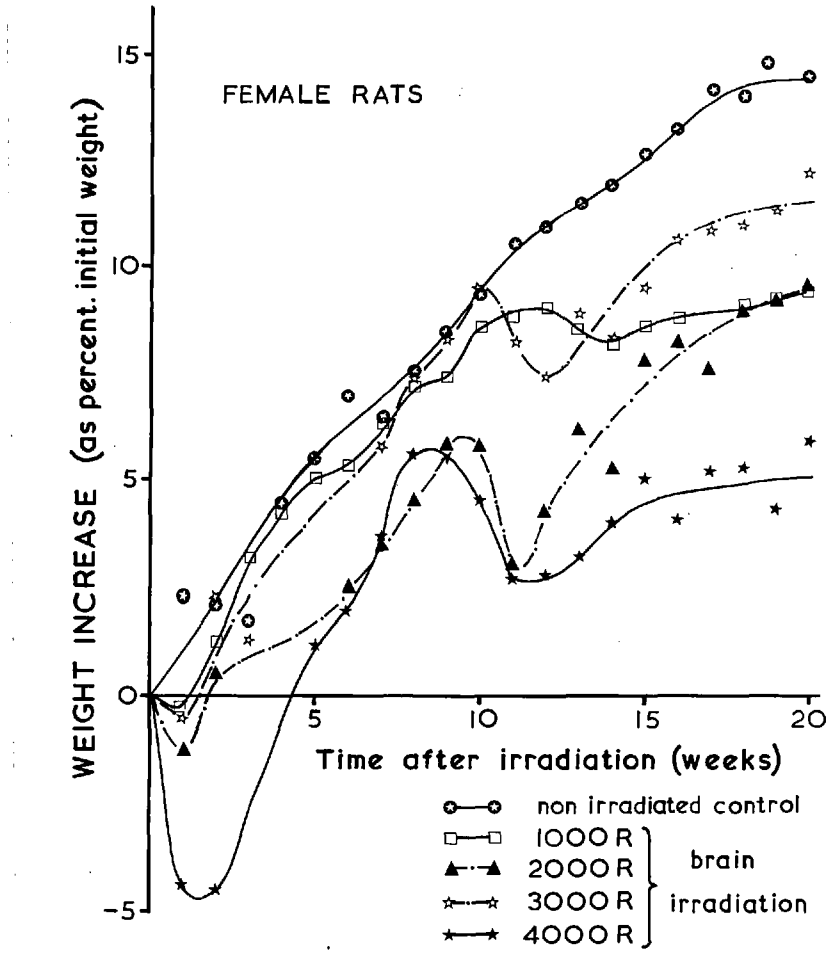


Figure 3. Mean weight increase for female rats following irradiation, weight increase being expressed as a percentage of the mean initial weight for the group.

depression in the growth curves for the 2,000 - 4,000R groups can be observed 2 - 3 months after irradiation, while in the 1,000R group there is only a slight dip in the curve.

Animals recover quickly from this weight loss and follow a growth curve approximately parallel to that of the non-irradiated controls, until a maximum weight is reached. This maximum weight is constant until death or shortly before death, depending on the dose of radiation administered.

In the animals receiving 4,000R a weight loss preceded death, this amounting to 60 to 100 grams, a loss of 30 and 33% of the maximum weights over a period of time ranging from 3 to 20 weeks. Over this period, changes occurred in the rats' normal behaviour. They became agressive when handled, attempted to bite or to jump out of the cage. They no longer responded when a hand was placed near the cage, possibly indicating blindness. Large patches of hair on the back and limbs were lost, and the remaining hair stood erect.

Most of the animals finally succumbed overnight, but in a single case (Rat 80) the animal was found comatosed with the eyes and nasal regions eaten away by the remaining animals in the cage and was therefore killed.

In the 3,000R group the picture was very similar. A weight loss of 50 - 135 grams or 23 and 45% of the animals' maximum weights,

over a period of 3 - 16 weeks prior to death was seen. The neurological signs were similar to those mentioned in the 4,000R group. One animal developed ulcers and scabs on the legs and feet to such an extent that it was killed. Another was found comatose and killed.

In the 2,000R group, four animals died suddenly with no weight loss, while the other two animals in the group lost 70 and 40 grams over a short period of 2 and 3 weeks, respectively, prior to death.

The animals in the 1,000R group died at approximately the same time as the non-irradiated controls.

2. Hypertensive animals - The hypertensive irradiated animals, unlike the normotensive irradiated group, showed no weight loss two to three months after irradiation. No conclusions can be drawn from this, as in the hypertensive group the majority of animals were males, (Table 3) making direct comparison of weight changes impossible because males grow at a faster rate than females.

With the highest dose (4,000R) five of the six rats in the group died after losing weight for a period of 6 - 8 weeks prior to death, (i.e., 20 - 130 grams or 7 - 42% of the maximum body weight).

In all ways a similar picture to the 4,000R group of normotensive rats. The sixth animal of the group, which was the first to die five months after irradiation, did so suddenly with no weight loss.

In the group receiving 3,000R, the last three animals to die after a latent period of $5\frac{1}{2}$ to 13 months, showed a weight loss prior to death (40 - 100 grams over a period of 4 - 8 weeks), while the first three animals in the group died without any weight loss. One of these latter three rats died suddenly while the other two became semi-comatosed for approximately two weeks prior to death. For example, rat 92 which died six weeks after irradiation:-

Ten days prior to death it was found in a hunched, motionless posture apart from spasmodic movements of the head to the right. It was very cold and the hair was ruffled. The pupils were small and the respiration rate slow, 76 respirations per minute. Two days after this it showed signs of improvement although on occasions it would be found hunched in the corner of the cage. However, ten days after the initial observations it was again deeply comatosed and unable to maintain an upright position, the limbs lacking muscle tension. The rectal temperature was recorded as 34.2°, as compared with a normal of 37.5° to 38°C., and the respiration rate was slow and irregular, 50 respirations per minute. After further deterioration throughout the day the animal was killed.

In the 2,000R group of hypertensive animals, all but one died suddenly without any weight loss, the single exception lost 100 grm. in the relatively short period of one week prior to death.

All the animals in the 1,000R group died suddenly.

The number of animals dying suddenly in the normotensive and hypertensive irradiated groups are given in Table 4.

TABLE 4

Number of Sudden ϕ Premature deaths/number of animals

	Irradiation dose (R)			
	1,000	2,000	3,000	4,000
Normotensives	6/6[b]	5/6	0/6	0/6
Hypertensives	5/5	6/6	3/6	1/6

b The animals in this group were not classified as having died premature deaths as they survived for the same length of time as the controls (Table 2)

ϕ Sudden death is defined as animals which die without warning or those losing weight over an interval of not more than two weeks prior to death.

C. Pathological findings in irradiated brain

The histopathological changes to be found in irradiated brains at the time of death can be broadly classified into the following three

groups:-

1. necrotic changes
2. vascular changes
3. other discreet changes, i.e., changes in the subependymal plate.

1. Large necrotic areas occurred mainly in the white matter, with some deposition of calcium in a few isolated regions. Gliosis was markedly absent in and around the necrotic areas.

The areas most commonly affected were the internal capsule (fig. 4) and the fibrium hippocampus (fig.5). The necrotic areas are nearly totally acellular with no noticeable glial proliferation around their borders. The necrotic area in the fimbria hippocampus occasionally extended into the hippocampus with a loss of nerve cells from the gyrus dentatus. The optic tracts and optic chiasma were often completely necrotic. (fig.6).

Only rarely were vascular lesions associated with these large necrotic areas which were always restricted to the white matter. No large areas of necrosis were ever found in the cerebral cortex. Microscopic foci of necrosis were found in the cerebral cortex but these were restricted entirely to the hypertensive group of animals.

In the hypertensive animals vascular changes were associated with the necrotic area. Figure 7 shows a typical necrotic focus around a

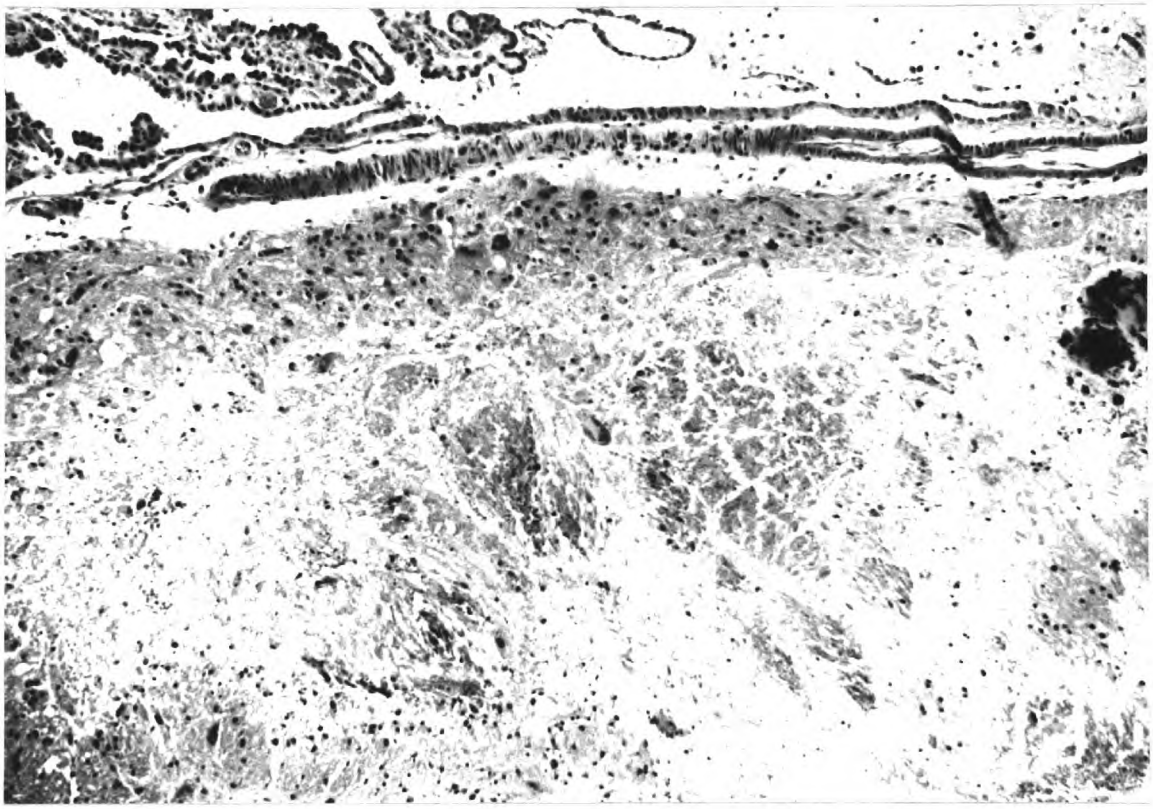


Figure 4. Necrosis of internal capsule. Nine month latent period following 4000R. H&E x110.

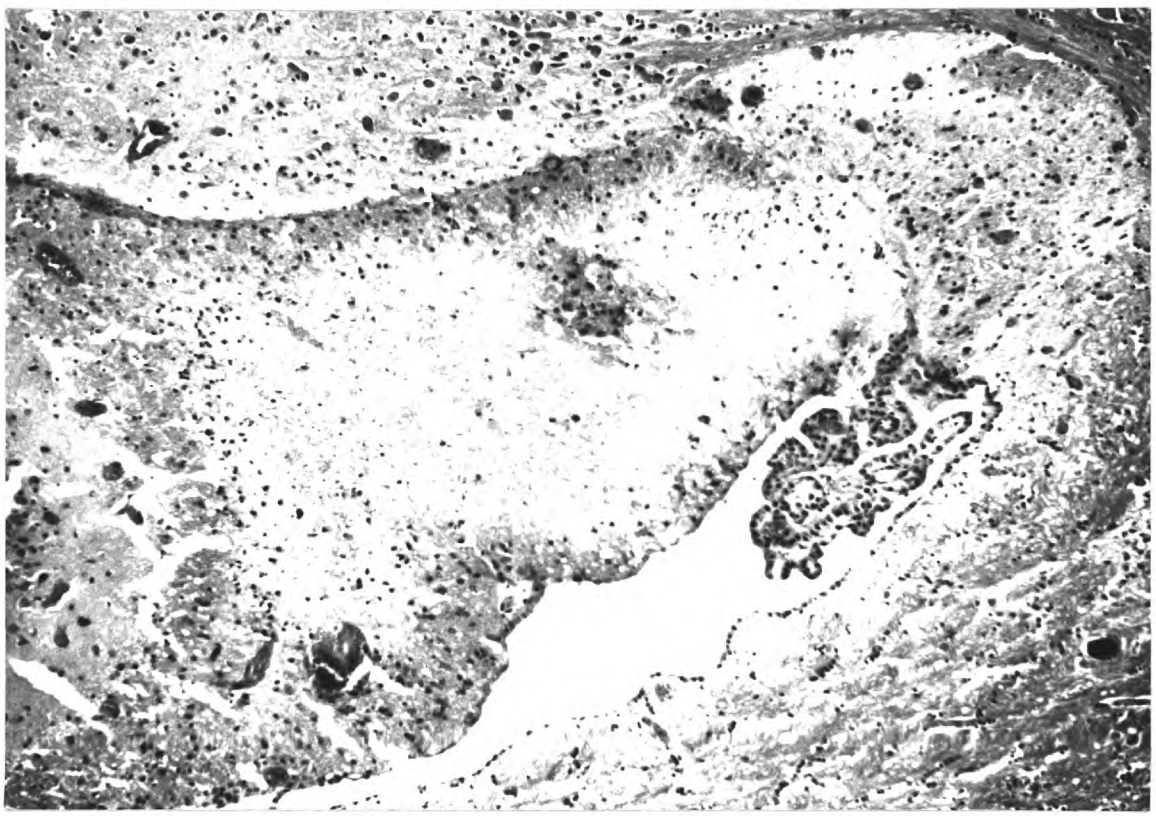


Figure 5. Necrosis of the fimbrium hippocampus. Nine month latent period following 4000R. H&E x110.



Figure 6. Necrosis of optic chiasma. Nine month latent period following irradiation dose of 4000R. H&E x110.

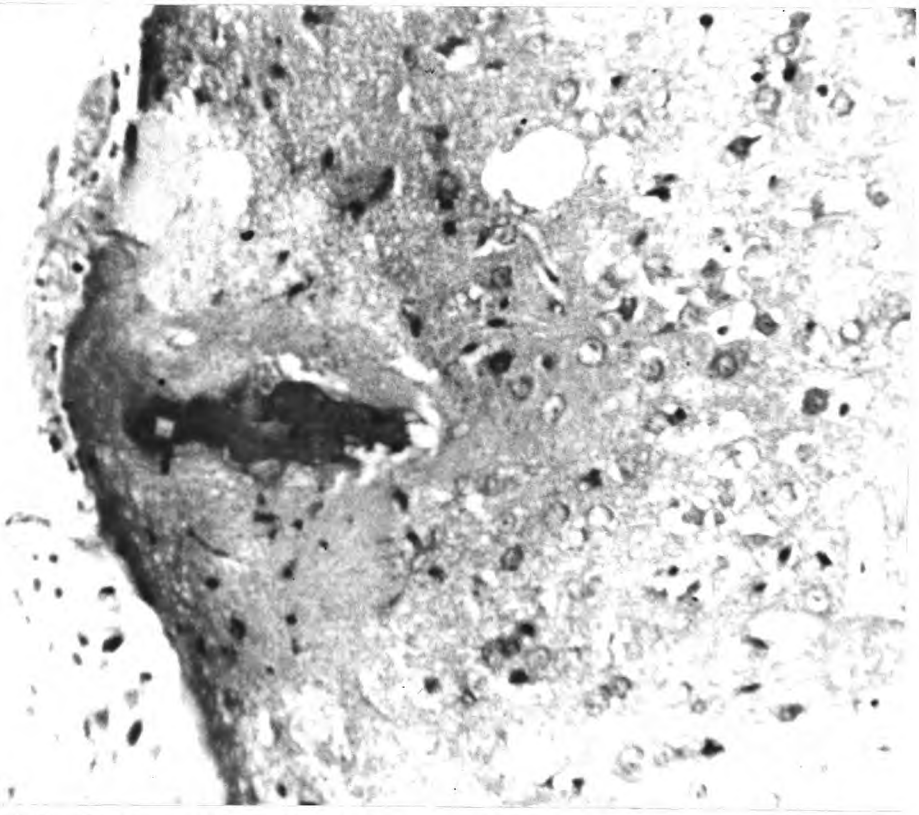


Figure 7. Small focus of necrosis, in the cerebral cortex, about a hyalinated blood vessel. Hypertensive animal dying 5 weeks after irradiation (3000R). H&E x300.

superficial hyalinated blood vessel in the cortex. Figure 8 shows slight oedematous loosening of neural tissues around a slightly dilated blood vessel. Thinning of cortical tissue was also encountered.

2. Discrete vascular changes were also observed and these could be classified into several broad groups:-

a. Haemorrhagic foci were encountered around small dilated blood vessels. Yellowish brown pigment was found in the glial cells surrounding the blood vessel. (Fig.9).

b. Fibrinoid necrosis of blood vessel walls was a very common feature, particularly in the thalamic and hypothalamic regions of the brain (fig.10) and were occasionally associated with what appeared to be microaneurysms. (Fig.11).

c. Round laminar bodies which contained some calcium may be termed amyloid bodies, were found in close proximity to blood vessels. In some cases, they appeared to occlude the vessels.(Fig.16).

d. Thrombosed blood vessels were occasionally found in normotensive and hypertensive irradiated animals. The example demonstrated (fig. 12) is a large thrombus in a cortical surface vessel which was clearly visible to the naked eye. An interesting feature associated with this thrombus was the small area of necrosis found a little more posterially to it in the brain (fig.13). Some of these thrombi were clearly not of very recent origin, for evidence of vessel

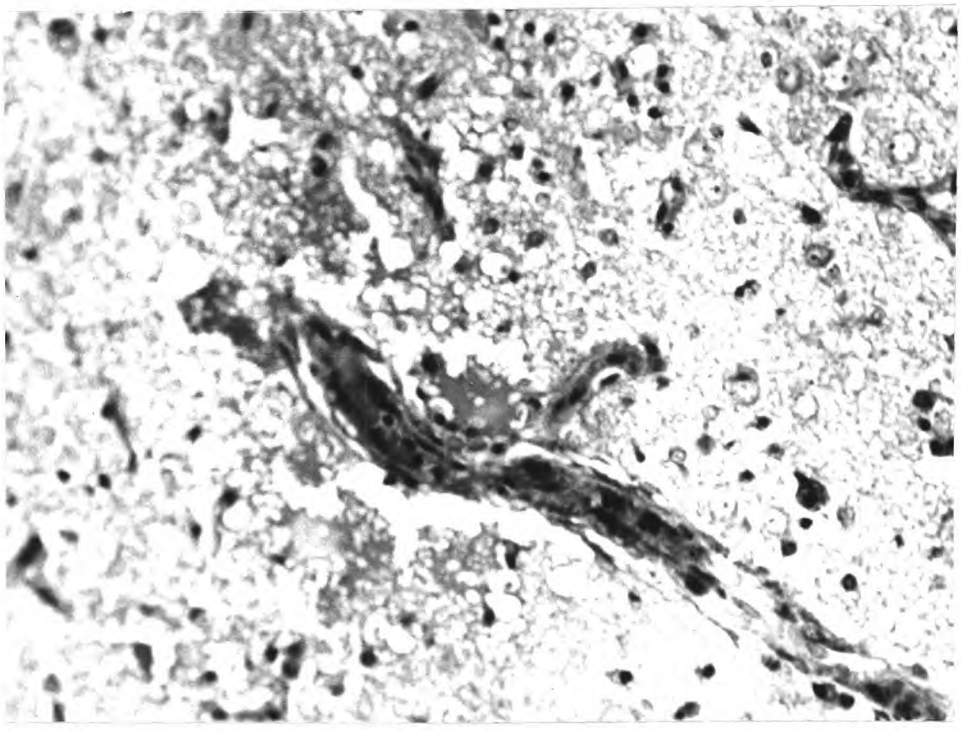


Figure 8. Oedematous loosening of neural tissue around a slightly dilated thin-walled blood vessel. Hypertensive animal dying 5 weeks after irradiation (3000R). H&E x300.

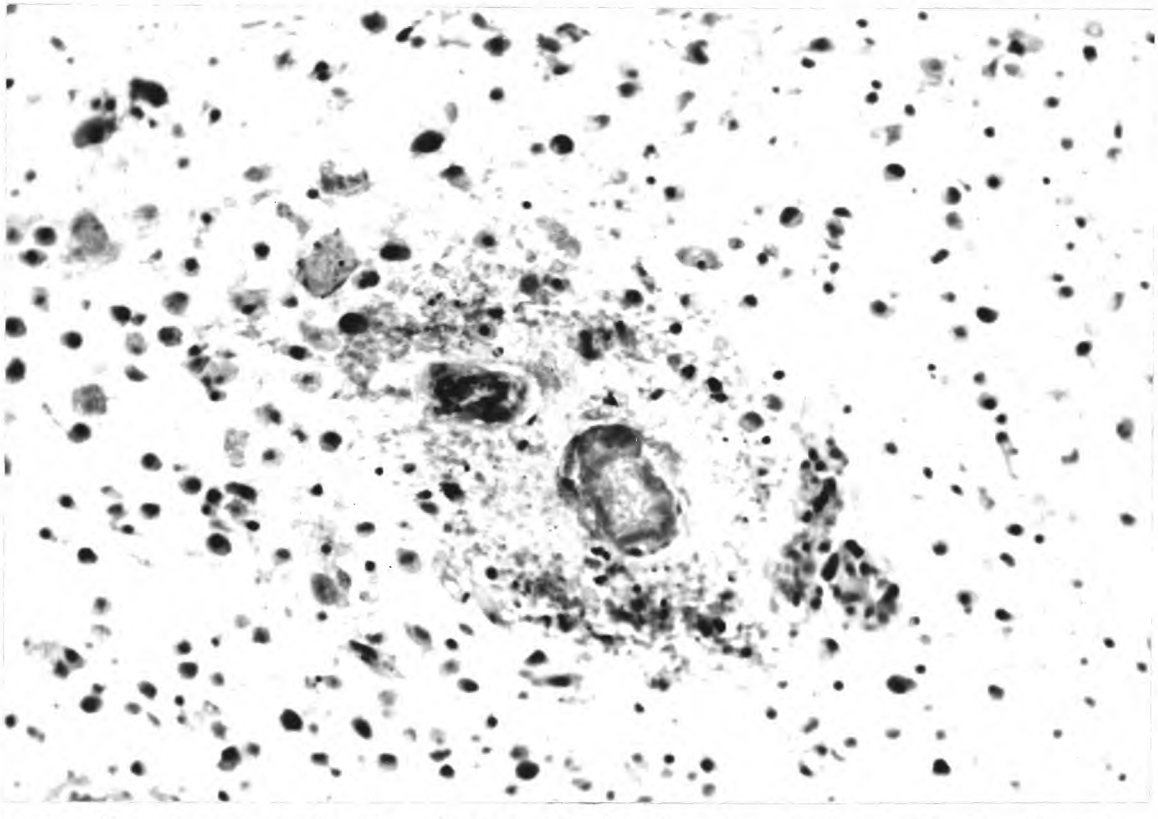


Figure 9. Haemorrhagic focus in thalamic region of the brain. Animal dying 10.5 months after irradiation (4000R). H&E x300.

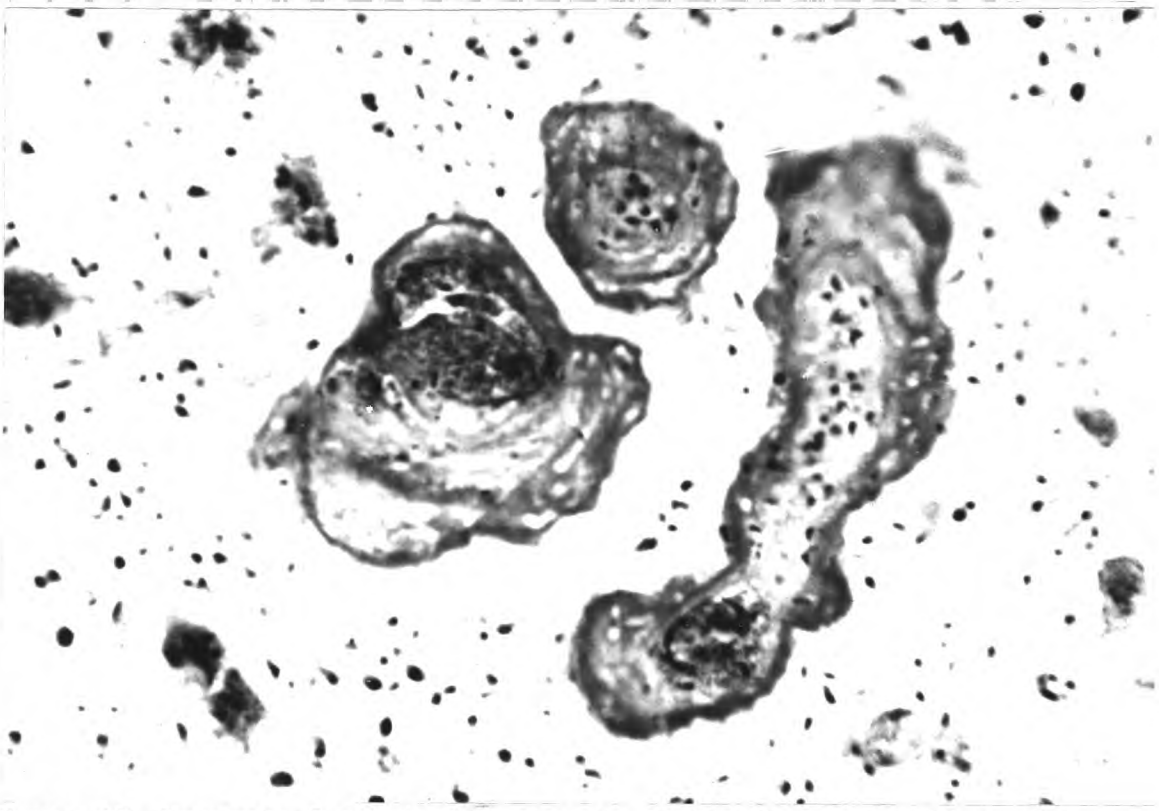


Figure 10. Pronounced fibrinoid necrosis of blood vessel wall in the hypothalamus. 10.5 month latent interval following 4000R. H&E x300.

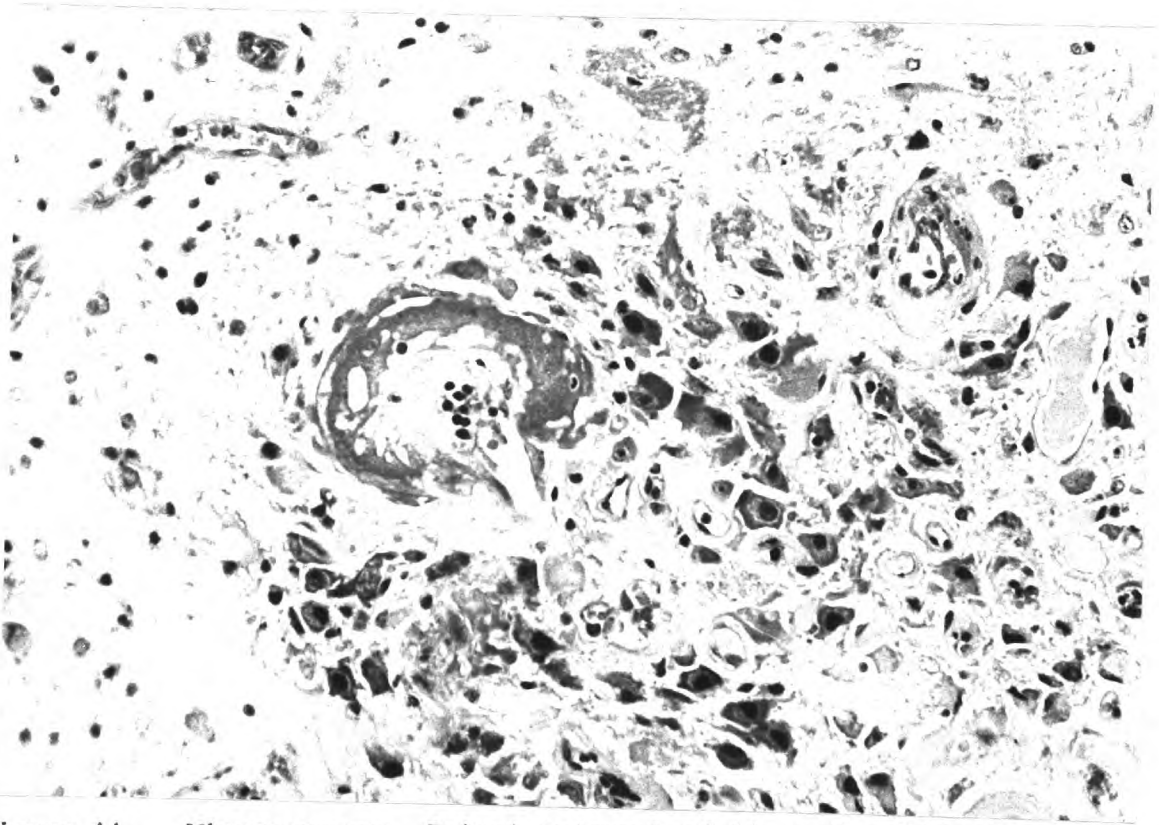


Figure 11. Microaneurysm. Latent interval of 11 months following 3000R. H&E x300.

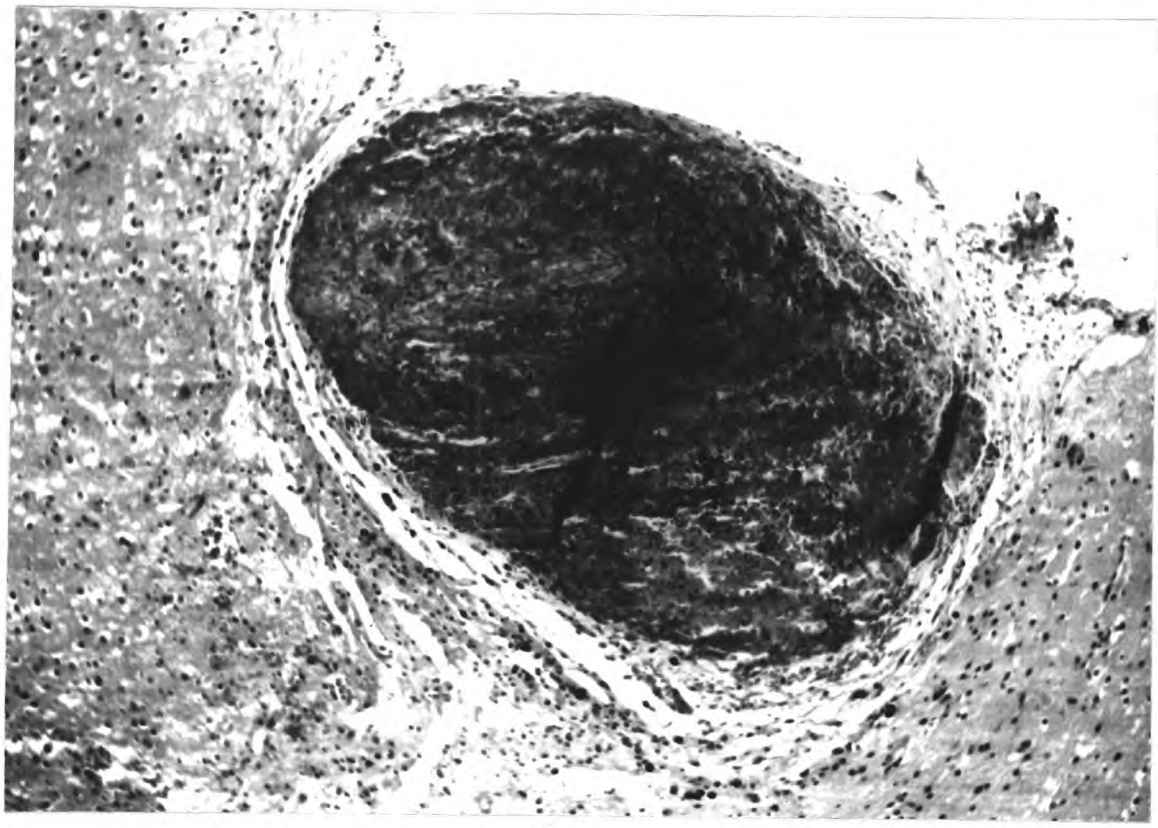


Figure 12. Large thrombosed blood vessel on the surface of the cerebral cortex. Hypertensive animal dying 15 months after irradiation (1000R). H&E x110.

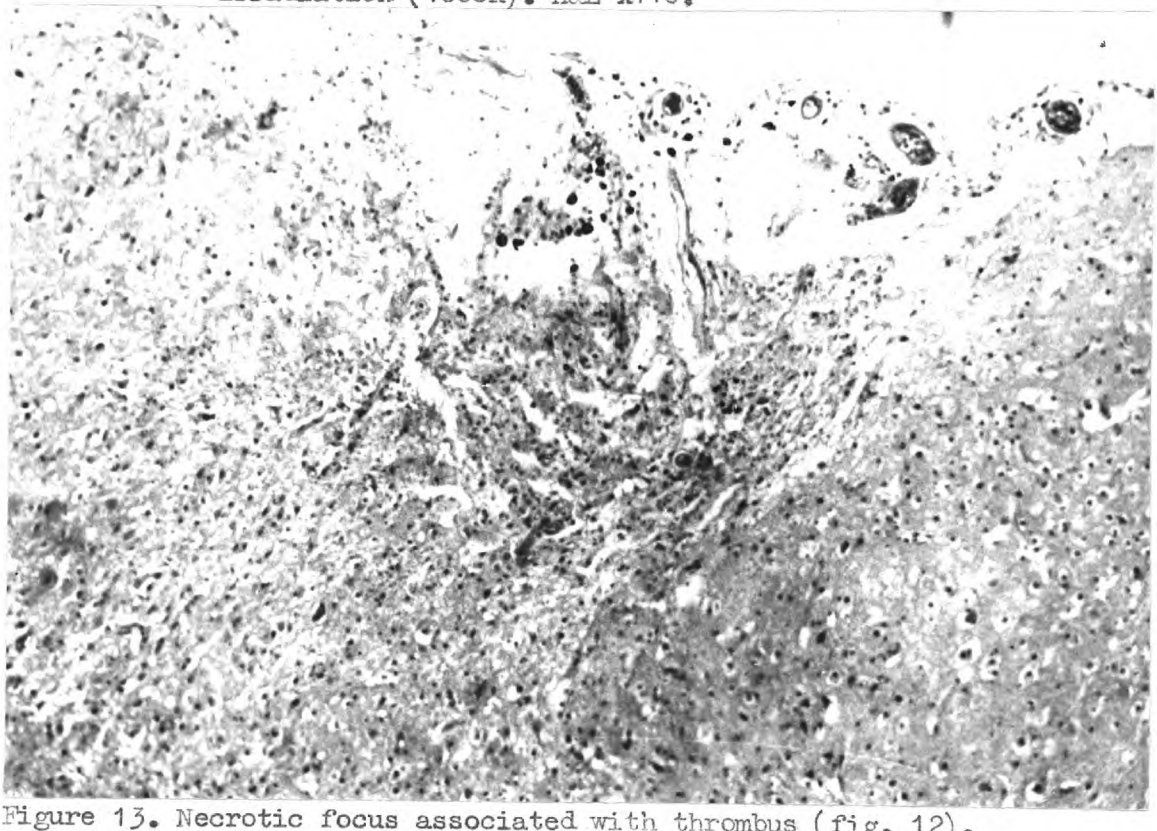


Figure 13. Necrotic focus associated with thrombus (fig. 12). H&E x110.

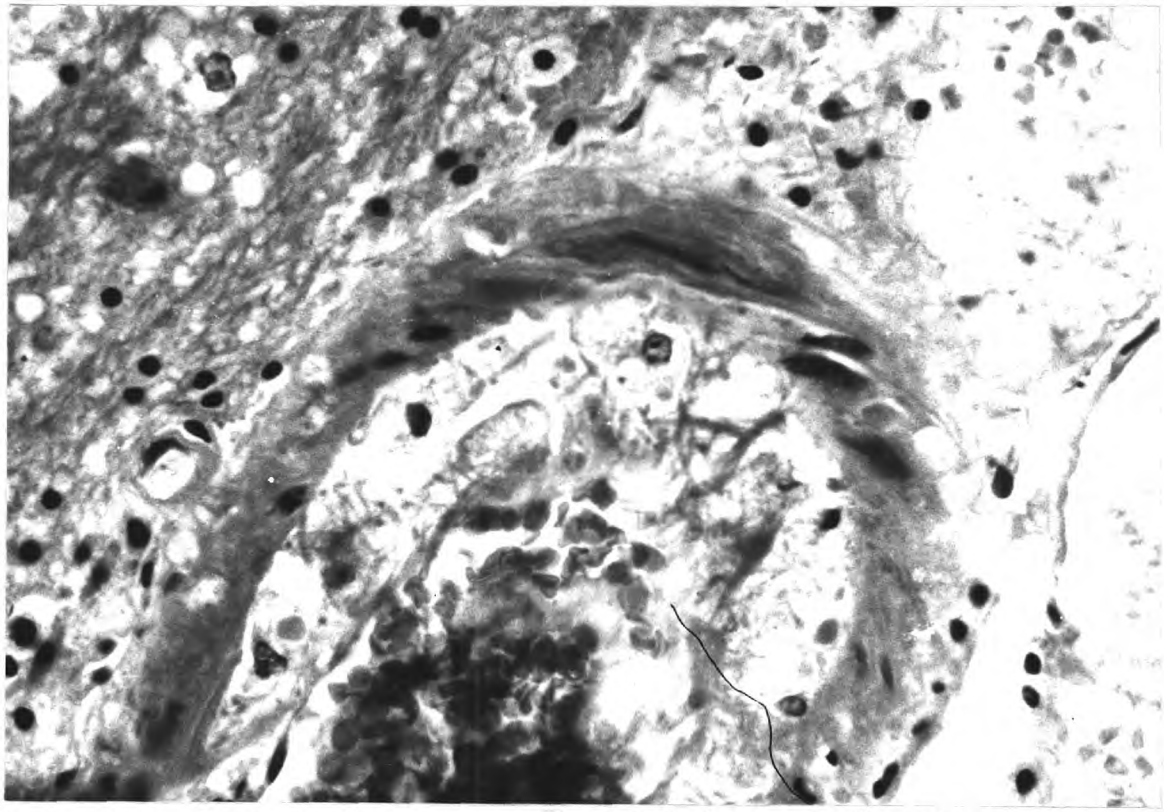


Figure 14. Macrophage reopening of occluded vessel. Hypertensive animal dying after a latent interval of nine weeks (3000R). H&E x700.



Figure 15. Thrombosed blood vessel in the choroid plexus. Latent interval 14.25 months following 2000R. H&E x110.

reopening could be observed with macrophage cells lining the vascular cavity (fig.14). Occlusion of vessels in the choroid plexus was another feature (fig.15).

e. Hyalinised blood vessel walls were, as stated in section 1 found on the cortex of hypertensive rats. Occasional vessels of this type were found in the normotensive animals in the thalamic region.(Fig.17).

3. The subependymal plate, which is a very distinct region of cells found around the anterior part of the lateral ventricles, exhibits striking changes the severity of which are clearly related to dose.

In the 4,000 and 3,000R irradiated groups the subependymal layer which is normally identified by its high nuclear density (fig.18), is not present and the subependymal region in close proximity to the lateral ventricles is almost anuclear. (Fig.19).

This is not the case of animals receiving doses of 1,000 and 2,000R, when the subependymal layer was always found to be intact. The example given in figure 20 is of a rat receiving 2,000R and dying 15½ months after irradiation. The subependymal plate in this case compares very well with that of the non-irradiated control animal (fig.18).

Five of the seven longest surviving rats (15 months) in the two highest dose groups, showed changes in the corpus callosum. In three of these five animals changes were severe with considerable

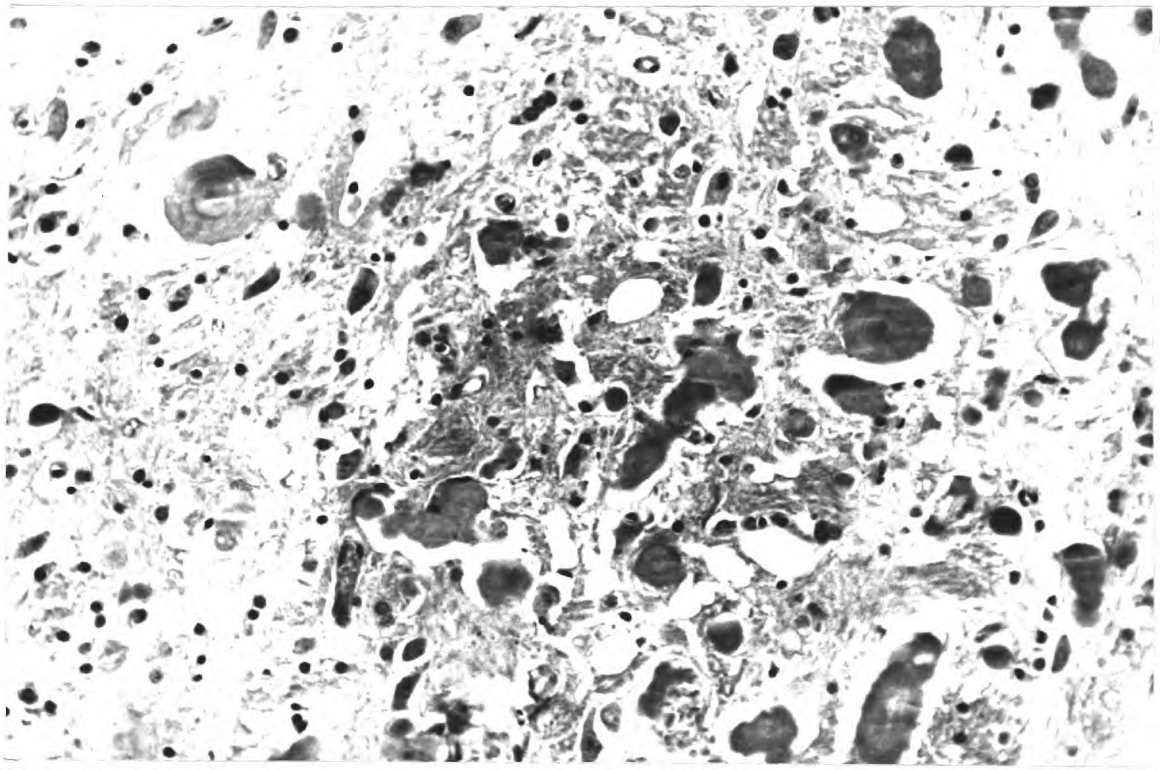


Figure 16. Small group of round laminar bodies found in the thalamic region of the brain. Animal dying 18.75 months after irradiation (3000R). H&E x300.

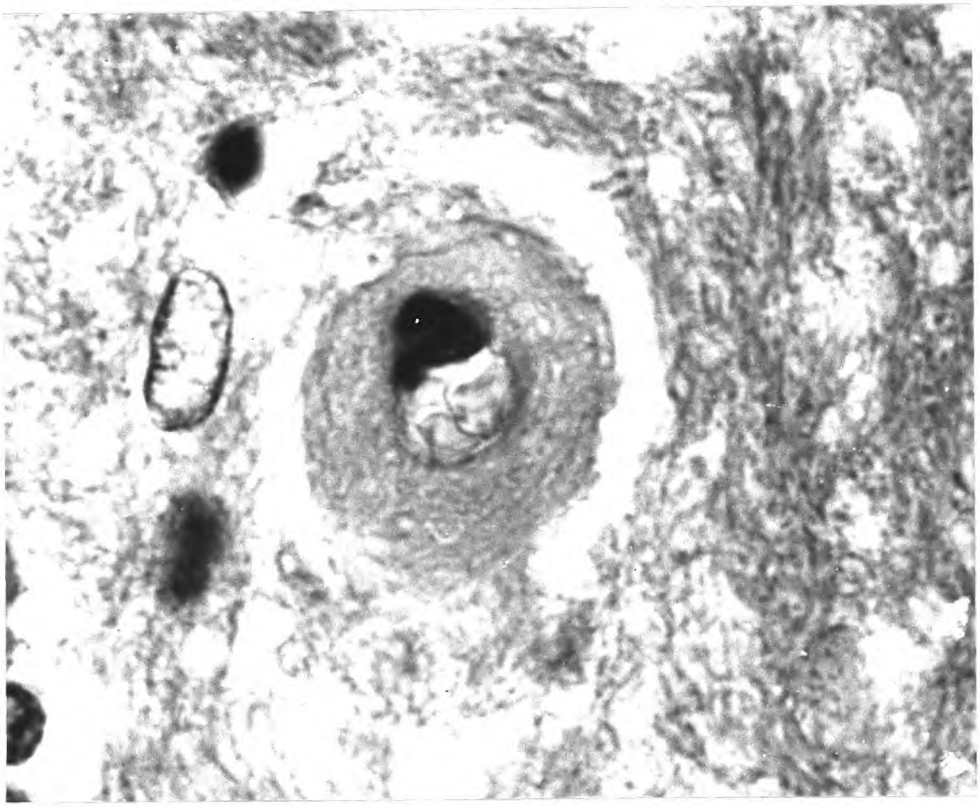


Figure 17. Hyaline change in small vessel in the thalamic region of an irradiated (4000R) brain. Latent interval 15 months. H&E x1600.

