Experimental brain death

1. Morphology and fine structure of the brain

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SUMMARY The morphological characteristics of brain death were examined in baboons and cats after artificial cerebral ischaemia. All animals showed autolytic changes in the brain, ischaemic neuronal changes, midbrain haemorrhages, focal necrosis of the brain-stem, demarcation at C 1/C 2 cord segment, and displacement of cerebellar tissue. Ultrastructural examination revealed extreme brain oedema, autolytic changes, and complete obstruction of capillaries by astrocytic and endothelial swelling and intravascular blebs. These data indicate that brain death develops in several stages. If the process starts in the supratentorial space it first leads to a breakdown of the cerebral circulation and to transtentorial herniation. As a result, midbrain haemorrhages develop and the infratentorial pressure begins to rise. The second stage is terminated by demarcation of the brain. The circulatory arrest is initially caused by venous compression but becomes irreversible when vascular obstruction develops.

Brain death may be defined as the survival of the organism after the complete and permanent breakdown of the cerebral circulation. This condition has been increasingly encountered since the development of intensive care treatment within recent years and has made the exact determination of 'death' extremely difficult. The most reliable sign of brain death thus far seems to be the arrest of the cerebral circulation as established by angiography. However, the mechanism which causes the circulatory arrest of the brain in conditions preceding brain death is far from being cleared up (Zander, Rabinowitz, and Tribolet, 1971).

The neuropathological characteristics of brain death in man have been extensively described by many authors (Bertrand, Lhermitte, Antoine, and Ducrot, 1959; Mollaret, Bertrand, and Mollaret, 1959; Mollaret and Goulon, 1959; Lindenberg, 1963; Kramer, 1964; Mantz, Storck, Tempe, and Hamann, 1966; Kimura, Gerber, and McCormick, 1968; Schneider, Masshoff, and Neuhaus, 1969; Käufer, Penin, Düx, Kersting, Schneider, and Kubicki, 1969; Walker, 1969; Mohandas and Chou, 1971; Zander *et al.*, 1971). Experimental studies deal-

ing with the morphological alterations occurring in brain death over a longer time period have not as yet been reported. Only Kramer (1970) has described the gross alterations in dogs after survival times of several hours. Nevertheless, experimental studies alone are able to show what morphological characteristics are necessary for the post mortem diagnosis of brain death. In human cases the underlying disease affecting the brain may modify these changes considerably. The following study, therefore, tries to elucidate the fundamental morphological changes which precede and follow brain death.

METHODS

For the experiments four adult cats (*Felis catus*, 2-4.5 kg) and six baboons (*Papio cynecephalus*, 18.5-25 kg) were used. The cats were anaesthetized with intramuscular pentobarbital sodium (50 mg/kg) and atropine (0.3 mg per animal). The baboons received 25 mg/kg pentobarbitone intravenously. After muscular relaxation with 1 ml./kg nortoxiferrin chloride, the animals were ventilated via endotracheal tube during the entire course of the experiments. Blood gases were determined regularly at least every 12 hours, and blood pH at intervals of at least six

hours. The respiratory minute volume was held constant, while the tidal volume and breathing frequency were slightly altered at intervals of several hours. Developing acidosis was compensated by intravenous sodium bicarbonate. If potassium in the blood diminished to values less than 3.0 m-mole/l., appropriate amounts of potassium malate were given intravenously.

Polyethylene catheters were inserted into the femoral artery and femoral vein and advanced into the abdominal aorta and inferior vena cava respectively. Pressures were monitored and recorded with calibrated pressure transducers. For recording the intracranial pressure a small balloon was placed epidurally over the parietal lobe.

All animals had an urethral catheter. The total water output was measured at intervals of six hours. Based on these outputs, sufficient amounts of intravenous and intra-arterial fluid (Rheomacrodex, isotonic saline solution, and 5% glucose in proportion 1:7:2 by volume) were administered in order to maintain normal water and electrolyte balance. The water balance was rechecked by weighing the animals.

