Cerebral Ischemia*

I. An Improved Experimental Method for Study; Cardiovascular Effects and Demonstration of an Early Vascular Lesion in the Rabbit

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Several investigators have developed methods of producing temporary cerebral ischemia and have measured the duration of ischemia compatible with life.\(^{5,8,12-14,17,18,22,23}\) Relatively few studies, however, have been made of the progression of the pathological changes produced by the ischemia, and little systematic attention has been given to determining the first changes responsible for the appearance of irreversible damage.

The results of several experimental studies suggest that failure to recover from a period of temporary ischemia may be due to changes produced by ischemia in the cerebral vasculature. Nervous tissue deprived of oxygen and glucose in vitro, where circulation of the blood was not a consideration, showed a high degree of recovery after deprivations lasting as long as 20 min.\(^{1,26}\) Prolongation of the permissible period of cerebral ischemia has been obtained in vivo by preadministration of anticoagulants.\(^{10,11}\) Necely and Youmans\(^{41}\) found that dogs could survive for much longer periods of ischemia when the brain was totally devoid of blood. However, no systematic study has been made, to our knowledge, of the patency of cerebral vessels during and following the period of ischemia.

The primary objective of the present study was to examine the pathophysiology of cerebral ischemia, with attention to changes in cardiovascular function and particularly to possible changes in the vasculature of the brain itself. A secondary objective was to develop a better preparation for the study of temporary cerebral ischemia in experimental animals. A total of 78 rabbits was used in the study.

Materials and Methods

Production of Ischemia. The studies were performed on rabbits weighing from 1.4 to 3.6 kg. Blood flow through the basilar artery was interrupted by a preliminary operative procedure, and ischemia was then produced by clamping the common carotids and inflating a pressure cuff around the neck to a pressure of 350 mm Hg.

To section the basilar artery, the rabbits were anesthetized with intraperitoneal Chloralose (50 mg/kg body weight) or pentobarbital sodium (30 to 40 mg/kg). A longitudinal incision 3 cm in length was made in the anterior cervical region, and with a self-retaining retractor, the trachea and esophagus were displaced to the left. The anterior border of the foramen magnum was exposed by separating the paired longus colli muscles. With small rongeours, bone was removed cephalad to allow a direct approach to the basilar artery, and the dura mater was opened. Two small tantalum clips were placed on the basilar artery just above the junction of vertebral arteries, and the vessel was divided between these. The dural opening was covered with a piece of gelatin foam sponge, and the incision was closed in layers with interrupted catgut sutures. In seven rabbits observed for 5 to 7 days after division of the basilar artery (but without clamping the carotids and applying the pressure cuff), there was no apparent neurological deficit.

Cerebral ischemia was produced at some time, varying from minutes to several days, after the basilar artery section. Mayfield clips were applied to both common carotid arteries of the anesthetized animal, and an infant's blood pressure cuff was placed around the neck and rapidly inflated to 350
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mm Hg. In some animals, the experiment was terminated at the end of the period of ischemia, and the brains were prepared immediately for histological examination. In others, the pressure cuff was deflated, the Mayfield clips removed, and the animals were observed for varying periods to determine the degree of recovery, or to follow post-ischemic changes in cardiovascular physiology and in the histological appearance of the cerebral vasculature.

Artificial respiration was begun as soon as spontaneous respirations ceased, and was continued until the experiment was terminated or spontaneous breathing resumed. The animals were respired with a simple Harvard piston-type pump which delivered room air by means of a tracheotomy tube. The tidal volume and rate were adjusted to exceed, slightly, the spontaneous respiration of the animal.

Physiological Measurements. Changes in respiration and in corneal and pupillary reflexes were recorded for all animals during and following the period of ischemia. In some experiments, continuous recordings of the electrocardiogram and electroencephalogram were made; and measurements of arterial and venous blood pressures were obtained by means of a Sanborn recording electromanometer using polyethylene catheters in the femoral artery and vein. The rabbits used in the physiological studies were anesthetized with Choralose, because it has been shown to have no effect on cerebral oxygen and glucose utilization and, in low dosage, provides adequate anesthesia without eliminating the corneal reflex. Succinylcholine chloride, 0.1 mg per kg body weight, was administered intravenously prior to the onset of ischemia to lessen the extensor rigidity that occurs at this time.

Histological Procedure. The two methods used to determine the patency of the cerebral vasculature after ischemia have been described in detail elsewhere. In one, the carotid arteries were perfused with a suspension of carbon black* at a pressure of 120 mm Hg.; the brain was then removed, fixed in formalin, cut into coronal sections, and examined grossly and microscopically for regions of impaired filling. The second method depended upon perfusing the brain thoroughly through the carotid arteries with mammalian Ringer's solution to displace blood from the patent vessels; the brain was then removed, fixed, and sectioned as above; the sections were stained with benzidine (using a modification of the Le phen-Pickworth technique as described by Mallory) to reveal blood sequestered in the nonperfused vessels.

Results

Completeness of Ischemia. The effectiveness of the procedure used to interrupt blood flow to the brain was tested by introducing various labeling substances into the systemic circulation during the cerebral ischemia and then examining the brain for their presence. In three animals, Micropaque barium sulfate† and in three animals aqueous Dionosil‡ were injected into the left auricle after the cerebral circulation had been occluded. The brains were removed, fixed in 10% formalin, and then radiographed. In none of them had the contrast material entered the cerebral vasculature. In two additional animals the heart was stopped after occluding the arteries to the brain and a suspension of carbon black particles was injected into the aorta at a pressure of 200 mm Hg. The entire systemic vasculature was stained black, but there was no evidence of carbon in the cerebral circulation under gross or microscopic examination. The completeness of the ischemia was further demonstrated by the abrupt appearance of a reproducible sequence of pathophysiological responses and by the consistent demonstration of irreversible damage after 5 minutes (see below).

Physiological Alterations with Ischemia. Within 30 seconds after clamping the carotid arteries and inflating the pressure cuff to produce the cerebral ischemia, both pupils dilated and lost their response to light. Urination and tonic spasm of all limbs were frequently noted. Spontaneous respiratory movements ceased in all animals in 30 seconds or less, and at this point artificial respiration was begun. The electroencephalogram became flat in 30 to 90 seconds.

Consistent changes in cardiovascular func-

* Prepared especially for biological use by Pelikan Werke, Guenther Wagner, Hanover, Germany.

† Made by Damancy and Co., Ltd., Ware, Herts., England. The particle size is between 0.5 and 3 µ.

‡ Made by Glaxo Laboratory Ltd., Greenford, Middlesex, England. A 50% suspension of propylidone. Most particles are between 3 and 15 µ in diameter, approximately 10% are between 15 and 30 µ, and 5% are larger, up to 100 µ.