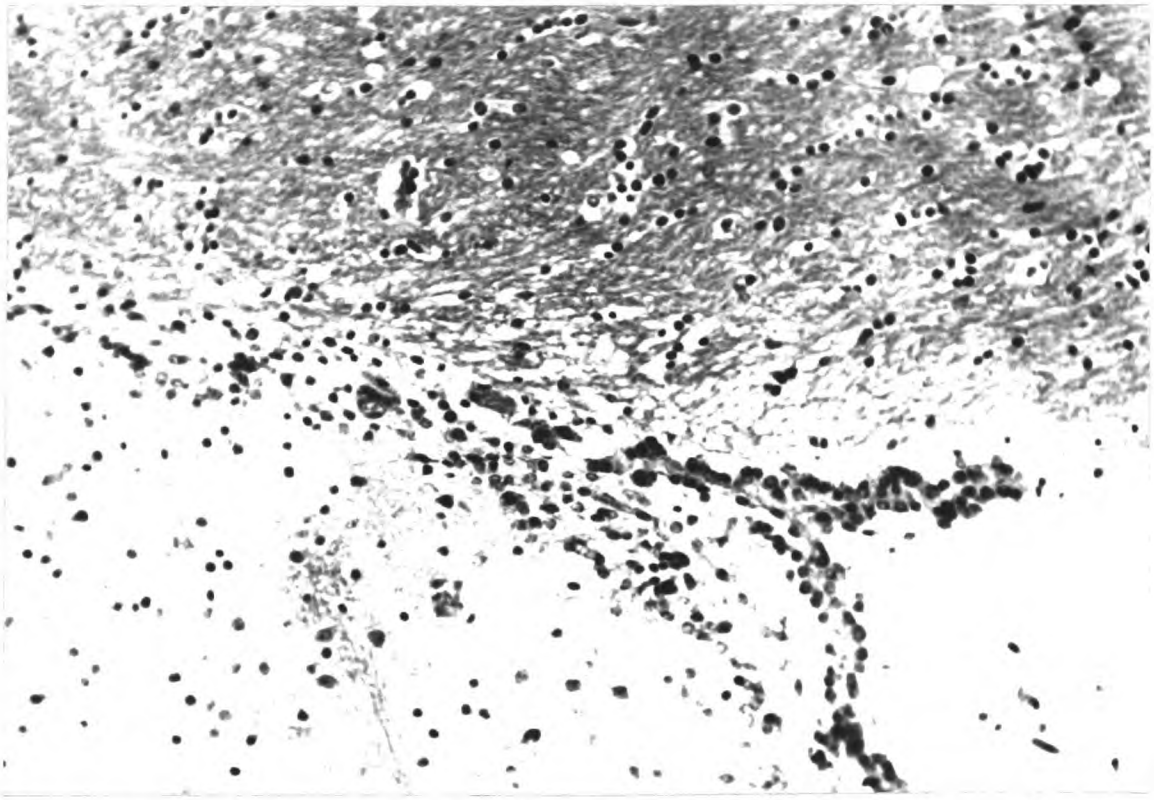


Figure 18. Subependymal layer, situated immediately under the ependyma on the lateral wall of the lateral ventricle. 26 month old rat. H&E x300.

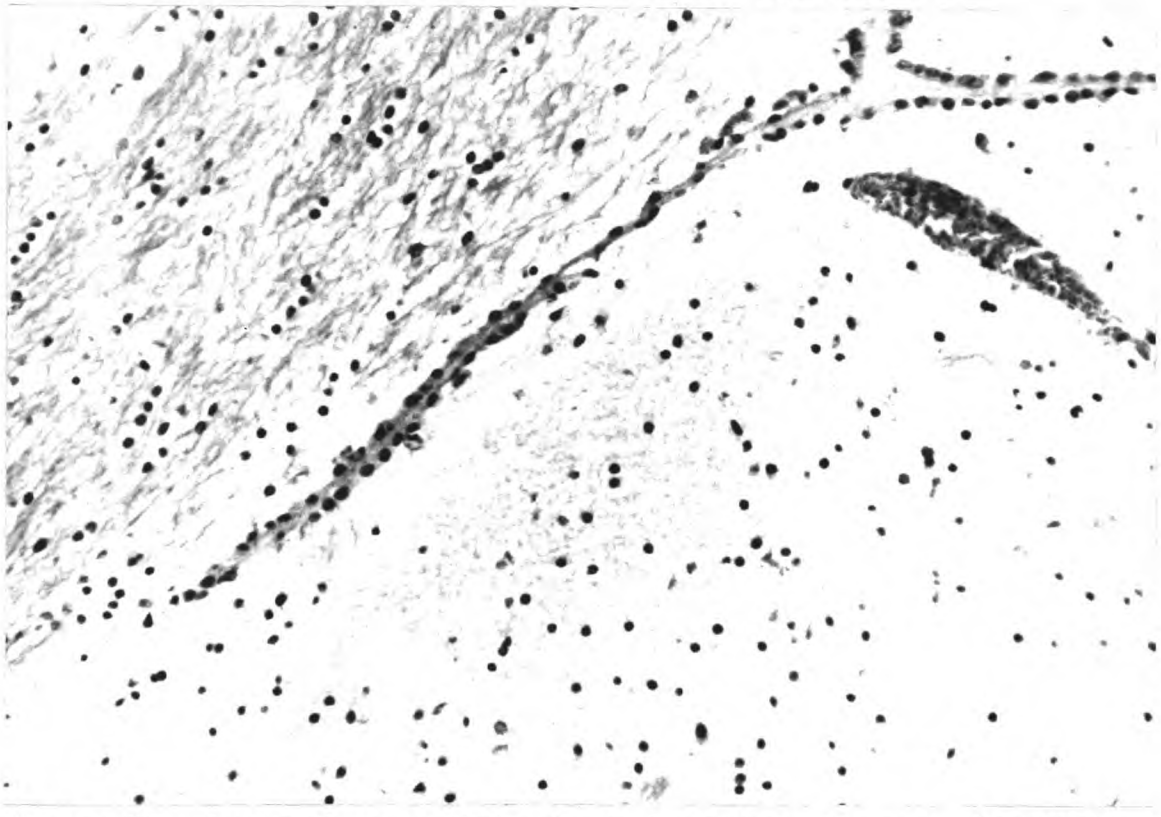


Figure 19. Lateral ventricle from irradiated brain (4000R). The area underlying the lateral wall is almost anuclear. Latent period 9 months. H&E x300.

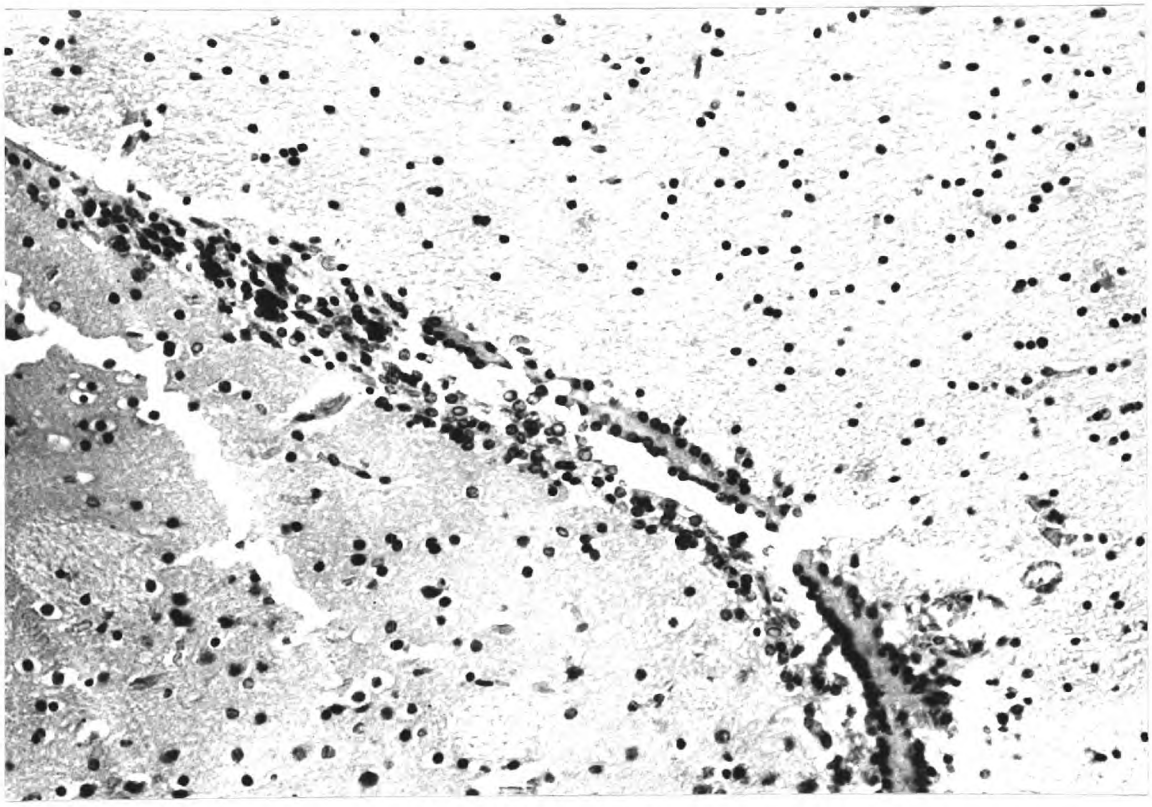


Figure 20. Apparently intact subependymal layer in 2000R irradiated brain. Latent period 15.5 months. H&E x300.

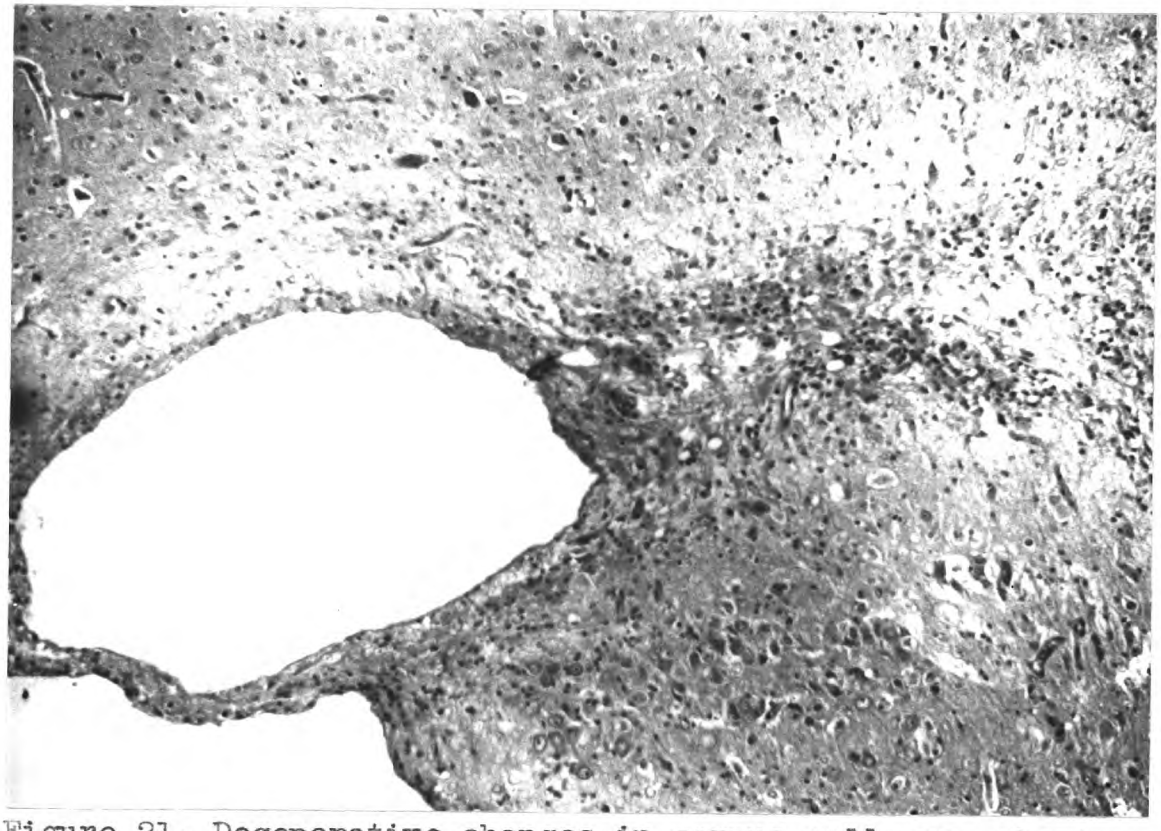


Figure 21. Degenerative changes in corpus callosum, showing large cystic space. Latent period 18.75 months following 3000R. H&E x110.

destruction of myelinated fibres. This results in the complete destruction of the organisation of the corpus callosum and the appearance of large cystic spaces (fig.21). In one case there has been a large infiltration of small dark round nucleated cells which are of doubtful cellular type and origin, (fig.22). In another brain, large abnormal multinucleate macrophages are observed in the corpus callosum (fig.23) which are in many ways similar to the abnormal macrophages encountered in wounded areas of previously irradiated brains (fig.46). Deposition of foamy cells are also encountered in the corpus callosum, (fig.24).

In a single animal in the 4,000R hypertensive brain irradiation group that died 15 months after irradiation, numerous abnormal mitotic figures were observed in the superficial regions of the cerebral cortex (fig.25). The number of mitosis amounted to between 10-15 per section. The mitotic figures were identified as abnormal astrocytes. They did not appear to be associated with any other abnormal features in the cerebral cortex.

In one other rat (1,000R hypertensive) a very extensive tumour of glial origin was found throughout the brain. The detailed histology of this and another radiation induced tumour are dealt with in Chapter 6.

D. Distribution of lesions in irradiated brains

The degree and site of delayed radiation changes in the irradiated brain varies according to the dose of radiation given. The hypertensive

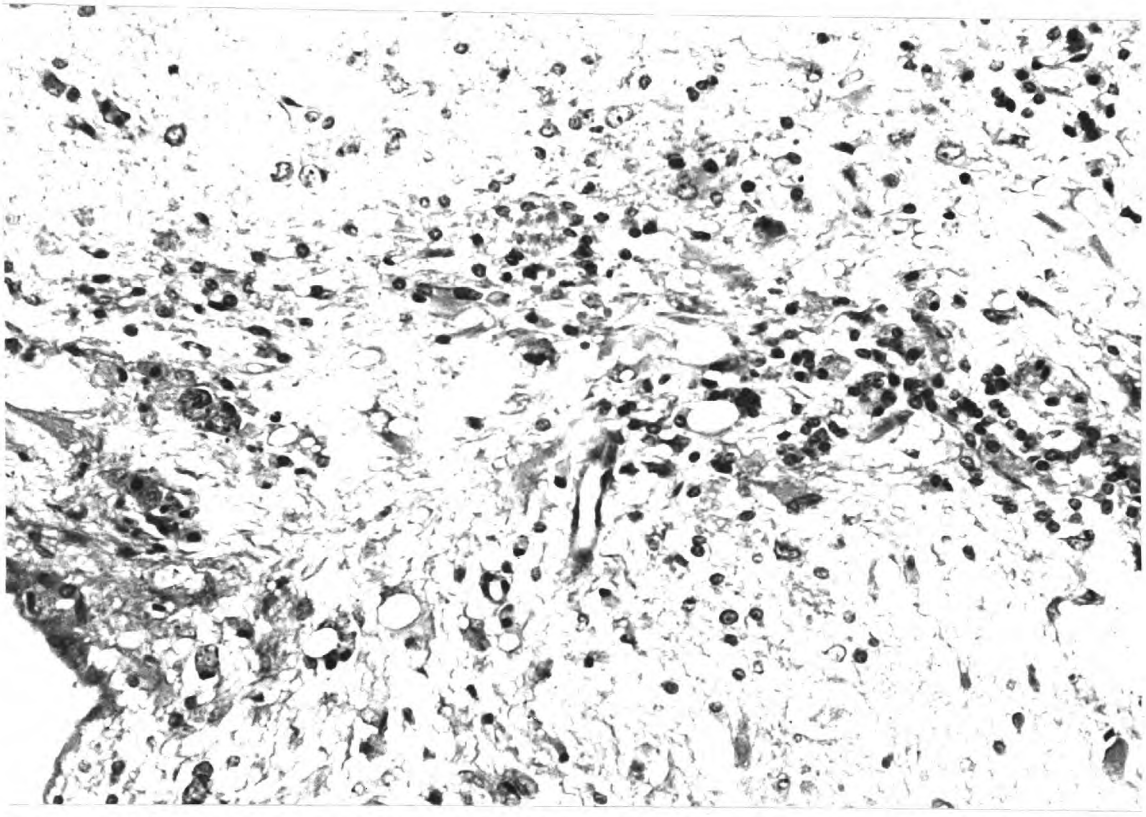


Figure 22. Degeneration of the corpus callosum. Increase in the number of small dark round cells. Latent period 18.75 months following 3000R. H&E x300.

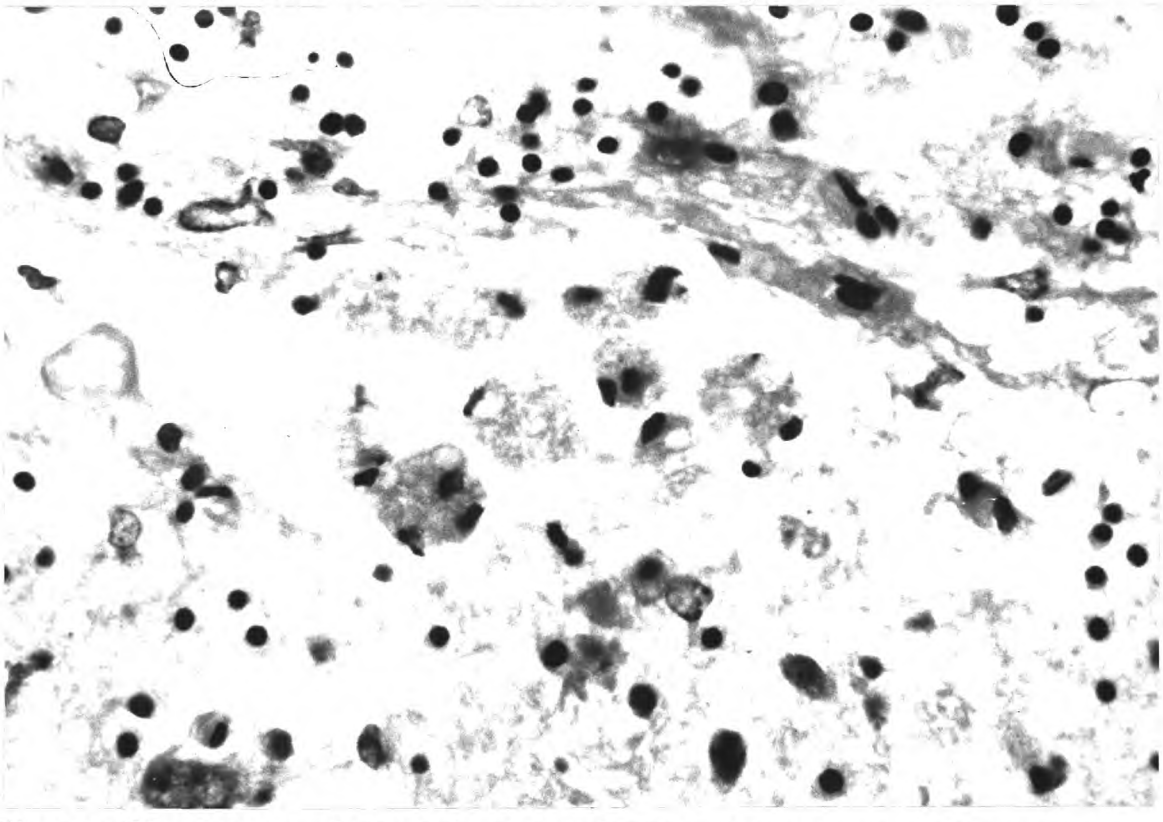


Figure 23. Large abnormal macrophages in corpus callosum. Hypertensive animal dying 18 months after irradiation (4000R).

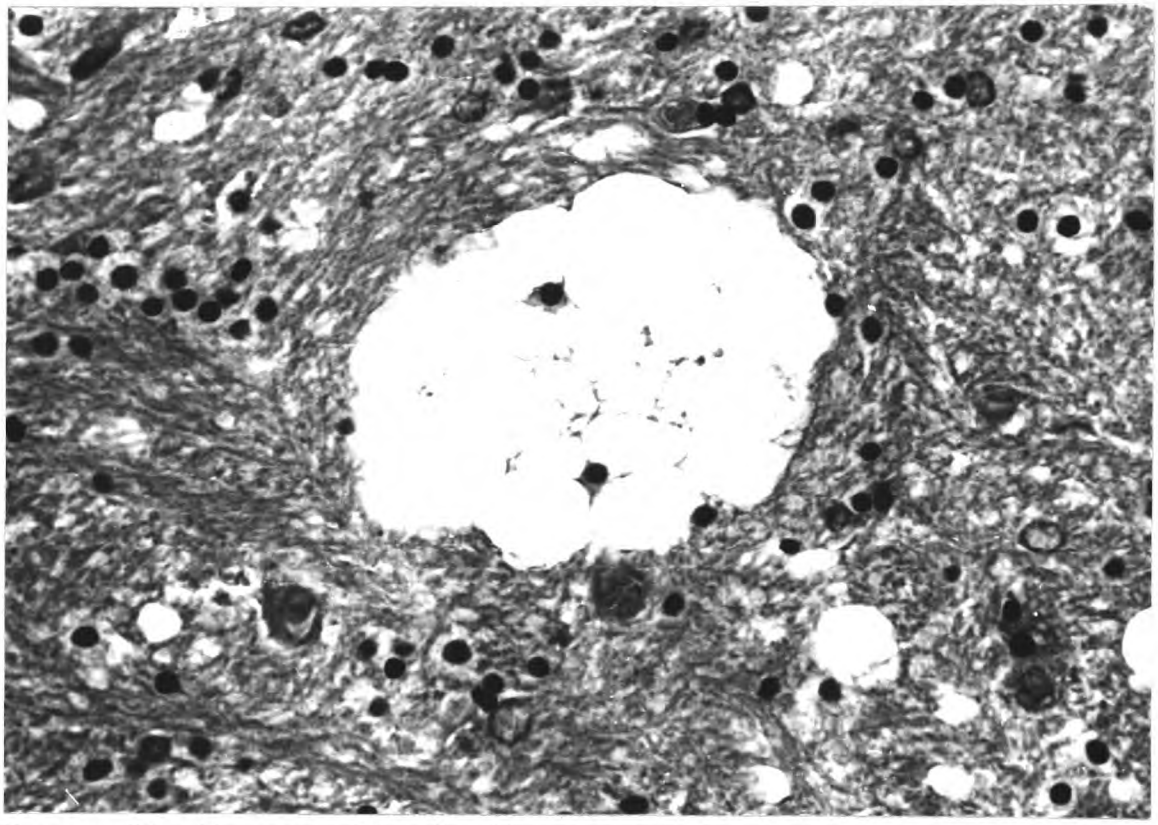


Figure 24. Foamy cells in the corpus callosum. Hypertensive animal dying 18 months after irradiation (4000R). H&E x700.

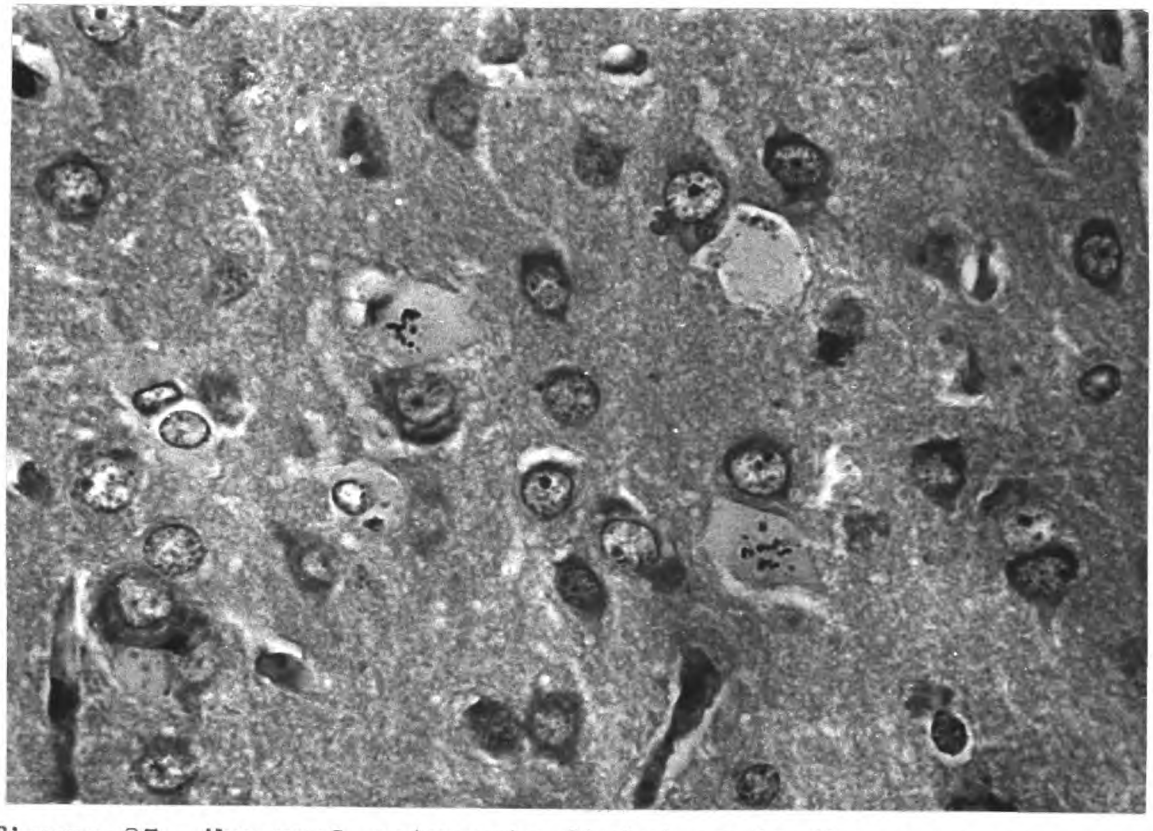


Figure 25. Abnormal astrocyte divisions in the cerebral cortex. Hypertensive animal dying 15 months after irradiation(4000R). H&E x700.

or normotensive condition of the animal also influences the type of lesion produced. Each group will be considered in turn.

1. Normotensive

4,000R (Figs.26 & 27)

Animals in this group survived for a mean latent period of 10 months after irradiation. Their brains showed the most marked histological changes with large cystic areas of necrosis restricted almost entirely to the white matter.

The large areas of necrosis were most commonly found in the internal capsule with all but one of the animals showing degeneration to some extent in this region. In one brain almost complete destruction of this region on the left side had occurred in the section examined. Both sides of the fimbria hippocampus were commonly affected (five out of six animals). In one animal the necrotic area had extended to involve the hippocampus.

Complete necrosis of the optic nerve and optic chiasma was observed in three animals of this group.

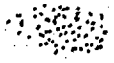
Small areas of necrosis were noted in the corpus callosum of the longest surviving rat in this group. It may be of importance to note that no necrotic lesions were observed in the cerebral cortex. The lesions that did occur were only occasionally associated with vascular damage, as was the case with rat 25.

Slight vascular damage did occur in regions well away from the large areas of necrosis already mentioned. Regions affected were the median preopticus nucleus (two cases) and the median dorsal

Key to Figures 26 - 51.

▲ Vascular lesions.

● Regions containing round bodies.

 Areas of necrosis.

 Sections not available.

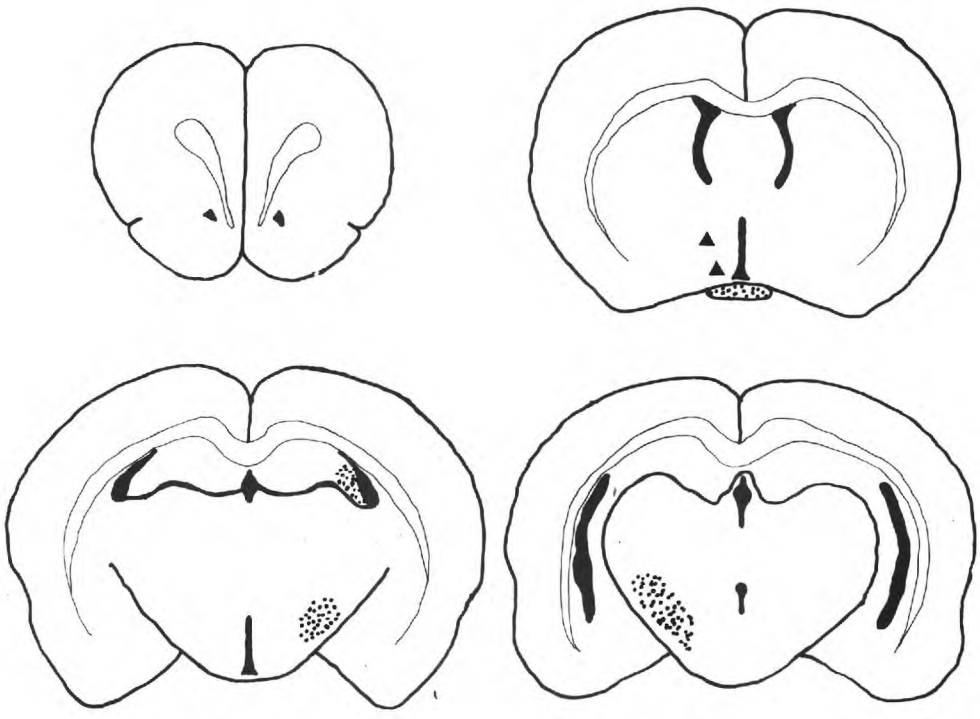


Figure 26. Distribution of lesions in 4000R irradiated brain after a latent period of 9 months.

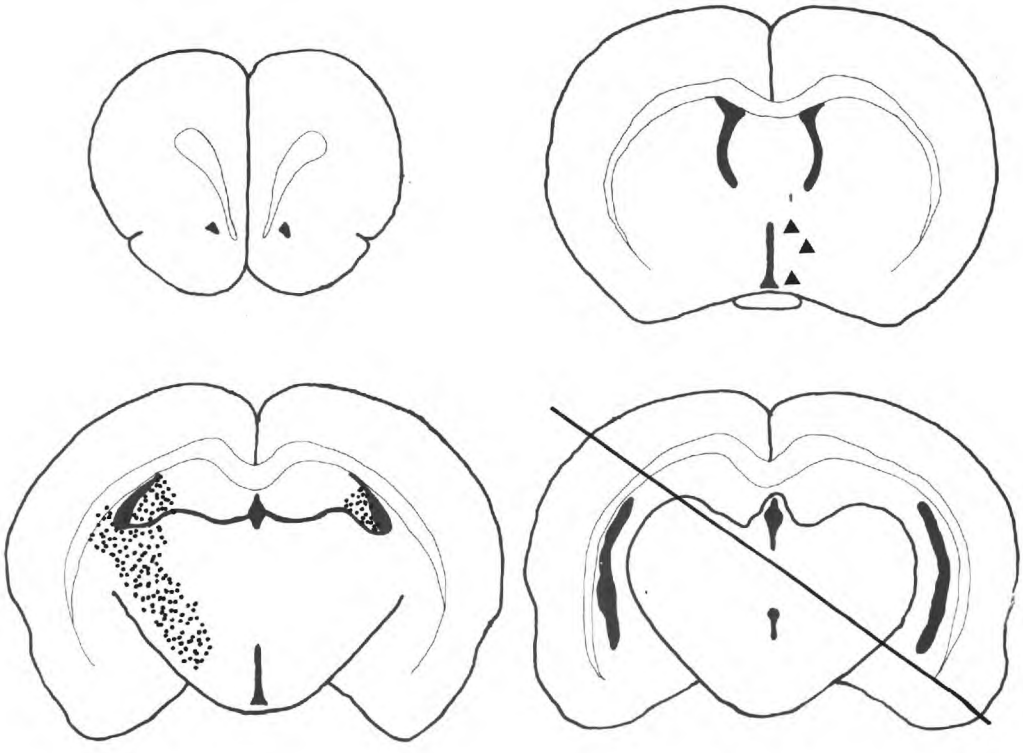


Figure 27. Distribution of lesions in 4000R irradiated brain after a latent period of 10.5 months.

nucleus (one case). Gliosis was not associated with any of these necrotic or vascular lesions.

3,000R (Figs. 28 & 29)

As in the previous group cystic areas of necrosis were present but not to the same extent. They were, as before, restricted to the white matter in the internal capsule and fimbria hippocampus and extended into the hippocampus and corpus callosum in the longest surviving animal of the group. The optic nerve and optic chiasma were affected in three animals of the group. Gliosis was not present in or around any of the necrotic regions.

Blood vessel changes were more evident than in the 4,000R group. Vascular damage was again restricted to the region of the median preopticus nucleus, the median dorsal nucleus in the hypothalamus, and the lateral thalamic nucleus. Amyloid bodies were evident in two brains.

In one animal there was evidence of vascular occlusion and necrosis of the choroid plexus in the lateral ventricles.

2,000R

Only slight vascular damage could be seen in 50% of the animals in this group, and this was mainly restricted to the choroid plexus. The remaining animals in the group showed no changes in the sections of the brain examined.

No abnormalities could be detected in any of the rats in the 1,000R group.

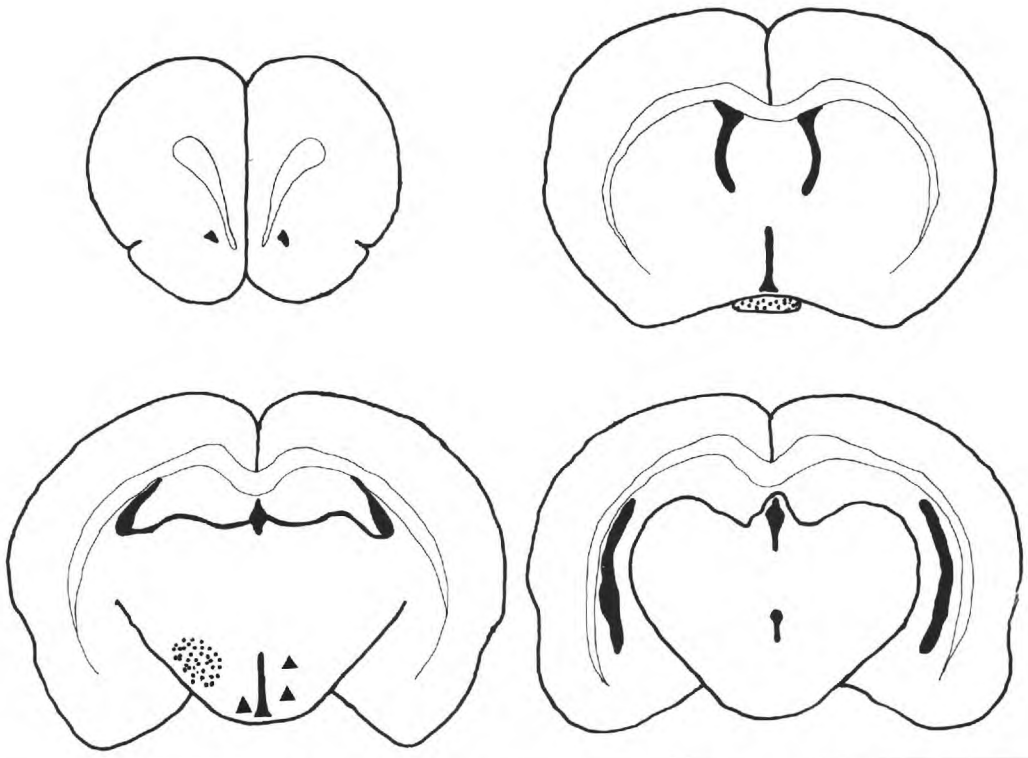


Figure 28. Distribution of lesions in 3000R irradiated brain after a latent period of 11 months.

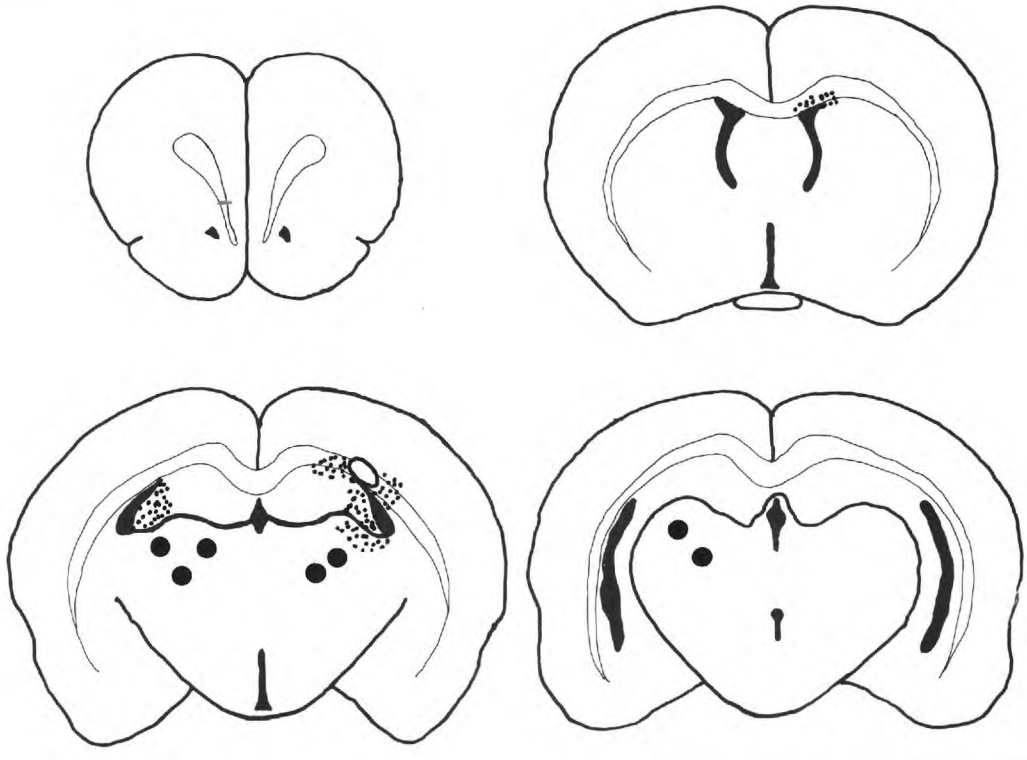


Figure 29. Distribution of lesions in 3000R irradiated brain after a latent period of 18.75 months.

2. Hypertensive

In this series of animals only those in the 4,000R group showed any of the large areas of necrosis that were found in the 3,000R and 4,000R normotensive groups.

4,000R (Figs 30 & 31)

The necrotic areas, as in the corresponding normotensive group of animals, were found only in the white matter and this in mainly the internal capsule and optic chiasma. In the longest surviving animals in the group spaces were found extensively throughout the corpus callosum. In an animal surviving for 15 months after irradiation almost complete destruction of the central white matter in the olfactory bulbs had occurred.

Blood vessel changes were also present in the thalamic and hypothalamic regions of the brain.

Only one animal in the group did not exhibit these gross irradiation changes. This was the first animal in the group to die, 5 months after irradiation. Thickening of the muscle wall of some superficial cortical arteries being the only abnormal feature.

3,000R

Damage of the vascular type only was detectable. This damage was restricted to the cortex and hypothalmic regions. Small focal areas of necrosis were often associated with these vascular lesions.

One animal died with marked internal hydorcephalus, the cause of which could not be determined.

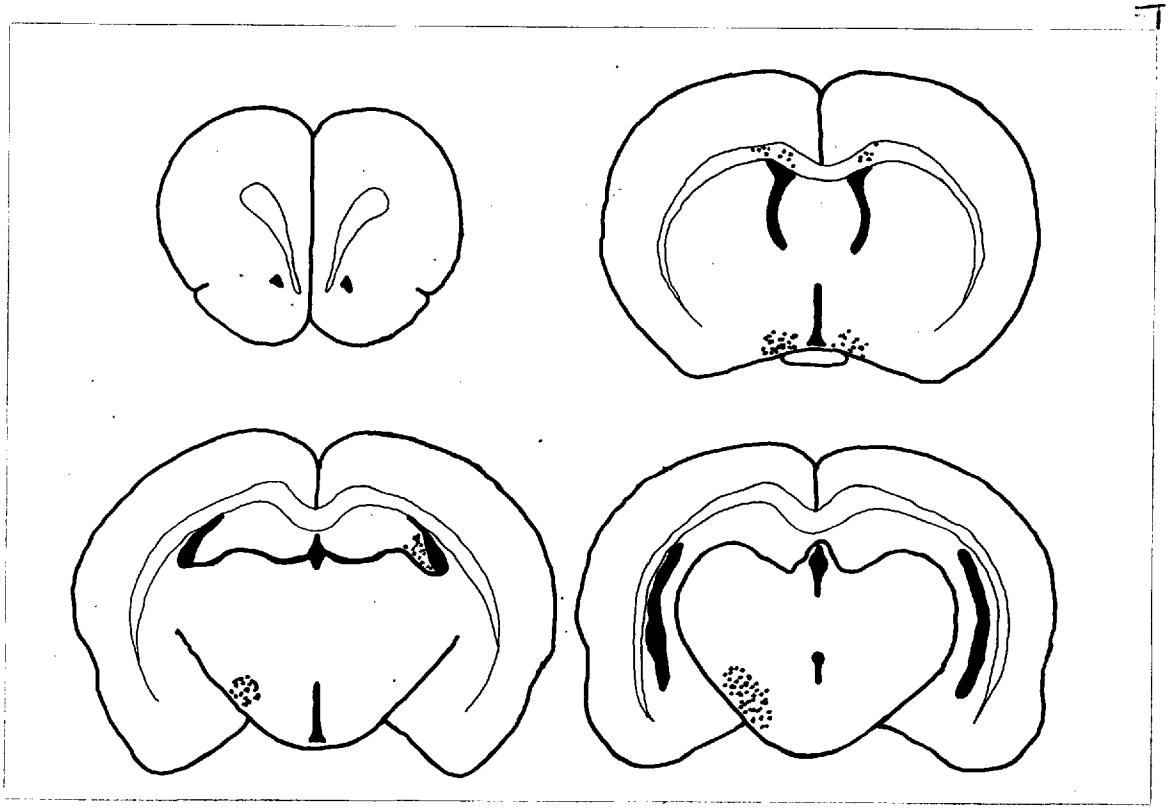


Figure 30. Distribution of lesions in 4000R irradiated brain of hypertensive animal after a latent period of 8 months.

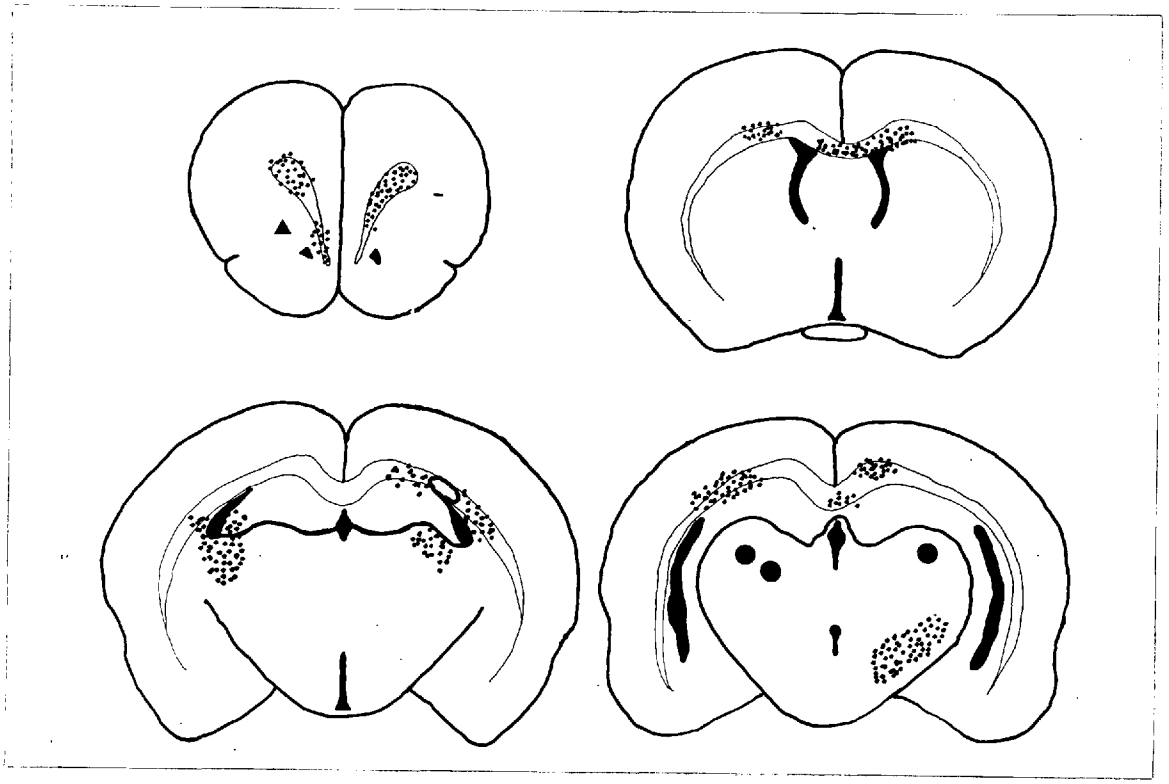


Figure 31. Distribution of lesions in 4000R irradiated brain of hypertensive animal after a latent period of 14.75 months.

2,000R

As in the preceding group the only damage found was of the vascular type and was mainly restricted to the highly vascular cerebral cortex. The vessels of the choroid plexus were also affected.

1,000R

Unlike the brains of the 1,000R normotensive rats where no changes were found, these animals showed vascular damage which was associated with small areas of necrosis.

One of the animals in the group died with severe internal hydrocephalus, possibly resulting from recent extensive haemorrhage in the base of the brain.

Thus latent cerebral irradiation damage in the normotensive animals seems to follow a recognisable pattern. In the 4,000R group necrotic areas are found in the deeper white matter. Vascular damage is restricted to the thalamic and hypothalamic regions. A similar picture emerges in the 3,000R group with more extensive vascular damage. In the longest surviving animal in these groups the corpus callosum is damaged. In the 2,000R groups only vascular damage is present.

In the hypertensive group of animals, the 1,000, 2,000 and 3,000R groups all show vascular damage with the occasional foci of necrosis. In the 4,000R group, white matter degeneration is again in evidence, the longer surviving animals showing damage to the corpus callosum, as in the normotensive groups.

CHAPTER III

THE EFFECTS OF PREVIOUS LOCAL IRRADIATION ON THE CEREBRAL RESPONSE TO INJURY

The following chapter gives an account of the preliminary results published previously (Hopewell & Wright 1967). The results have been amplified with the addition of a cell labelling technique similar to that used by Spector et.al. (1965, see Chapter 7) and by the use of high dose rates of electrons to eliminate effects due to long irradiation periods necessitated by our X-rays source. Cerebral lesions were produced by freezing from the outside surface of the skull (see Chapter 7). This method reduced the risk of cerebral infection and avoided mechanical disturbance of tissues. It produced a uniform saucer shaped lesion on the surface of the brain 2 - 3mm across and 0.5 - 1.0mm. deep. The lesion was clearly depicted by the incorporation of trypan blue which is phagocytosed by the macrophages in the lesion. Histologically a marked cellular reaction was observed in the initial area of necrosis which was maximum by the seventh day. In the brains that had been previously irradiated this cellular reaction was reduced, the reduction being dependant on the dose of prior irradiation. The pathogenesis of the cellular reaction resulting from freezing injury will be discussed later in this chapter.