Since all animals tended to develop an increasing arterial hypotension, intra-arterial noradrenaline was given by an infusion pump at a concentration of 0.01–0.1 mg/ml. as soon as the blood pressure fell below 80 mmHg. The infusion pump was controlled by an electronic control system which kept the blood pressure from exceeding 120 mmHg or dropping below 80 mmHg.

In two cats cerebral ischaemia was produced by injecting an isotonic saline solution into the sub-arachnoid space. But, since this technique required too much fluid and thus presumably interfered with normal haemodynamic functions, it was abandoned. In all other animals brain oedema was caused by inflating an epidural balloon (different from that used for recording intracranial pressure) until the intracranial pressure equalled the arterial pressure. Cerebral ischaemia lasted for exactly 20 minutes. During the experiments the electroencephalogram (EEG), electrocardiogram (ECG), arterial pressure, venous pressure, and intracranial pressure were constantly recorded. Body temperature was controlled and kept constant (36.5–38.0° C) by means of a heating blanket. Cerebral ischaemia during the initial compression and in the phase of brain death was confirmed in the baboons by the cerebral xenon clearance method.

At the end of the experiments, samples for histological examination were taken while the animal was still alive. Small pieces of the parietal cerebrum including grey and white matter were fixed immediately after excision in cooled 5% buffered glutaralde-

hyde and prepared for examination with the electron microscope. The brain and the spinal cord were then removed, fixed in formalin, and embedded in celloidin.

Four baboons served as controls. They were treated as above, but in two cases cerebral compression was not so severe as to produce complete ischaemia. The intracranial perfusion pressure was not lowered to less than 10 mmHg. These animals again showed EEG activity after 24 hours. In the other two baboons, intracranial pressure was elevated for more than 20 minutes to the level of the arterial pressure by an epidural balloon; but these animals were killed within the next three hours.

In our experiments the increased intracranial pressure, caused either by fluid injections or by an epidural balloon led to cerebral ischaemia lasting for 20 minutes. Immediately after relieving the experimental ischaemia, the intracranial pressure fell to normal but then rose again quickly. Within minutes values equal to the systemic arterial pressure were reached. At this moment the cerebral circulation again ceased, as confirmed by the xenon clearance technique or by measuring the intracranial pressure (details to be reported by Matakas, Eibs, and Waechter (in preparation)).

The Table summarizes how long the animals were allowed to survive after cerebral ischaemia.

RESULTS

GROSS MORPHOLOGY The morphological changes of brain death were identical in all animals. The brain appeared extremely swollen when the skull was opened. The dura mater was tense. At

FIG. 1. Cat. Twenty-four hours after cerebral compression with an epidural balloon (20 minutes). Confluent haemorrhages at the base of the midbrain and in medial parts of the occipital lobe.

FIG. 2. Baboon. Six hours after cerebral compression with an epidural balloon (20 minutes). Small round haemorrhages in the midline of the diencephalon.

FIG. 3. Baboon. Same case as in Fig. 2. Pressure cone and necrosis of the cerebellar tonsils. The roof of the 4th ventricle is also necrotic. Softening of the spinal cord at C1/C2 segments.

the site where the epidural balloon had been inflated flat epidural and subdural haematomas were found, which, however, were so small that they had not led to a compression of brain tissue. The cortex of the underlying region showed some small perivascular haemorrhages. Bilaterally a temporal transtentorial herniation was evident. The ventricles were narrowed to small clefts as a result of brain oedema. In the medial portions of the occipital lobe, in the midbrain, and in the anterior pons small perivascular, round, or longitudinal haemorrhages were observed (Fig. 1). In the pons they were found either in the midline or in the lateral regions, usually in the caudal portions. Occasionally they extended into the hypothalamus (Fig. 2).

In the border zone between the brain-stem and spinal cord the tissue was softened and showed a greyish colour (Fig. 3). In animals which survived 24 hours or more these regions were completely necrotic. The tonsils of the cerebellum either

showed a pressure cone (Fig. 3) or had disintegrated in those animals which had survived 24 hours or more. In the case of cerebellar necrosis, the subarachnoid space of the cervical spinal cord contained cerebellar detritus (Fig. 4). In two animals the roof of the 4th ventricle was also necrotic (Fig. 3).

