Evidence for Early Anoxic-Ischaemic Cell Damage in the Rat Brain

LEVINE[1] observed that it was difficult to maintain a rat in deep anoxia by exposure to nitrogen for 20 min or more. High mortality and undamaged brains in the survivors were attributed to the fact that the sensitivity of the medullary centres approximated to that of the remainder of the brain. This difficulty was overcome by unilateral common carotid artery ligation followed by exposure to nitrogen or to nitrous oxide, and resulted in a largely ipsilateral ischaemic-anoxic necrosis of the forebrain.

In this investigation the application of standard neuropathological techniques to the LEVINE preparation has made it possible to demonstrate early ischaemic cell change in the brains of animals killed at the end of a 40 min period of exposure to nitrogen. This consistent observation is of interest in view of the fact that others have believed that it is necessary to use histochemical techniques[2-4] and the electron microscope[5] in order to detect the earliest effects of ischaemia upon the nerve cell. Further, in contrast to the view that brain damage rarely follows exposure to nitrogen alone, ischaemic nerve cell change has been demonstrated in animals killed at the end of such exposures.

Perfusion-fixation with FAM (40% formaldehyde, 1 part; glacial acetic acid, 1 part; absolute methanol, 8 parts), a fixative developed by DAVID[6], gave optimal staining of paraffin sections with cresyl violet. These were entirely free of artefact and particularly hyperchromatic neurones. The brain was left in situ for 1–4 h. Paraffin sections cut at 7 and 12 μ were stained with cresyl violet, cresyl violet and Luxol fast blue, hematoxylin and eosin, Mallory's phosphotungstic acid and hematoxylin, and with PAS and hematoxylin.

Young adult, female, white Wistar rats (Carshalton strain) weighing 140–160 g were used. Blood flow in the right common carotid artery was interrupted by ligation for survival periods over 4 h. For survival periods under 4 h, an artery 'clasp' (Figure 1) was applied. This was removed at the end of the survival period and restoration of blood flow was verified under an operating microscope. After interruption of arterial flow, the animal was placed in a Perspex tube through which nitrogen flowed at a rate of 5 l/min.

Convulsions usually occurred within 15–25 sec and apnoea within 60 sec. After artificial respiration the animal was returned to the tube. Exposure to nitrogen together with several periods of apnoea totalled 40 min. Most animals exhibited a left hemiparesis and circled to the left.

Of 53 animals, 21 (40%) died during exposure to nitrogen. Of 32 survivors the brains were histologically abnormal in 25. In most animals killed at 24 h, there was obvious swelling of the ipsilateral hemisphere. Typically (Figure 2a), necrosis in the ipsilateral hemisphere involved the neocortex (apart from the upper para-median portion), more or less of the pyriform cortex, the hippocampus (particularly H.l. and the endfolium), the striatum, thalamus and amygdaloid nucleus. In all these regions there was a loss of neurones and typical ischaemic cell change in the remainder.

This distribution of necrosis corresponds to that portion of the hemisphere whose blood supply is dependent upon one internal carotid artery and its branches. The para-median neocortex is supplied by the opposite internal carotid artery via the unpaired anterior cerebral

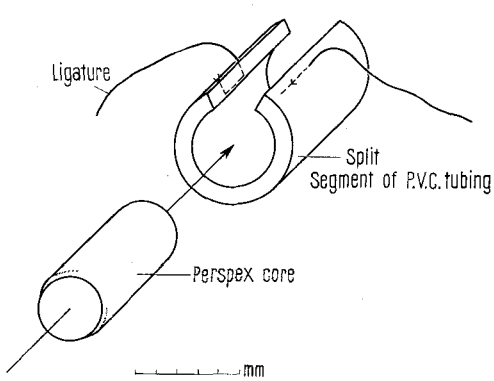

Fig. 1. The 'clasp' is passed around the right common carotid artery the lumen of which is temporarily occluded by inserting the plastic core and tying the ligature.

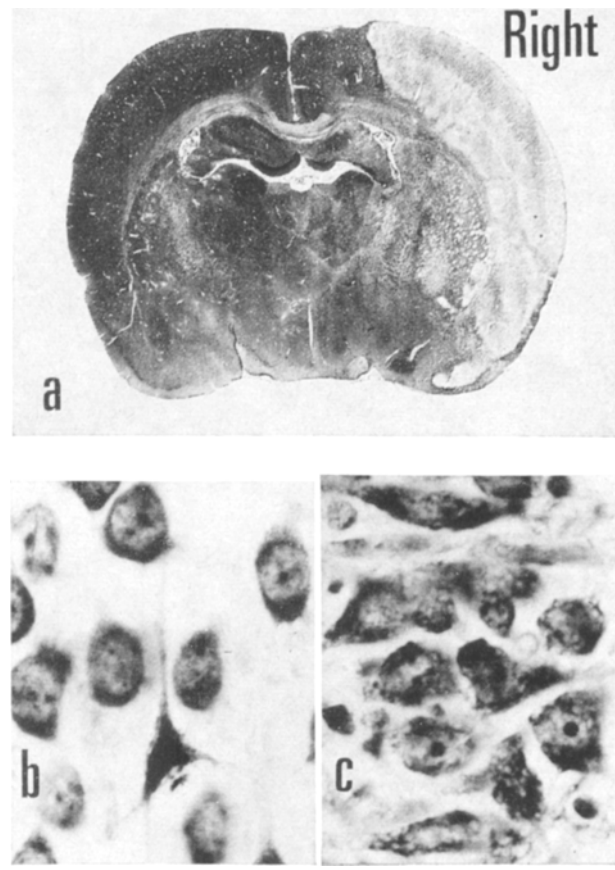

Fig. 2. (a) Levine preparation. Survival 24 h. Mallory's phosphotungstic acid hematoxylin. × 8. (b) Levine preparation. Survival $^1/_2$ h. Ischaemic cell change with encrustations in pyramidal neurone of ipsilateral neocortex. Cresyl violet. × 1065. (c) Levine preparation. Survival '0' h. Microvacuolation of cytoplasm of neurones of ipsilateral hippocampus. Cresyl violet. × 1020.