A. Results

Cell counts were made within the lesion. To minimise

subjective errors, these cell counts were made by using the following standard procedures. Counts were carried out where the lesion was 0.5mm. in depth. Using a squared ocoular eye-piece and oil immersion lens (magnification 1,050) a proportion of cells were counted in four consecutive areas (0.86 x 0.86mm.) perpendicular to the surface of the brain (fig.32). The eye-piece was subdivided into 64 smaller equal squares by a squared graticule (8 x 8) and cells in alternate columns of squares (1 x 8), perpendicular to the surface of the brain were counted. Thus the cells in 32 small squares (half the field area) were counted. The number of cells was estimated by counting nuclei in the stipulated area.

Up to five consecutive serial sections were mounted on a slide and from these a minimum of two sections were counted. Attempts were made to avoid large blood vessels and obvious artifacts which would clearly produce an erroneous result.

In the example of cell counts demonstrated (Table 5) the field areas, A, B, C and D are arranged from the depth to the surface of the lesion. The mean cell count is calculated from the six rats in each group and expressed as a percentage of the mean cell count for the non-irradiated controls (230 cells). One of the features illustrated by this table is the uniformity of cells within the counted area, a feature that was not anticipated. All groups in the 220KV X-ray experiments are made up of six animals.

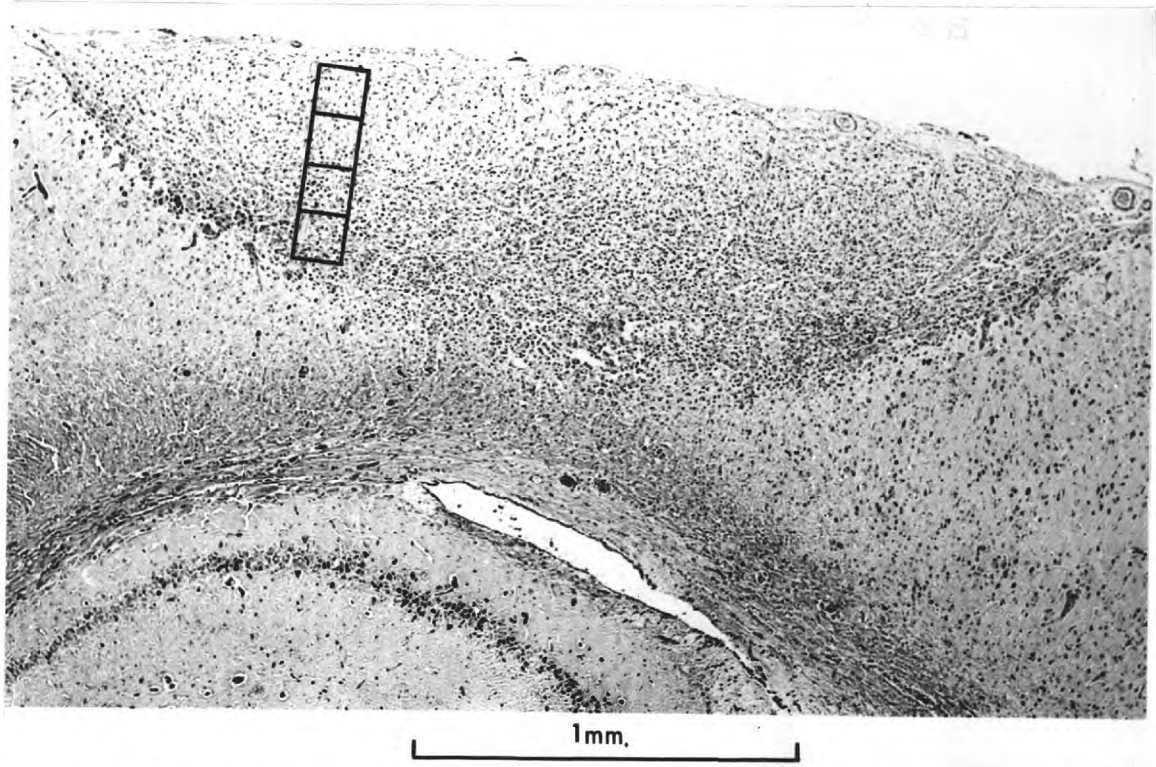


Figure 32. Lesion on the left cerebral hemishere of the brain of the rat, indicating the four areas (0.86x0.86) used for cell counts.

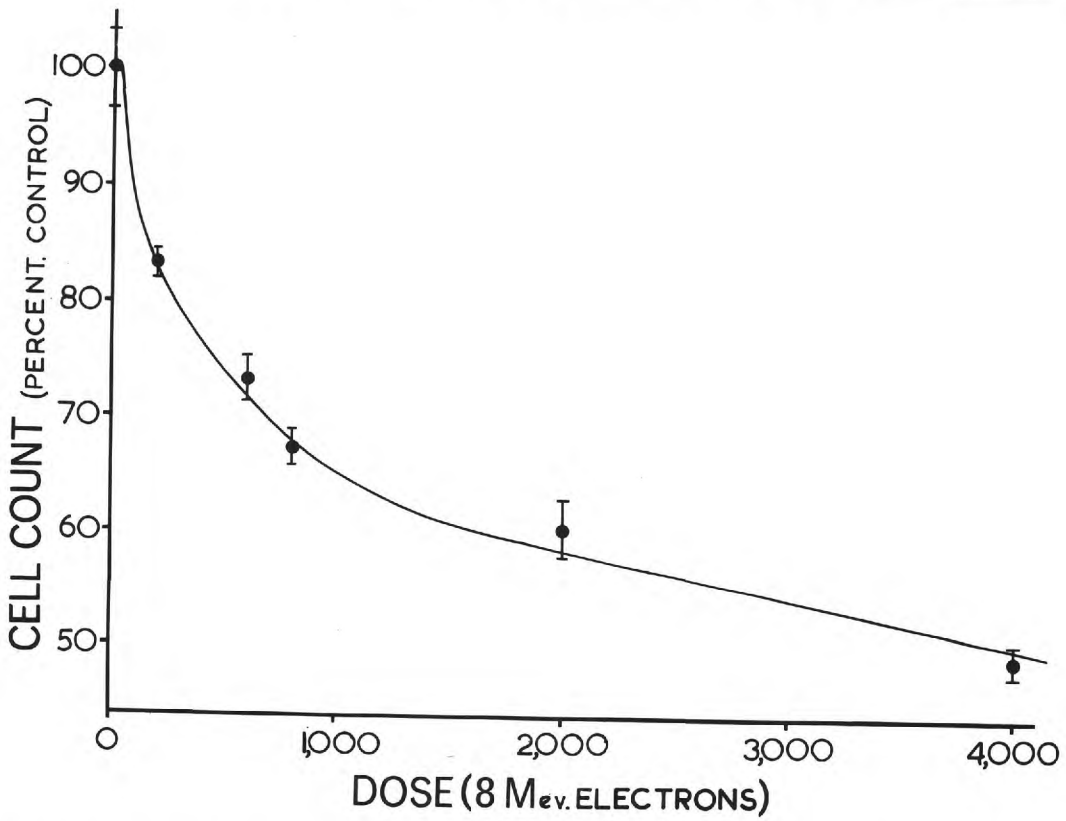


Figure 35. Cell counts/unit area of lesion, expressed as percentage of the controls, plotted against the dose of 8Mev. electrons.

TABLE. 5

Cell Counts in 7 Day Brain Wound. Injury Made 3 Days After 600R Irradiation.

Rat No.	1	2	3	4	5	6	Mean cell count per field area.
Field area	Section Number.						
	1 2	1 2	1 2	1 2	1 2	1 2	
A	43 49	40 42	33 41	44 45	35 38	37 41	40.67
B	39 40	44 45	46 36	45 41	44 42	43 37	41.83
C	45 43	35 44	46 36	35 35	33 38	34 39	38.58
D	43 46	47 39	35 40	49 34	36 37	39 36	40.08
Total cell count.	170 178	166 170	160 153	173 155	148 155	153 153	161.1
Mean cell count.	174	168	156.5	164	151.5	153	161.16

Mean Cell Count as percentage of non-irradiated control - 70.07%

The results are shown graphically in fig.33. It can be seen that the cell counts fall off rapidly with doses up to 800R but that with higher doses the fall off in cellular reaction reduced. The maximum reduction is approximately 50% with a previous irradiation dose of 4,000R.

If the log of the cell counts is plotted against the exposure dose on a linear scale the dose response can be expressed as two straight lines (fig.34). An interpretation of these two lines is that they represent two cell populations. It appeared possible that the shallower slope might represent an anoxic cell fraction possibly occurring as a result of mild hypothermia at the time of irradiation. This in turn being a consequence of long irradiation periods. For example it took 50 minutes to give a dose of 4,000R with the Maximar 220kV X-ray machine. A rat's body temperature can fall from approximately 37° to 31°C in this time.

Because of the difficulties of controlling body temperature using conventional 220kV X-rays the experiment was repeated using 8 MeV electrons from the linear accelerator at the Post Graduate Medical School, Hammersmith.

This enabled all doses to be administered within a similar time interval of 1 - 2 minutes so that all animals were irradiated under similar physiological conditions.

The results obtained (fig.35) are similar to the previous results using X-rays which discounts the hypothesis that mild

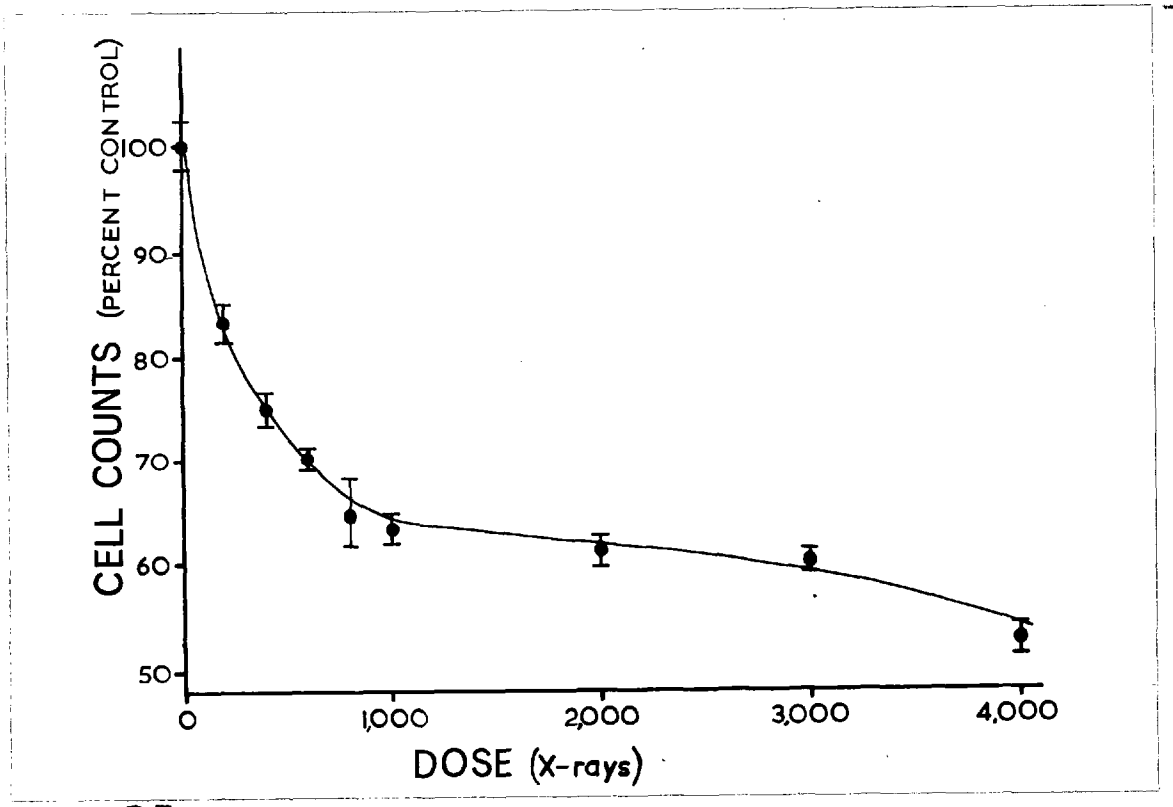


Figure 33. Cell counts/unit area of lesion, expressed as a percentage of the controls, against dose of 220kV X-rays.

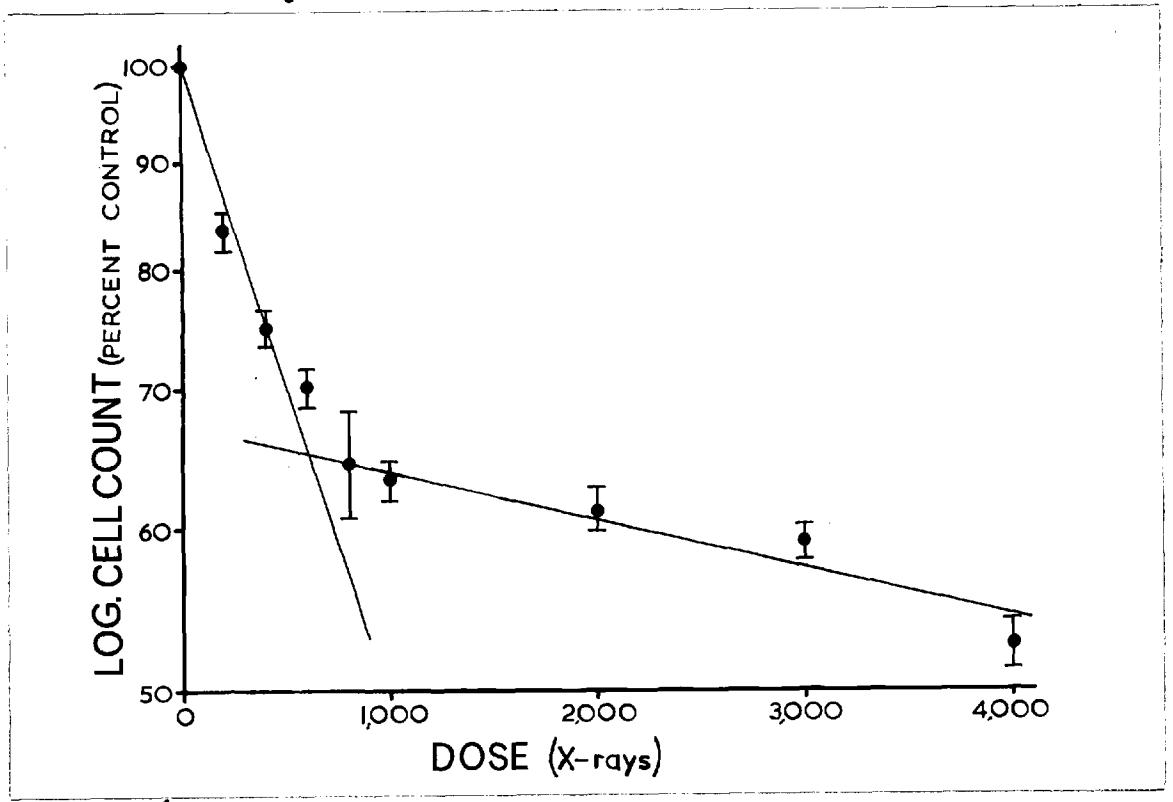


Figure 34. Semi-logarithmic plot of cell counts/unit area of lesion, expressed as a percentage of the controls, against dose of 220kV X-rays.

hypothermia produces an anoxic fraction of cells.

All the results so far cited have been for animals irradiated three days prior to injury. In a further study using fewer doses the interval between irradiation and injury was varied (table 6). This produced no significant variation in the response obtained even when an interval of six months had elapsed.

The simplest explanation of these results is that local cells are damaged on irradiation and that this radiation damage is manifest when the cells are stimulated to divide by injury. They then either fail to divide or die. It may also follow that the cell population of the cerebral cortex is a static one because the reduction in cellular reaction is not affected by increasing the interval between irradiation and injury. Radiation damage it would appear, is "stored up" until the cells are stimulated to divide.

For this hypothesis to be true we must postulate that the majority of brain macrophages are of local origin and not derived from the general circulation.

B. Pathogenesis of cerebral lesions

A better understanding of the pathogenesis of the cellular reaction to cerebral injury in normal and irradiated brain was obtained from the study of the brain lesions of a series of

TABLE. 6

Exposure Dose (R)	Cell counts per unit area of lesion as a percentage of non irradiated control			
	Latent Period Between Irradiation and Injury.			
	3 Days	3 Weeks	3 Months	6 Months
200	83.47±1.73 (6)	84.3±2.30 (3)	81.9 (2)	-
400	74.96±1.55 (6)	73.13-2.21 (5)	74.39±3.37 (5)	72.21±1.69 (5)
2000	61.12±1.49 (6)	61.83±1.80 (7)	62.30±2.08 (5)	62.26±1.87 (5)

The figures in brackets indicate the number of animals used in each group.

animals supplied with tritium labelled thymidine. This isotopically labelled DNA precusor was administered either prior to injury or after injury one hour prior to death (see Chapter 7).

Pairs of animals for each thymidine treatment were killed at intervals of 1, 3 and 7 days after injury for both normal and previously irradiated brains (2,000R). This scheme provided us with a comprehensive picture of the cellular reaction to injury in normal and previously irradiated brain up to the time (7 days) used extensively in our previous investigation.

DAY 1

On the first day after injury the damaged area was necrotic and apart from polymorphs and the occasional palely stained nucleus it was completely devoid of cells. In the control non-irradiated lesion given a single injection of thymidine prior to death, a few labelled cells were to be found in the periphery of the necrotic area both in the meninges (fig.36) and in the adjacent uninjured brain. Polymorphs were very common in all parts of the lesion. The lesion was not distinct, its borders gradually merging with the normal tissues of the brain. In the comparable pair of locally irradiated animals, the lesions were similar and labelled cells were to be found about the periphery of the lesion. Only occasional labelled cells were to be found within the previously defined (fig.32) cell counting areas (Table 7).

When animals had been injected with H^3 thymidine prior to

TABLE 7

Total cell counts per unit area of lesion, with its associated cell labelling index

	Time after injury (Days)											
	1				3				7			
	A		B		A		B		A		B	
	Total	Lab	Total	Lab	Total	Lab	Total	Lab	Total	Lab	Total	Lab
CONTROL	22.5	2	24.25	0	165.5	10.5	163	30.5	200	4	209	5
	23	4	25.5	0	176	7.5	147	22.5	208	3	229	3
IRRADIATED	24	3	27	0	102.5	7.5	119	2	122	11	113	2
	21	6	25	0.5	108.5	10.0	124.5	1	134	8.5	124	4

A - H^3 thymidine prior to injury B - H^3 thymidine after injury

Eight animals were examined at each time interval after injury. Of these four were previously irradiated (2,000R) and four acted as non-irradiated controls. Each group was further sub-divided into animals receiving H^3 thymidine either before or after injury.

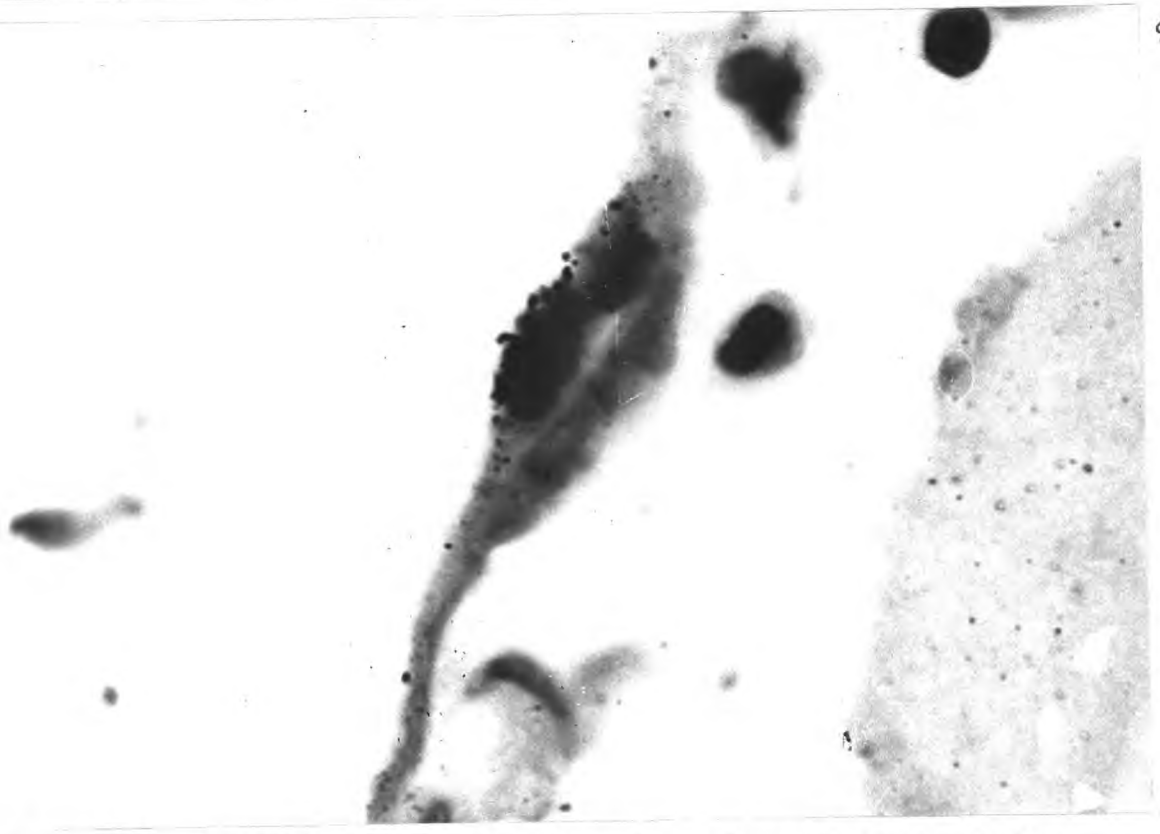


Figure 36. Labelled spindle shaped meningeal cell at the edge of the lesion, one day after injury. H^3 thymidine given 1 hour prior to death. Mayers x1600.

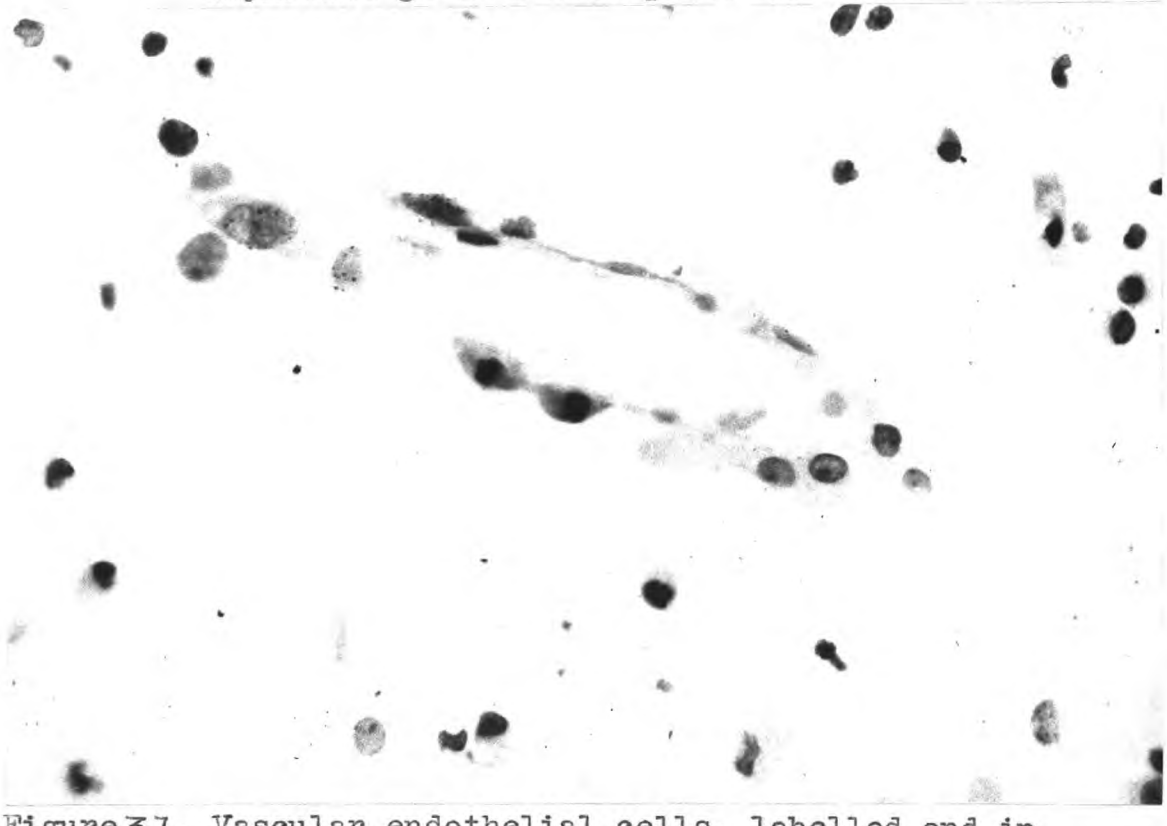


Figure 37. Vascular endothelial cells, labelled and in mitosis 3 days after injury. H^3 thymidine given 1hour prior to death. Mayers x700.

injury and killed on the first day after injury labelled mononuclear cells were to be found within the necrotic area. Labelled cells were usually associated with groups of polymorphs. A few were found within, or in close proximity to vessels of the intact meninges. This was a feature that contrasts markably with the numerous spindle shaped meningeal cells labelled in this region, in the previous groups. Polymorphs and labelled cells possibly form the main cellular components in the counted area at this time (haemotogenous cells). No significant differences between irradiated and normal lesions could be detected.

DAY 3

Three days after injury the appearance of the normal and irradiated lesions were strikingly different. In the control lesion given a single dose of H^3 thymidine one hour prior to death numerous labelled cells and large numbers of mitotic figures were observed. Labelled cells again predominated about the periphery of the lesion. This was in the spindle shaped cells of the meninges and in cells in the brain parenchyma, in close proximity to the lesion. The majority of these cells had small dark round nuclei characteristic of microglia although a few with large pale nuclei were possibly astrocytes. Mitotic figures and labelled cells were also present in the lesion. Vascular endothelial cells were also commonly labelled or in mitosis (fig.37). The centre of the lesion was necrotic although there had been some invasion by polymorphs and the occasional labelled cell.

In marked contrast in the comparable irradiated lesions, labelled cells and mitotic figures were rare (table 7). The occasional small group of 3 or 4 labelled cells were to be found in and around capillaries. The cells may be macrophages entering from the blood and synthesising DNA in preparation for division (fig.38). A few abnormal mitotic figures were to be found (fig.40).

In the animals receiving H^3 thymidine before injury, labelled cells were found in the lesion but not in such large numbers as when the isotope was administered after injury one hour prior to death. A few mitotic figures were labelled although the majority were unlabelled (fig.39). No vascular endothelial cells were labelled although labelled cells were found in close proximity to capillaries. Only a few cells about the periphery of the lesion were labelled.

In previously irradiated brain, the number of labelled cells in the lesion, when H^3 thymidine was given before injury, were similar to the numbers found in the lesion of non irradiated brain (table 7). Abnormal mitotic figures and cells with 4 or 6 micronuclei (micronucleated cells) were found in the irradiated lesion (figs. 41 & 42). These changes are often associated with radiation damage which infers that these cells are of local origin.

DAY 7

By the seventh day in the control non irradiated lesion, the cellular density had reached a peak and the previously necrotic

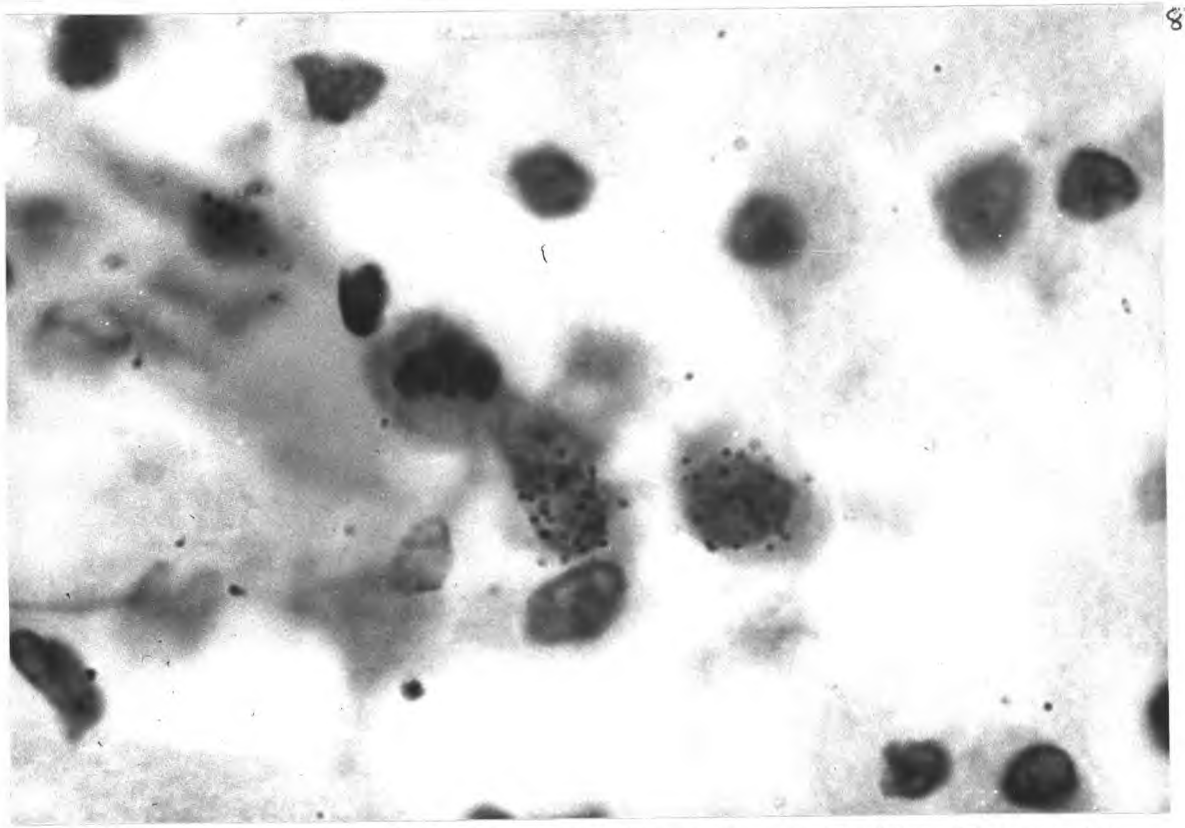


Figure 38. Two large labelled cells and a mitotic figure in an irradiated (2000R) lesion close to a dilated capillary. The may be blood macrophages synthesising DNA and dividing in the brain lesion. H^3 thymidine given 1 hour prior to death, 3 days after injury. Mayers x1600.

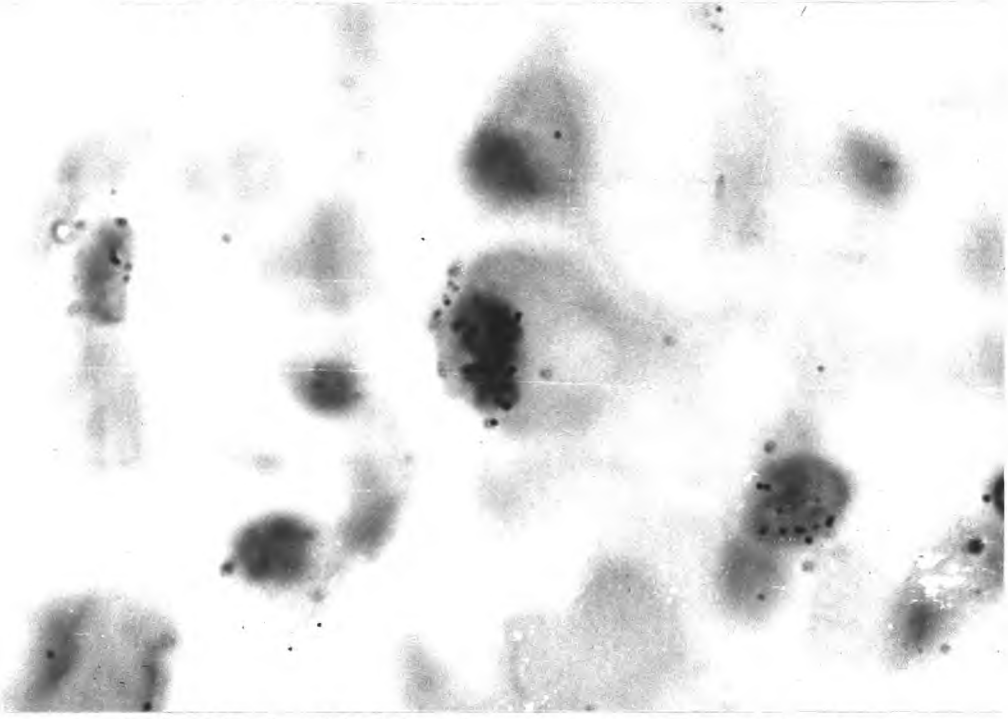
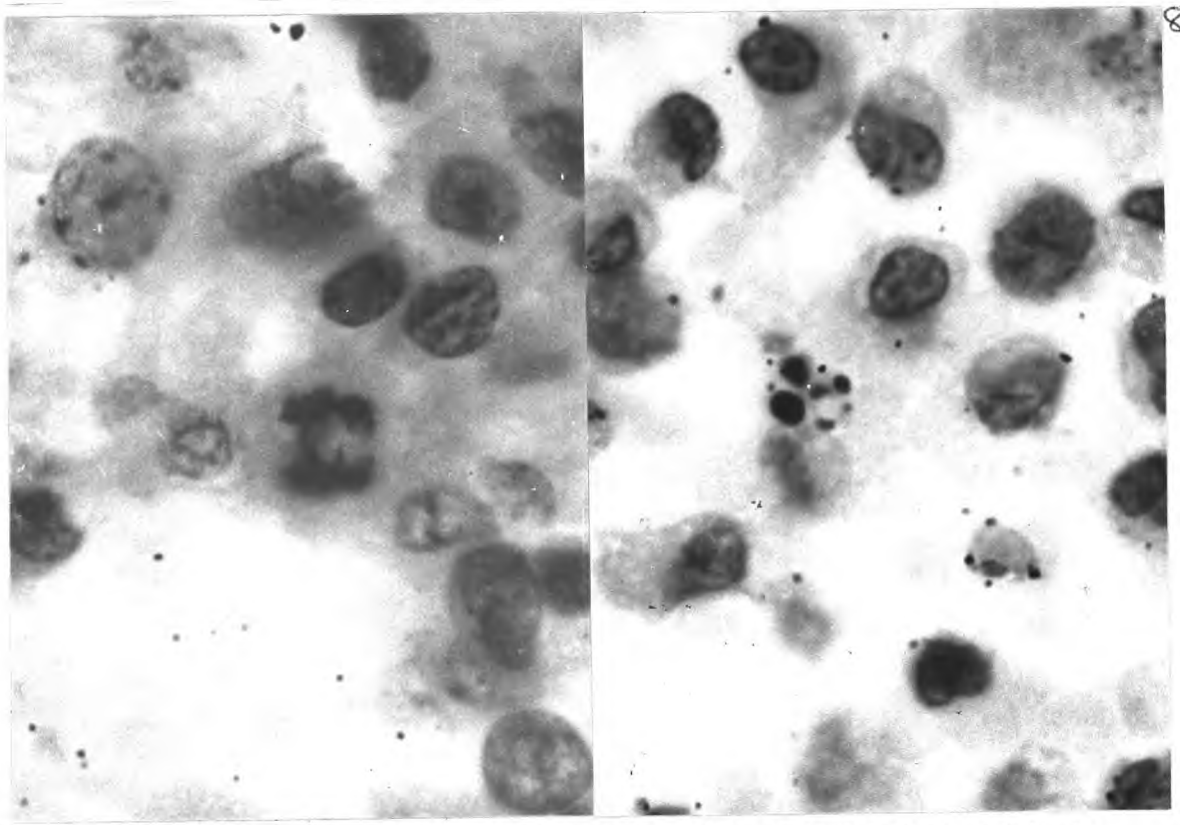


Figure 39. Labelled mitotic figure in irradiated cerebral lesion, three days after injury. H^3 thymidine given prior to injury. Mayers x1600.



Figures 40 & 41. Anaphase bridge and micronucleated cell in irradiated (2000R) cerebral lesion, 3 days after injury. Mayers x1600.

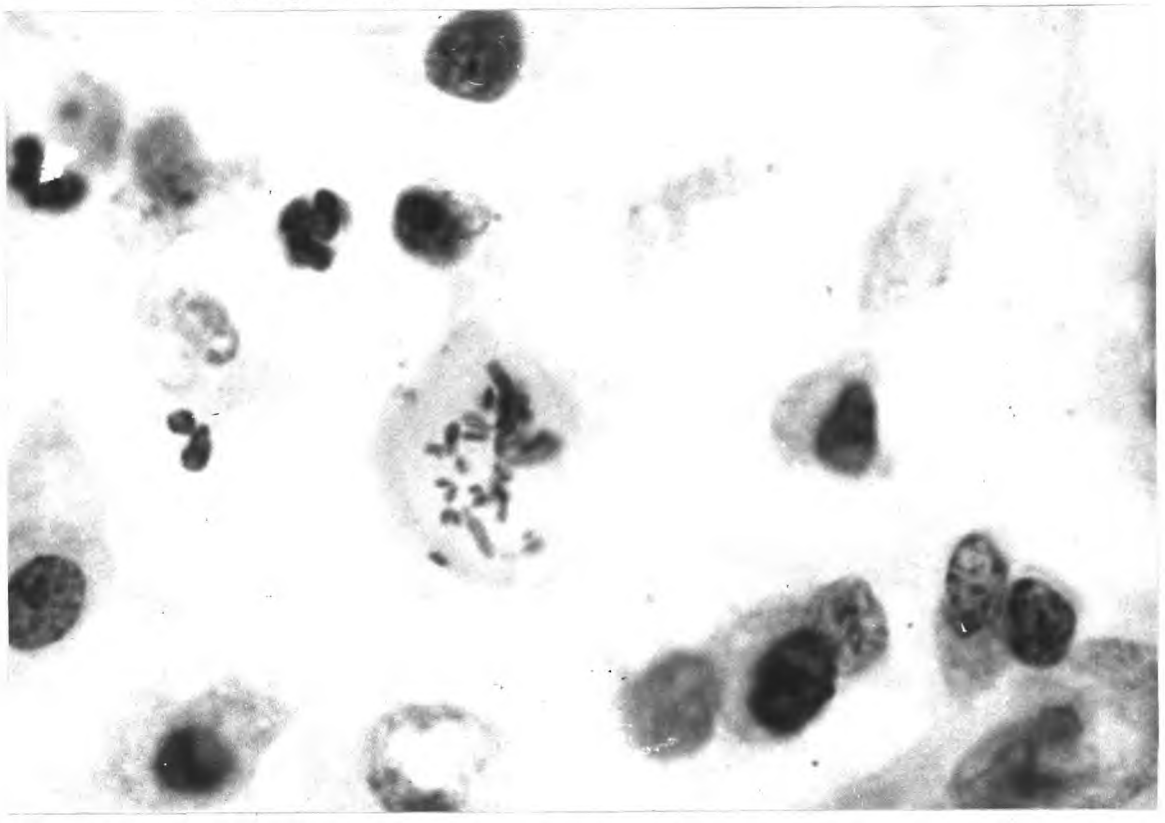


Figure 42. Astrocyte, abnormal mitotic figure in an irradiated (2000R) cerebral lesion, 3 days after injury. Mayers x1600.

area was filled with cells (fig.43). Few labelled cells and only the occasional mitotic figure was to be found when H^3 thymidine was given one hour prior to death. In the irradiated lesion, cells were larger and less numerous (fig.44) and many mitotic abnormalities and "micronucleated" cells were found. Small areas of necrosis were still found in the centre of the lesion of previously irradiated animals although the size of the necrotic areas varied.

When H^3 thymidine was given prior to injury, few labelled cells were found in the unirradiated lesion on the seventh day, while in the irradiated lesion a somewhat higher number of labelled cells were found (table 7). The labelled cells had by this time a much reduced grain count.

In the unirradiated lesion produced by freezing two well defined morphological cell types were to be found. The first of these types had a large pale nuclei, characteristic of astrocytes. The second cell type, which was the most numerous, had a small dark round nuclus, characteristic of microglia. However, with the knowledge that some cells enter the lesion from the blood stream and that vascular endothelial cells proliferate in response to injury we must conclude, that the cells with small dark round nuclei pepresent a mixed cell population of the above cell types with microglia (fig.45). All these types having this nuclear characteristic.

The number of cells present in the irradiated lesion depends

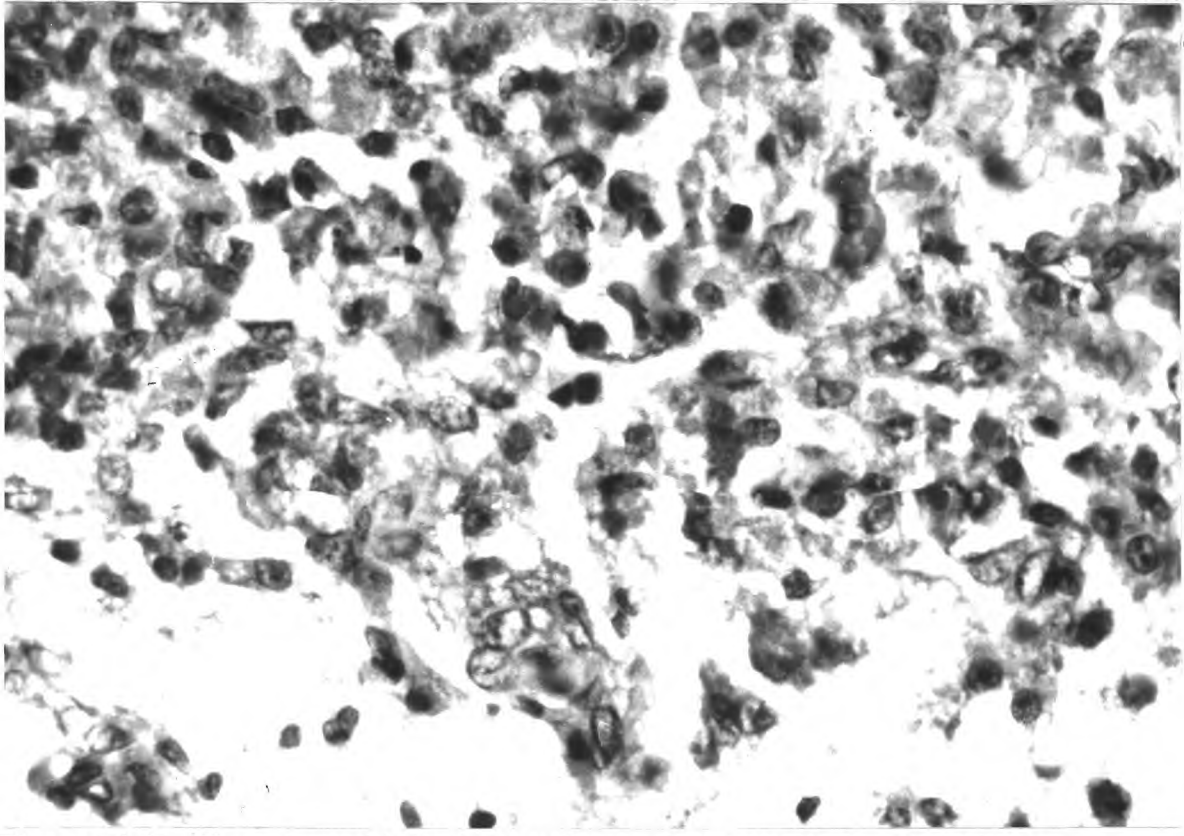


Figure 43. Non irradiated cerebral lesion 7 days after injury. H&E x700.

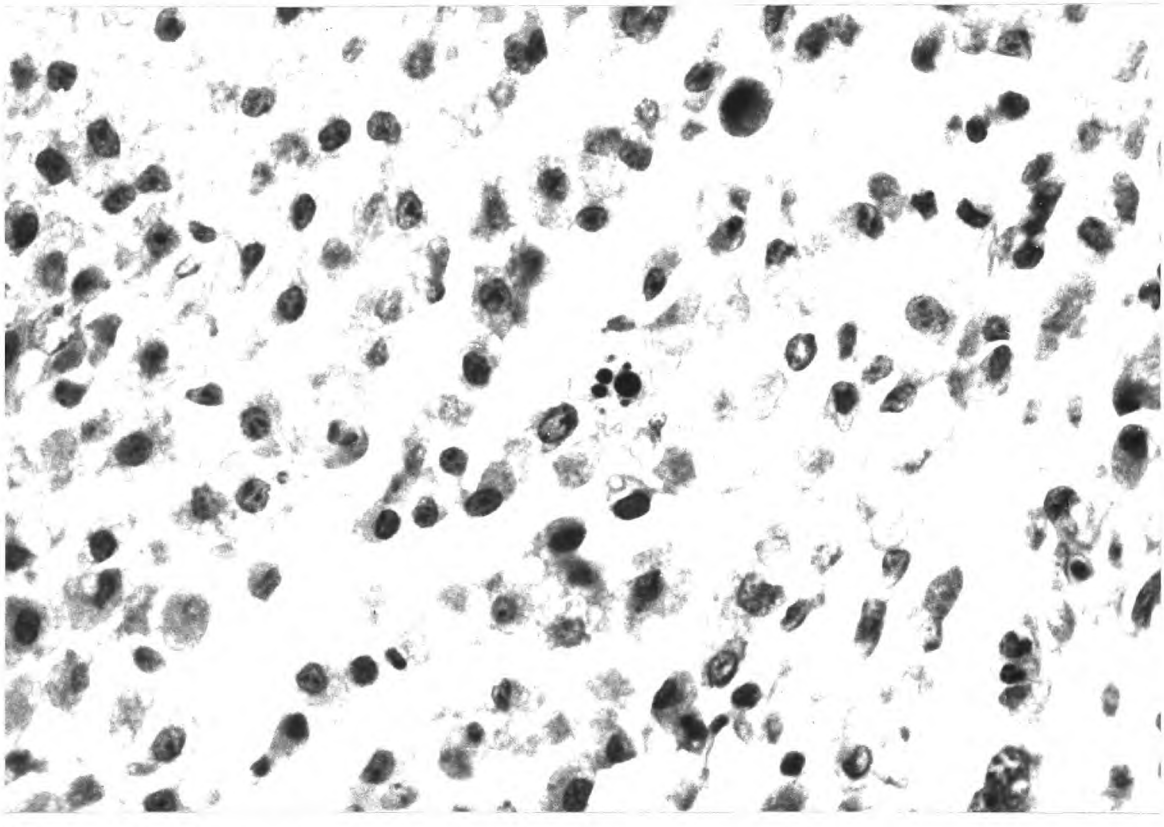


Figure 44. Irradiated (4000R) cerebral lesion 7 days after injury. Irradiation given 3 days prior to injury. H&E x700.

