SUMMARY Cerebral infarction was produced in paralyzed, ventilated rats by a 30 min period of right common carotid artery occlusion combined with systemic hypoxia (Pao₂ 21–25 mm Hg). After 30 min the arterial clamp was removed and the animals were reoxygenated and allowed to survive for 1 min (6 animals), 30 min (12 animals), or 1½ to 2 h (6 animals). The animals were reanesthetized and sacrificed by perfusion-fixation with paraformaldehyde-glutaraldehyde.

Light and electron microscopy revealed ischemic cell change in neurons in the ipsilateral cerebral cortex, striatum and hippocampus. These changes were mild to moderate in the early post-ischemic period and severe in the late post-ischemic period. Cerebral infarction was present in one of the 30 min survivors and in all of the 1½ to 2 h survivors. Electron microscopy showed platelet thrombi in the infarcted brain in 3 of the 7 animals with infarcts, and in an area of very severe ischemic cell change in a fourth animal. They were not present in areas of brain showing only mild to moderate ischemic cell change. These findings showed that platelet thrombi form in association with cerebral infarcts and suggested that they are induced by tissue necrosis rather than by neuronal ischemic cell change alone.

ABNORMALITIES in platelet function following acute cerebral ischemia have been described in man and experimental animals, but the histological identification of platelet aggregates has been reported only rarely in cerebral infarction. The relationship between the formation of platelet thrombi and the presence of either ischemic neuronal damage or tissue infarction is not clear. Accordingly, this report describes the ultrastructural appearance of platelet thrombi associated with experimental cerebral ischemia in the rat and discusses the possible role played by these thrombi in the maturation of the infarct. The alterations in vascular permeability to protein tracers in this model have been described elsewhere.

Materials and Methods
The experimental protocol has been described in detail previously. Briefly, adult male Wistar rats were paralyzed and ventilated with a mixture of 70% N₂O-30% O₂. A tail artery cannula was inserted for continuous monitoring of blood pressure and periodic sampling of arterial blood gases. Temperature was maintained at 37° C. Unilateral cerebral infarction was produced by a combination of right common carotid artery occlusion and systemic hypoxia (Pao₂ 21–25 mm Hg) for 30 min. The arterial clip was then removed, the animal reoxygenated and allowed to survive for 1 min (6 animals), 30 min (12 animals), and 1.5 to 2 h (6 animals) following the hypoxic-ischemic insult. At the time of sacrifice, the animal was reanesthetized and perfused-fixed through the ascending aorta with 1:4 (1 min) and full strength (20 min) Karnovsky’s fixative (4% paraformaldehyde-5% glutaraldehyde, 2010 milliosmoles). The brain was removed the following day and cut into 2 mm coronal sections. Small pieces of corpus striatum and cortex were taken from both hemispheres, post-fixed in osmium tetroxide, dehydrated through graded alcohols, and embedded in Araldite. Semi-thin sections were stained with toluidine blue and appropriate areas selected for ultra-thin sectioning. The grids were stained with uranyl acetate and lead citrate and examined with a Siemens Elmiskop I electron microscope.

The remaining coronal sections of the cerebral hemispheres and a transverse section of cerebellum and pons were embedded in paraffin and stained with hematoxylin-eosin and cresyl violet for light microscopy.

Controls included normal animals (5 animals), hypoxia alone (5 animals), and carotid occlusion alone (5 animals).

Results
1. Controls
The brains were free of red blood cells save for rare focal areas of inadequate perfusion in some animals. The normal animals and carotid occlusion controls were unremarkable when studied by both light and electron microscopy. The hypoxic controls and the contralateral hemisphere of the experimental animals contained rare ischemic cell change (ICC) in the neurons in the striatum, cortex or hippocampus, characterized by shrunken, eosinophilic cytoplasm and pyknotic nuclei. No intravascular thrombi were seen.

2. Experimental Animals
Animals sacrificed after 1 min of survival showed a small number of ischemic neurons in the ipsilateral striatum, middle cerebral artery (MCA) cortex, and hippocampus. Animals sacrificed after 30 min of survival showed mild to moderate numbers of ischemic neurons. Two animals in this group had severe ICC (all neurons) in the striatum, and one of these had an early infarct as well. All 6 sacrificed after 1.5 to 2 h of survival had severe ICC and all 6 also had infarcts in the ipsilateral cortex and/or striatum (fig. 1).