The controls showed no signs of brain oedema. In one animal there was blood in the subarachnoid space of the medulla and upper cervical spinal cord, which, however, had not led to any compression of the nervous tissue. There were no haemorrhages of the midbrain and no necrosis.

MICROSCOPIC EXAMINATION Throughout the entire brain scattered perivascular diapedetic haemorrhages were found around various types of small vessels. These haemorrhages were more extensive in regions where they were visible macroscopically. In the cerebral cortex, in the

FIG. 4. Cat. Forty-eight hours after cerebral compression by fluid injection (20 minutes). Spinal cord at C5/C6. Cerebellar detritus in the subarachnoid space. Spongy degeneration of the white matter. H and E.

TABLE

SURVIVAL TIMES OF ANIMALS AFTER CEREBRAL ISCHAEMIA

	Hours	Number of animals
	6	2 (baboons)
	12	1 (cat)
	24	1 (cat)
	48	2 (cats)
Controls	2-3	2 (baboons)
	24	2 (baboons)

ganglia, and in the brain-stem most of the nerve cells were shrunken and hyperchromatic (Fig. 5). These changes were marked in the 6th and 7th layers of the cerebral cortex. There was an intense vacuolation of the smaller neurones in these regions. The nerve cells of the cerebellum showed no changes.

In tissue areas neighbouring the line of demarcation at C1 cord segment the microglia had proliferated. There were some neurophages but no infiltration of leucocytes. The spinal cord at C1 segment and the cerebellar tonsils in animals which had survived for 24 hours or more were necrotic.

In the controls numerous nerve cells were shrunken and hypochromatic. The perivascular space was widened.

FIG. 5. Baboon. Twelve hours after compression with an epidural balloon (20 minutes). Cortex of the frontal cerebrum. Numerous shrunken neurones. Celloidin. Nissl, $\times 100$.

ULTRASTRUCTURE There was a strict correlation between survival time and the development of ultrastructural changes. The latter will therefore be described with respect to the time course.

Six hours after cerebral ischaemia there was severe cerebral oedema of the grey and white matter. In the cortex this was predominantly localized in the astrocytes. Astrocytes, oligodendrocytes, and numerous cytoplasmic processes of the neuropil were swollen and contained masses of vacuoles which appeared empty. The astrocytic swelling was marked in the perivascular regions (Fig. 6). The basement mem-

brane, though generally intact, appeared to be broken at some points. The chromatin of all cell nuclei had condensed. The perinuclear cisterna as well as the endoplasmic reticulum of all cell types, but predominantly of oligodendrocytes, were occasionally swollen at circumscribed regions. The ribosomes had often conglomerated. Mitochondria were swollen and showed only few cristae. In many of them a single granule with a diameter of about 60 nm was observed. The synaptic vesicles of neuronal cytoplasmic processes had crowded into clusters so that the individual vesicles had lost their sharp profile.

FIG. 6. Baboon. Twelve hours after compression with an epidural balloon (20 minutes). White matter of the cerebrum. The perivascular astrocytes are swollen and contain filaments. A swollen oligodendrocyte at the bottom. The endothelial cells are swollen so that the capillary lumen is narrowed to a small cleft. The rest of the tissue appears rather normal. $\times 3,450$.

In the white matter the oedema was localized predominantly in astrocytes and in the intercellular space, which was moderately widened (Fig. 6).

The astrocytic oedema was marked in the perivascular areas in the cortex as well as in the white matter. In only a few cases it appeared to have compressed the capillaries. The endothelium of blood vessels was swollen, had a watery appearance, and contained numerous vacuoles, which because of their size for the most part did not appear to be pinocytotic vesicles. The endothelial swelling had obstructed the lumen of most of the vessels to a small cleft with a diameter of 1 nm or less. In some cases the

endothelium formed balloon-like protrusions that were developing blebs. In cases where the vascular lumen was wider it was filled with deformed erythrocytes. Other vessels, either capillaries, arterioles, or venules, contained blebs.