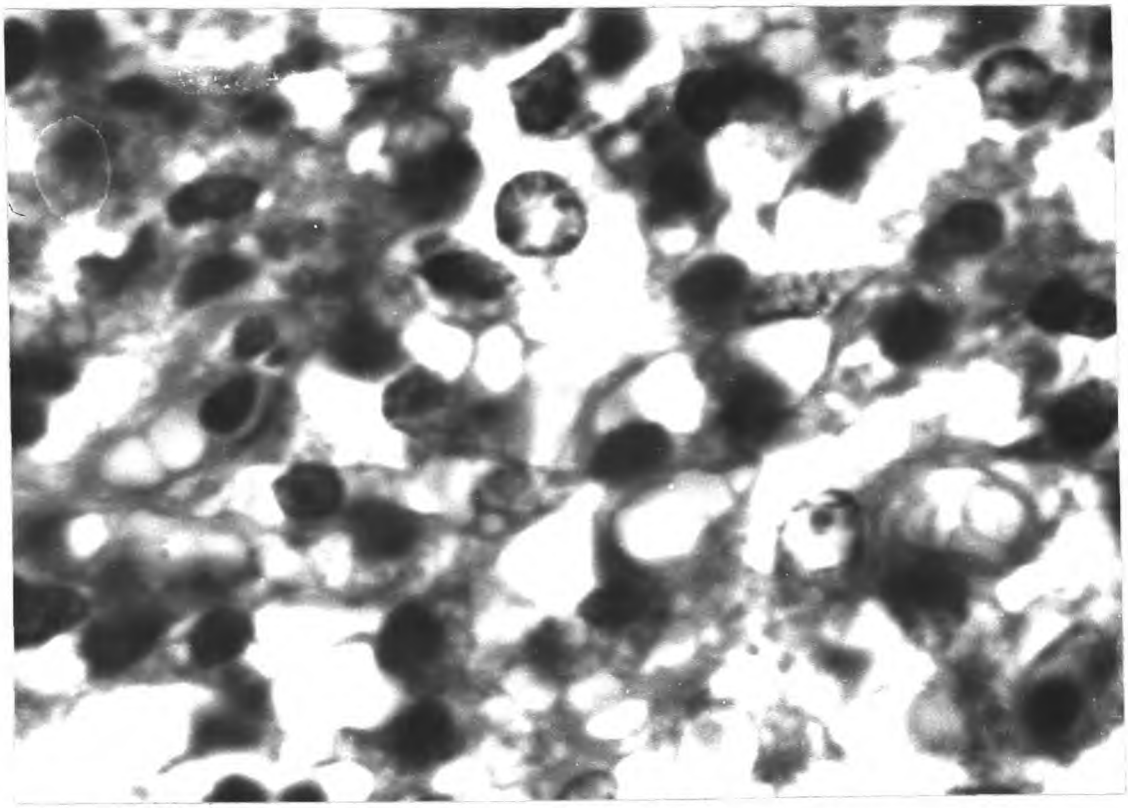


Figure 45. Non irradiated cerebral lesion, showing the two well defined cell types (see text). H&E x1600.

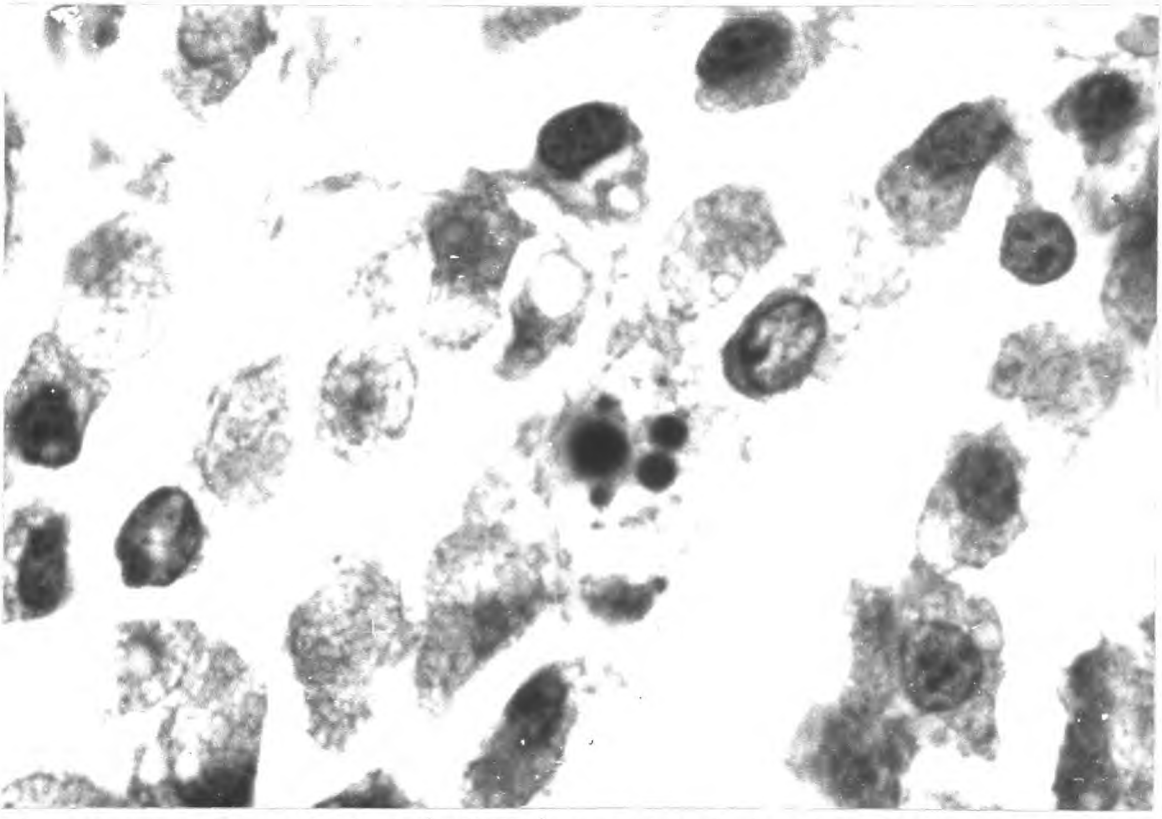


Figure 46. Irradiated (4000R) cerebral lesion 7 days after injury. Shows enlarged cells and a micronucleated cell. H&E x1600.

on the dose of prior irradiation. Most of the cells in the lesion are larger with rounded cytoplasm. There is evidence of irradiation damage as "micronucleated cells" and mitotic abnormalities are found (fig.46).

In summary it would appear, that following an injury produced by freezing of a superficial area of the cerebral cortex, there is an initial cellular reaction about the periphery of the lesion, not only of the neuroglial elements, microglia and astrocytes, but also of vascular endothelial cells and spindle shaped meningeal cells. Following this there is a migration of cells into the necrotic area where they undergo further cell multiplication. This cell multiplication, as indicated by cell labelling and mitotic activity, reached a peak on the third day after injury. By the seventh day, cellular debris had been removed and the area was filled with cells. The function of the various cell types reacting in response to injury is suggested as follows: microglia it would appear, by the nature of their granular cytoplasm, play a phagocytic role. Vascular endothelial cells, which proliferate in response to injury, may also transform into phagocytes. However the primary function of new endothelial cells will be the production of new capillaries which are found throughout the lesion on the seventh day after injury. The function of astrocytes in the lesion was not clear, no evidence of phagocyosis was to be seen and so it was believed that they may play a part at a later stage, in the formation of a scar. The mitotic activity in the spindle shaped cells of the meninges

was primarily for the replacement of damaged parts of this layer. It was however possible that these cells may play a part in scar formation at a later stage. No evidence of transformation of spindle shaped cells into rounded phagocytic cells was found.

The appearance of labelled cells in the lesion when H^3 thymidine was only given prior to injury, indicated that some circulatory white cells enter the lesion from the blood. They must also divide locally to a limited extent as the occasional labelled mitotic figure was found. However, the numbers are small and they are not by the seventh day, the principal source of brain macrophages.

The results show that although haemotogenous cells predominate on the first day after injury the principal cellular components thereafter are of local origin.

It is interesting to note that in the irradiated lesion the number of haemotogenous cells had increased by the seventh day as compared with the number in the non-irradiated lesion (table 7). This increased participation of haemtogenous cells may be a result of the reduced local cellular reaction.

CHAPTER IV

THE EFFECTS OF PREVIOUS X-IRRADIATION ON INTRACEREBRAL INFECTION WITH BORDETELLA PERTUSSIS

This study was undertaken conjointly with G.J.Adams, Department of Applied Immunology, Wright-Fleming Institute.

A. Bacteriological Investigation

We have used strain B.1772 of B. pertussis, which is of low virulence for mice when injected intracerebrally.

The work of Adams (1968) like that of Iida (1963), has indicated that ciculatory leucocytes may play a part in the eradication of low virulence B. pertussis infections from the brain. This hypothesis was based on the enhancement of low virulence infections by cortisone. Cortisone was injected subcutaneously 2 hours before and 1 and 2 days after intracerebral infection with B.1772 B. pertussis. In these animals the number of bacteria in the brain continued to increase after the second day and animals were dying on the fifth day with bacterial counts of 107 to 10^8 viable bacteria. The proposed explanation of these results was that mild leucopepenia, produced by cortisone, brings about the enhancement of the low virulence B. pertussis infection.

This phenomenon was investigated further using 400R whole

body irradiation instead of cortisone, to reduce the white cell count. In addition, because of our belief that the majority of brain macrophages were of local origin, we repeated the experiment with the heads of the mice shielded with lead. This avoided irradiation damage to local brain cells, damage that would become evident should local cells be stimulated to divide by intracerebral infection. The overall reduction in the white cell count was not significantly affected by shielding the head (Table 8). This is consistant with the knowledge that the stem cell population of these cells is situated in the bone marrow of the body.

The difference in response between the two irradiated groups was very marked (fig.47) and provides very good evidence, for the view that the mechanism responsible for the eradication of this infection is of local origin. Animals were infected with 5×10^4 viable organisms three days after irradiation. After an initital reduction in the bacterial counts after 1 hour there was a rapid increase in the bacterial numbers. In the unirradiated mice and those receiving irradiation to the body only, the bacterial count reached a maximum 24 - 48 hours after infection and then rapidly disappeared from the brain, 5 out of 10 brains being nearly sterile by the fourth day. In the other irradiated group, where the whole animal including the head was exposed, bacterial counts continued to rise and the brains had a mean viable count of 8×10^5 on the fourth day. This contrasts with a viable count of 9×10^2 in

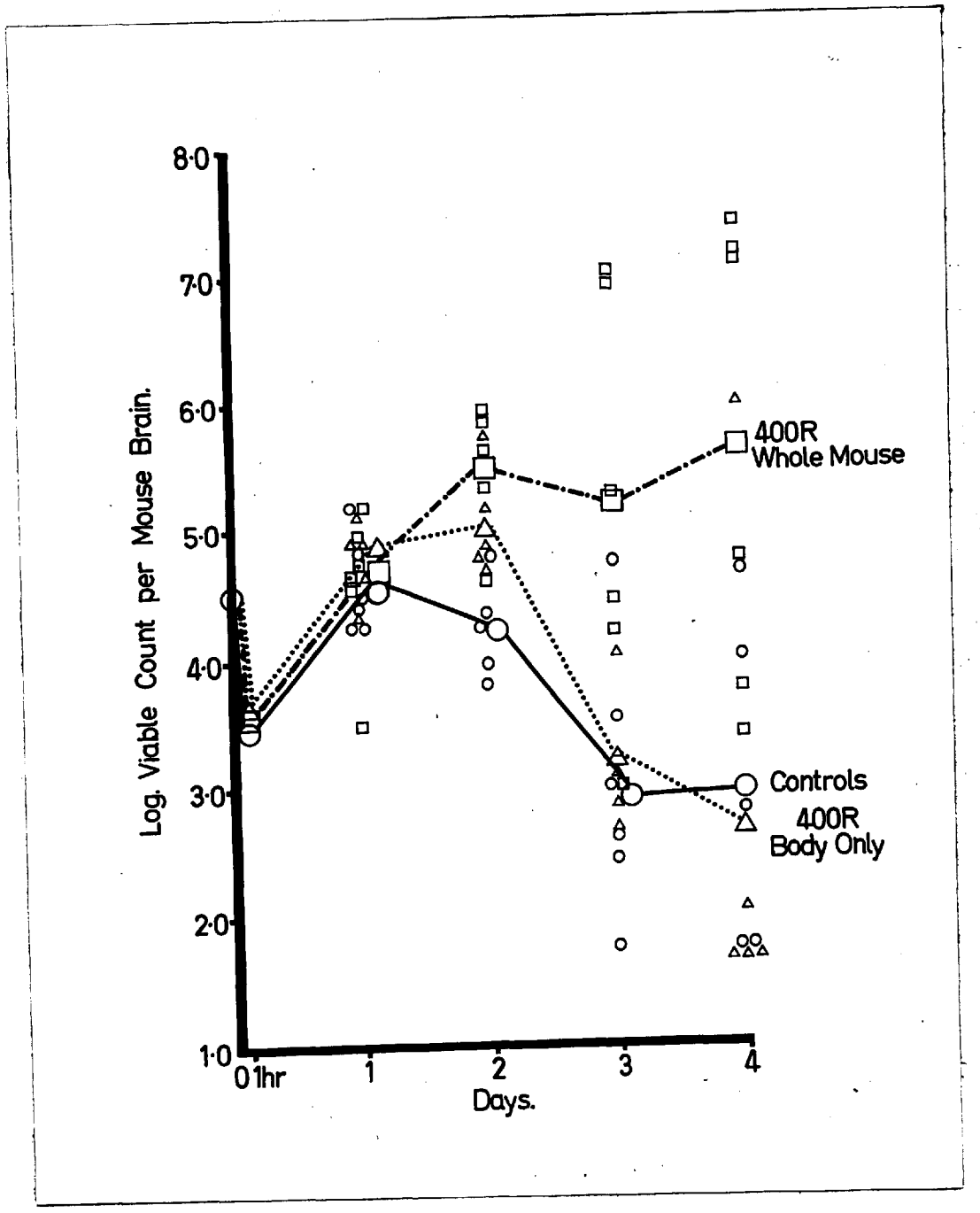


Figure 47. Intracerebral infection with B. pertussis. Animals were infected 3 days after irradiation.

TABLE 8

Animal group	White cell count per mm^3. of blood. Time after infection (days) 1	2	3	4
Control	4700	4266	5608	5950
400R whole mice	210	290	190	350
400R bodies only	470	580	630	710
1,000R heads only	1600	2600	4300	2540
Controls	2900	3800	3500	2560

White cell count per $mm.^3$ of blood taken from the branchial artery of mice. Mice were infected with B. pertussis three days after irradiation and white cell counts taken from 1 - 4 days after infection.

the control unirradiated animals and 7×10^2 for the irradiated animals with the heads shielded.

In both irradiated groups the white cell count was substantially reduced. The difference being that when the head was irradiated bacterial infection was prolonged, but when the head was protected against irradiation damage the mouse was still able to eradicate the infection from the brain even though the circulating white cells were depleated. This is a result which clearly indicates that a local mechanism is acting in the eradication of low virulence, B. pertussis infection from the brain.

In an attempt to correlate this phenomenon with our previous observations concerning the suppression of the cellular response to cerebral injury by previous irradiation a series of experiments were undertaken irradiating the heads of mice only. Two radiation doses were selected; 1,000R possibly the lowest limit at which delayed radiation changes occur, and 400R a sub-lethal dose.

In the first series of experiments, animals were irradiated to the heads only, while the bodies were shielded. This was done three days prior to infection, with 2.4×10^5 viable organisms. Bacterial counts in all three groups followed a similar pattern over the first twenty-four hours (fig.48), then in the two irradiated groups the bacterial multiplication was prolonged, while in the control group of animals the bacterial count remained at a constant level (3×10^5) for three days, before declining on the fifth day. Meanwhile, in

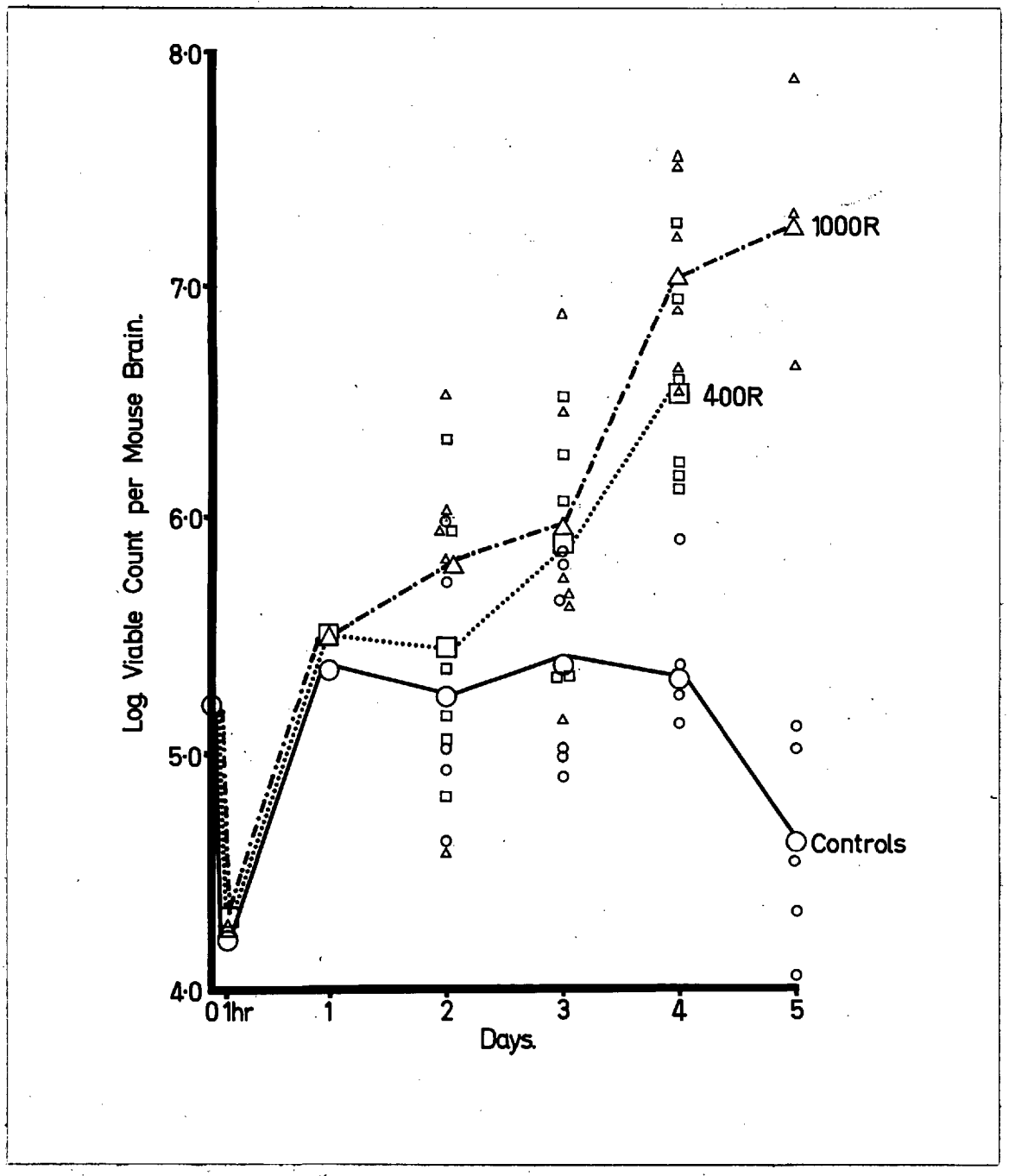


Figure 46. Intracerebral infection with B. pertussis. Animals were infected 3 days after irradiation to the head only.

the two irradiated groups bacterial counts had reached between 10^7 to 10^8 viable organisms and animals were dying. A slight dose dependence was noted in animals similarly irradiated and infected and allowed to survive after the fourth day. 10 out of 12 animals died in the 1,000R group and 7 out of 12 animals died in the 400R group. Deaths occurred on the fourth to fourteenth day after infection. Only a single animal died in the control infected group of animals.

As with previous experiments on the response of the brain to injury we next increased the time interval between irradiation and infection to three weeks. In this case the mice were challenged with a slightly smaller initial inoculum of 8×10^4 viable organisms. The response obtained followed a very similar pattern to the previous series of experiments. In all three groups the infection followed a similar pattern for the first 24 hours after which the two irradiated groups showed a continued bacterial multiplication while in the control group the count declined to give a mean bacterial count of 7×10^2 on the fourth day (fig.49). This was in marked contrast to the two irradiated groups where the bacterial counts had reached 6×10^6 and 5×10^5 for the 1,000R and 400R groups respectively.

There is hint of a possible late recovery in the 400R group, the mean bacterial count on the fourth day being slightly lower than on the third. The differences are however small and so could be due to chance.

In the third and final experiment in this series we allowed

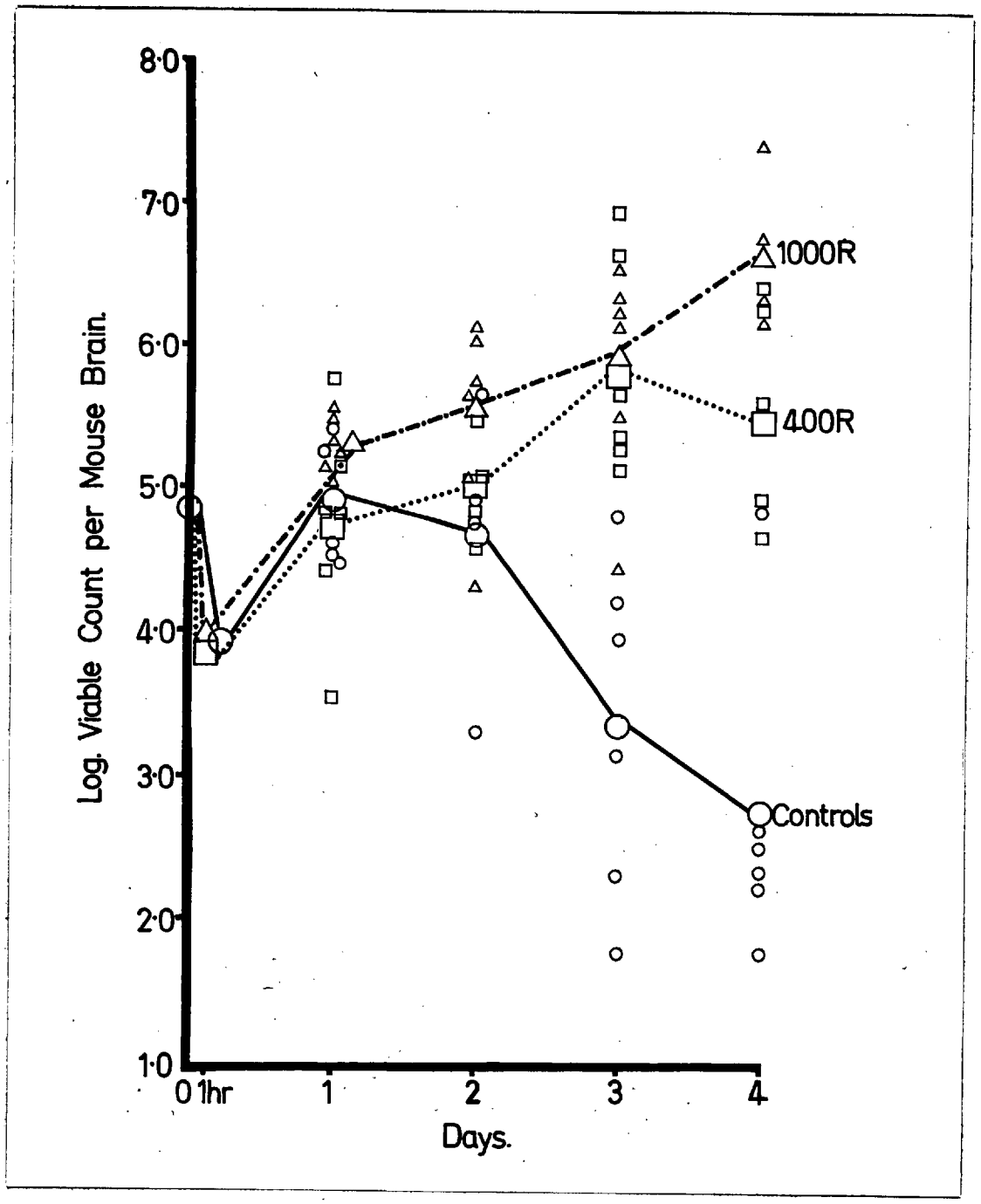


Figure 49. Intracerebral infection with B. pertussis. Animals were infected 3 weeks after irradiation to the head only.

a three month latent period between irradiation and infection. In this case the mice were infected with 1.5×10^6 viable organisms and as in all previous experiments the infection for all groups followed the same course for the first 24 hours (fig.50). After this time the mean count in the control declined. In the two irradiated groups the bacterial multiplication was prolonged, reaching a maximum in the 400R group by the third day, when it differed from the control count by a factor of 20. After the third day, recovery occurred and by the fifth day the count only differed from the control by a factor of four. This is in marked contrast to the 1,000R group where the bacterial count was greater than the control, at this time, by a factor of 500.

This suggests that some recovery has occurred with doses of 400R and the brain has partly been able to repair the irradiation damage. In the 1,000R group no recovery appears to occur in the three months over which this study was extended.

Possible recovery mechanisms will be discussed later.

B. Histological Studies

Histological studies were undertaken on a series of control and irradiated infected brains. Although no quantitative evaluation of the cellular reaction was possible (because of the sampling problems involved) we were able to follow the course of the infection in qualitative terms.

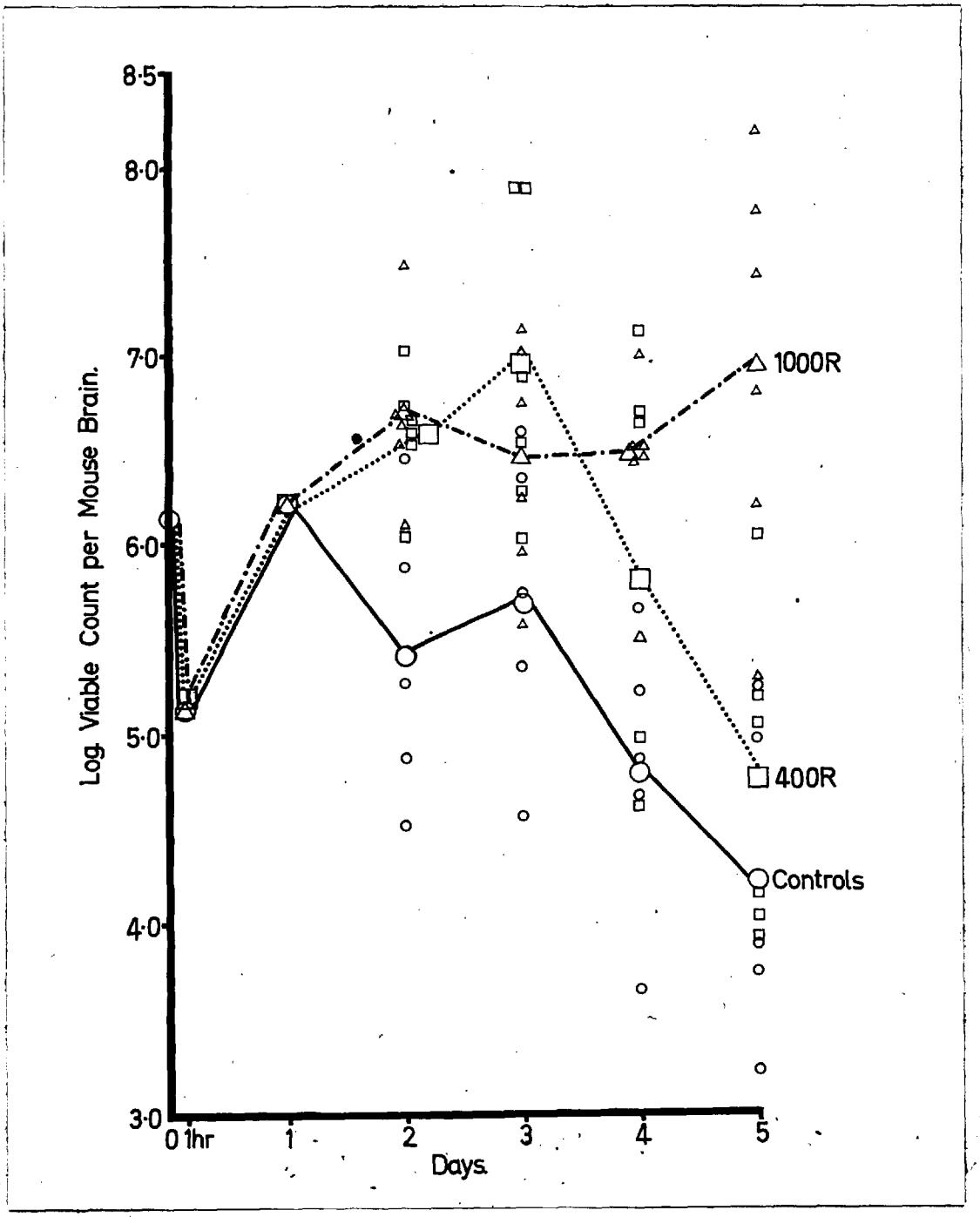


Figure 50. Intracerebral infection with B. pertussis. Animals were infected 3 months after irradiation to the head only.

The brains of mice infected intracerebrally with B. pertussis, of both control and irradiated groups, were examined histologically at intervals of 6 hours and 1, 2, 3, 4 and 7 days after infection with approximately 10^5 viable organisms. Two to four brains were examined in each group for each time interval. Colcemid, which arrests mitoses in metaphase, was injected subcutaneously 4 hours prior to death.

As little as 6 hours after infection, mitotic figures were observed in the controls and irradiated groups. These mitotic figures were found in three places; in close proximity to the lateral ventricles, in the ventricular space and finally, a considerable number appeared to be between ependymal cells. (figs. 51 & 52).

From the first day of infection onwards, histological variation in the brains of animals in the same group was considerable. This is perhaps indicative of the wide variation in bacterial counts observed with similarly infected animals.

In the control infected animals, mitotic activity of brain cells was continued on the first and second days but by the third day some brains were apparently normal. Small clumps of mononuclear cells, believed to be of local origin, were observed in the ventricles. Polymorphs were observed in the choroid plexus when the bacterial counts in the brain were highest.

In irradiated infected brains many abnormal mitotic figures

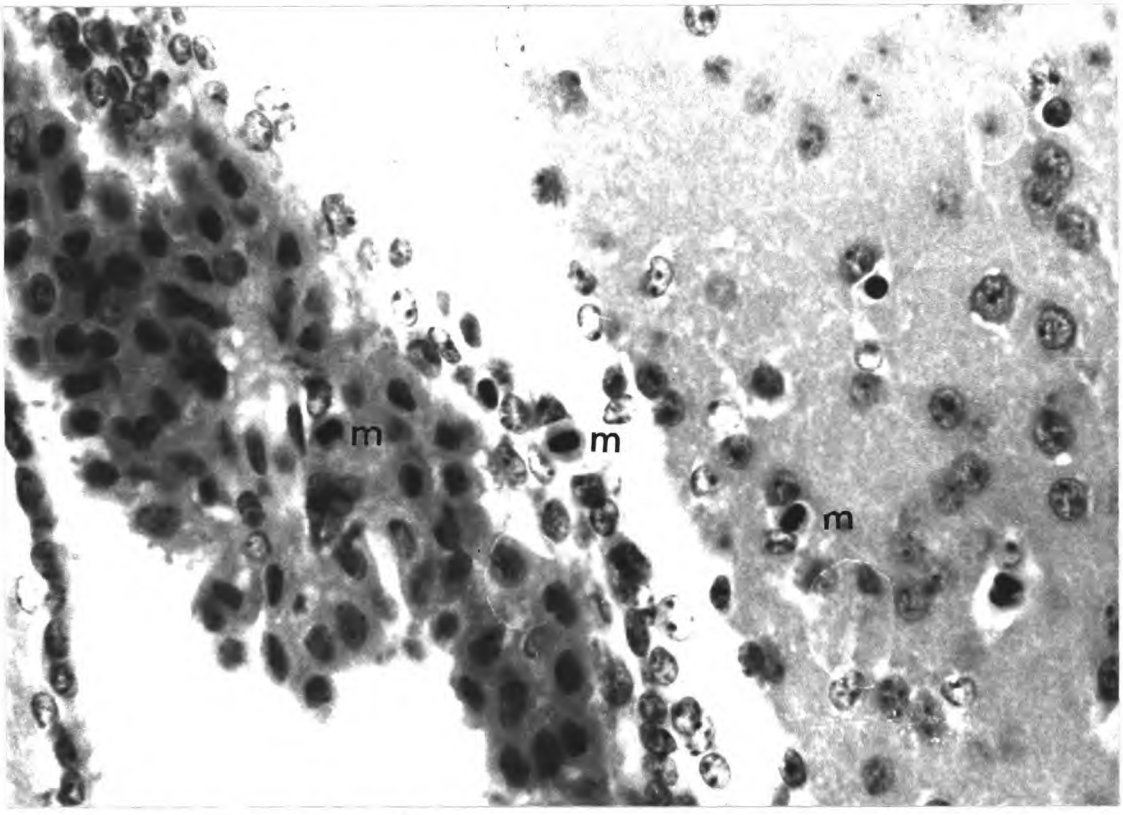


Figure 51. Mitotic figures(m) in the brain, between ependymal cells and in the ventricle 6 hours after infection with B. pertussis. H&E x700.

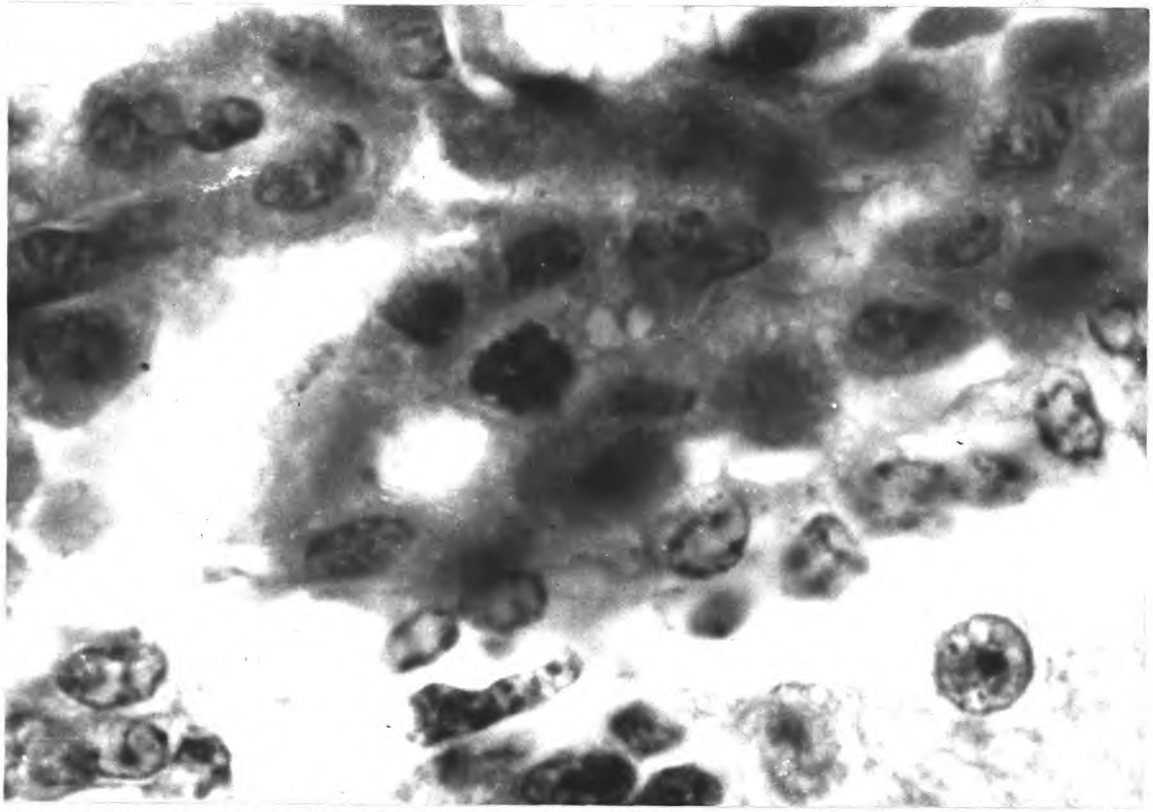


Figure 52. Mitotic figure in the choroid plexus of the lateral ventricle 6 hours after infection with B. pertussis. H&E x1600.

and cells with many micronuclei are found. For example figure 53 shows a brain irradiated with 400R on the third day of infection, an anaphase bridge and micronucleated cell are observed in the lateral ventricle. Cells with several small nuclei, "micronucleated" cells, were found in the brain in close proximity to the ventricles (fig.54).

The observation of mitotic activity on the fourth day was perhaps indicative of the prolonged high level of infection, again some of the mitoses are abnormal. Figure 55 shows an abnormal mitosis in a brain on the fourth day of infection, the animal having previously received 1,000R local irradiation.

In the brain-irradiated mice, as in the non-irradiated controls, polymorphs were found within the choroid plexus by the second or third day. After this time, as the bacterial numbers continued to increase, there was a gradual infiltration of both the ventricular space and cerebral parenchyma by polymorphs.

By the fourth to the seventh day most of the brains of previously irradiated mice developed a marked internal hydrocephalus (fig.57), which may have resulted in the death of the animals. It is at this time that colonies of bacteria are clearly evident on the ependymal lining of the ventricles (fig.56).

The above histological observations support the view that the principal mechanism for the eradication of low virulence B. pertussis

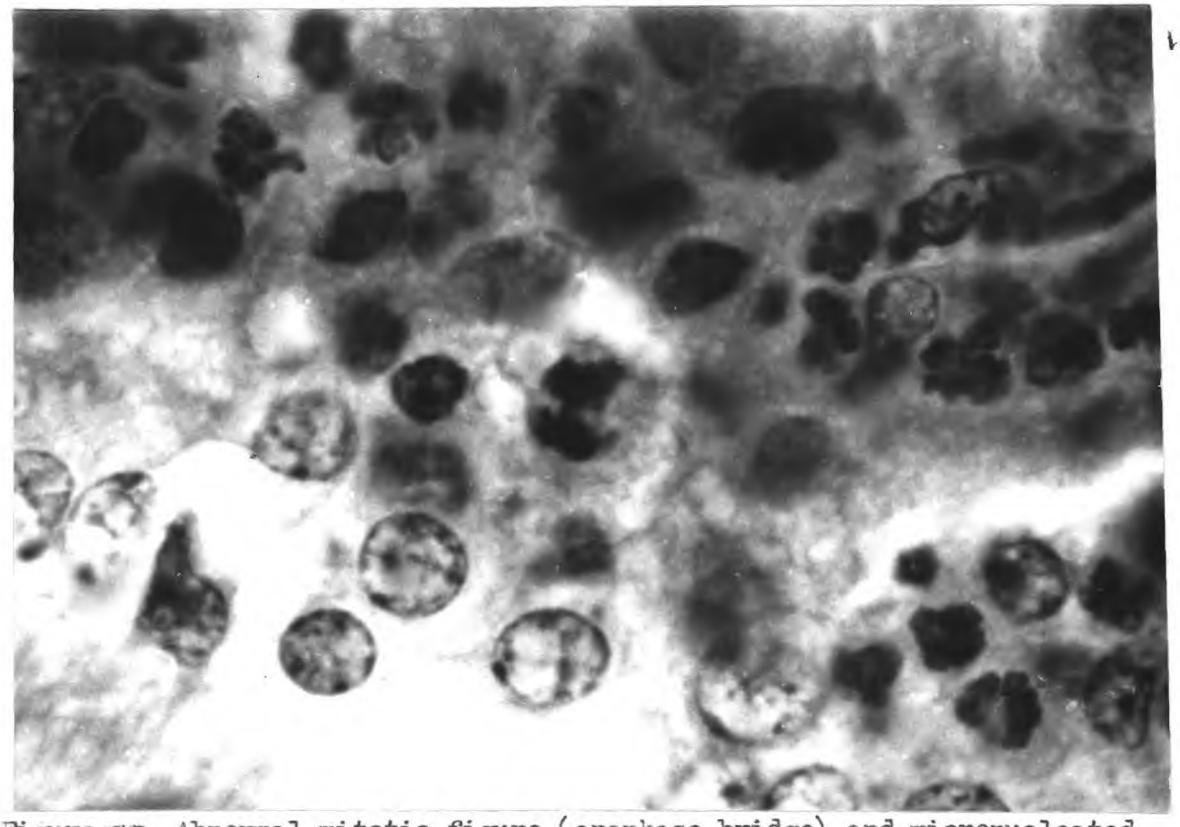


Figure 53. Abnormal mitotic figure (anaphase bridge) and micronucleated cell in the lateral ventricle 3 days after infection of irradiated brain (400R) with B. pertussis. H&E x1600.

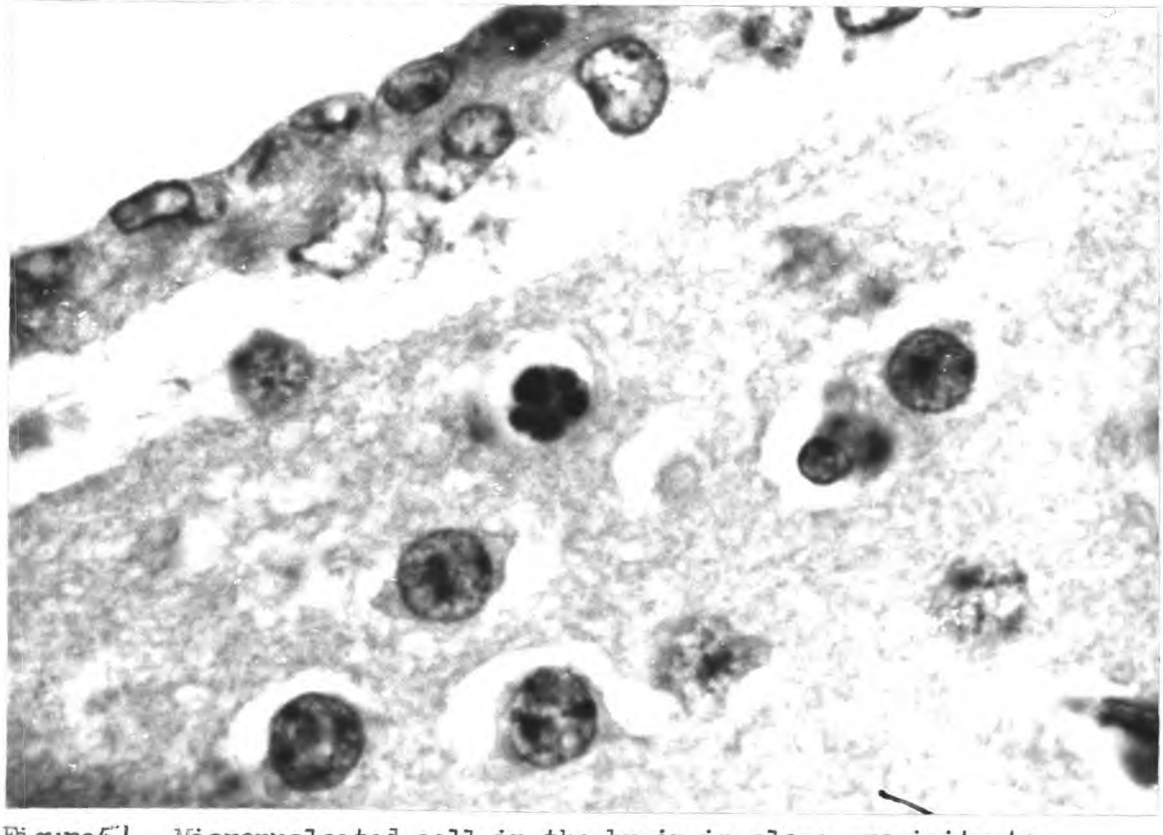


Figure 54. Micronucleated cell in the brain in close proximity to the lateral ventricle 2 days after infection of irradiated (400R) brain with B. pertussis. H&E x1600.

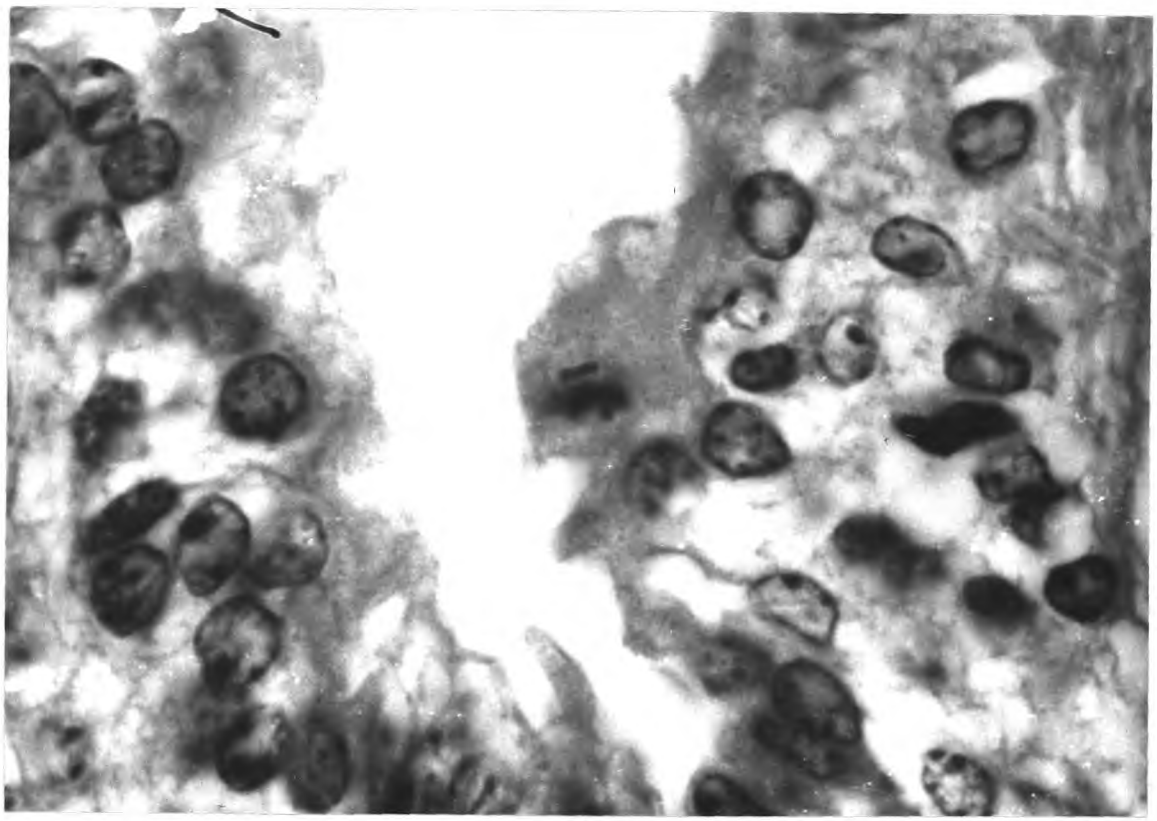


Figure 55. Large abnormal mitotic figure in the lateral ventricle of an irradiated brain (1000R) 4 days after infection with B. pertussis. H&E x1600.