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artery so that the sharp medial edge of neocortical necrosis represents the boundary zone or 'watershed' between the territories of the anterior and middle cerebral arteries (ACA and MCA). A typical 'watershed' lesion as well as a marked loss of neurones in the outer layers was seen in the contralateral neocortex of 1 of 6 animals killed at 24 h.

As neuronal damage was unequivocal after a survival of 4 h, intervals between 24 and 4 h were omitted and animals surviving 3, 2, 1 and $^1/_2$ h were studied, as well as 6 killed at the end of the 40 min exposure to nitrogen ('0' survival). A narrow band of finely spongy tissue along the line of the ipsilateral ACA/MCA 'watershed' was recognizable in animals surviving from 4 to $^1/_2$ h and contained neurones in stages of ischaemic change. Encrustations of the Golgi network were visible here and also in scattered neurones in the neocortex and pyriform cortex after a survival of $^1/_2$ h (Figure 2b). The earliest evidence of ischaemic damage, visible in 2 animals after-'0' survival, took the form of microvacuolation of neuronal cytoplasm in scattered cells in the outer layers of the neocortex, in the pyriform cortex and in many cells of the hippocampus (H.l. and endfolium Figure 2c). Ischaemic cell change with encrustations was visible in the hippocampus after 1 h. Ischaemic damage in the thalamus, striatum and amygdaloid nucleus was less frequent and severe than in the neocortex.

In the contralateral neocortex and pyriform cortex of animals surviving from '0' h upwards, ischaemic cell change had reached the same stage as in the ipsilateral hemisphere but was less extensive. Typical lesions in the contralateral ACA/MCA 'watershed' were seen in three animals killed after 1, 3 and 4 h.

Of 28 animals exposed to nitrogen alone by the method already described, 11 (39%) died during the exposure. Of the 17 survivors the brains were histologically abnormal in 14. Ischaemic cell change occurred bilaterally in the neocortex, always sparing the ACA territories. Sharply-defined lesions in the ACA/MCA 'watersheds' were seen in two animals after 4 and 24 h. Ischaemic cell change was also seen in the pyriform cortex and hippocampus, but only occasionally in the basal ganglia. In the neocortex microvacuolation of neurones was visible after '0' survival and encrustations of nerve cells in animals surviving $^1/_2$ h and more.

The occurrence of lesions in neocortical 'watersheds' suggests that a major reduction in overall cerebral blood-flow had occurred[7,8], most probably due to myocardial hypoxia. A similar reduction in cerebral blood flow is the most probable explanation for the damage in the contralateral neocortex observed when exposure to nitrogen followed unilateral carotid ligation.

In the 'LEVINE preparations', swelling of the ipsilateral hemisphere was recognizable from $^1/_2$ h onwards, but was not detected before 3 h by MACDONALD and SPECTOR[9]. SPECTOR[2,3,10] has employed the contralateral hemisphere as a control in biochemical and enzymatic studies. In the present series, ipsilateral lesions, no more severe than those illustrated by SPECTOR[10], were often associated with variable contralateral alterations.

In contrast to the demonstration of ischaemic alterations in neurones from '0' survival onwards in the present series, the earliest evidence of similar changes has been minimal shrinkage of neurones and sponginess of tissue after 1 h obtained by ZEMAN[11], ischaemic cell change in the caudate nucleus after 3 h and in the neocortex and hippocampus after 4–6 h obtained by BECKER and BARRON[4], and in the hippocampus after 10–18 h obtained by SPECTOR[3].

Zusammenfassung. Es wird gezeigt, dass Schädigungen von Nervenzellen nach dem Aussetzen in eine Stickstoffatmosphäre, mit oder ohne Abklemmung der Carotis, histologisch erkennbare Veränderungen ergeben.

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Effects of Angiotensin II and Epinephrine Infusion on Juxtaglomerular Cell Granularity and Arterioles of the Rat Kidney[1]

In general, when a kidney is exposed to hypertensive blood pressure levels either in vivo or in vitro the granularity of the juxtaglomerular cells decreases[2–5], whereas kidneys protected from excessive elevation of blood pressure show an increased number of granules in juxtaglomerular cells. Angiotensin both increases aldosterone secretion by the zona glomerulosa of the adrenal cortex[6,7] and directly promotes vasopressor activity on intravenous infusion[8–13]. Indeed, ASSALI and WESTERSTEN[12] have suggested that angiotensin II is the most potent known constrictor of peripheral arteries, particularly the renal arteries.

The present study was performed to observe the changes in granularity of the juxtaglomerular apparatus in a kidney exposed in vivo to high blood pressure that was maintained by administration of angiotensin II or epinephrine.

16 female Sprague-Dawley rats weighing 100–150 g were divided into 2 groups of 8 rats each. The left kidney of each rat was removed under ether anesthesia and 2 h later infusion was begun with the rats now anesthetized with Nembutal (0.06–0.08 ml/100 g body weight).

In group 1 synthetic angiotensin II was infused for 1 h through a polyethylene tube (PE 50) inserted in the jugular vein. The dosage and rate were controlled manually in order to maintain systolic blood pressure above 180 mm Hg. The blood pressure was measured directly from the carotid artery through PE 60 poly-