At 12 to 48 hours after cerebral ischaemia, all these changes had developed even further, but were qualitatively identical with those described above. The karyoplasm of all cells had either condensed into large pieces or had disintegrated. Ribosomes, endoplasmic reticulum, and a Golgi apparatus could only rarely be recognized. All membrane structures were destroyed. Only a number of mitochondria were fairly well pre-

FIG. 7. Cat. Twelve hours after cerebral compression with an epidural balloon (20 minutes). Nerve cell from the cerebral cortex. Extreme shrinkage. Only mitochondria are recognizable. Pericellular oedema. $\times 10,000$.

served. Most of the cells contained numerous vacuoles. The cytoplasm of glial cells was extremely swollen and contained large, apparently empty areas. The nerve cells were often shrunk and filled with an electron dense material (Fig. 7).

The cytoarchitecture had disintegrated. A wide intercellular space had isolated cytoplasmic processes, which in most cases could not be identified. The swelling of the perivascular glial cells was extreme. The capillary lumen was always obstructed either by endothelial swelling (Fig. 6), by tightly wedged erythrocytes, or by

blebs (Fig. 8). The blebs contained small particles, but no identifiable structures. In large vessels, up to five blebs were found on cross-section (Fig. 8). The basement membrane seemed to be intact.

In total 115 vessels (nearly all of them capillaries) were observed on electron microscopic pictures of all animals except the controls. Only 28 vessels were not obstructed. They were all found in circumscribed regions, in most cases in the medulla. Fourteen capillaries were not completely obstructed but had a narrowed lumen. All other vessels were completely ob-

FIG. 8. Cat. Twelve hours after cerebral compression by fluid injection (20 minutes). Vessel is completely obstructed by blebs. Blebs are separated from the endothelium by a membrane. $\times 4,000$.

structed either by impacted erythrocytes (26), by blebs (23), by endothelial swelling (nine), or by a mixture of all (15).

In those two control animals where artificial ischaemia was not complete a moderate oedema was observed. The intercellular space of the white substance was slightly wider than normal. All astrocytic processes were swollen and had a watery appearance. The oligodendrocytes were also swollen. Some capillaries showed a marked swelling of the endothelium with narrowing of the capillary lumen (Fig. 9). In a few cases capillary blebs were observed. The other vessels observed, however, were unaltered. The cytoarchitecture was normal and there were no changes in nerve cells. In the two other animals with severe brain oedema which survived only a few hours, all characteristics of brain oedema

were found. Forty-eight capillaries were observed in these animals. Only 10 of them were obstructed, either by impacted erythrocytes or by endothelial swelling. Two obstructed capillaries contained blebs.

DISCUSSION

The breakdown of the intracranial circulation in any type of severe brain oedema is a process which occurs in several stages. The first impairment is caused by a compression of the superficial cerebral veins (Hekmatpanah, 1970). This results in increased venous pressure, which leads to a reduction of cerebral perfusion pressure, and thus—beyond a certain limit—to a reduction or even breakdown of the cerebral blood flow (CBF).

FIG. 9. *Baboon. Twelve hours after incomplete cerebral compression with an epidural balloon (20 minutes). Eight hours after cerebral compression EEG activity reappeared. Capillary of cerebral cortex with extreme endothelial swelling. The neuropil appears normal. Moderate swelling of perivascular astrocytes. $\times 13,500$.*