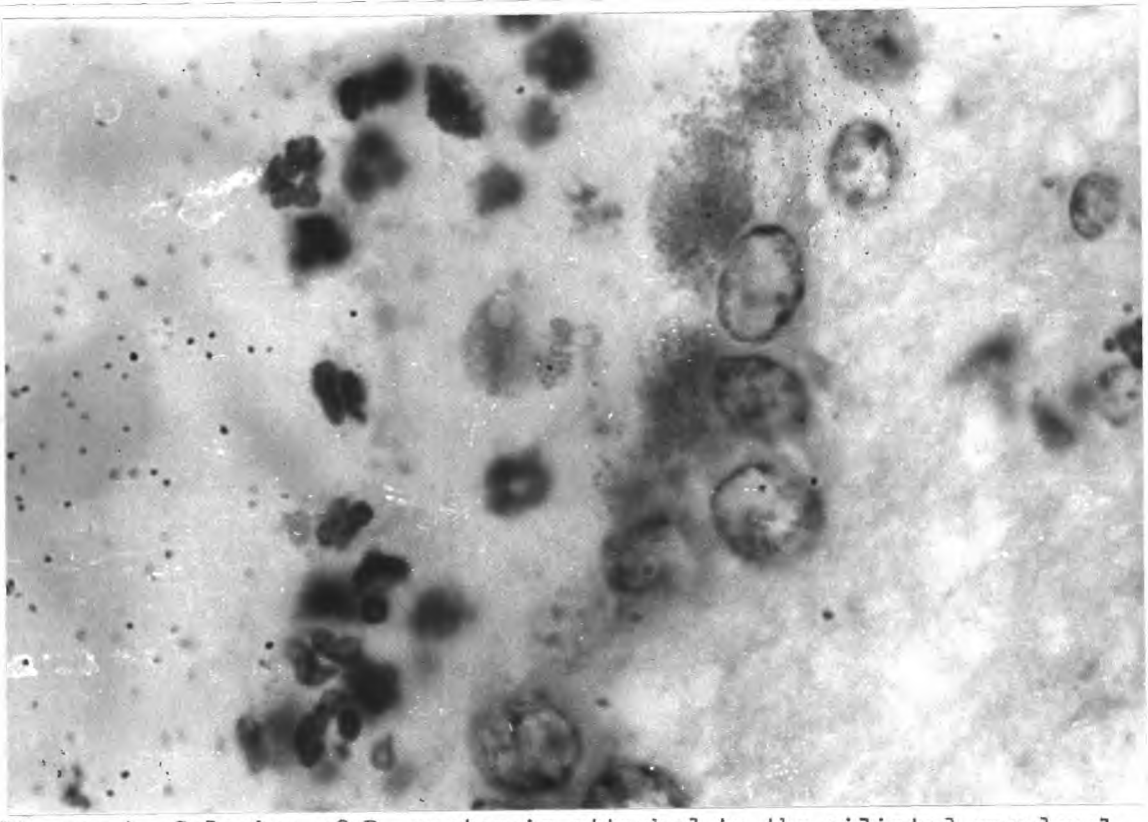


Figure 56. Colonies of B. pertussis attached to the ciliated ependymal lining of the lateral ventricle. Mouse dying of infection 5 days after challenge. Mayers x1600.



Figure 57. Dilated lateral ventricle in head irradiated (400R) mouse dying 5 days after intracerebral infection with B. pertussis H&E x110.

infection from the mouse brain is of local origin. The observations show that intracerebral infection stimulates cell division in the brain. Dividing cells were observed in the brain between ependymal cells and in the ventricles and so it would appear that migration of cells takes place from the brain into the ventricles.

In the irradiated brains, mitotic abnormalities as well as micronucleated cells are observed. These features are often associated with irradiation damage. These abnormalities result in cell death thus reducing the ability of the brain to eradicate low virulence B. pertussis infection.

CHAPTER V

THE OXYGEN EFFECT AND BRAIN IRRADIATION

A. Introduction

The embryonic and foetal central nervous system, in marked contrast to the adult, is very radiosensitive (Rugh 1962) and many abnormalities developed even with very low doses. Hicks et. al. (1954, 1957) showed that the primitive neuroblasts and spongioblasts were selectively damaged by ionising radiation in foetal life. The degree and extent of irradiation damage decreased with the age of the foetus but increased with the dose of irradiation. The general thesis was that the radiation dose became less and less effective as gestation progressed.

This increasing radioresistance continued into postnatal life until in the adult rat brain a dose of 1,000R produced no detectable changes and animals appeared normal even into old age.

The time sequence during which radiation resistance develops in the neonatal period has been fully investigated by Yamazaki et.al. (1962). Irradiation in the first 3 - 4 days after birth produced severe neurological changes over a wide range of radiation doses (125 - 1,000R). Five days after birth only 50% of the animals receiving a dose of 500R developed a lack of co-ordination and

weakness of the hind limbs from the third month after irradiation. By the fifteenth day after birth no neurological signs occurred even after a dose of 1,000R.

These findings were associated with a reduction in body growth and head growth. Mortality was also dependant on the age of the rats and dose of irradiation.

The weight of the brain stem, cortex and cerebellum was reduced following the irradiation of the brain of two day old rats with 750R (Schjeide et.al.1962).

Because of the high radiosensitivity of the nestling rat brain as compared with the adult this age was selected for demonstrating the oxygen effect in the central nervous system. The known suppression of brain weight being a useful indicator of radiation effect.

B. Half Brain Irradiation

Brains were irradiated on the seventh day after birth. This age was selected for several reasons:-

1. It enabled a wide range of irradiation doses to be used without the risk of high mortality. (Yamazaki et.al. reported approximately 90% mortality with 1,000R whole brain irradiation given between the first and fourth day, but after this time the mortality rate following 1,000R dropped to a very low value).

2. At seven days, the brain is still relatively undifferentiated

and still only about one-third of its adult weight, which is reached at approximately 8 - 10 weeks of age (fig.86).

In addition only the left half of the brain was irradiated, the other side being used as a control for evaluating the radiation effects. This avoided comparing the irradiated half brain weight with that of normal non-irradiated controls of comparable age, as these animals are heavier than animals in the irradiated group (table 9). This was observed by Yamazaki et.al.(1962) following whole brain irradiation of young rats. This would have introduced another factor which would have been difficult to control as brain weight is related to body weight (Donaldson 1908). This may account for the lower half brain weight in the non-irradiated controls as compared with the normal half brain weight (table 9).

Groups of five one week old rats were irradiated with doses of 200 - 1,000R to the left side of the head. The right side of the brain was shielded with lead. The irradiated animals were enclosed in a sealed vessel to which gas was supplied at various oxygen concentrations i.e. 7%, 20% (air), 100% and 300%. The gas in all experiments was supplied at 3 litres/min and an interval of five minutes was allowed for equilibrium to be reached before exposure to X-rays.

Animals were killed 1, 3 and 7 weeks after irradiation. The brain was exposed by removal of the parietal bones and fixed by immersion in 1% acetic acid in 10% formal saline solution. After

TABLE 9

Half brain weights and standard error of non-irradiated controls and half brain irradiated one week old rats (1,000R under 1 atm.oxygen).

AGE OF RATS (WKS)	Normal non-irradiated 12 ♂	1,000R half brain irradiation (1 atm. oxygen)	
		Non-irradiated control (Right) 5 ♂	1,000R (Left) 5 ♂
2	0.566±0.01 (31.3±1.15)	0.519±0.02 (19.1±2.31)	0.464±0.02
4	0.712±0.01 (64.7±2.65)	0.608±0.02 (47.0±2.55)	0.550±0.01
8	0.864±0.01 (209±11.6)	0.668±0.01 (130.5±21.4)	0.590±0.01

♂ Number of animals used in each group. ( ) Mean weight of rats in grams.

fixation for a minimum period of 48 hours the brains were carefully dissected out and with extreme care sliced into two halves along the mid line. The two halves of the brain were quickly blotted dry and weighed seperately on a torsian balance. The radiation effect was expressed as the weight of the irradiated half of the brain (left) as a percentage of the non irradiated half (right).

The changes in brain weight recorded were small (fig.58 and table 10) with a maximum reduction of 15.5% between the two halves of the brain in animals irradiated with 1,000R under 300% (hyperbaric) oxygen and killed three weeks after irradiation. With such small changes in brain weight variation in radiation effects with different oxygen tensions was correspondingly smaller with some overlap of groups when the standard errors were incorporated. The results do however show a consistant trend over the whole range of doses used for all time intervals investigated, after irradiation. This is demonstrated most clearly by the dose response curves obtained for animals killed three weeks after irradiation. In figure 58 the two extremes, 7% and 300% oxygen, have been plotted out with their associated standard errors. If the difference between these two extremes of oxygen tension are considered together then they are significantly different within the 5% limit, i.e., $P<0.05$. (The detailed analysis of the results are given at the end of this chapter).

The dose modifying factor between the two extremes of oxygen

TABLE 10

Mean half brain weight as a percentage of non-irradiated control.

	DOSE	OXYGEN CONCENTRATION (percent)			
	(R)	7	20	100	300
A	200	98.1±1.09	95.2±1.18	94.3±0.97	96.0-0.21
	400	95.3±1.50	92.0±1.58 (6)	90.8±0.72 (7)	90.5±1.93
	600	92.3±0.87 (4)	92.0±0.56	90.7±1.73	87.5±0.57
	1000	90.1±1.27	89.7±1.82	87.4±2.18	84.5±2.39
B	400	93.5±1.88	92.2±2.64	88.3±1.67 (6)	88.2±1.33
	1000	91.0±1.43 (4)	-	88.3±0.70	87.7±1.96
C	400	89.7±1.30	93.8±1.29	90.2±1.93	87.47±1.80
	1000	93.9±1.80	92.4±1.53	89.5±0.79	-

A: Rats killed at 4 weeks old
B: Rats killed at 8 weeks old
C: Rats killed at 2 weeks old

} All animals irradiated at 1 week old.

Each percentage is the mean of 5 animals except where indicated ( ).

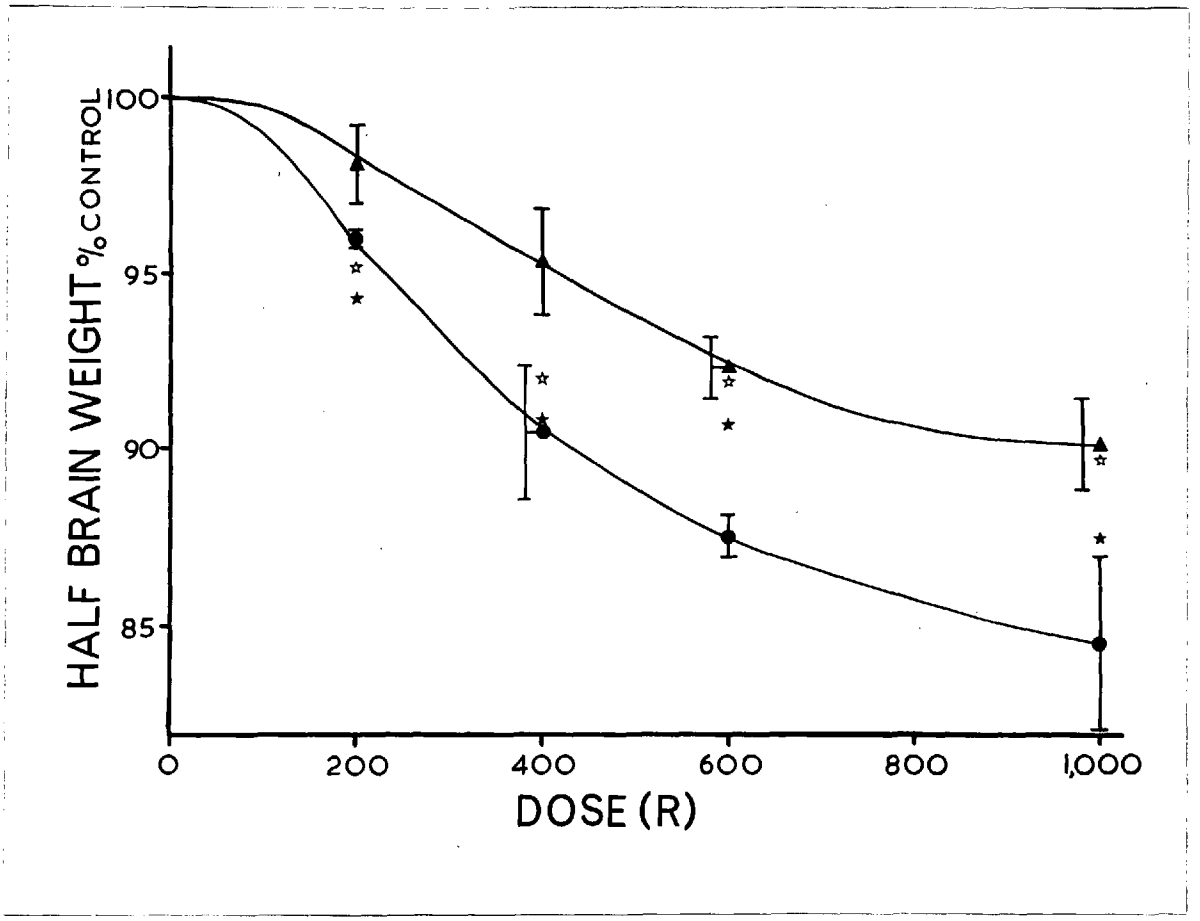


Figure 58. Irradiated half brain weight (left half) as a percentage of the non irradiated control (right half) against dose of 220kV X-rays, irradiation being given under different oxygen tensions. The associated standard errors are given for brains irradiated under atmospheres of 7% and 300% (hyperbaric) oxygen.

▲ 7% oxygen

☆ 20% oxygen

★ 100% oxygen

● 300% oxygen.

tension varies as the two dose response curves are not parallel. The dose modifying factor increasing with irradiation dose. Taking two intermediate points on the 300% oxygen curve, dose modifying factors of 1.85 and 2.1 respectively were found as compared with 7% oxygen. Thus it would appear that the dose modifying factor for 7% oxygen as compared with 300% oxygen is of the order of 2 for irradiated developing brain.

Thus, as in other tissues, low oxygen tensions will protect against radiation damage, while high oxygen tensions, in particular hyperbaric oxygen, will sensitise the developing central nervous system to radiation damage. The latter was suggested by Van den Brenok et.al. (1968) for the adult human C.N.S., after the evaluation of 357 patients receiving irradiation of the spinal cord, under hyperbaric oxygen conditions.

C. Hair growth as an indicator of radiation effects

The reduction in the amount of hair growth after irradiation also provided a visual demonstration of the variation in radiosensitivity with oxygen tension. This was only observed in the intermediate dose range (400 - 600R).

In the example cited, the 3 animals of the same litter were irradiated at one week of age with a dose of 400R, and observed 1 week later (fig.59). The middle animal in the group was irradiated in air and showed slight reduction in hair growth. The



Figure 59. Reduction in hair growth one week after irradiation (400R) as an indicator of variation in radiosensitivity with oxygen tension.



Figure 60. Variation in the amount of hair growth, 4 weeks after irradiation, as an indicator of increased dose due to scattered radiation.

animals on the left and right of fig. 59 were irradiated under the two extremes of oxygen concentration, 7% and 300%. In the latter the reduction in hair growth is more pronounced, while in the animal irradiated in an atmosphere of 7% oxygen, no obvious reduction in the amount of hair growth can be detected. Thus we conclude, as was shown for the effects of these oxygen tensions on the radiosensitivity of brain, that low oxygen tensions protect against radiation damage, while high pressure oxygen sensitises the nestling rats epidermis, to radiation damage.

Following irradiation with 1,000R, the reduction in hair growth was most marked with eventual epilation. This occurred with irradiation under all oxygen tensions, no differential in response being observed. This dose did however provide us with an interesting biological indicator of an increased radiation dose, resulting from lower energy scattered X-rays and photoelectrons.

One week after irradiation (1,000R) the side of the head exposed to X-rays is clearly defined by the reduction in hair growth. (Fig.103). In the following week hair loss continued so that by the end of the second week little or no hair was found on the irradiated side of the head. Regrowth of hair was slow and it was interesting to note, that just to the left of the mid line hair regrowth was more delayed than the remaining part of the irradiated side of the head. This probably indicates a higher exposure dose in this region because of the formation of photoelectrons and lower energy X-rays from the edge of the lead

shielding (Robertson 1956). These lower energy radiations, in particular the photoelectrons, will loose their energy in the first few millimetres of tissue, thus giving a higher dose in this region.

APPENDIX

Statistical test

The significance of the response to irradiation between the two extremes of oxygen tension was determined using the standard error of the difference between the two means.

$$\text{S.E. difference between 2 means} = \sqrt{SE_1^2 + SE_2^2}$$

The results for each group are given below:-

Dose (R)	% Diff. between the 7% 300% oxygen subgroups	SED	$\frac{\%\ \text{Diff.}}{\text{SED}}$	P.
200	2.1	1.11	1.89	< 0.06
400	4.8	2.44	1.96	< 0.05
600	4.7	1.04	4.59	< 0.001
1,000	5.6	2.71	2.07	< 0.04

Three of these four results are significantly different within the 5% limit and the fourth is nearly so. The results taken as a whole do prove statistically significantly different within the 5% limit.

CHAPTER VI

CEREBRAL CARCINOGENESIS

A. Introduction

In this study we have set out to investigate the effects of chemical carcinogens on two implantation sites within the brain. In each case, cerebral tissue was in contact with the carcinogenic pellet, but they were positioned so that in one group the pellet was in contact with the meninges, while in the second group the ependyma of the lateral ventricles was involved. These two groups will, in the following paragraphs, be referred to as "superficial" and "deep" respectively (fig.61).

The experiment was also arranged to test the effects of two carcinogenic types, namely methylcolanthrene and benzpyrene, and also the importance of sex in the induction of cerebral tumours.

Forty rats of the Sprague Dawley strain, (20 males and 20 females) aged 4 - 6 weeks received intracerebral implants of carcinogen. The pellets of carcinogen, weighing 3 - 7 mg. were obtained by cutting 3mm discs of carcinogen in half, thus giving a maximum pellet size of 3mm by 1.5mm.

Under chloral anaesthesia the left parietal bone of the skull was exposed, and with the aid of a power operated dental burr, a small hole was produced to allow easy passage of the carcinogenic pellet. After a small incision had been made in the overlying dura,

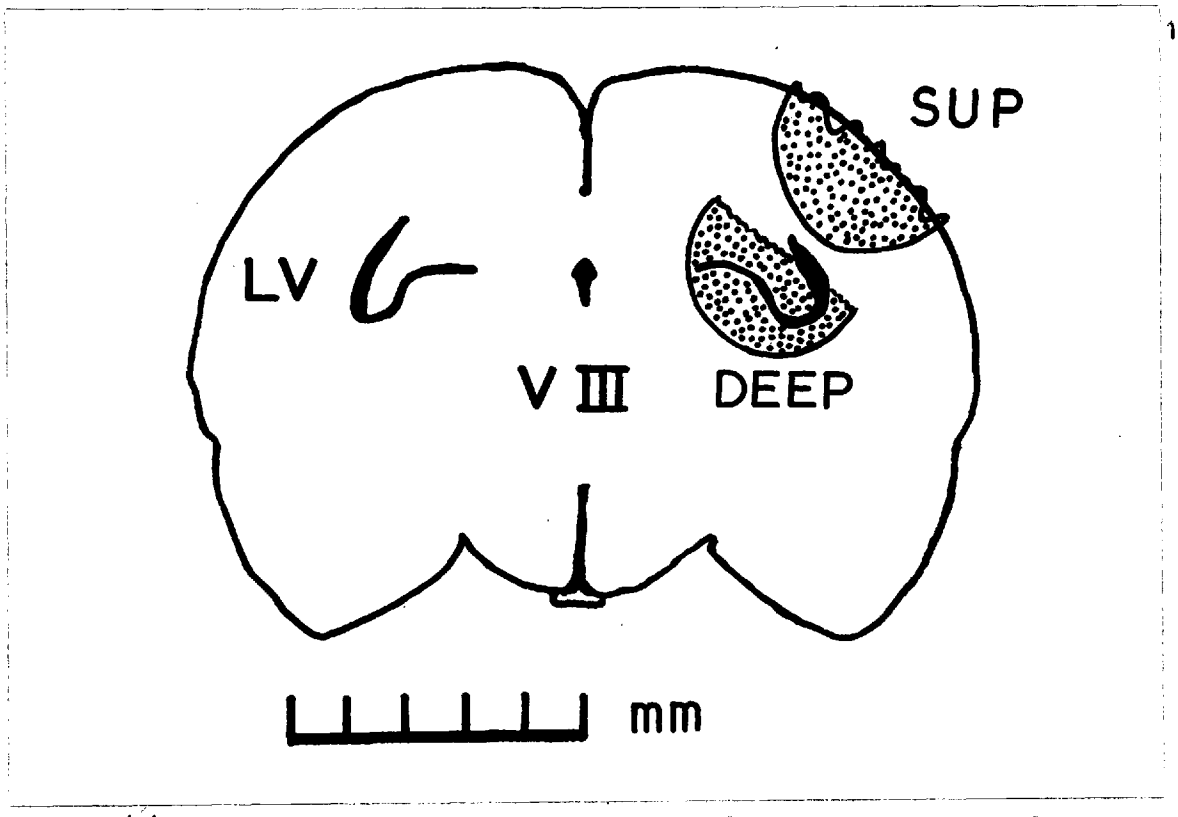


Figure 61 . Diagram showing position of deep and superficial carcinogenic pellets.

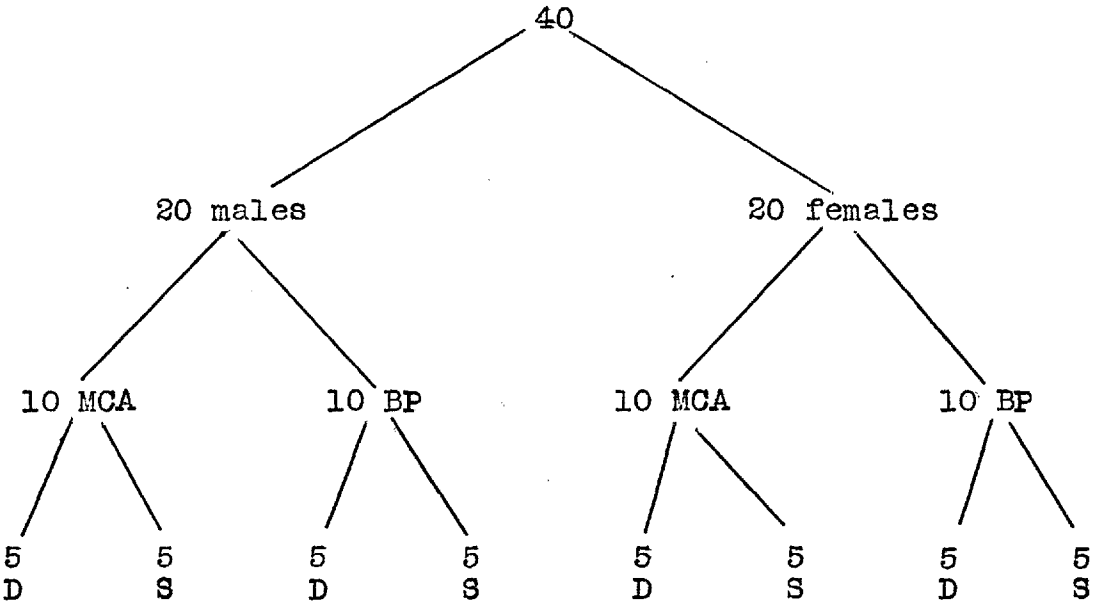


Figure 62 . Distribution of animals.
MCA- methylcholanthrene, BP- benzpyrene,
D- deep, S- superficial.

pellets were implanted into the exposed cerebral cortex according to the following standard procedure:-

1. "Superficial" implantation.

The pellet was placed on the surface of the brain and pushed very carefully into the brain substance until the pellet was flush with the meninges.

2. "Deep" implantation.

The pellet was held in a fine pair of forceps and inserted into the brain to a standard depth of 3 - 4mm.

The implants were arranged to give eight separate groups of five animals. For each sex, ten were given methylchlanthrene and ten benzpyrene, and each of these were further subdivided to give five with deep and five with superficial pellets (fig.62). The animals were then observed at regular intervals for signs of neoplasms. On the death of the animals the heads were removed. The brains were exposed and fixed in 1% acetic acid in 10% formal saline. A general post-mortem examination was carried out on 75% of animals to ascertain causes of death other than cerebral tumour.

After fixation the brains were dissected and sliced coronally. The carcinogenic pellet was removed at this time.

B. Results

The results obtained over the $2\frac{1}{2}$ years duration of the experiment were clear. In the group of twenty animals where the

pellet of carcinogen was implanted deeply fifteen tumours developed; in the twenty animals where the pellet was placed superficially only four tumours developed. In two of these "superficial" animals the pellet was found to be deeply situated at autopsy so in further analysis of the results they will be considered with the group of animals with deep pellets. On this basis the results are summarised in figure 63. This shows a 77% (17/22) incidence of tumours in the deep group and 11% (2/18) in the superficial group. A more detailed analysis of the results is given in the histograms (figs. 64 - 71).

1. Carcinogen type (figs. 64 & 65).

Using methylcolanthrene eight out of twenty animals produced tumours and of these seven were from the "deep" group. In a similar group of animals where benzpyrene was used, eleven tumours were induced and ten were from deeply situated pellets. The numbers are too small to allow any firm conclusions to be drawn as to differences between the two carcinogenic types.

2. The importance of sex (figs. 66 & 67)

Considering all the tumours nine were in males and ten in females; clearly no significant difference. If the results are examined more closely with particular reference to the time of development of tumours from deeply situated pellets an interesting fact emerges. In the "deep" group nine male and seven female "glial" tumours developed, (with a single choroid plexus tumour in another female). However in the first thirteen months seven tumours

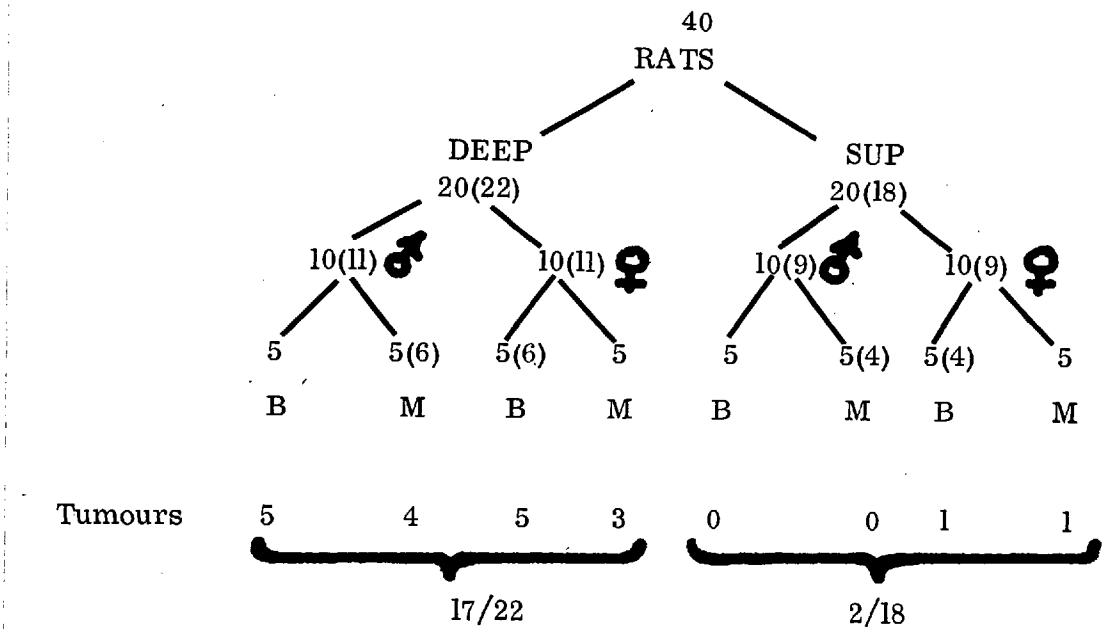


Figure 63. Summary of results showing distribution of experimental brain tumours.

Intracranial Tumours: Rats

Ependymoblastoma	6
Glioblastoma multiforme	5
Spongioblastoma	1
Oligodendroglioma	1
Astrocytoma	1
Mixed oligo./astro.	2
Choroid plexus tumour	1
Meningeoma	1*
Unclassified	1*
	19

* Carcinogens superficial

Table 11. Type and incidence of experimental intracranial tumours in rats.

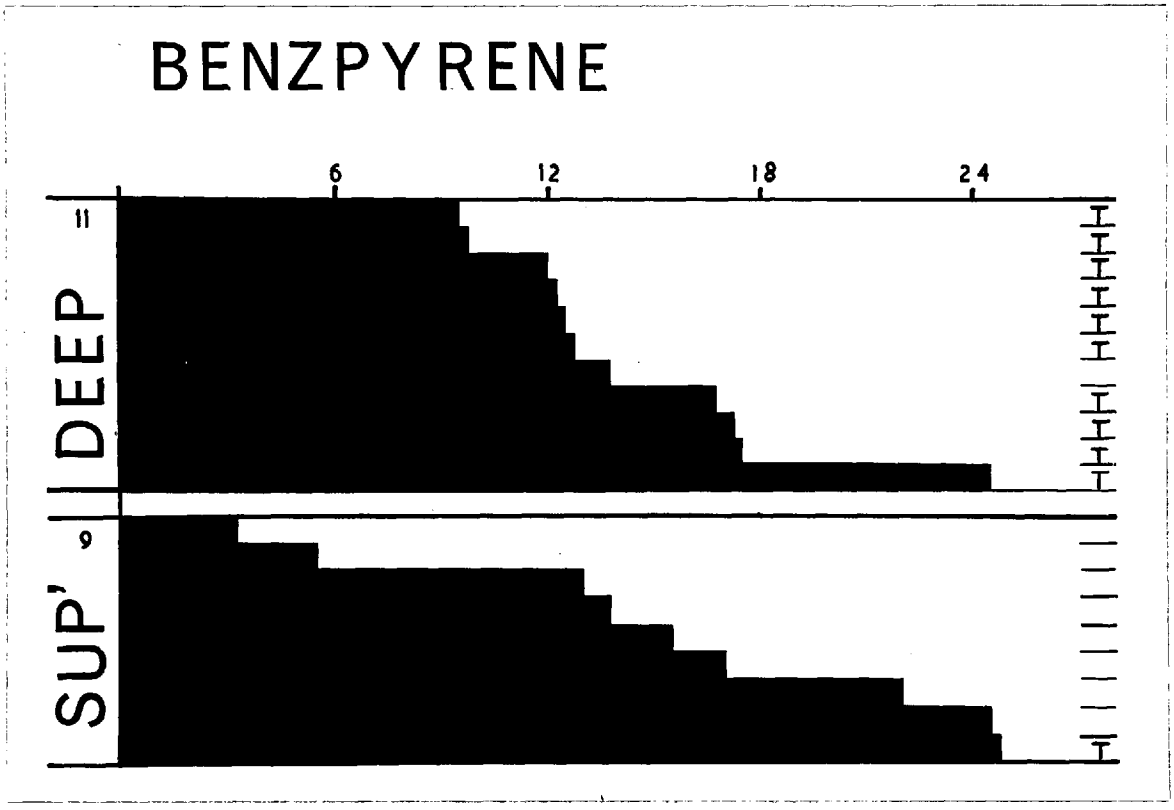


Figure 64. Intracranial tumours produced with benzpyrene.

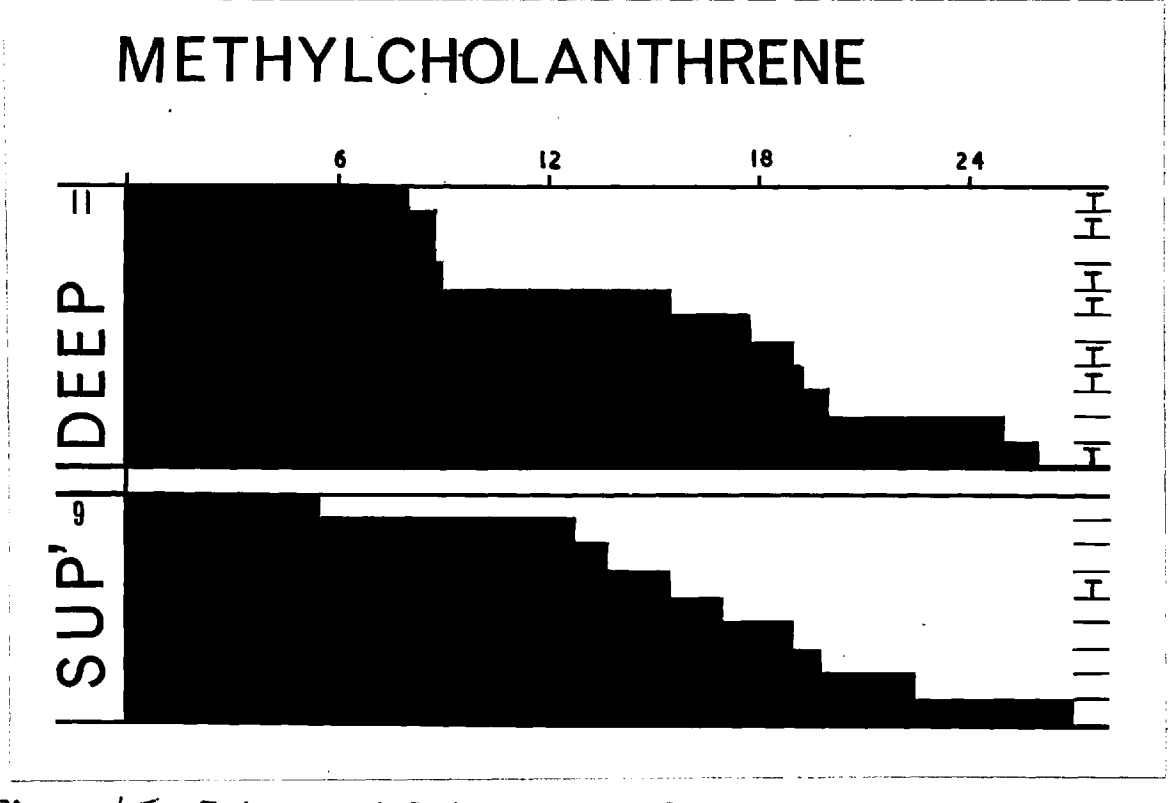


Figure 65. Intracranial tumours produced with methylcholanthrene.

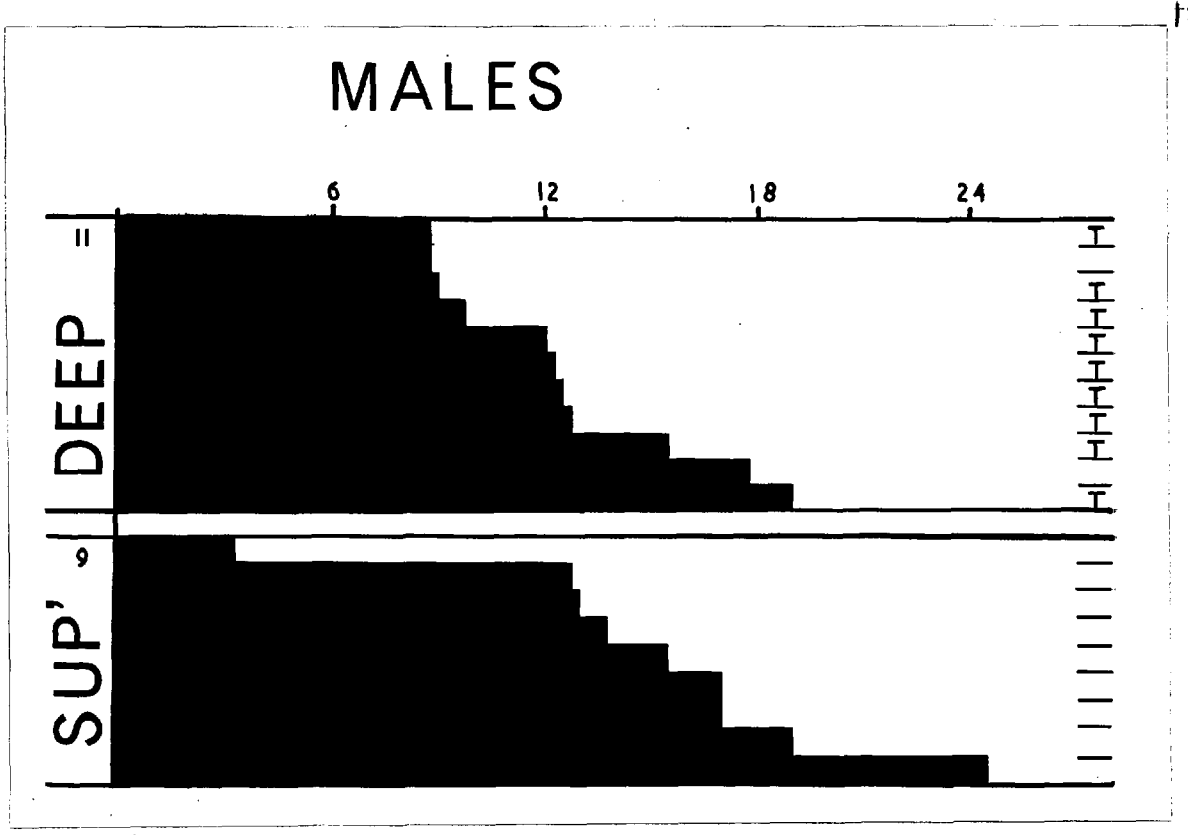


Figure 66. Intracranial tumours in males.

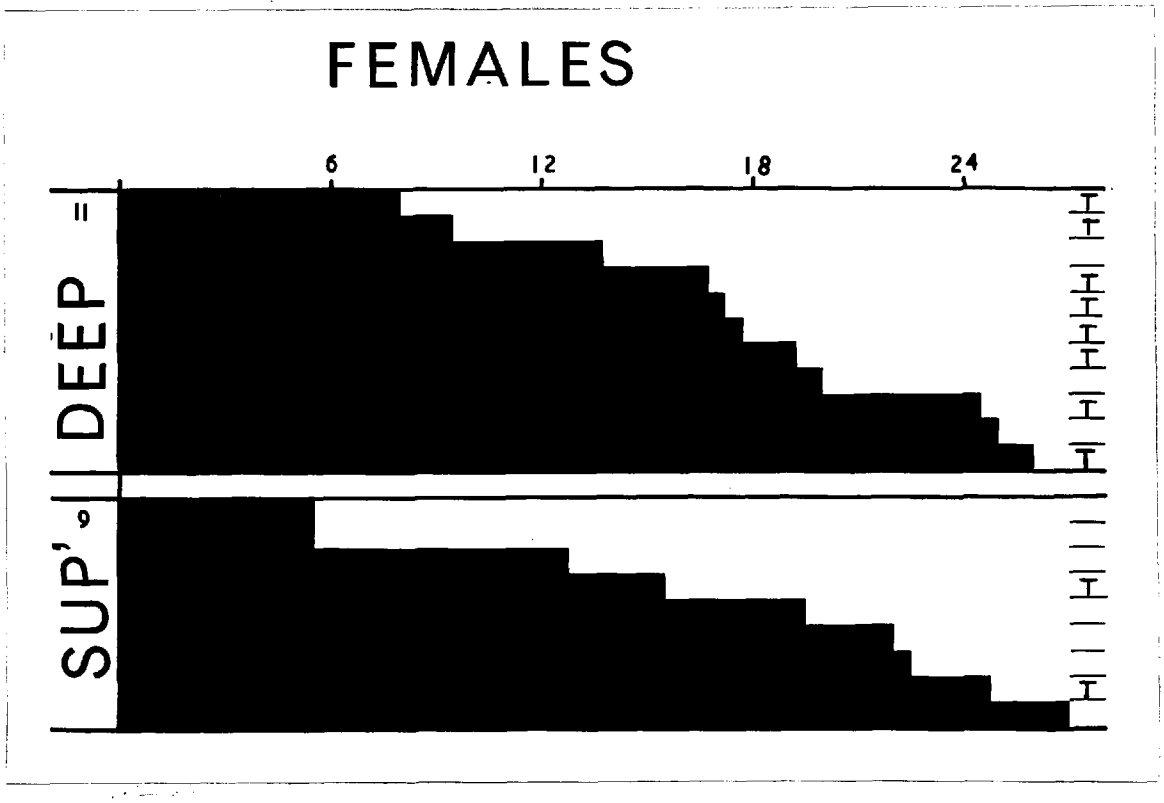


Figure 67. Intracranial tumours in females.

developed in males but only two occurred in females. On the other hand in the second thirteen month period the position was reversed with five tumours in females and only two in males. Thus it appears that gliomas are more common and develop earlier in males than females. Later in this chapter other evidence will be discussed in support of the view that cerebral glial tumours are more common and tend to develop earlier in males than in females.

3. Effects of premature death

In all long term experiments of this type the health of the experimental animals and the risk of a premature death from other causes must be considered. The influence of premature intercurrent death can best be interpreted by examining each group closely.

In the male benzpyrene group (fig.68), 100% (5/5) tumours developed where the pellets were implanted deeply but in the corresponding group of superficial animals no tumours developed (0/5). The mean tumour bearing age in the deep group was approximately 12 months. In the superficial group all but one of the animals (which died prematurely with a cerebral abcess) lived longer than this 12 month period. In the methylcolanthrene group of males (fig.69) 66% (4/6) tumours developed in the deep group of animals while all the superficial animals lived beyond the mean tumour bearing age, i.e., 13 months.

In the two female groups (fig.70 & 71) both deep and superficial animals lived for a similar length of time, and so it could be

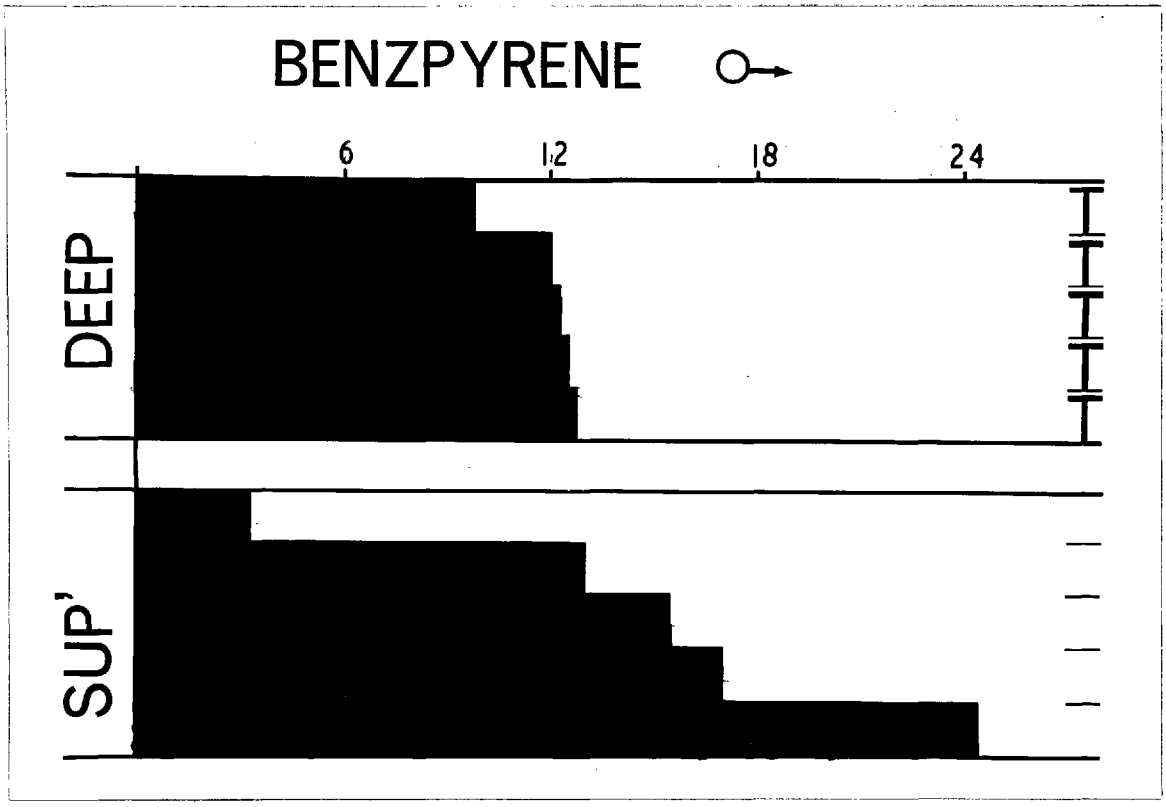


Figure 68. Intracranial tumours in males produced by benzpyrene.

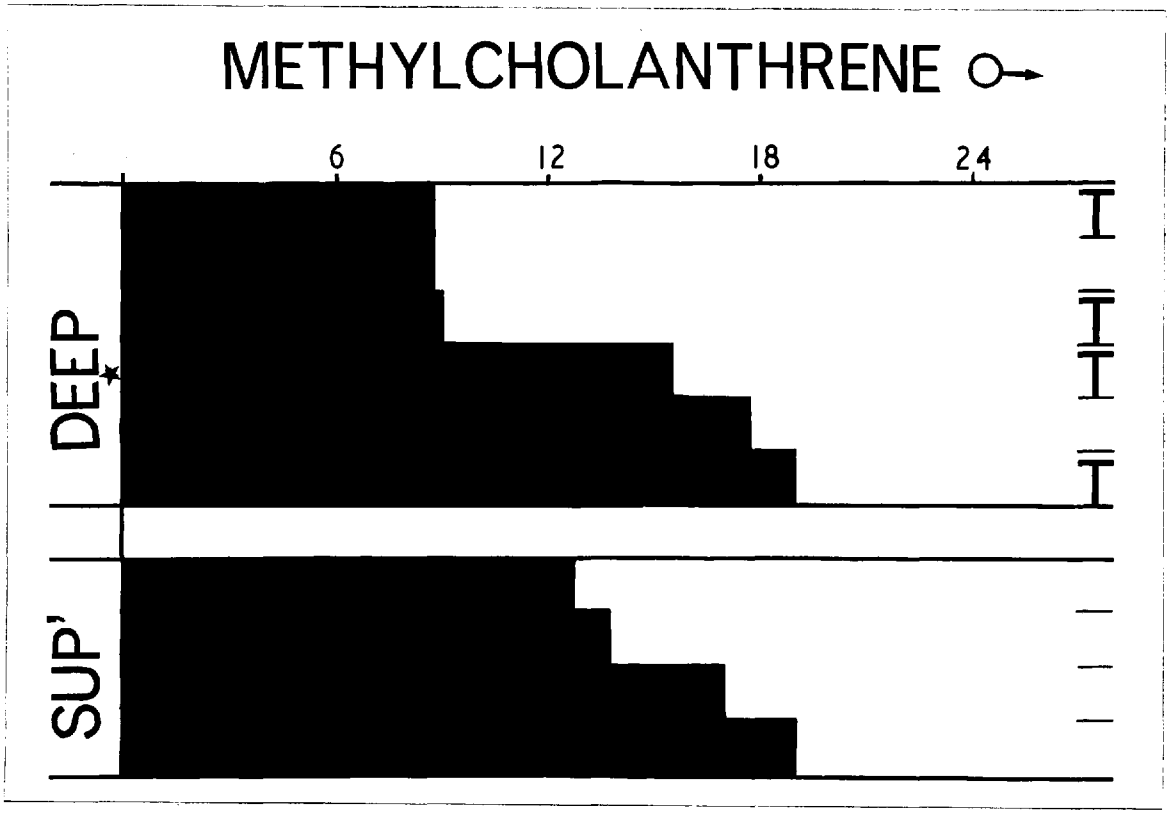


Figure 69. Intracranial tumours in males produced by methylcholanthrene.
★ Carcinogen deep in error.

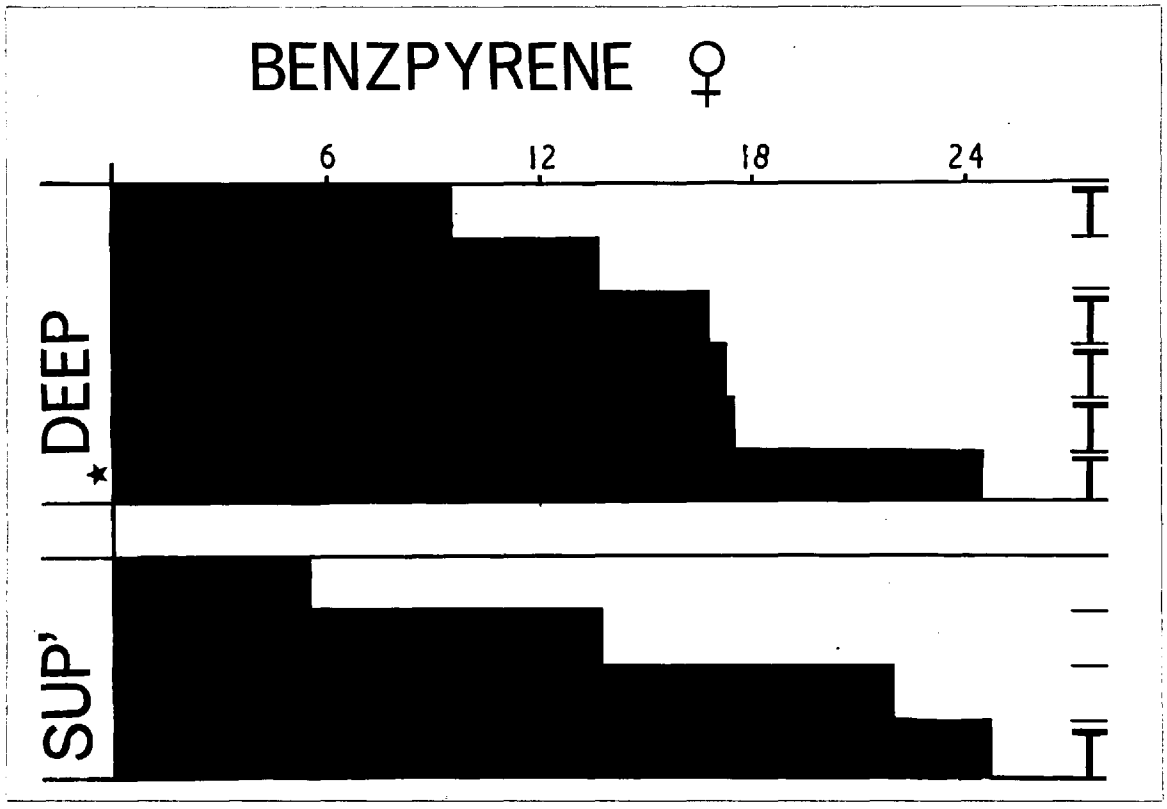


Figure 70. Intracranial tumours in females produced by benzpyrene.
★ Carcinogen deep in error.

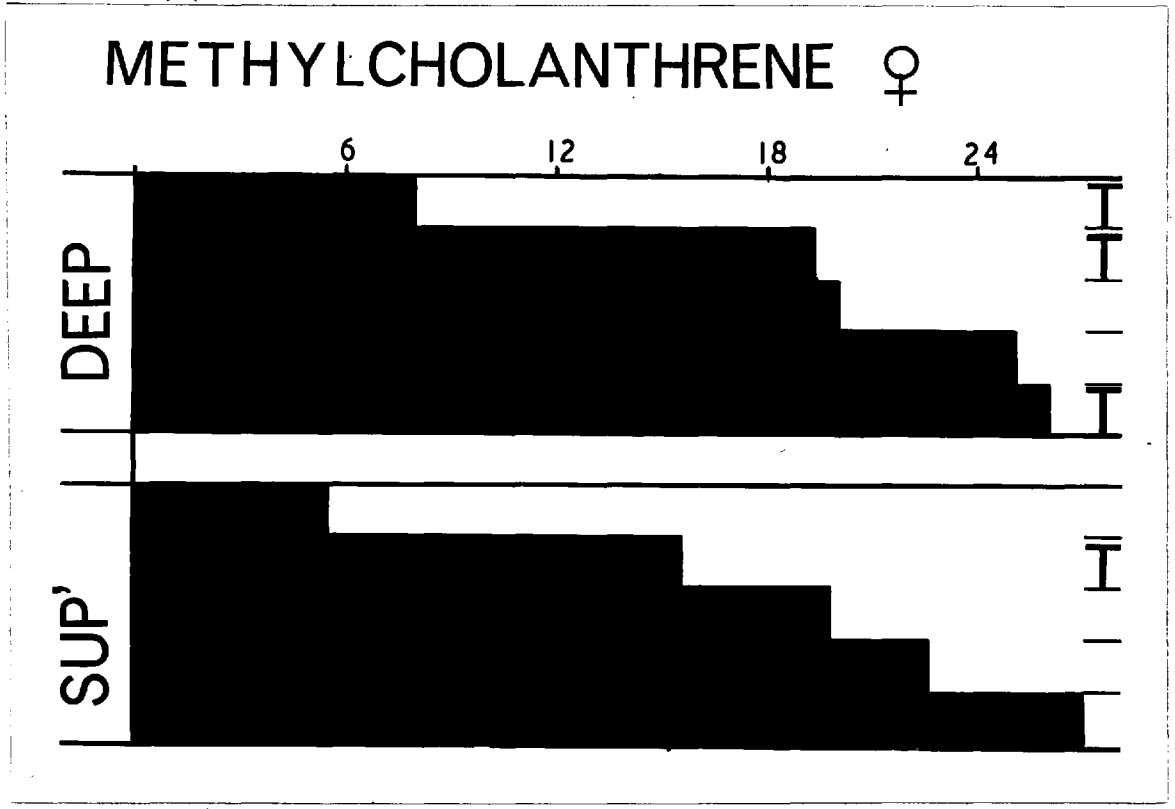


Figure 71. Intracranial tumours in females produced by methylcholanthrene.

argued that both groups are just as likely to have developed cerebral tumours.

It might be argued that fewer tumours developed after an implant of a superficial pellet because the animals died too early and that tumours would have developed had the animals lived longer. Other workers have pointed out however that meningeal sarcomas and other sarcomas have a shorter induction period than gliomas.

Thus, it appeared that the site of implantation was of the utmost importance in the induction of cerebral tumours in rats:-

1. When themeninges were involved only the occasional sarcoma develops:

2. In a midway position between the meninges and the lateral ventricles no tumours were induced (Delorme):

3. When the carcinogen pellet was implanted deeply so that it involves the lateral ventricles a high proportion of tumours were induced and most of these were of glial origin.

C. Histological observations

The types of tumours induced varied between different animals and in some cases between different parts of the tumour mass in the same animal. All tumours produced from deeply implanted pellets were identified as being of glial origin with a single exception. Of the two tumours originating from a superficially situated pellet one was classified as a meningeoma (of two types) and the other,

markably different from any other tumour, remained unclassified.

The tumours were grouped together as shown in table 11. In some cases the tumour was said to be of single cell origin while in others, small isolated regions of other possible tumour types were occasionally found. However, a tumour was only identified as truly mixed if its parts formed a major contribution to the total tumour mass or if two cell types were clearly intermingled throughout the tumour mass as in the case of the mixed-oligoastrocytoma.

The small anomalies mentioned above will be discussed in the detailed description of representative tumours which is to follow.

Rat 219

This animal was found dying 260 days after implantation of 2.4 mg. of methylcholanthrene into the deep position. It exhibited extensive neurological symptoms with lack of motor co-ordination and "righting" responses. Respiration rate was slow. The animal was killed. When the parietal bones were removed a slight separation of the sagittal suture was interpreted as being indicative of increased intracranial pressure in life.

After fixation the brain was sliced and the pellet located; it clearly involved the left lateral ventricle. The tumour mass was very extensive, infiltrating into much of the thalamic region of the brain and extending posterially through the inferior colliculus region into the cerebellum. The tumour was restricted to the side of the carcinogenic implant but the opposite half of the brain was

distorted by the tumour mass. Encroachment into the ventricular space was minimal.

The greater part of the tumour was made up of large round or oval cells with grossly pleomorphic nuclei (fig. 72). The cells were to a large extent unipolar with the nucleus situated to one side of dense clear cytoplasm. Bizzare mitotic figures were a common feature. The cells were closely packed and where necrotic areas had developed, cells tended to palisade with the cytoplasmic tails pointing into the necrotic space. This is an arrangement of tumour cells commonly observed around blood vessels (fig.73). The occasional thrombosed blood vessel was observed within the tumour mass. This tumour was classified as a glioblastoma multiforme.

RAT 201

The rat was found dead 15½ months after implantation of methylcholanthrene. The pellet at autopsy was found, protruding into the lateral ventricle (fig.74). The tumour was large and extended from the floor of the ventricles onto the surface of the brain. The remaining brain was grossly distorted due to the large size of the tumour. In other sections, tumour can be found extensively overlying the meninges.

The cells were of medium size with regular, pale nuclei showing a fine chromatin pattern. The cells were moderately packed and showed a tendancy to lie in sheets particularly in the tumour mass

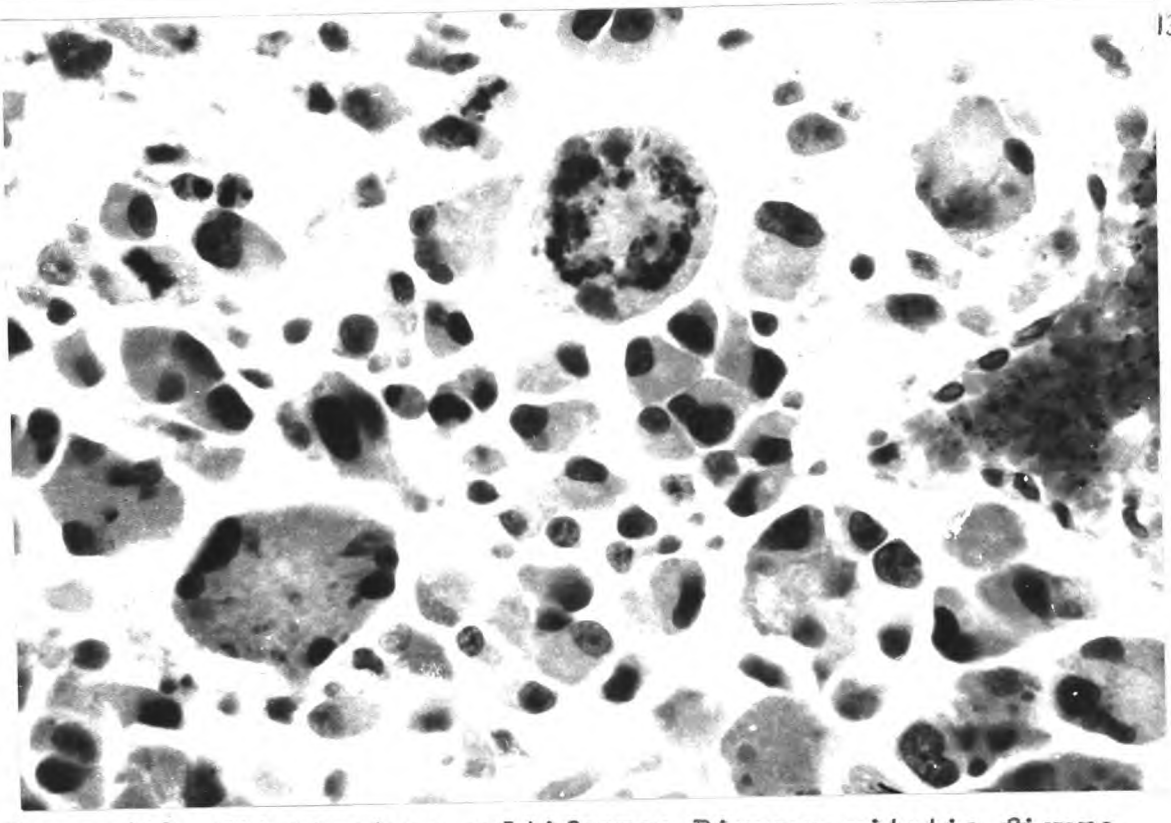


Figure 12. Glioblastoma multiforme: Bizzare mitotic figure and multinucleate cells. H&E x1600.

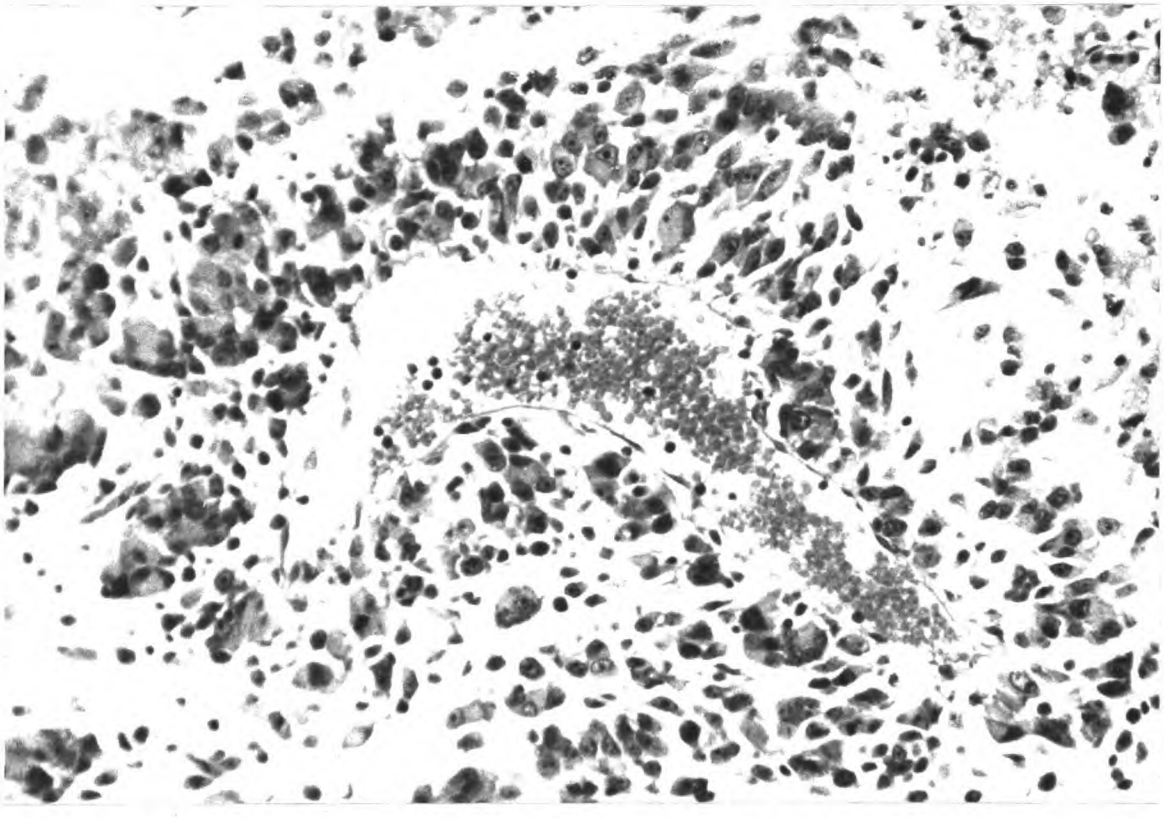


Figure 13. Glioblastoma multiforme: Palisading of cells around dilated blood vessel. H&E x700.



Figure 74. Slice of brain showing position of carcinogenic pellet and cerebral tumour.

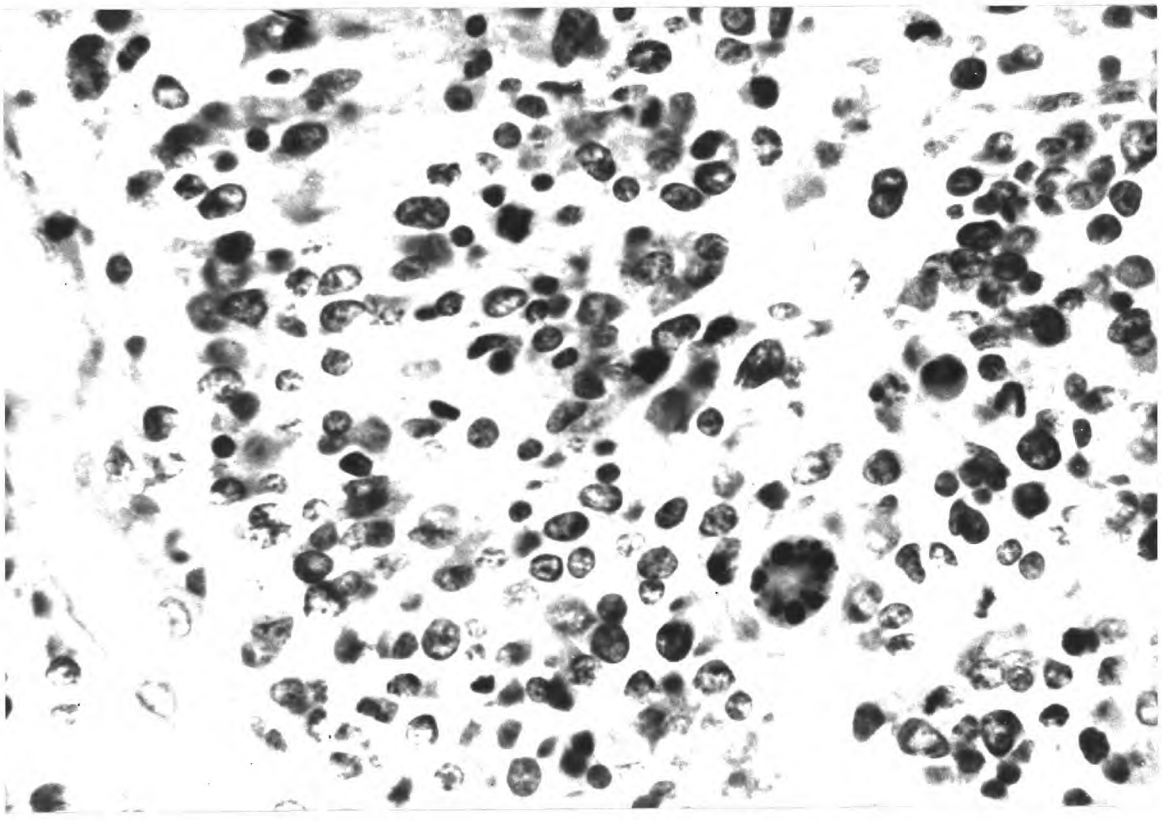


Figure 75. Ependymoblastoma: Shows multinucleate cell and the organisation of cells into layers. H&E x700.

that had extended over the meninges (fig.75). The cells also tended to palisade around small areas of necrosis. Occasional multinucleate and micronucleated cells were observed as well as bizzare mitotic figures. This tumour was classified as an ependymoblastoma.

RAT 211

Died 15 months after a superficial implant of methylcholanthrene, the pellet at post mortem was found to be implanted on the surface of a large white tumour mass.

Histological observations revealed that the tumour mass was made up of two very distinct tumour types which bordered on each other but did not to any large extent intermingle. The most characteristic feature of one of these tumours was the interlacing bundles of long narrow spindle shaped cells (fig.76). The nuclei were pale, narrow and rod shaped, with a fine chromatin structure and a single nucleolus. The cells were elongated and resembled fibroblasts. The tumour was not highly vascular and there was a large central area of necrosis. This could be termed a fibroblastic meningioma.

The other tumour was very uniform in appearance, the cells being slightly polygonal although in many cases the cells were not clearly defined. The nuclei were relatively large and centrally placed within the cell. They had a fine chromatin net with a

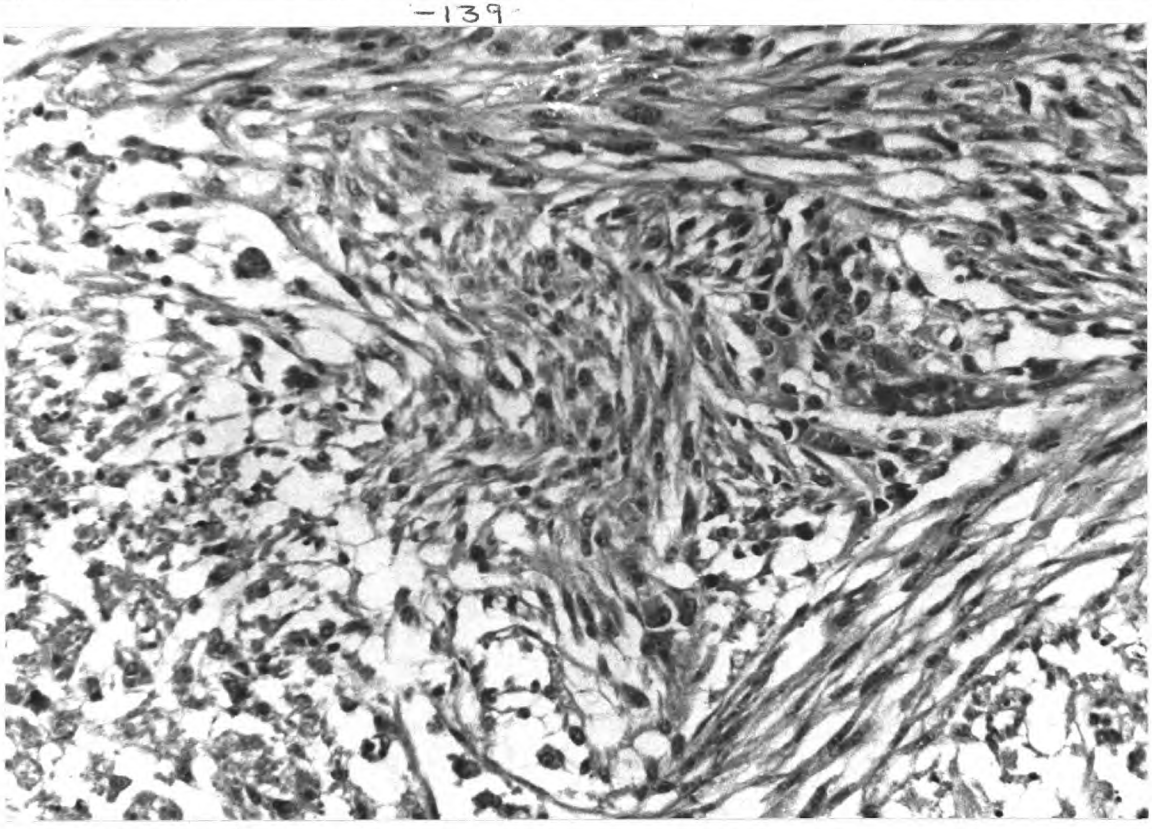


Figure 76 . Fibroblastic meningioma. H&E x300.

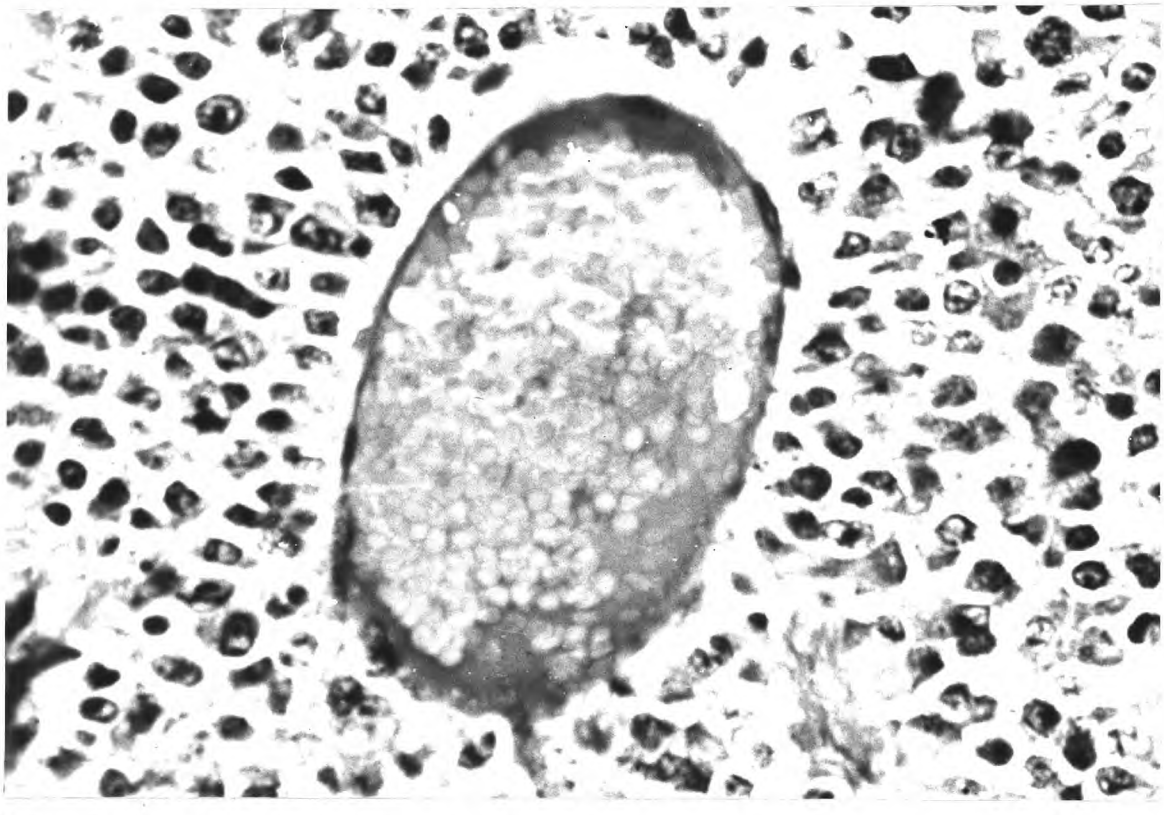


Figure 77. Syncitial meningioma: Shows slight palisading around dilated vessel. H&E x700.

variable number of nucleoli.

The cells exhibit slight palisading around the numerous dilated capillaries found within the tumour mass (fig.77) and around small areas of necrosis.

This tumour could be described as a syncitial meningeoma.

RAT 206

The animal was found dead 270 days after an implant of methylcholanthrene into the deep position. The pellet was found in contact with the roof of the lateral ventricle after death.

Histologically we observed a large cystic tumour occupying the fronto-parietal region of the brain. The tumour was made up of two types of cells; one with a large pale nucleus with abundant dendritic cytoplasm, presumably astrocytes, and the other cells with a small dark round nuclei were probably oligodendrocytes. The two cell types are in approximately equal proportions (fig.78).

There were numerous necrotic areas, some filled with clear fluid. The cells with large pale nuclei tend to palisade around these necrotic areas (fig.79). There were a few pleomorphic areas. The tumour could be called a mixed oligo-astrocytoma.

RAT 203

This rat received a pellet of methylcholanthrene deeply implanted into the brain. Evidence of raised intracranial pressure

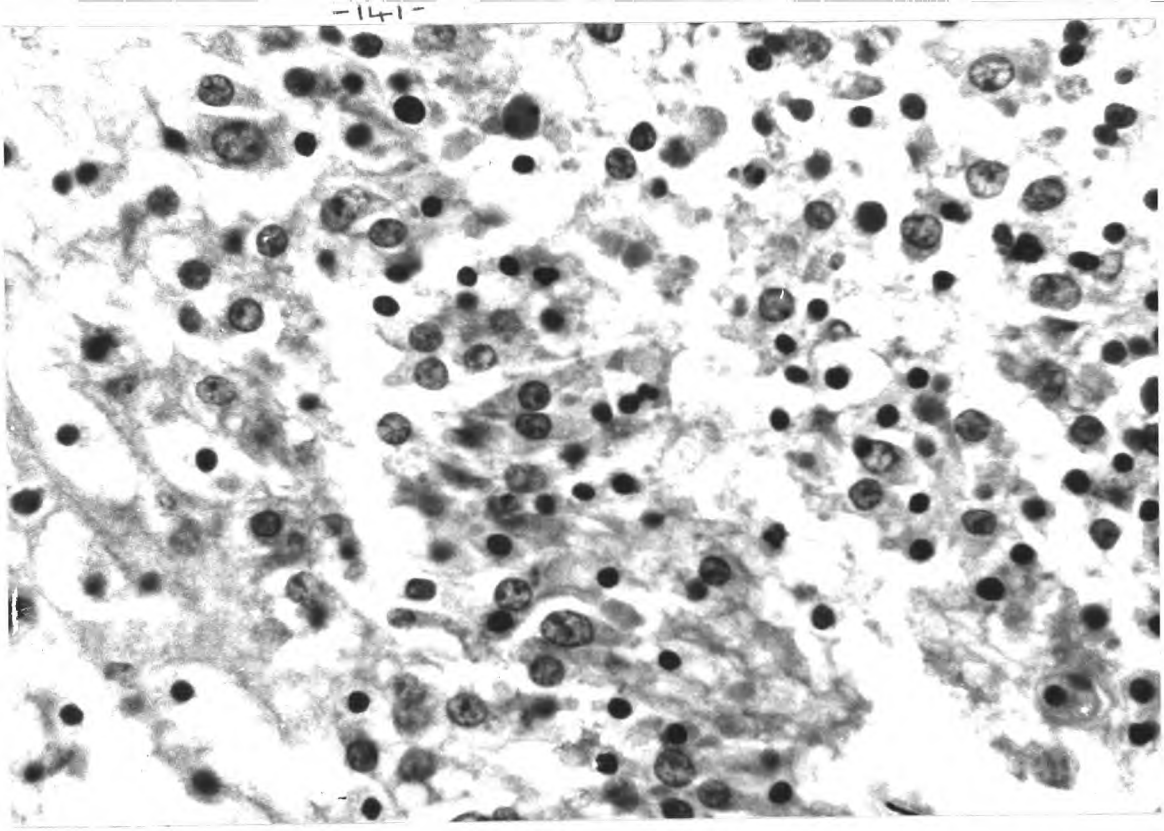


Figure 78. Mixed oligo-astrocytoma. H&E x700.

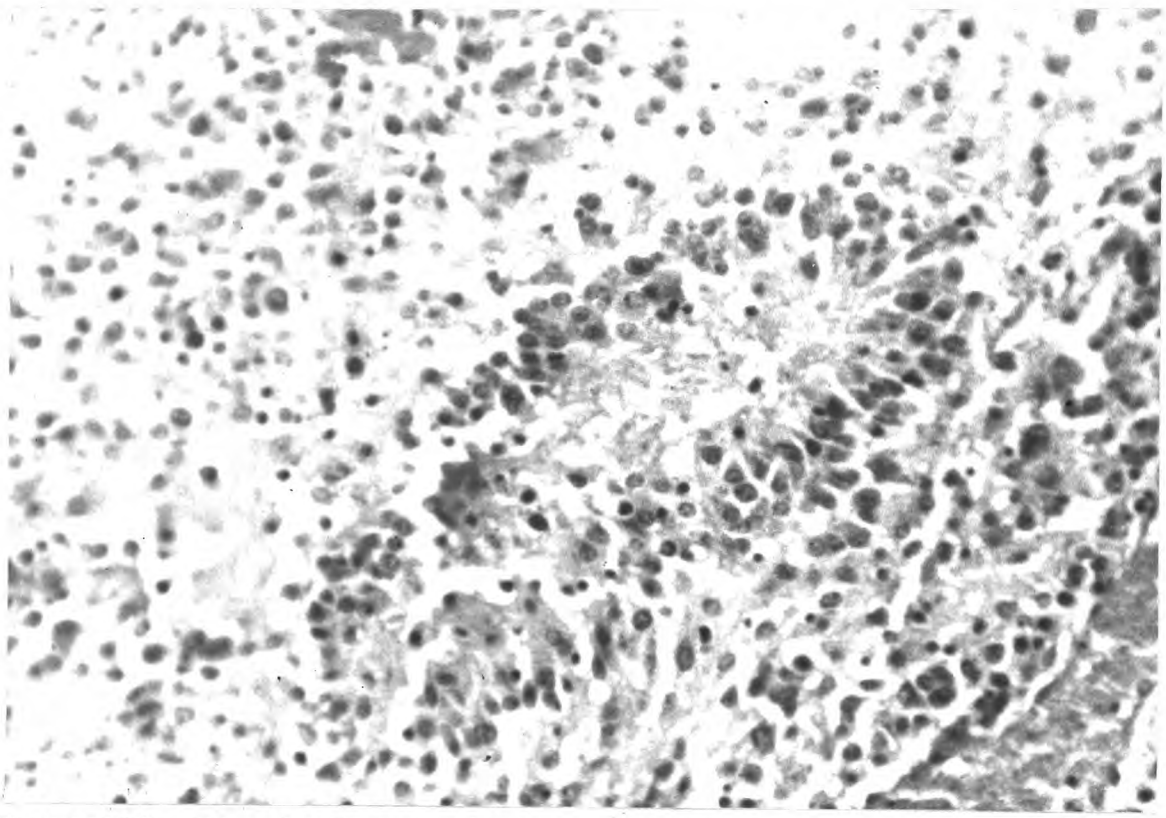


Figure 79. Mixed oligo-astrocytoma: Palisading of astrocytes around a necrotic area. H&E x300.

was present at death, with marked separation of the parietal bones along the mid line.

This very extensive tumour occupied the area between the roof of the lateral ventricles and the surface of the brain. The tumour extended through the front parietal region into the cerebellum where the tumour cells were more closely packed than in the other regions (fig.80).

The small, oval, central nuclei were surrounded by highly vacuolated cytoplasm, showing many processes (fig.81). This highly vacuolated cytoplasm gives the tumour a very spongy appearance. This is a feature which has been lost to a large extent in the cerebellum. Pleomorphism was another feature of the tumour. Large areas of necrosis existed around which palisading occurred.

This tumour was classified as a spongioblastoma.

RAT 236

This animal died 12 months after a deep intracranial implant of benzpyrene. Post mortem revealed a very extensive tumour centred on the left lateral ventricle.

The tumour was made up of small round cells with dark, round, often pyknotic nuclei, characteristic of oligodendrocytes. The tumour mass was relatively avascular and large areas of necrosis were a common feature, with the palisading of cells around them. Another characteristic feature was the orientation of the tumour

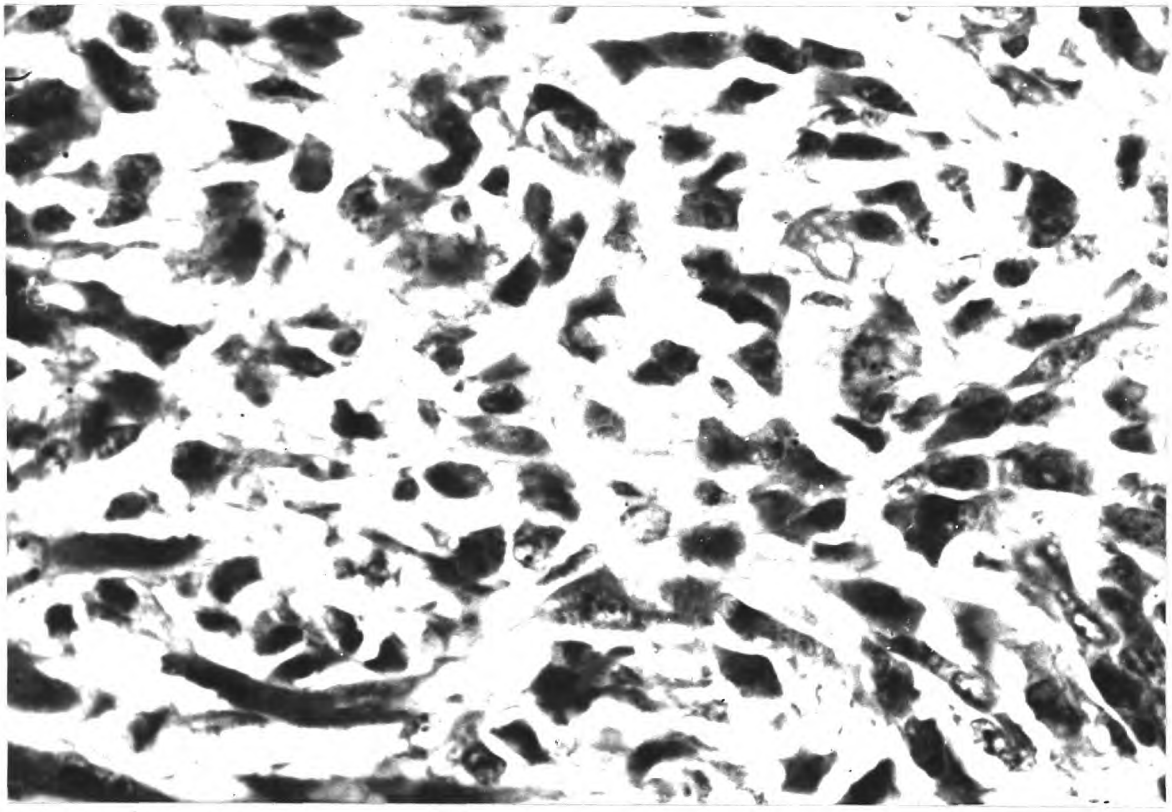


Figure 80. Rat 203: Closely packed tumour cells in the cerebellum. H&E x700.

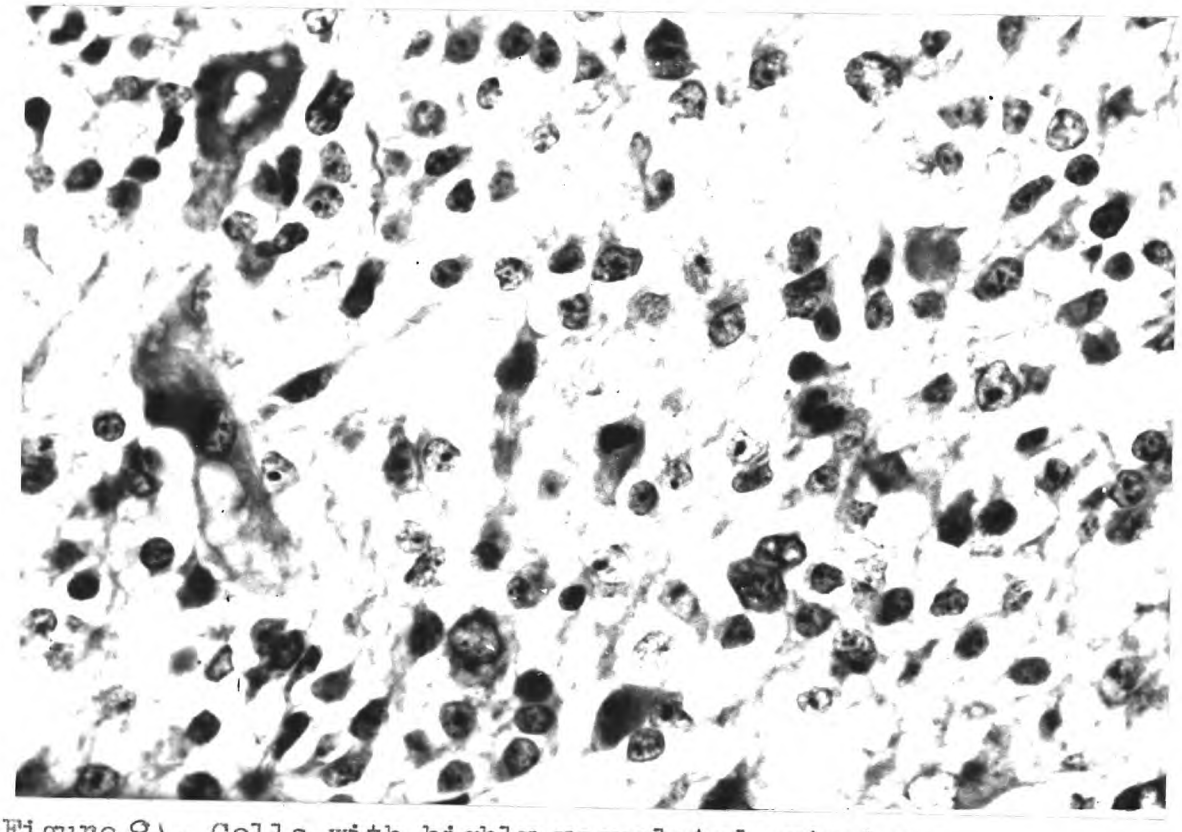


Figure 81. Cells with highly vacuolated cytoplasm characteristic of a spongioblastoma. H&E x700.

cells into rows, in much the same way as oligodendrocytes are found along myelinated nerve fibres (fig.82).

This tumour is classified as an oligodendrocytoma.

RAT 216

The animal was found dead 26 months after implantation of methylcholanthrene. The pellet was found in an enlarged lateral ventricular space surrounded by tumour which was almost entirely restricted to the ventricular space with only limited infiltration into the surrounding brain. A most striking feature was the large dilated capillaries and numerous blood spaces. This tumour clearly involved the choroid plexus (fig.83).

Nuclei were small and round, similar to the normal epthelial cells of the choroid plexus, although the normal single layered arrangement of these cells was lost. These cells were intermingled with a considerable amount of supporting stroma of vascularised connective tissue.

In the tumour infiltrating the brain, the small nucleated cells tended to have highly vacuolated cytoplasm.

For reasons of position and similarities with the normal choroid plexus, this can be described as a tumour of the choroid plexus.

D. Conclusions

If the cumulative tumour number in the deep group of rats is

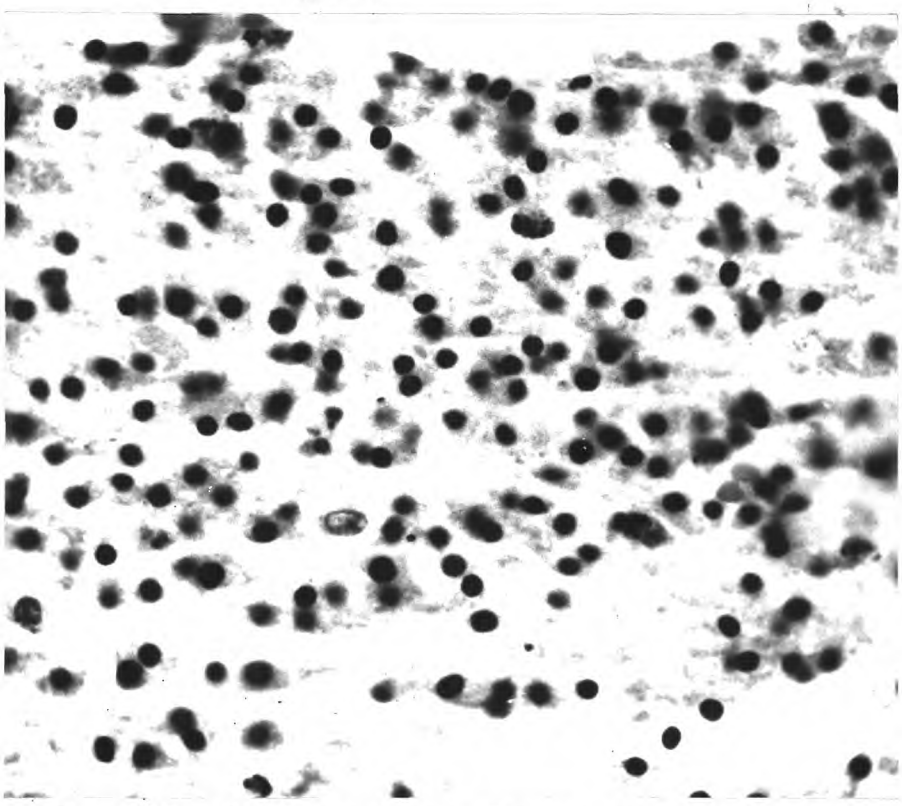


Figure 82. Oligodendrocytoma: Shows organisation of cells into rows. H&E x700.