If brain oedema is primarily confined to the supratentorial space, the reduction of CBF first occurs in the cerebrum. However, transtentorial displacement of brain tissue leads to the so-called 'Axialverschiebung' (Riessner and Zülch, 1939), which in turn causes cerebellar herniation. This herniation, either by venous compression or by obstruction of CSF drainage, leads to a pressure rise within the infratentorial space and thus initiates the same process as took place in the supratentorial space. A phase difference between supra- and infratentorial oedema is well demonstrated by the morphological manifestations of brain death. Whereas in the cerebrum no cellular reaction to tissue injury was able to develop because the circulation was stopped so quickly, in the brain-stem the presence of necrosis, neurophages, and a slight glial proliferation indicated that a phase of impaired but still present circulation preceded circulatory arrest. In the cerebrum only vacuolation and ischaemic neuronal changes were found. In man they are predominantly but not exclusively observed in the brain-stem (Johnson and Yates, 1956; Kramer, 1964; Mantz *et al.*, 1966). According to Vanderhaegen and Logan (1971) they develop only when cerebral circulation persists for a limited period of time after anoxia. Jellinger (1966) observed ischaemic neuronal changes three hours after the anoxic injury. Many authors have observed this discrepancy between autolytic changes without any cellular reaction in the cerebrum and the changes in the brain-stem in brain death (Kramer, 1964; Kimura *et al.*, 1968; Zander *et al.*, 1971). The fact that the circulation also finally stopped in the infratentorial space is proved by the line of demarcation of the upper cervical spinal cord, which terminates any vital reaction of the brain (Schneider, Masshoff, and Neuhaus, 1967). This biphasic development of intracranial circulatory arrest has also been observed in man by angiography (Heiskanen, 1963; Bücheler, Käufer, and Dux, 1970).

However, ischaemia of the brain-stem is not the last stage of brain death. In those animals which survived for more than 12 hours, cerebellar herniation was so extreme that necrosis and destruction of the cerebellar tonsils with displacement of cerebellar tissue into the spinal subarachnoid space had occurred. Although the

process of oedema usually stops at the foramen magnum, the spinal cord as well is affected by brain death. The cerebellar detritus and possibly a pathological composition of the CSF exert a noxious influence on the spinal cord (Schneider and Matakas, 1971). In our experimental model we observed a marginal oedema of the spinal cord and alterations of the spinal nerve cells in the upper segments. Similar alterations have been observed in man (Schneider *et al.*, 1967; Schneider *et al.*, 1969; Schneider, 1970).

The midbrain haemorrhages are of special interest. These probably arise as a result of the biphasic development of extreme brain oedema. In intracranial hypertension venous blood pressure is at least as high as the intracranial and tissue pressures (Noell and Schneider, 1948; Matakas, Leipert, and Franke, 1971). If, therefore, the intracranial pressure rises to the level of the systemic blood pressure, any circulation would have to cease. But the increase of intracranial pressure alone is not sufficient for the development of haemorrhages. The identity of extra- and intravascular pressures would prevent any rupture of vessels. If, however, a pressure gradient exists between different parts of the intracranial space, then the compression of cerebral vessels would not be equal in all parts of the brain. For example, if the veins are compressed in a circumscribed region, the blood pressure within them will quickly rise to the level of the arterial pressure. In this case of circumscribed stopped blood flow, the arterial driving pressure is transferred through the capillaries to the point of venous compression. If the tissue pressure, acting on the outer surface of the veins, remains low, a high positive pressure gradient between the lumen of veins and the environment results. This gradient certainly facilitates venous haemorrhages, since the veins are not built for such high pressures.

If intracranial hypertension begins to develop in the supratentorial space, the tissue pressures of the upper parts of the pons and the midbrain initially remain normal or are only moderately elevated. Similar pressures should be recorded in the venous vessels traversing these tissue compartments. But since the upper part of the pons and the midbrain are drained by veins running to the vena Galeni (Huang and Wolff, 1965; Hassler, 1967; Holdorff and Cervos-Navarro,

1971), these veins will be obstructed either at the incisura of the tentorium or by compression of the vein of Galen. This may explain why mid-brain haemorrhages always accompany lethal brain oedema.