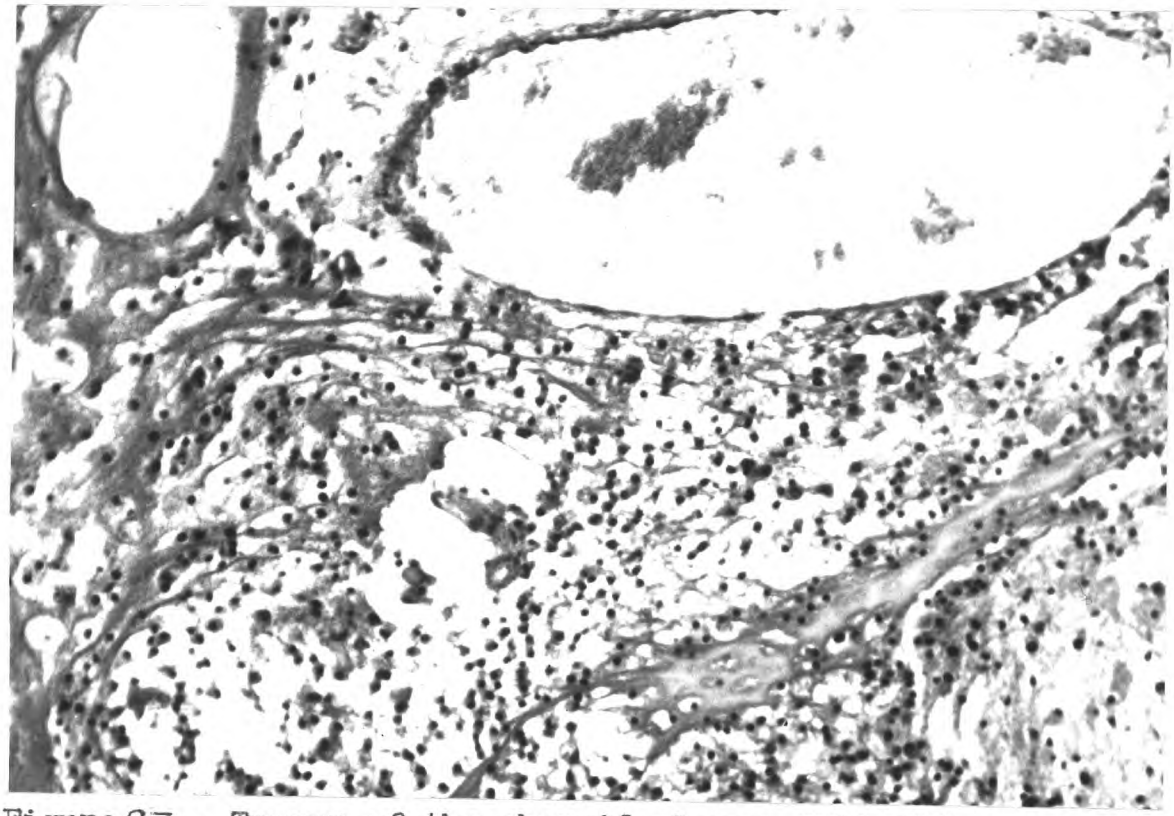


Figure 83 . Tumour of the choroid plexus. H&E x300.

plotted against time for males and females seperately then two clearly distinct curves are obtained (fig.84). The incidence of superficial tumours is plotted in a similar fashion for comparison illustrating that more tumours develop from deeply situated pellets.

The curves for male and female tumours indicate that tumours in males tend to develop earlier than in females. This correlates very well with the incidence of glial tumours in man. If the results of Penman and Smith (1954) for adult gliomas are plotted graphically in the manner used for the incidence of experimental tumours, a pair of similar curves are produced (fig.85).

Some of the conclusions arrived at by the above authors also agree with our findings. Male cerebral glial tumours are more common than in females in the ratio of 4:3. This is a point they emphasise, adding that they know of no author having found a predominance of glial tumours in females. They also state that in the case of their predominant tumour type, glioblastoma, the highest frequency appears one decade earlier in males, 40 - 49 years as opposed to 50 - 59 years in females.

This predominance of glial tumours in males is also found in children, as was reported by Bodian and Lawson (1952, Table 12).

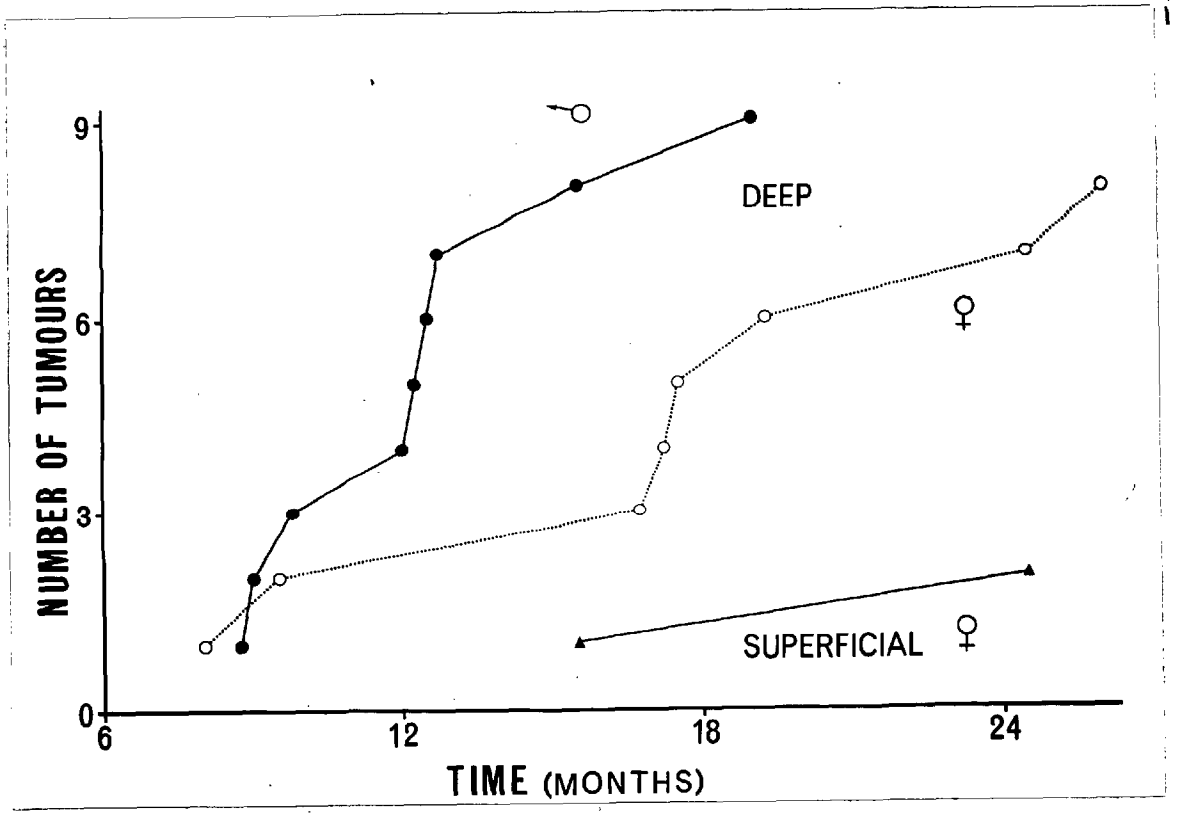


Figure 84. Cumulative number of experimental cerebral tumours, in male and female rats, against time in months.

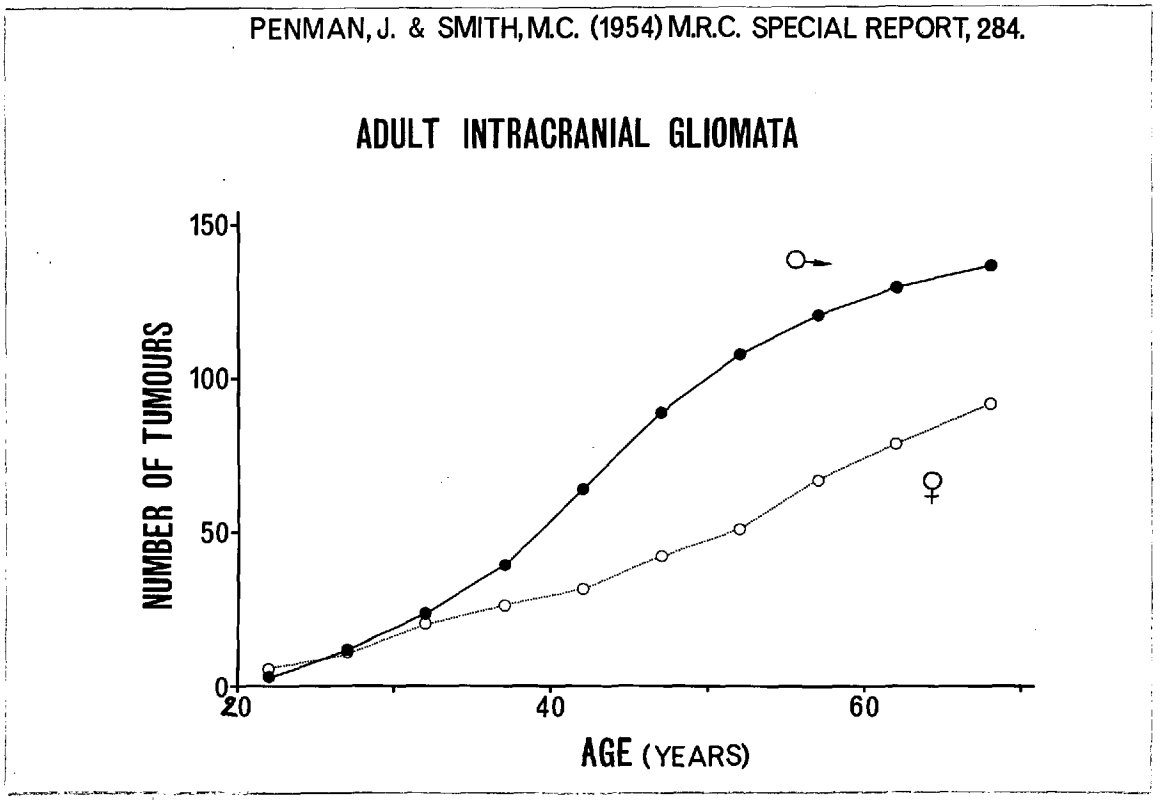


Figure 85. Cumulative number of glial tumours in man against age in years.

TABLE 12

Intra cranial glial tumours in children

	Male	Female	Total
Medulloblastoma	34	17	51
Astrocytoma	32	23	55
Subependymal glioma	3	6	9
	69	46	115

The simplest explanation of the predominance of glial tumours in males is that they are in some way testosterone dependant, a view that was expressed by Penman and Smith to explain the high incidence of glial tumours in the male. The continued growth of the male rat brain may be of importance or at least a related phenomenon (fig.86).

In addition to its possible role in the induction of cerebral tumours, testosterone was also shown to be important in the growth of transplanted cerebral tumours (Autsyn and Yablonovskaya, 1965). These workers showed that castration of male rats will reduce the percentage "take" of several types of intracerebrally transplanted tumours in non syngeneic animals. The protective effects of castration disappeared after the administration of testosterone.

The final question to be answered is, why do gliomas only develop when the carcinogenic pellet is implanted into the deep

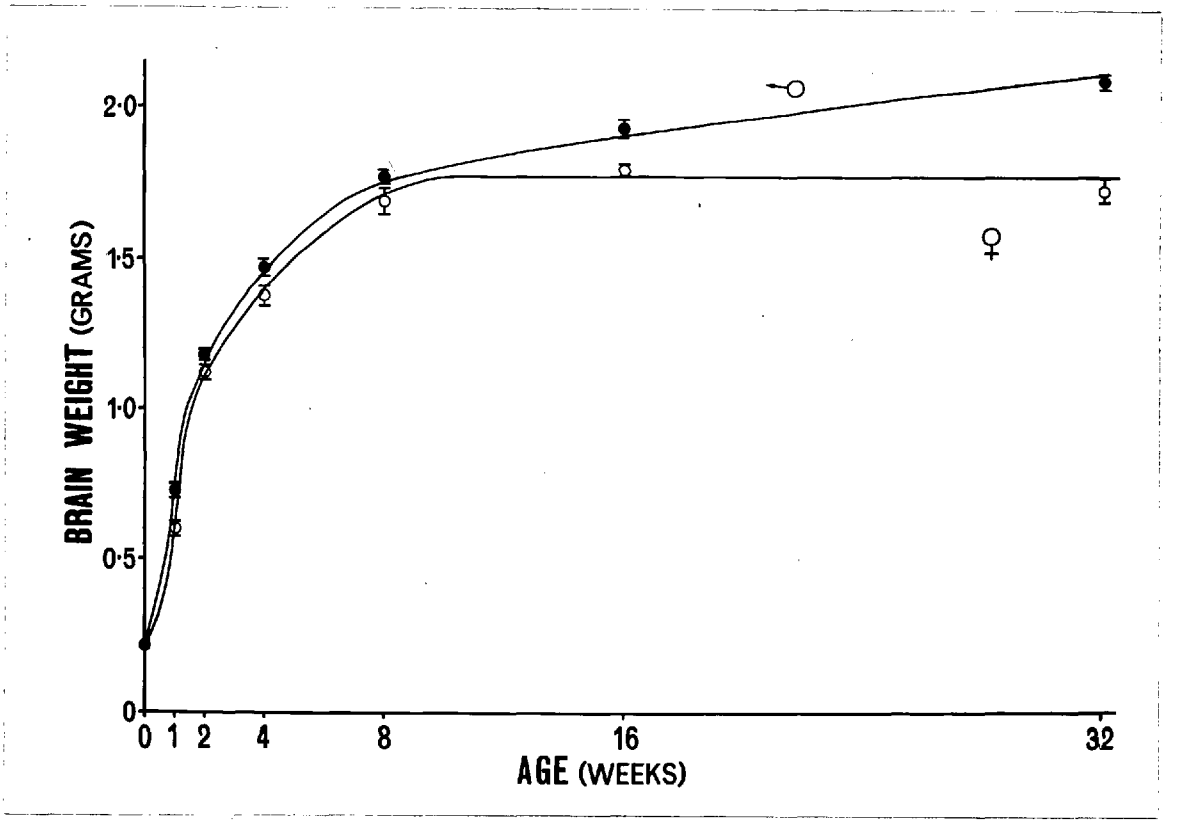


Figure 86. Comparison of the growth, by weight, of male and female rat brain (S/D strain) after birth. In the female growth of the brain is maximal by the tenth week after birth, while in the male the brain continues to grow.

position? The possible explanation of this is that in this position the pellet is in contact with cells of the subependymal layer. This is a primitive layer of cells which are probably mitotically active throughout the life of the animal (Allen 1912), and are therefore prone to tumour induction. The subependymal layer has been suggested (Globus & Kulhenbeck, 1942; 1944; Kulhenbeck 1947) as the site of origin of several glioma types in man.

E. Radiation Carcinogenesis

In approximately 70 rats irradiated with various doses and surviving for at least three months after irradiation, two intracerebral tumours developed. A few of these animals survived for periods of up to two years after irradiation, although the mean survival time was less than one year.

RAT 75

This animal, which was hypertensive, died 19 months after cerebral irradiation, 1,000R. A large, very diffuse, tumour was found throughout the brain. Anteriorly it was found on the left side of the brain, in the region of the caudate nucleus, between the fibres of the corpus callosum and extensively in the hypothalamus. Areas of necrosis were present in this latter region. The left half of the corpus callosum and the brain parenchyma around the fourth ventricle was heavily infiltrated with tumour. The white matter tracks of the internal capsule showed only slight infiltration.

The tumour also extended back into the cerebellum where involvement of the ventricular spaces was much more in evidence.

The cells were small and round with small dark round nuclei. Pyknotic and micronucleated cells were often found. In some cells the nuclear chromatin was separated into two or three parts within an oval nucleus (fig.87). This type of cell was most common in the cerebellum and was only found occasionally elsewhere. These cells show no distinct organisation other than to aggregate around blood vessels (fig.88).

Identification of this tumour was difficult, as it does not readily fit into any common tumour group. The term microglial tumour may be applied.

RAT 854

This animal received 400R three months prior to cerebral injury and death. The tumour at autopsy, appeared as a blue patch (trypan blue) on the surface of the brain. It was slightly larger than, and on the opposite side of the brain to the frozen cerebral lesion.

Histologically the tumour occupied a position between the roof of the lateral ventricle and the meninges, although some invasion of the hypocampus did occur. Cells are small and slightly elongated with small oval nuclei and only occasional mitotic figures.

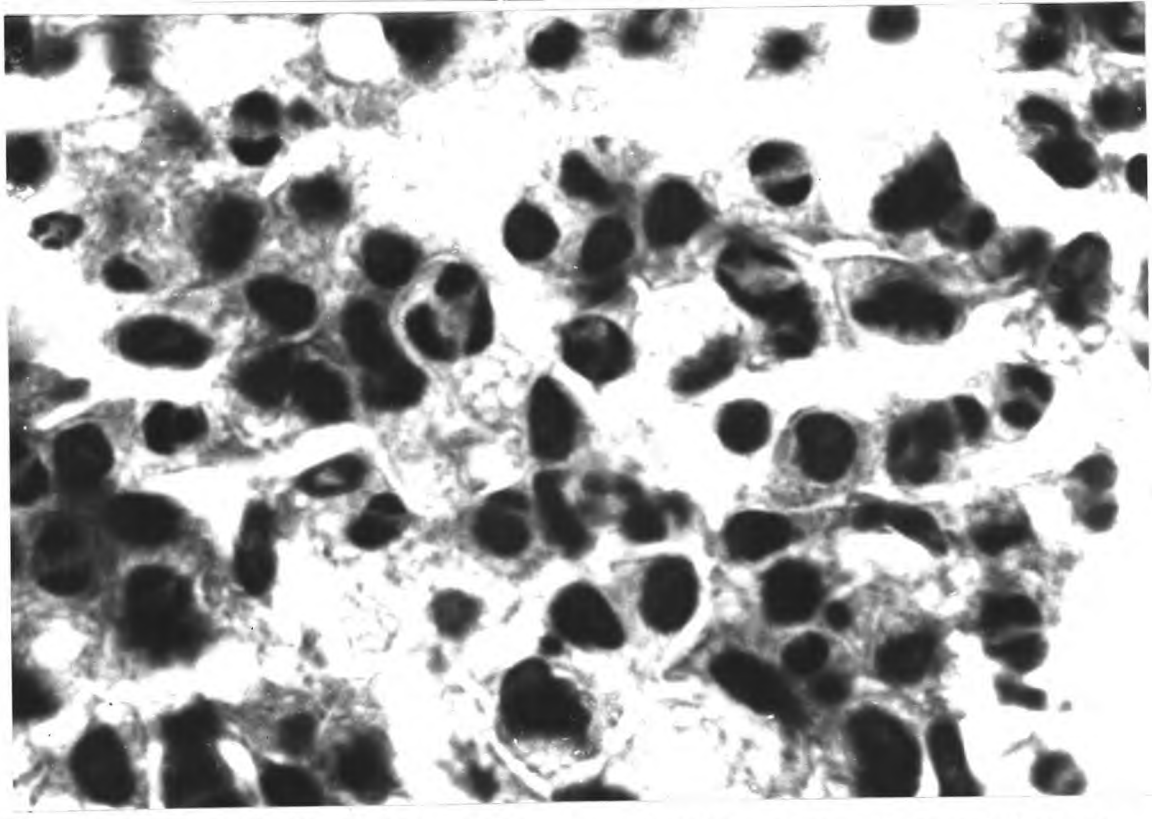


Figure 87. Rat 75. Radiation induced cerebral tumour, shows subdivision of nuclear chromatin into two or three parts. H&E x1600.

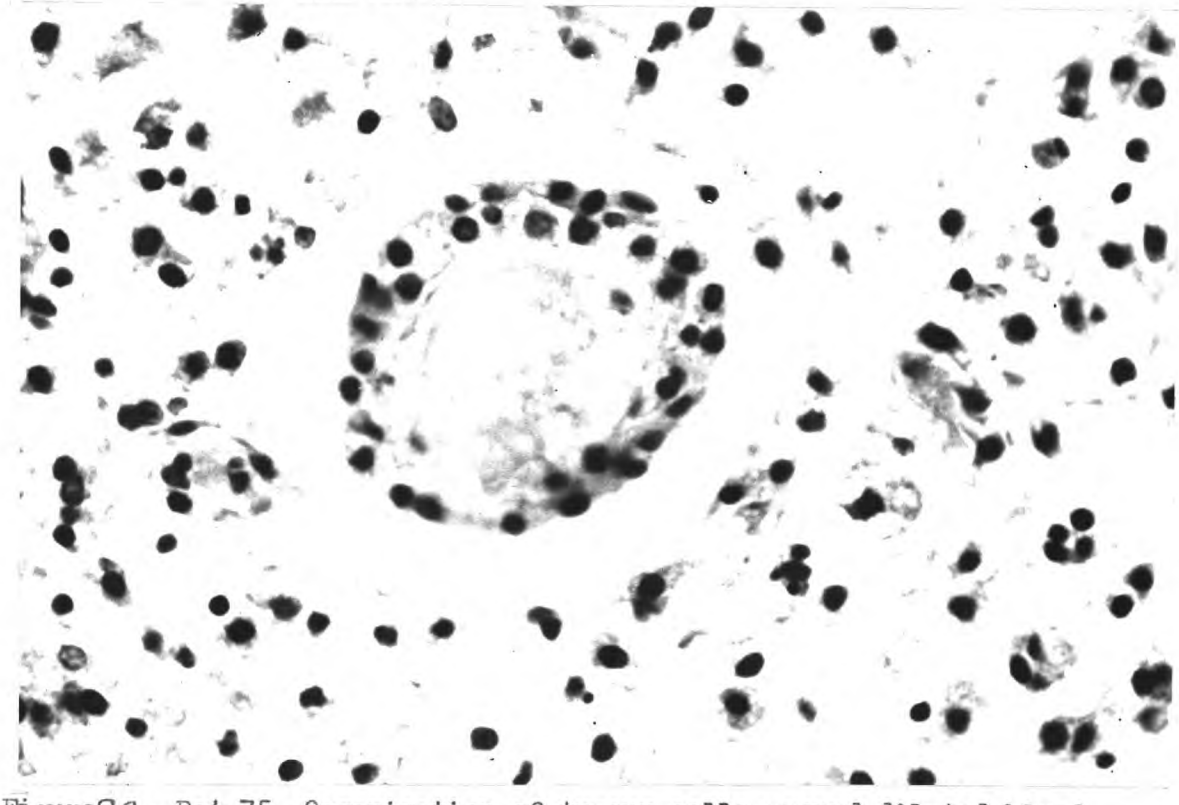


Figure 88. Rat 75. Organisation of tumour cells around dilated blood blood vessel. H&E x700.

Small areas of necrosis occur with palisading of cells around them (fig.89). Cells also aggregated around blood vessels (fig.90).

This tumour was not classified.

Thus it would appear that radiation is slightly carcinogenic to nervous tissue.

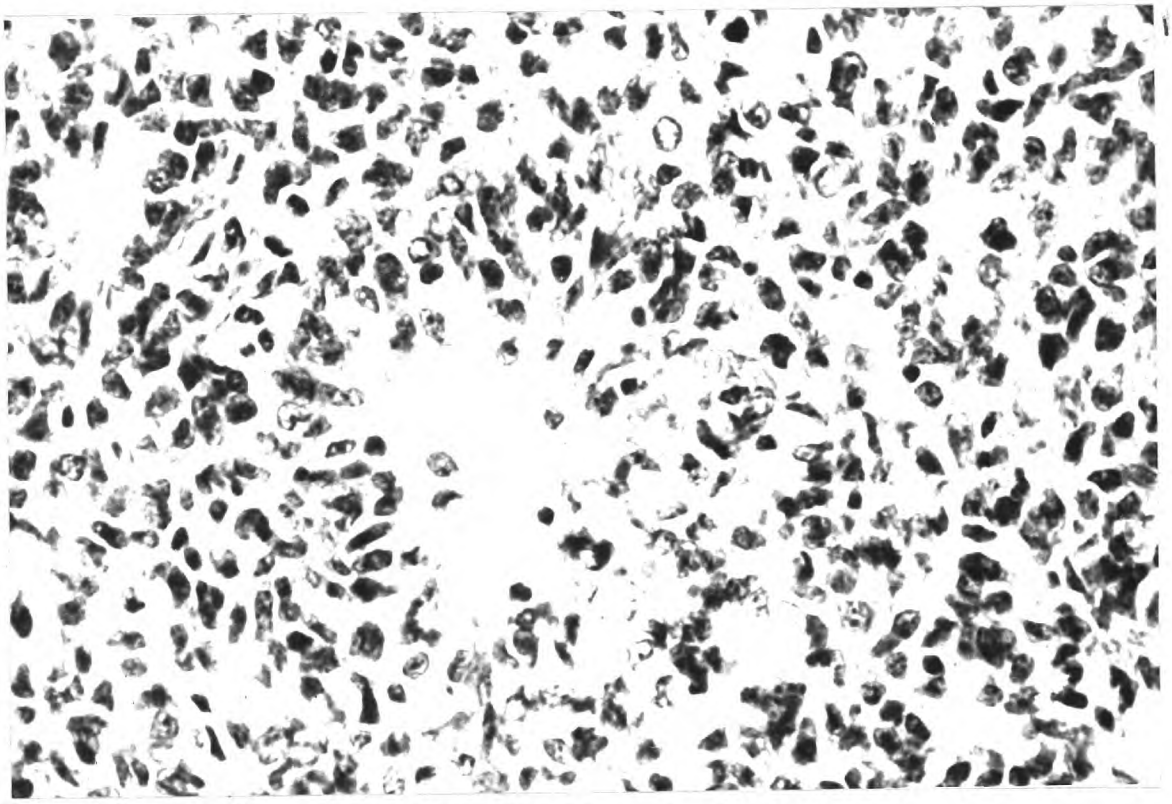


Figure 89. Rat 854. Palisading of cells around a necrotic area.
H&E x700.

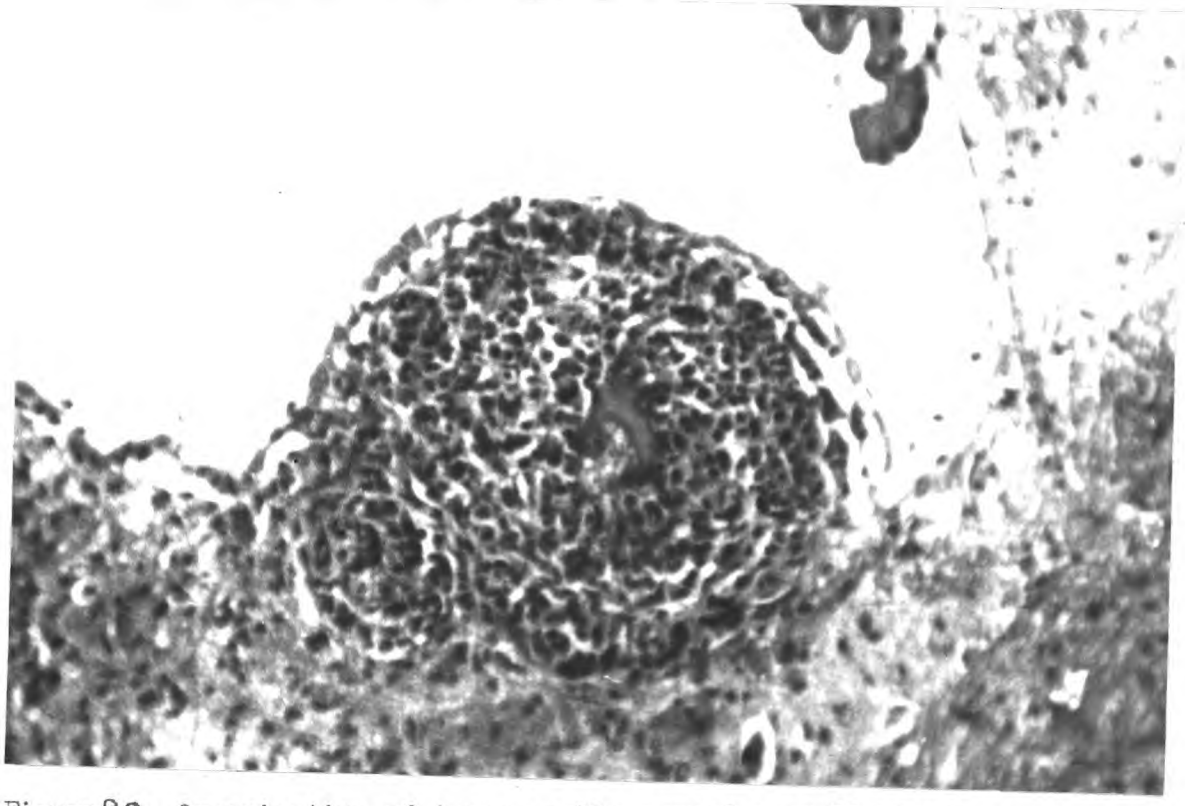


Figure 90. Organisation of tumour cells around a small blood vessel.
H&E x300.

CHAPTER VII

MATERIALS AND METHODS

The animals used in the following investigations were as follows:-

Wistar Rats - for normotensive and hypertensive brain irradiation (adults)

Sprague Dawley Rats - Cerebral injury (adults)
Cerebral carcinogenesis (weanlings)
Half brain irradiation (litters, 7 days)

Theilers original albino mice - B. pertussis infection (young adult females)

Anaesthetics:-

Rats; Chloral hydrate 300 mg/Kilo body weight, 3% solution I.P.

Mice; Sodium pentabarbitone (Nembutal) 60 mg/Kilo body weight, 1% solution I.P.

A. Irradiation techniques

In all irradiation experiments where only a small area of tissue was exposed care was taken to ensure that the animal was held firmly while the irradiation was in progress. Shielding was suitably placed to protect other areas against direct exposure and to reduce exposure by scattered radiation. A layer of lead 2mm thick was considered suitable for this purpose in the 220 kV

X-ray experiments, for this will reduce the dose to the shielded parts to less than 1%. In the electron experiments 1.6cms of aluminium was used.

1. Adult Rats (180-250 grms)

The heads were firmly supported in a specially constructed hinged perspex clamp (fig.92). The two upper incisors were placed through the hole in the lower half of the clamp and the upper half of the clamp was gently tightened down on top of the head. With the head firmly clamped the rat could be fixed into position in the irradiation box (fig.91).

The perspex box was fitted with an air inlet and outlet so the animal could be supplied with air in its confined position throughout the irradiation. The rectal temperature of the animal was recorded throughout the irradiation with an electric thermocouple type thermometer. Air flow was maintained at 1 litre per min. by a Prockter pump.

The animals were irradiated through the side of the head via a semi-circular port 1.2cms. diameter (fig.93). This method had the added advantage of avoiding irradiation of the respiratory tract and alimentary canal which is very important in animals receiving high doses and expected to survive for long periods of time.

The two points of the semi-circular irradiation port were aligned between the posterior corner of the eye and the ear, these being two positions which enabled the irradiation port to be

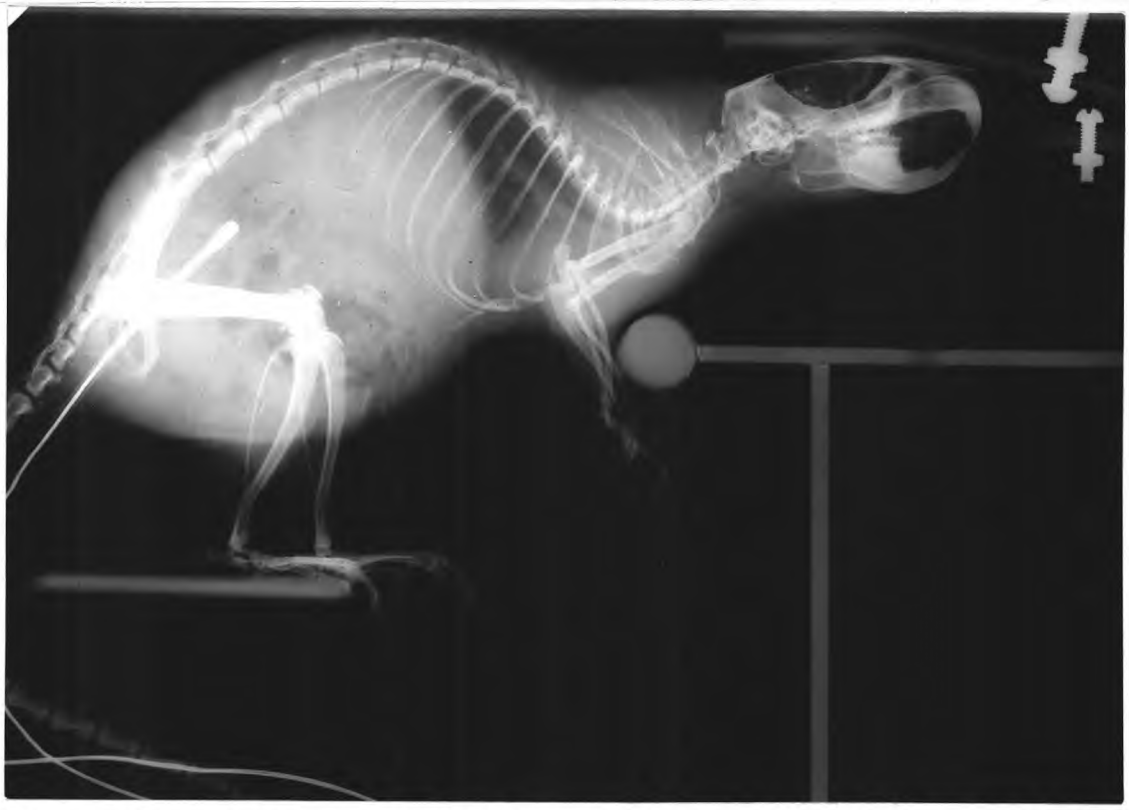


Figure 91. Radiograph showing position of rat in irradiation apparatus.



Figure 92. Radiograph of rat skull showing position of circular irradiation field (2cm. diameter) used with 8Mev electrons. The positioning of the hinged clamp, used to secure the head, is shown.



Figure 93. Radiograph of rat's skull showing position of semi-circular irradiation field (diameter 1.2cm.) used with 220kV X-rays.

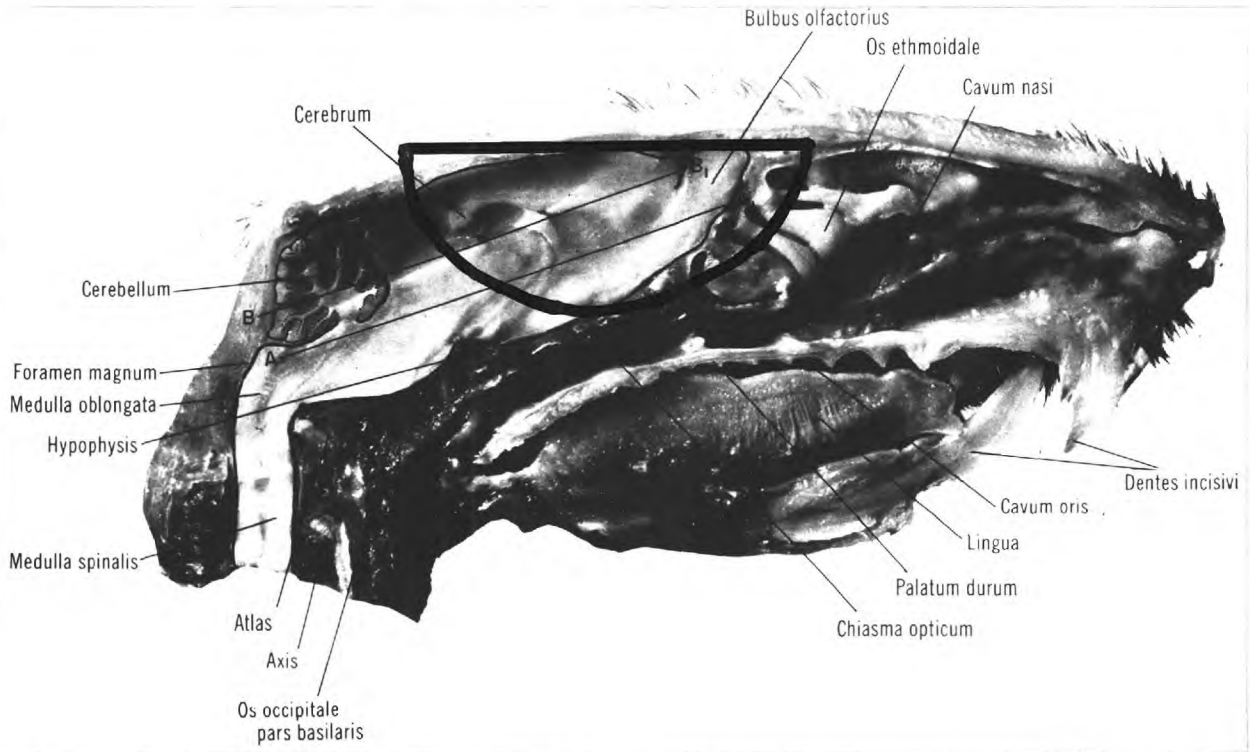


Figure 94. Sagittal section of a rat's head indicating the region of the brain irradiated with 220kV X-rays (cf. figure 93).

CRAIGIE (1963)

positioned accurately. The region of the brain irradiated with 220 kV X-rays is shown in fig.94. This was the olfactory lobes and the majority of the cerebral hemispheres. The cerebellum was not irradiated.

2. Adult Rats (180-250 g) 8 MeV electrons

The animals were restrained as mentioned in the previous section but in this case the animals were fixed horizontally in a partitioned perspex box. Irradiation was given laterally through the side of the head, through a 2cm. diamter hole in aluminium shielding (fig.92). Animals were in this case irradiated lying on their backs and not as in the previous experiments with 220kV X-rays, on their sides.

3. Suckling Rats (10-15 grms)

In this case it was essential that the animal was secured very firmly as it was intended that only half the animal's brain was irradiated. It was not necessary to anaesthetise these small, 7-day old animals.

The apparatus for securing the rats was constructed from thin flexible tempered copper strips that were bent over the neck, behind the ears and over the snout. The rest of the body was held down with further strips of copper. The head was thus held firmly and the brain irradiated through the top of the calvarium.

Positioning of the lead shielding over the right side of the

head was performed with extreme care. The edge of the lead being placed on an imaginary line between the tip of the snout and the mid-point between the ears. The remainder of the body below the base of the skull was shielded by a tunnel of lead.

A very useful means of checking the accuracy of the irradiation was to examine the animals one to two weeks after irradiation. At this time in a majority of the animals, a considerable difference in the amount of hair growth on the irradiated and non-irradiated sides of the head was observed. An animal irradiated on the seventh postnatal day with 1,000R and observed one week later is shown in fig. 95.

4. Young adult mice (15 - 20 grms)

For all the 400R irradiations (to the head only, body only, and whole body irradiation) a cylindrical box, divided into seven radially arranged compartments was used. This box was a suitable size for fixing into the cone of the X-ray machine, so that the whole box was in the irradiation field. The various combinations of irradiations could then be formed by placing 2mm lead over the head or bodies of anaesthetised animals.

A similar apparatus to that used in the irradiation of suckling rats was used for the irradiation of mice with the higher dose (1,000R), although in this case the animals were anaesthetised (Nembutal 60mg/kilo, I.P.). This apparatus reduced scattered radiation to the body, which proved a problem when the previous

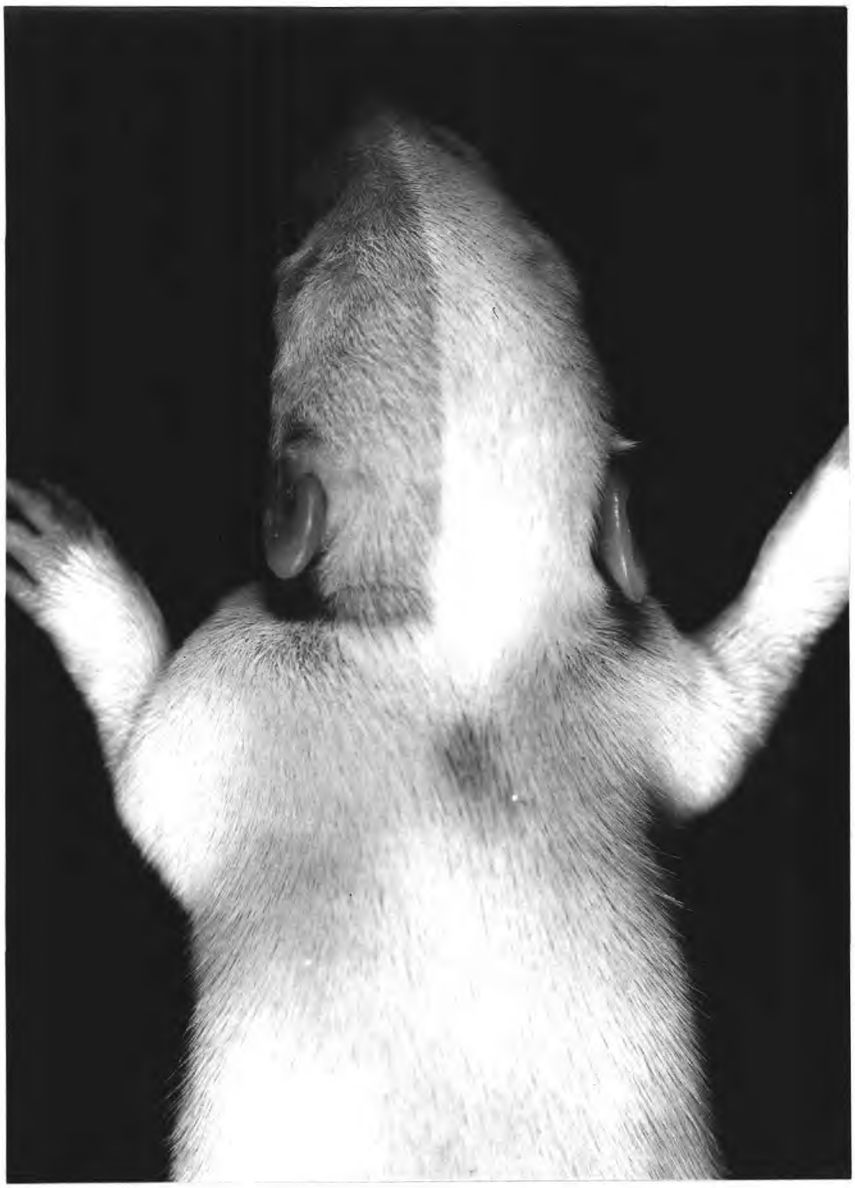


Figure 95. Two week old rat showing reduced hair growth on left side of head one week after irradiation (1,000R). The well defined area indicates that lead shielding was placed accurately, on the mid line, at the time of irradiation.

apparatus was used for this higher irradiation dose. Exposure of the body to scattered radiation was indicated by a gradual reduction in the leucocyte count following irradiation.

B. Dosimetry

As far as possible in all experiments the dose of radiation administered was monitored with a Baldwin Farmer Sub-standard dosimeter. The probe of this instrument was inserted into a "Mix-D" tissue equivalent phantom and placed in the X-ray field in a comparable position to the irradiated animals. This material has equivalent scattering properties with regard to X-rays as tissue. Where continuous monitoring with the Baldwin Farmer Dosimeter was not possible, as in the case of the pressure chamber experiments, a transmission ionisation chamber was used in conjunction with a vibron electrometer.

For all practical purposes the X-ray field can be considered to be in the form of a cone emitted from the focal spot of the X-ray tube. Thus, all points of equal radius from the central axis of this cone will receive approximately the same exposure dose.

In all experiments therefore, all animals or the parts of animals to be irradiated were placed on a circle the centre of which was the central axis of the irradiation field. In like manner, when continuous exposure measurements were being taken with the Baldwin Farmer Dosimeter the probe was placed on this same circle.

When the above type of continuous monitoring was not possible a transmission type ionisation chamber was placed in the radiation field above the apparatus. The ionisation current was measured with a vibron electrometer with 240 volts (collecting voltage) across the chamber.

The electrometer was calibrated by comparing a constant reading on the electrometer with the exposure dose recorded with a Baldwin Farmer Dosimeter at the required position in the irradiation apparatus. From the exposure dose recorded the time required for any particular exposure dose can be calculated. The electrometer can then be used as a check that the dose rate does not alter (constant electrometer reading as before) and any slight adjustments made in the X-ray set controls made to keep it so.

Dosimetry for the electron experiments were carried out in a similar way to that of Wright and Bewley (1960).

C. Induction of hypertension

Hypertension was induced by a modification of the technique first used by Koletsky (1959). Under chloral anaesthesia, a median dorsal incision was made through the skin of the back. By reflection of the skin to either side the kidneys could be exposed through a small secondary incision in the muscle wall. Fat was carefully dissected away from the renal artery and vein. On the right side ligation of both the artery and vein resulted in infarction of the whole kidney. On the left side a small

infarct was produced by ligation of the inferior branch of the left renal artery (figs. 96 & 97), this was the only modification to the Koletsky method (anterior renal artery) and produced a milder hypertension.

One week post operatively an infarct involving the posterior third of the left kidney was very clearly defined. The totally infarcted right kidney was large and pale at this time (figs. 98 & 99).

The blood pressure of animals was measured pre operatively and two to three weeks post operatively using the method of Byrom (1963). When the blood pressure had reached a level of 140mm. Hg or above the animals were irradiated.

At death the heart was weighed as an extra check on the degree of hypertension in life. The mean heart weight was doubled for a rise in blood pressure of 62mm Hg present for 2 - 22 months (table 13).



Figure 96. Operative ligation of the inferior branch of the left renal artery.

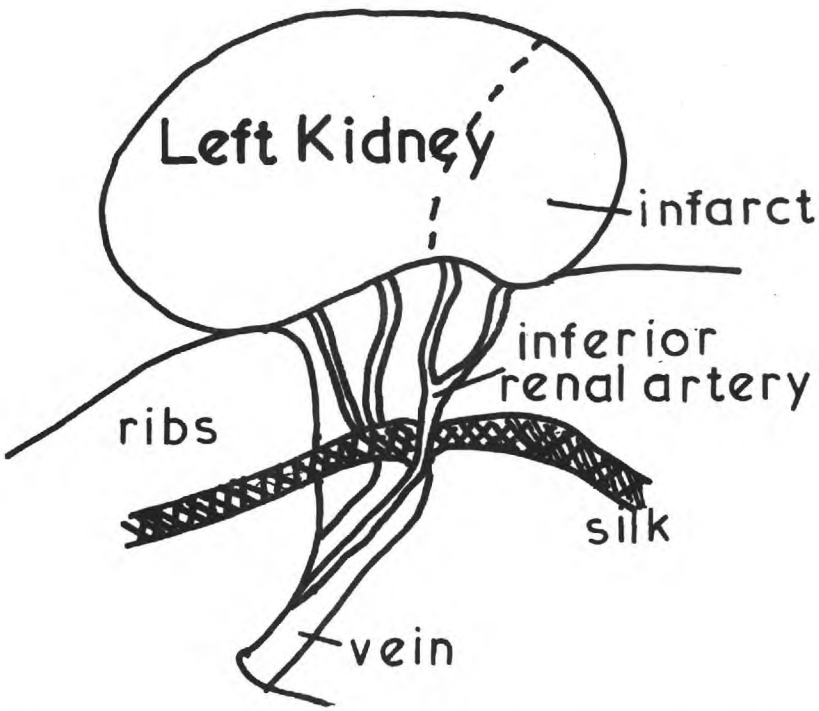


Figure 97. Diagrammatic representation of the operative area, showing the three branches of the left renal artery the inferior branch being ligated.

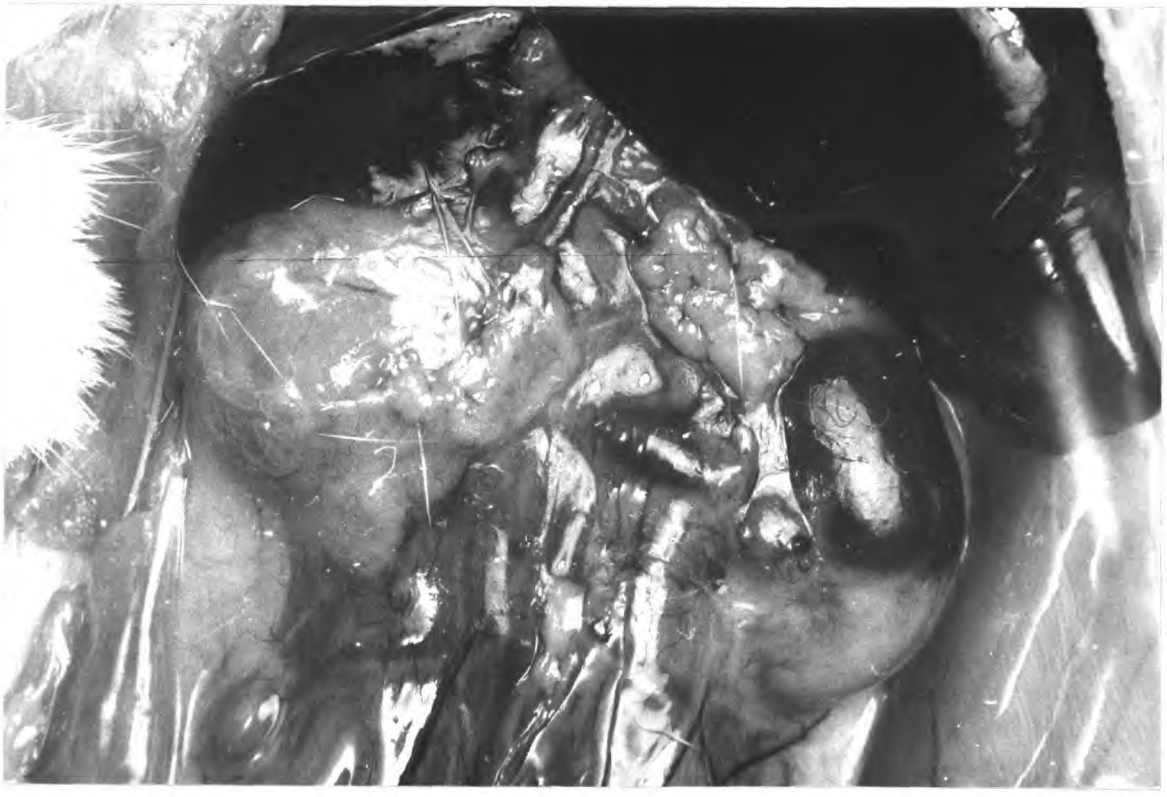


Figure 98. Post-mortem dissection of rat killed one week after ligation of the inferior branch of the left renal artery and total infarction of right kidney, showing necrotic right kidney and partial necrosis of left kidney.

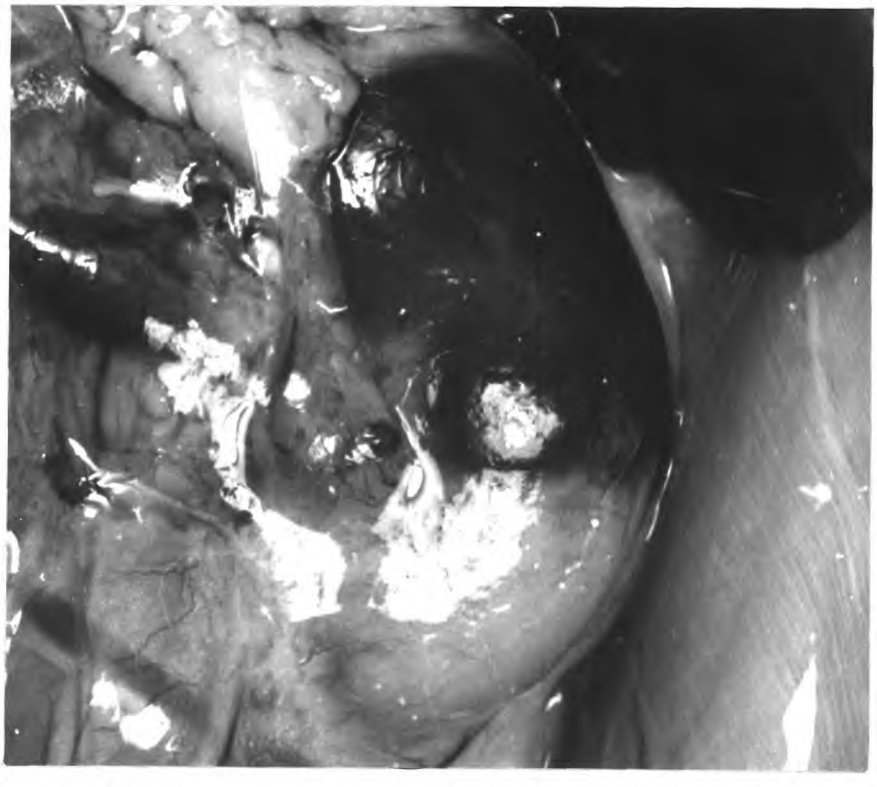


Figure 99. Partial necrosis of left kidney (as above).

TABLE 13

	Normotensive	Hypertensive
Heart weight (grams)	0.64 ± 0.03 ●16	1.26 ± 0.09 ●17
Blood Pressure (mm. Hg.)	92.8 ± 1.1 ●20	154 ± 2.0 ●29

● Number of animals.

Correlation of heart weight and blood pressure for normotensive and hypertensive animals.

D. Cerebral injury

Cerebral lesions were produced by freezing from the outside surface of the skull. The simple applicator consisted of a brass cylinder 3.8cm diameter and 10.2cm long with a small copper applicator tube 2 - 5 cm long of 3mm O.D. at one end. The end of this probe was sealed with silver solder and filed flat. The whole apparatus was then insulated with a layer of cotton wool held in place with adhesive tape. The tip of the applicator tube was left exposed. The apparatus was then filled with a freezing mixture of Dry ice and isopropyl alcohol at -72°C.

Prior to injury the rat was anaesthetised and the left parietal bone of the skull exposed by a small median incision of the skin overlying the skull. The periosteal membranes were scraped away and excess blood removed from the exposed bone with sterile swabs. This was essential, if rapid attachment of the freezing probe was to be attained. Supporting the head under the lower jaw, the exposed parietal bone was held against the cold tip of the applicator tube. A few drops of saline were added and within seconds a firm attachment had been obtained between the skull and the freezing apparatus (fig.100).

The time of application was dependant upon the size of the animal, varying from 15 mins for a rat under 200g to 20 mins for a rat of 350g and over.

After injury the skin incision was closed by two silk sutures. Bleeding was slight and no infection resulted. At the time of injury the rats were given a single injection of 3% trypan blue in water intraperitoneally at a dose of 15mgs/kilo body weight. This was to facilitate finding the lesion at autopsy. Under normal conditions trypan blue is not taken up by the brain as it is unable to cross the blood brain barrier. Thus, when the brain was injured this barrier is broken down and trypan blue was taken up at the site of injury (fig.101).

At a predetermined time after injury the animals were killed with an overdose of Nembutal, the parietal bones removed and the



Figure 100. Cerebral lesions were produced by freezing from the outside surface of the skull. The probe of the freezing apparatus is shown attached to the exposed left parietal bone.



Figure 101. Slice of brain showing saucer shaped lesion on the surface of the cerebral cortex.

coronal slice of brain containing the lesion removed. This slice of brain was fixed by immersion in a 1% acetic acid in 10% formol saline solution.

E. Bacteriology

Bacterial strain

The B 1772 strain of B. pertussis has been used throughout this study. This strain is of low virulence for mice when injected intracerebrally. It has an LD50 of the order of $10^{6.5}$ viable organisms. A challenge dose of less than this produces a transitory infection with the brains ultimately becoming sterile.

Medium

Bacteria were grown on Bordet-Gengou (B.G.) medium

A - Peeled sliced potatoes 2500g.

'Analar' glycerine 200ml.

Distilled water to make up to 10 litres.

The potatoes were dispersed in a gauze bag and cooked in the glycerine - water until they were soft. The bags were removed from the fluid and allowed to drain. The volume was then made up to 10 litres.

B - Sodium chloride (Analar) 170g.

Distilled water to make up to 10 litres.

The two solutions were mixed, added to Bacto Agar (2g per 100 ml.) and autoclaved for 30 mins at 10lb.p.s.i.

Two-layer plates were used, the bottom layer consisting of 20ml of the base and the top layer with 12ml of 20% horse blood added to the base.

Casamino Acid Solution

Casamino acid 40g.

Sodium chloride 16g.

Distilled water to make up to 4 litres.

This was adjusted to pH 7.4 and then autoclaved for 20 mins at 10 lbs.p.s.i.

Bacterial Cultures

The bacterial strain was grown from the freeze-dried state on B.G. medium at 34°C. for 72 hrs. The second 18hr subculture from this was used for the mouse challenge.

All suspensions and dilutions were made up in 1% casamino acid solution.

A suspension of bacteria was injected intracerebrally at a point likely to traverse the ventricles. 0·025ml was injected from a 0.25ml syringe, via a 7mm. 26 SWG needle.

Bacterial Counts

Each mouse brain was removed aseptically and added to 2ml of 1% casamino acid in a universal bottle.

The brains were homogenised at half speed in a M.S.E. homogeniser

for one minute. The homogenate was then centrifuged at 15000 r.p.m. for 90 sec. in an International Refrigerated centrifuge. Decimal dilutions of the supernatant were made in 1% casamino acid and the bacterial counts estimated by the method of Miles and Misra (1938) on B.G. medium.

Three drops of each of three suitable dilutions were spread on each plate and bacterial counts were made where possible from drops containing 40 - 80 organisms.

Leucocyte counts

Blood was taken (0.5ml) from the brachial artery and diluted in 0.95ml of 2% acetic acid solution which was coloured light blue with gentian violet. The dilution fluid lyses the red cells and a total white cell count was made on $0.1mm^3$ of blood in a Neubauer Cell. The number of cells counted x 200 gives the number of white cells per mm^3 of blood (Dacie and Lewis, 1963).

F. Cell labelling and Autoradiographic Technique

Thymidine is a specific precursor of chromosomal D.N.A. and is incorporated into the cell nucleus when D.N.A. synthesis occurs. For autoradiography tritium labelled thymidine is injected in the form of thymidine 6-T(n) (Radiochemical Centre, Amersham). The cells synthesising D.N.A. in preparation for division will incorporate the radiochemical and thus nuclei will become labelled with tritium.

Thymidine 6-T(n) has a specific activity of 5.0 curies/mM and a

concentration of 1mc/cc. The tritium label is attached to the pyrimidine ring of thymidine. Tritium, which has a half life of 12.5 years, decays to helium by the emission of nuclear electrons. Electrons emitted by tritium have an average energy of 6KV and a maximum range of 6μ in tissue.

Two series of rats were injected with tritiated thymidine intraperitoneally in a dose of 1μC per gram body weight.

(a) Three injections were given at six hourly intervals so that the last injection was given 12-15 hours prior to cerebral injury. This is similar to the method employed by Spector et.al.(1965) for labelling a high proportion of white cells in the blood before the initiation of an inflammatory reaction. All free thymidine is incorporated within one hour of the final injection and so no circulating precursor remains available for utilisation by cells in response to the inflammatory insult. Thus it follows that any labelled cells found in the lesion must have originated from the blood unless recycling of the labelled precursor occurs.

(b) In the second series of experiments only a single injection of tritiated thymidine was administered after injury and one hour prior to death. This will be incorporated into cells synthesising D.N.A. in the lesion. No interference is encountered with labelled cells from the blood as none will be circulating in the blood at this time.

The correlation of counts from these two groups enable us to make some assessment as to the proportion of cells of haematogenous

and local origin in the cellular reaction to cerebral injury.

Histological technique

The specimens are dehydrated and embedded in paraffin wax in the normal way.

Duplicate serial sections for coating with nuclear emulsion were cut at 5μ, floated out on distilled water and picked up on specially coated slides.

Coating solution:-

1. Boiled distilled water 100ml.
2. Add gelatin 0.5 grms.
3. Cool and add 0.05-0.1 grm of chrome alum.
4. Filter and apply thinly to well cleaned slides.

Sections were allowed to dry slowly for 24 hours after which they were dewaxed, hydrated and washed for 30 min. The slides were allowed to dry until they were just damp.

The remaining procedure must be carried out in a dark room with a dull red safety light.

The nuclear emulsion (K2,Ilford) is removed from its packing and heated in a water bath at 60°C until liquid. In this state it can be diluted with an equal volume of distilled water and poured into a warmed Coplin jar maintained at 60°C.

Pairs of slides were placed back to back in the emulsion for

2 - 3 secs. After drying the slides were placed in a light-tight box, exposed at 4°C for periods of 3 and 6 weeks and then developed.

Slides were developed for 5 min. in glass slide racks with Kodak D 19B developer and fixed until clear with Johnsons Fixsol. After washing the sections were stained, dehydrated and mounted.

G. Staining Methods

Ehrlichs Haematoxylin and Eosin Stain (H & E)

1. Xylol to remove wax.....2 mins
2. Absolute alcohol.....2 mins) To remove xylol
3. 95% Alcohol.....2 mins)
4. Place slides in Ehrlichs Haematoxylin to stain nuclei, for 20 min.
5. Wash in running tap water till blue
6. Differentiate in 1% acid/alcohol for a few seconds
7. Wash till blue again and examine under the microscope
   Nuclei should be blue while most other structures should be colourless.
8. Wash in running tap water for a further 15 min.
9. Place slide in 1% eosin for 1 - 2 min.
10. Differentiate eosin in running tap water for several minutes
    At this stage the nuclei should be blue while the cell cytoplasm should be pale pink.

11. Dehydrate sections in 95% alcohol.....2 min.

Absolute alcohol 1.....2 min.

Absolute alcohol 11.....2.min.

12. Place section in xylol until cleared - the section should now be transparent.

13. Wipe excess xylol and mount in Dammar Xylol.

Results:

Nuclei.....blue

Collagen.....pale pink

Muscle & RBC's.....bright pink

Mayer's Haemalum

Used to stain nuclei in Autoradiographs.

Constituents of Mayer's:-

Haematoxylin	1 gm.
Distilled water	1000 ml.
Sodium iodate	0.2 gms.
Citric acid	1 gm.
Chloral hydrate	50 gms.
Potash alum	50 gms.

Staining method:-

1. Dewax in xylol.....2 mins.
2. Remove xylol in absolute alcohol.....2 mins.
3. Wash in distilled water.
4. Stain with Mayer's Haemalum.....5 mins.
5. Blue in running tap water.

6. Dehydrate in 95% alcohol.....2 mins.

absolute alcohol.I.....2 mins.

absolute alcohol.II.....2 mins

7. Clear in xylol and mount in Dammar xylol.

H. Pressure Apparatus (figs.102 & 103)

The apparatus was a modification of that used at the ERRU MRC Royal Post Graduate Medical School, Hammersmith and the Paterson Laboratory, Christie Hospital and Holt Radium Institute, Manchester 20.

The apparatus was constructed from 1.2cm. mild steel to produce a chamber 6.3cm deep and 30.5cm in diameter. The 1.2cm thick perspex lid was clamped to the chamber by a 1.2cm steel lid, and 8 bolts placed around the circumference of the lid. A 10.7cm diameter irradiation port was provided in this upper steel lid. A large synthetic rubber "O" ring produced an effective seal between the chamber and the perspex lid.

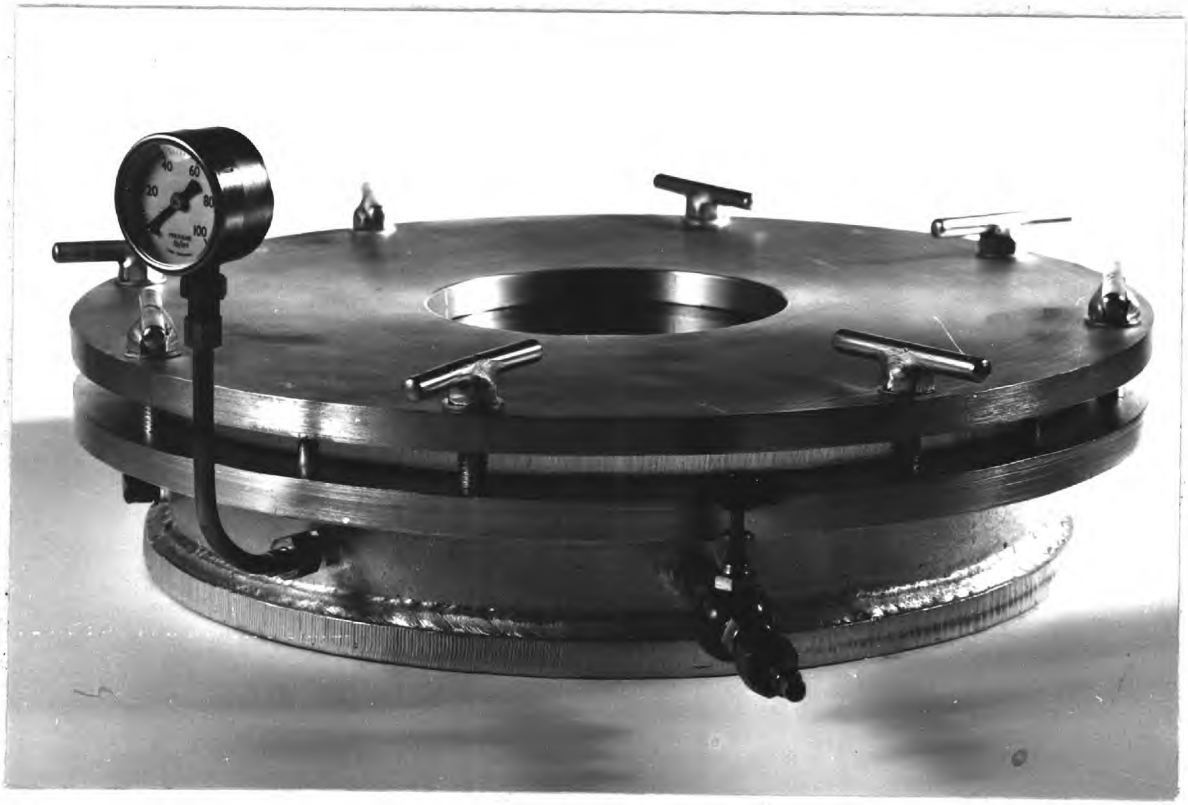
The chamber was fitted with inlet and outlet needle valves, pressure guage and pressure safety valve. A 1.2cm. diameter hole was provided for insertion of the Baldwin Farmer probe in the preliminary dosimetry. This hole can be closed off after use with a large bolt.

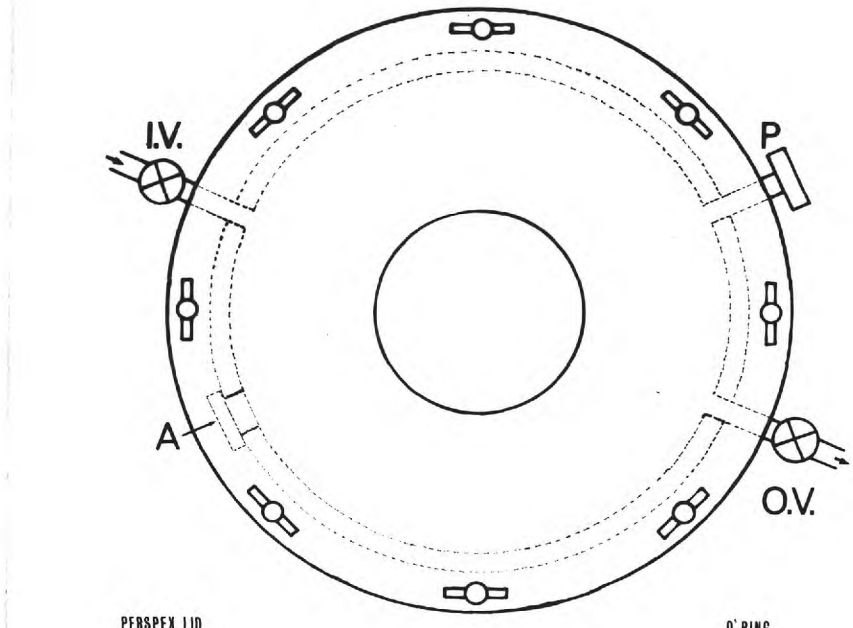
Five animals, held by special copper holders attached to a perspex disc which could be lifted in and out of the chamber with ease, were irradiated on each occasion.

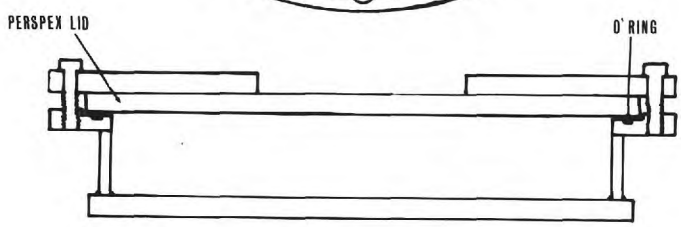
Figure 102. Hyperbaric oxygen irradiation chamber.

Figure 103. Diagram of hyperbaric oxygen irradiation chamber.

I.V. - inlet needle valve.
O.V. - outlet needle valve.
P - pressure gauge.
A - inlet for Baldwin Farmer Dosemeter probe.



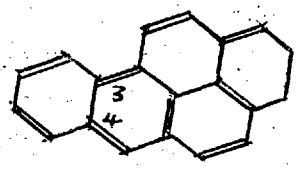

I.V.
P
A
O.V.


PERSPEX LID
O'RING

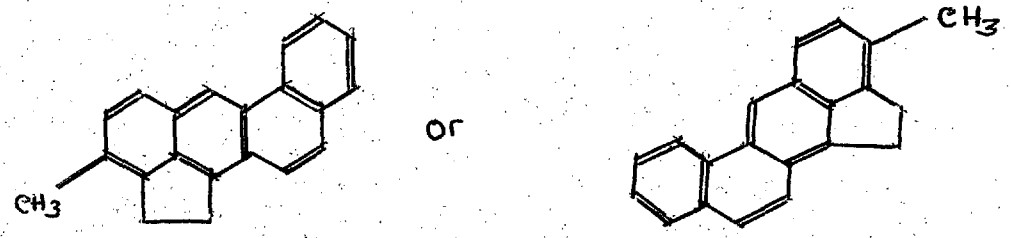
I. Carcinogens

The two carcinogenic types used in pellet form were:-

3-4 Benzpyrene



20 Methylcholanthrene



CHAPTER VIII

DISCUSSION

A. Latent cerebral irradiation damage

This study has confirmed that for normotensive animals doses of 1,000R produce no apparent morphological changes in the brain and animals live into old age. With higher doses, 2,000 - 4,000R, after a latent period of many months, morphological changes do occur and animals die earlier than controls. The mean latent period between irradiation and death is clearly dose dependant although considerable variation occurs in the length of the latent period for animals in the same irradiation dose group. For example, with a dose of 4,000R the latent period varies from 6 to 15½ months.

In this latent period between irradiation and death, or the appearance of terminal neurological signs, the rats showed no evidence of brain irradiation other than a weight loss recorded 2 - 3 months after irradiation. A possible explanation of this is that it represents some change in the vascular bed of the brain producing a non-lethal cerebral disturbance and a loss of appetite in the rat. Evidence of vascular impairment due to irradiation after this time period being provided by the work of Assoher et.al. (1961) from the irradiation of mesenteric arteries and by Sams (1965) following the irradiation of bone marrow in the mouse tibia. The latter showed that with doses of 2,000R, bone marrow of the

mouse tibia quickly recovered from local irradiation damage, but this recovery was not maintained and nine weeks after irradiation there was a further degeneration of the marrow cavity. This it was considered was due to an impairment of the vascular system.

Assoher (1961) also quoted an interval of 2 months before the sensitisation of irradiated blood vessels to hypertensive damage. He postulated that after this length of time post irradiation changes occurred in blood vessels. However he was unable to find any histological evidence to support this view.

The present findings showed that with doses of 3,000R and 4,000R, large areas of necrosis were found in the brain. These necrotic areas were principally found in the white matter and were in a majority of cases not apparently associated with any vascular damage. This is an observation that has been previously reported by other workers (Arnold et.al.1954, Innes and Carsten 1961). In marked contrast, animals that died after 2,000R showed no damage to white matter and only vascular irradiation lesions occurred. Vascular lesions were also found in animals receiving the two higher doses, but these were restricted to the thalamic and hypothalamic regions of the brain which have numerous blood vessels. The view that the high vascularity of the hypothalamic region accounted for its radiosensitivity was postulated by Haymaker (1962). From this view point, one would expect that the other highly vascular region of the brain, the cerebral cortex, would also show vascular damage. This however is not the case, and following all doses of

radiation the cerebral cortex is apparently devoid of morphological changes of either the vascular or necrotic type. It would appear that vascular lesions are more extensive following 3,000R than any of the other doses.

Perhaps related to this marked variation in morphological damage, is the variation in neurological signs prior to death. With the two higher doses animals became disturbed and lost weight for 3 - 20 weeks prior to death. This could be due to the slow degenerative changes observed in the white matter. Of the normotensive animals receiving the lower radiation dose of 2,000R most died suddenly without warning and only a few showed slight terminal neurological symptoms. This is possibly indicative of a sudden vascular accident resulting from vasoualr irradiation damage.

In the 3,000R and 4,000R irradiation groups no correlation was apparent between the duration of the neurological symptoms and the extent of white matter damage. The irradiation damage did however show some correlation with the length of the latent period. This was particularly marked in the 4,000R group. The first animal (Rat 80) died after 6 months and exhibited marked neurological symptoms but only had degenerative changes in the optic chiasma. With increasing latent period necrotic areas appeared in the internal capsule and the fimbria hypocampus and in the last animal to die (after 15 months) there were in addition degenerative changes in the corpus callosum.

In the hypertensive groups of animals radiation doses of 1,000 - 3,000R shorten the latent interval between irradiation and death. The pathological changes found on the death of the animals were only vascular or vascular-induced and so it would appear that with these doses hypertension accelerates the appearance of vascular irradiation damage. This confirms the findings of Asscher and Anson (1962) who observed silimar changes following the irradiation of the spinal cord in hypertensive animals. Our experiments showed that irradiation damage to blood vessel walls were, as might be expected, found in the highly vascular regions of the brain; the hypothalamus, cerebral cortex and choroid plexus. The appearance of vascular lesions in the cerebral cortex, contrasts markably with the lack of such lesions in the normotensive irradiated animals. Hypertension also lowers the threshold dose for vascular radiation damage.

Perhaps more interesting is the apparent inhibition of the hypertensive effects with the higher dose of 4,000R. The animals live for approximately the same length of time as the 4,000R normotensive controls. Some extensive necrosis in the white matter is also seen. As in the corresponding normotensive animals the extent of the white matter damage is in proportion to the length of the latent period. The effects of hypertension on accelerating the appearance of vascular radiation damage appears to have lost its effect.

The simplest explanation of these results is that there are two modes of radiation injury, one vascular, which is most effective with doses of 2,000 - 3,000R for normotensive rats. The other type of radiation damage is an indirect action on white matter resulting from damage to the glial supporting cells. This being most clearly seen with doses of 3,000 - 4,000R. Thus in hypertensive animals the vascular irradiation damage with doses of 2,000 - 3,000R was accelerated but with a dose of 4,000R no shortening of the latent period occurs.

This hypothesis may be explained in cellular terms. It would appear that hypertension and vascular irradiation damage are in many ways supplementary. It has been demonstrated that irradiation will sensitise blood vessels to hypertensive damage (Asscher et.al. 1961) and hypertension will facilitate vascular irradiation damage (Asscher and Anson 1962). From these findings of Asscher it would appear that hypertension and radiation affect blood vessels by a similar mode of action. Some of the end results are the same, for example, fibrinoid necrosis and hyaline change of arteries and arterioles.

The observations of Byrom (1963) that hypertension initially causes a localised constriction of retinal arteries in the rat may be important. These changes precede and may produce the vascular lesions so typical of hypertension.

Similar changes, possibly in the muscle elements of the vascular wall, could be envisaged in irradiated arteries. In

moderately irradiated vessels a slow depopulation of these cells will occur because irradiated cells die on attempting division. A few remaining viable cells could multiply in response to this cell loss and produce clones or clumps of cells at irregular intervals along the blood vessel. These clumps of cells or clones could produce similar vascular constrictions to those observed in hypertension and from this the ensuing vascular irradiation lesions. With high doses of radiation (4,000R) the number of viable cells will be reduced and the mitotic replacement of cells suppressed, so that the vascular irradiation phenomenon seen with moderate doses is lost. This hypothesis is based on the fact that irradiated cells die when they attempt mitosis. Using this hypothesis the effects of hypertension can be explained. Crane and Dutta (1963) have demonstrated an increase in cell labelling with H^3 thymidine in the muscle and endothelial cells of vessel walls with hypertension. This increase in cell labelling indicates an increase in the mitotic turnover which would in turn result in an acceleration of vascular irradiation damage. A comparable example can be quoted for skin (Shewell and Wright 1967), in which irradiation damage was shown to develop earlier if mitotic stimulants such as formalin were applied after irradiation, or conversely later if the mitotic activity was depressed by keeping the animals in the cold.

The findings of several workers (Smart and Leblond 1961, Hommes and Leblond 1966) have shown that the neuroglial population of the

adult brain is not a static one, but that a slow mitotic replacement of cells occurs. Thus, when irradiated cells die on attempting division a gradual depletion of neuroglial supporting cells would occur. This possibly results in the marked glial disturbances observed by Zeman (1963) in white matter prior to the onset of radiation necrosis.

The high radiosensitivity of white matter may be explained by the findings of Hommes and Leblond (1967) for it would appear that in white matter the mitotic replacement of glial cells is greatest. The cerebral cortex, which shows no delayed radiation necrosis, was reported (Hommes and Leblond 1967) to have the lowest glial mitotic turnover. Further evidence will be provided later to support the view that the cerebral cortex has an apparently static cell population.

The observations from our own experimental material that the subependymal plate is completely devoid of cells following doses of 3,000 - 4,000R may provide another explanation of delayed radiation necrosis. It is these doses which produce radiation necrosis of white matter while with the lower doses where such necrosis does not occur the subependymal layer remains intact. The findings of Smart and Leblond (1961) suggests that newly formed differentiated or partly differentiated neuroglia, migrate out along the white matter tracks of the corpus callosum. Thus removal of this stem cell population may have detrimental effects

on the areas of brain they supply. Damage to this stem cell population may explain why radiation necrosis in white matter appears to progress with increasing latent interval. Clearly, the effects of radiation on the mitotic activity and migration of cells from the subependymal plate requires further investigation.

Thus, it is reasonable to conclude that two mechanisms are at work following the irradiation of brain. The predominance of one or the other depending on the dose of irradiation. This hypothesis suggests two other possible lines of investigation.

1. What are the effects of irradiation on the mitotic activity and migration of cells from the subependymal plate?

2. Will irradiation produce similar changes in the retinal vessels of the eye as were produced by hypertension (Byrom 1964)?

Local Brain Macrophages

The effects of previous local irradiation on the reaction of brain to freezing injury and to infection with B. pertussis provide evidence to support the view that brain macrophages are mainly of local origin and are not derived solely from the general circulation. Our general view is that microglia are the principal source of brain macrophages. The phagocytic action of these cells being demonstrated by Hortego (1912) following cerebral injury and by Lebowich (1934) after cerebral inoculation with colloidal solutions and bacteria.

The general hypothesis to explain the effects of previous irradiation on cerebral injury and infection is that local cells, of which the majority are microglia, are damaged on irradiation. This damage is latent until the cells are stimulated to divide, by injury or infection, when they fail to divide or die. This would inevitably result in a reduction in the cellular reaction to injury or an inability of the mouse brain to eradicate the B. pertussis infection. Evidence of mitotic stimulation was reported following both types of cerebral insult. In previously irradiated brain, as might be expected, mitotic abnormalities were found. Comparable examples to this have been quoted for thyroid (Doniach 1955), liver (Weinbren et.al.1960) and peripheral nerve (Cavanagh 1968). These have all demonstrated irradiation abnormalities following mitotic stimulation at various intervals of time after irradiation.

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nerve (Cavanagh 1968). These have all demonstrated irradiation abnormalities following mitotic stimulation at various intervals of time after irradiation.

For this hypothesis to hold true we must assume that the majority of brain macrophages are of local origin and not derived from the general circulation.

This view is supported by our cell labelling study, which confirms that after the first day, the majority of the cells reacting to a superficial cerebral injury, are of local origin. The study also showed that a few haemotogenous cells enter into the cellular reaction and that in the lesion of a previously irradiated brain their participation was increased. This it was postulated, was a direct consequence to the reduced local reaction and this may to some extent explain the dose response curve obtained (fig.84).

The conclusions reached as a result of the present experiments are not in keeping with the findings of Konigmark and Sidman (1963) who using an H^3 thymidine cell labelling technique reported that at least 70% of 'brain' macrophages were of general circulatory origin. A possible explanation we can offer for this apparent inconsistency is that the lesion used by Konigsmark and Sidman was small (produced by a 21 guage hypodermic needle) as compared with the large freezing lesion used in the present study. This smaller lesion may produce a correspondingly smaller local reaction, as no scar developed and the needle track disappeared with time. A

mechanical lesion as opposed to a freezing injury may also evoke a different response.

Another possibility is that recycling of H^3 thymidine occurs. Free labelled DNA being liberated from labelled haemotogenous cells dying in the wound area. This is one of the explanations offered by Konigsmark and Sidman to explain the appearance of labelled endothelial cells in the lesion (H^3 thymidine had only been given prior to injury). To quote directly from their paper "A leucocyte transforms directly into an endothelial cell, or a macrophage might empty its debris into a vascular lumen and then remain in the vessel wall as an endothelial cell, or finally endothelial cells and 'microglial' cells might receive labelled DNA from cells dying in the wound area".

In our experiments when H^3 thymidine was given before injury no labelled vascular endothelial cells were observed although labelled cells were found within the vascular lumen and in the brain in close proximity to vessels.

Recovery from irradiation damage

In the two methods used to demonstrate irradiation damage to glial cells some very interesting differences occurred when the time interval between irradiation and cerebral insult was increased. With injury to the cerebral cortex, no recovery or accentuation of the cellular reaction was apparent over an interval of six months. Thus it would appear that the glial cell population of the

cerebral cortex shows little or no mitotic cell replacement so that the irradiation damage remains dormant until the cells are stimulated to divide by injury. This view of a static cell population could explain the lack of radiation necrosis in the cerebral cortex.

In marked contrast, when mouse brains were infected with B. pertussis three months after irradiation (400R), there appeared to be some recovery from the irradiation damage. With a higher dose of 1,000R, no recovery from irradiation damage seemed to occur, at least in the three months of this study.

In B. pertussis intracerebral infection the bacterial colonies (attached to the ciliated lining of the ependyma) are in close proximity to the mitotically active cells of the subependymal plate. The mitotic activity of this layer will initially be depressed by irradiation. If the cells of this layer react like a normal stem cell population, then the viable undamaged subendymal cells that remain after a dose of 400R will continue to divide, replacing the layer and producing new viable neuroglial cells in much the same way as bone marrow after irradiation. These new cells will migrate out into the brain parenchyma and replace non viable cells damaged by irradiation. Thus, when the mice are infected 3 months after irradiation some degree of recovery is apparent. Another possible explanation is that the eradication of the infection is solely the function of the subependymal layer. If this view is correct, then any recovery in the ability of the brain

to eradicate B. pertussis infection from the brain will be an indicator of recovery of the subependymal layer from the radiation damage. A combination of both these hypotheses is an equal possibility. Clearly, a study of the effects of radiation on the subependymal layer and its rate of recovery from a whole range of doses might elucidate this point.

Cerebral Carcinogenesis

The main factors regarding implantation site and the importance of sex in cerebral carcinogenesis have been discussed in the relevant chapter. Thus, here it is only necessary to discuss the main point that glial tumours are only produced by chemical carcinogens if the carcinogenic pellet is implanted deeply within the brain so as to be in contact with the subependymal layer. Implantation of the carcinogen into the cerebral cortex, which has been shown to have a relatively static glial population, will produce no glial tumours. Not only are cells of the subependymal layer mitotically active but they are also undifferentiated, which possibly accounts for the diversity of tumour types produced.

From all these studies it would appear that the cells of the subependymal plate, which have until now received little attention, should be more extensively studied for their possible role in both normal and pathological processes.

SUMMARY AND CONCLUSIONS

1. For normotensive animals, doses of 1,000R produce no morphological changes in the brain and animals have a normal life span.

2. Doses of 2,000R only produce vascular damage, although this type of damage is not restricted to this dose level.

3. Doses of 3,000 and 4,000R produce selective necrosis of white matter, the extent of which depends on the length of the latent period.

4. Hypertension accelerates the appearance of vascular irradiation damage, with doses of 2,000 - 3,000R, and lowers the threshold dose to 1,000R.

5. Vascular damage in hypertensive irradiated animals is restricted to the highly vascular regions of the brain.

6. Hypertension appears to loose its effects following higher doses of 4,000R and animals die at the same time as the 4,000R normotensive rats.

7. Two modes of radiation damage are proposed for the brain; vascular damage which is most effective between doses of 2,000 - 3,000R and damage to the glial supporting cells. This is most effective between doses of 3,000 - 4,000R.

8. Both theories of radiation damage are based on cell death occurring at mitosis. The slow mitotic replacement of cells in the brain accounting for the long latent period between irradiation and the onset of damage.

9. It was suggested that radiation and hypertension could act on blood vessels by similar modes.

10. Radiation effects on the subependymal layer may be a factor in the process of delayed radiation necrosis of white matter.

11. The majority of cells, reacting in response to a freezing cerebral injury are of local origin. The predominant cell type being microglia, although astrocytes, meningeal cells and vascular endothelial cells also take part.

12. Only a few circulatory cells enter into this cerebral reaction to injury and to some extent they divide locally.

13. Even with doses as small as 200R previous local irradiation of the brain will depress the local cellular reaction to injury. This reduced local reaction possibly results in an increased haemotogenous cell reaction.

14. The cellular reaction to injury was independant of the time between irradiation and injury. The reduced cellular reaction was dose dependant.

15. The cellular population of the cerebral cortex is a relatively static one and this may explain the lack of delayed radiation necrosis in this region.

16. B. pertussis infection is limited to the ventricular spaces of the brain. The organisms are found attached to the ciliated lining of the ependyma.

17. The mechanism responsible for the eradication of B. pertussis from the mouse brain is of local origin.

18. Local irradiation of the brain with doses as small as 400R will depress the local defence mechanism to B. pertussis infection.

19. B. pertussis infection stimulates a local cellular reaction with mitotic figures visible in the ventricles between ependymal cells and in the brain in close proximity to the ventricles. Mitotic abnormalities are to be found in previously irradiated and subsequently infected brains.

20. Unlike the reaction of brain to a freezing injury in the cerebral cortex, the reaction of the brain to this ventricular infection with B. pertussis is not completely independent of the time between irradiation and infection.

21. The partial recovery of the brain from lower doses of irradiation is possibly a function of the subependymal plate, either directly or indirectly.

22. Irradiation depressed the postnatal growth of the brain. The amount by which growth was suppressed was dependent on the irradiation dose.

23. Using half brain irradiation it was possible to show a consistant variation in the radiation response for different oxygen tensions. Low oxygen tensions were shown to protect, and high oxygen tensions were shown to provoke radiation damage. A dose modifying factor of approximately 2 was quoted between the two extremes, 300% oxygen and 7% oxygen.

24. Reduction in hair growth in suckling rats provides a simple indicator of the oxygen effect in these mammals.

25. Experimental gliomas are only produced when the chemical carcinogen is placed deeply in the brain so as to impinge on the subependymal layer.

26. Gliomas are more common and tend to develop earlier in males than females. The possible importance of testosterone in the induction of glial tumours was discussed.

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EFFECT OF PREVIOUS X-IRRADIATION ON THE RESPONSE OF BRAIN TO INJURY

By

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Effect of Previous X-irradiation on the Response of Brain to Injury

THE normal adult mammalian central nervous system shows no early or late morphological effects after a single dose of X-irradiation up to 1,000 rads[1,2] and even with doses of 2,000–4,000 rads there is a delay of many months before damage appears[3,4].

In spite of this absence of morphological change after moderate doses of radiation we have shown that the cerebral glial reaction to injury is diminished after both small and larger doses (200–4,000 rads) and, furthermore, that this change seems to be independent of the time between irradiation and injury.

Brains of Sprague–Dawley rats, of both sexes, 3–4 months old were irradiated, under chloral anaesthesia (300 mg/kg intraperitoneally), to single doses of X-rays of 200–4,000 rads. Irradiation was given laterally to both cerebral hemispheres through a semi-circular port of diameter 18 mm, in 2·5 mm of lead shielding. Doses were given at 90–95 r./min, 220 kV, 15 mamp, with 0·5 mm of copper and 1·0 mm of aluminium as added filtration.

At intervals of between 3 days and 6 months after the irradiation a small superficial area of the brain was injured by freezing through the calvarium. This method was used because it gave regular results without the necessity of opening the skull and thereby avoiding the risk of infection. For this a cooling device was used which consisted of a copper tube, filled with a mixture of solid carbon dioxide and alcohol, at one end of which there was an applicator tube 3 mm in diameter. This tube was placed in contact with the exposed left parietal bone of the skull for a period of 15–20 min depending on the size of the animal. A single intraperitoneal injection of 1 per cent w/v trypan blue (0·5 ml./100 g body weight) was administered at the time of injury so that the exact location of the lesion was obvious when the brains were removed 1 week later.

The animals were killed 7 days after injury with an overdose of sodium pentobarbitone ('Nembutal'). The cerebral hemispheres were removed and fixed in 1 per cent acetic acid in 10 per cent formal saline. The slice of brain containing the centre of the lesion was slowly dehydrated in alcohols and embedded in paraffin wax. Sections 5μ thick were cut and stained with Ehrlich's haematoxylin and eosin and mounted in the usual way.

The normal response to this freezing injury was necrosis followed by cell division and infiltration commencing at

the periphery of the lesion. This was clearly marked at 3 days, and 7 days after the injury the saucer shaped area was uniformly populated with cells (Fig. 1). They were approximately 2·5 mm in diameter and 0·5–1·0 mm deep. To minimize subjective errors cell counts were made to the following standard procedure. Counts were always made in an area where the lesion was 0·5 mm thick. Using a squared graticule eyepiece and an oil immersion lens (magnification 1,050) cells were counted in four consecutive areas 0·086 × 0·086 mm, perpendicular to the surface (Fig. 1). The number of cells was estimated by counting to the nearest whole nucleus found within the graticule area.

Up to five consecutive serial sections of each lesion were mounted on a slide and of these a minimum of two sections were counted. The mean number of cells counted in each section for controls was 230.

The results, for rats irradiated 3 days before injury, are shown in Fig. 2. The numbers of cells/unit area are expressed as a percentage of the non-irradiated controls.

With selected doses we varied the period between irradiation and injury and observed a similar reduction in the cellular response, although so far the numbers of animals in these groups have been small (Table 1).

The simplest explanation of this is that glial cells do not show radiation damage until they are stimulated to divide, when they either fail to divide or die. This "storing up" of radiation damage is possibly caused by the slow turnover of glial cells in normal conditions, so that replacement does not take place to any extent. Similar examples can be cited in such organs as the liver, skin and thyroid[5–7] where the radiation damage can be accelerated by the introduction of factors which influence the rate of mitotic activity. The observations[8] that the latent period before death after irradiation of the spinal cord could be greatly reduced by hypertension could have a similar basis.

The hypothesis that previously irradiated local glial cells die on attempting cell division is dependent on the assumption that most of the cells originate endogenously. The findings that a considerable number of the macrophages in brain lesions are haematogenous[9] seem to dis-

Table 1. CELL COUNTS/UNIT AREA OF LESION

Exposure dose (r.)	Cell counts/unit area of lesion as percentage of control. Latent period between irradiation and injury: 3 days	3 weeks	3 months	6 months
200	84·5 ± 1·4 (5)	84·3 ± 2·5 (3)	81·9 (2)	—
400	75·5 ± 1·6	—	—	68·5 (2)
2,000	61·1 ± 3·5 (5)	62·5 ± 2·5 (4)	58·5 (2)	62·5 (2)

Results are expressed as a percentage of controls, with standard errors when three or more animals were used. Figures in brackets indicate the number of animals used in each group.

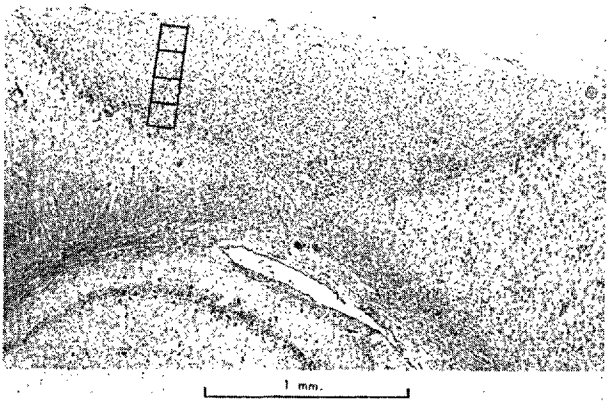


Fig. 1. Lesion on the left cerebral hemisphere of the brain of the rat, indicating the four areas (0·086 × 0·086 mm) used for cell counts, where the lesion is 0·5 mm thick.

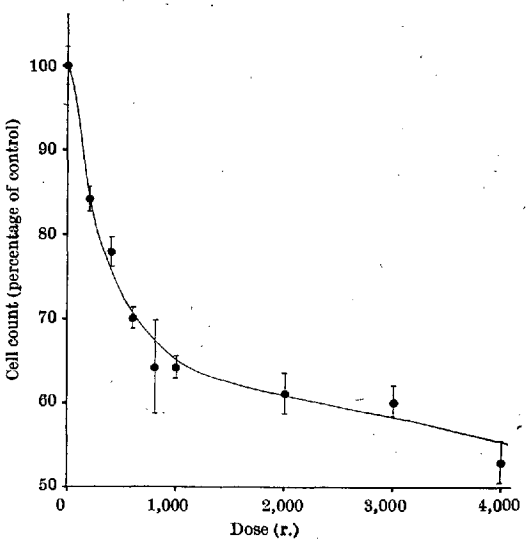


Fig. 2. Cell counts/unit area of lesion, expressed as percentage of the controls, plotted against the dose of 220 kV X-rays.

agree with our results. Thus further work is in progress to determine whether our results can be completely explained by a reduction in local glial cell proliferation, or whether some more complicated mechanism may be operating.

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