The occurrence of midbrain haemorrhages indicates the end of the first stage of developing brain death. If no treatment stops the process, this stage is inevitably followed by progressing hypertension in the infratentorial space. This second, infratentorial phase is terminated by demarcation at C1/C2 cord segments. But, although this region also constitutes a border line between compartments with high and low pressures, no haemorrhages occur. Apparently this is due to the fact that venous vessels run from the intracranial into the spinal space, but not vice versa. Haemorrhages in the basal ganglia, as observed in our cases and in clinical studies (Scheinker, 1945; Jellinger, 1967), may develop similarly to those in the brain-stem. Our experimental studies (to be published) revealed that transtentorial herniation may take place before complete circulatory arrest in the cerebrum. Thus the vena Galeni may be obstructed at a point of time where intracranial circulation has not yet ceased completely.

Our explanation not only confirms the observation of those authors (Bagley, 1923; Cannon, 1951; Lindenberg, 1963; Krayenbühl and Yasargil, 1965) who suggest that compression of the vena Galeni is the cause of venous haemorrhages but may also explain why in some cases arterial haemorrhages occur (Hassler, 1967). Additional factors, such as distortion and laceration, obviously may facilitate and accentuate these haemorrhages. However, two factors must be present to explain the localization and the occurrence in time of brain-stem lesions: a pressure gradient and a persisting blood flow in those regions where the haemorrhages occur. The observation of Klintworth (1968) that a high blood pressure facilitates the development of brain-stem haemorrhages is one of the facts supporting this hypothesis.

The impairment and cessation of the cerebral perfusion is initially caused by venous compression. However, anoxia following venous compression produces alterations of the vascular system which obstruct the capillary lumen. These alterations consist of swelling of the endo-

thelium and the development of capillary blebs. In addition, the swelling of perivascular astrocytes may possibly compress the capillaries to a significant degree, as was pointed out by Luse and Harris (1960), Hills (1964), and Wolff (1964). But according to our observations this does not seem to be a mechanism of major importance. These workers, moreover, did not evaluate the consequence of this phenomenon for the cerebral circulation. Capillary blebs are not specific for the brain in as much as they may also develop in other organs after various noxious agents (Büchner and Onishi, 1968; Donath, Mitschke, and Seifert, 1970; Backwinkel, Schmitt, and Themann, 1971; Vitak, 1971), but they do have a special importance for the cerebral no-reflow phenomenon (Ames, Wright, Kowada, Thurston, and Majno, 1968; Chiang, Kowada, Ames, Wright, and Majno, 1968). As shown by our experiments, this phenomenon develops to a maximal degree with the occurrence of brain death, in which case only few vessels appeared capable of allowing any further perfusion. But although the no-reflow phenomenon occurs within minutes, the extent to which it develops is dependent on the duration of ischaemia (Cantu and Ames, 1969). The control animals, which also revealed vascular changes, proved that only a quantitative difference exists between temporary brain oedema and brain death. When cerebral blood flow is first stopped by venous compression the vessels are obstructed by impacted erythrocytes. Very soon the endothelium begins to swell and after some hours blebs develop from the swollen endothelium.

The vascular changes indicate that cerebral ischaemia is irreversible, not only because of an anoxic injury but also because of complete vascular obstruction. This marks the absolute limit of any therapeutic effort. Therefore, the optimal treatment of cerebral ischaemia must have its emphasis in avoiding these cerebral vascular changes (Hossmann and Sato, 1970). Decompression of the brain by craniotomy has a prospect of success only when it is done before the development of these changes.

The ultrastructural characteristics of neurones and glial cells in brain death are those of brain oedema and postmortem autolysis. The oedema reached a maximum degree but was not essentially different from that of the control animals.

The autolytic changes were similar or equal to those which have been observed in autolysis after complete death (Karlsson and Schultz, 1966; David, Marx, and David, 1971).

As the main result of our experimental data, we find that all the morphological signs of brain death are a consequence of the intracranial circulatory arrest. These must be distinguished from the apallic syndrome, which we choose to define as a syndrome in which most of the nerve cells are destroyed, but cerebral circulation persists. Kramer (1963) has described a case which may be subsumed under this condition. If the primary pathological process starts in the supratentorial space, the circulatory arrest develops in three stages. The data available thus far are not sufficient to determine the time intervals between the different phases. It seems probable that they depend on several factors—for example, the degree and type of primary injury, haemodynamic conditions, etc.

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