Red blood cell stasis was seen in the infarcted areas.
of brain and in the striatum of the one 30 min animal with severe ICC.

In 3 of the animals with infarcts and the one 30 min survival animal with severe ICC, the 1 μ semi-thin sections showed amorphous debris within small vessels. Electron microscopy of these areas revealed platelet or platelet-red blood cell thrombi in many of the vessels (fig. 2). Fibrin was associated with some of the thrombi. Dense granules were occasionally seen within the platelets. The poor fixation of the infarcted tissue, due to the presence of intravascular thrombi, made it difficult to characterize further the fine structure of the
Platelet thrombi were seen only in the areas of infarction or very severe ICC. They were not present in areas of mild to moderate ICC nor were they present in the contralateral hemispheres or the 3 groups of control animals.

**Discussion**

When cerebral infarction is produced by temporary occlusion of a major blood vessel there is a gradual evolution of the histological changes during the period...
of recirculation, starting with a small number of neurons showing ICC and ending in infarction in which all tissue elements, including glial cells and blood vessels, are necrotic. Ito et al.\textsuperscript{11} characterized this evolution as “maturation” of cerebral infarct and demonstrated that in the gerbil model of infarction the rate of maturation was related to the intensity of the ischemic insult. They concluded that the ultimate fate of the tissue was determined at the end of the ischemic period. A similar maturation in ischemic brain damage is described above in our present model of experimental infarction in the rat.

However, it is not known whether some of the secondary effects of cerebral infarction, such as edema or vascular occlusion, influence the maturation of an infarct and whether therapeutic manipulations can alter the evolution of neuronal ischemia into tissue necrosis or reduce the total infarct size.

In the present model early cerebral infarction is associated with intravascular platelet thrombi. The platelet thrombi were seen in 3 out of 7 animals with infarcts and in the one animal with severe ICC. Two of these animals were sacrificed after 30 min survival, and 2 after 1.5 to 1.45 h of survival. Vascular occlusion, characterized by distension of small vessels by packed red blood cells (and, on electron microscopy, platelets), was seen only in infarcted tissue and not in areas showing only mild to moderate ischemic neuronal damage. Although we cannot exclude the possibility that platelet thrombi were present in the early postischemic period and were washed out during the perfusion-fixation, the high osmolality of the fixative used makes this unlikely.

This observation suggests that platelet thrombi do not form until tissue necrosis has developed. In contrast, Waltz and Sundt have reported the presence of white thrombi “suggestive of platelets” within meningeal veins and venules within minutes following occlusion of the middle cerebral artery in cats.\textsuperscript{12} This early appearance of platelet thrombi may be related to the method of infarct production (permanent occlusion versus temporary occlusion) or to the different vessels examined (meningeal not parenchymal).

The absence of platelet thrombi in 3 brains with infarcts may represent sampling artifact. Alternatively, their absence may indicate that these thrombi are present for only a short period and are subsequently dissolved. This may explain the failure to visualize such thrombi in many previous ultrastructural studies of experimental cerebral infarcts,\textsuperscript{13-20} including that of the gerbil,\textsuperscript{11, 16} in which in vivo platelet aggregates have been demonstrated using radioactive serotonin labeling.\textsuperscript{6}

The stimulus for the production of platelet thrombi is not known. Dougherty et al.\textsuperscript{1} postulate that tissue ischemia may release a number of platelet-activating substances. Alternatively, platelet aggregation may be secondary to damaged endothelium of necrotic or pre-necrotic vessels. Our observations showing platelet thrombi in areas of tissue necrosis, including vascular necrosis, but not in areas of brain with ischemic neurons yet having intact neuropil and blood vessels, suggests that infarction is necessary for the production of platelet thrombi.

Antiplatelet drugs recently have been shown to be of value in the treatment of cerebrovascular disease in man.\textsuperscript{21, 22} Although antiplatelet drugs have not been used in the treatment of experimental cerebral infarction, there is evidence that they prevent platelet aggregation in experimental myocardial ischemia.\textsuperscript{23} The use of antiplatelet drugs in experimental cerebral infarction in the present model of combined hypoxia-ischemia currently is being studied in our laboratory.

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References

Influence of Sex on Cerebral Ischemia Following Bilateral Carotid Occlusion in Spontaneously Hypertensive Rats: A Metabolic Study

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SUMMARY Cerebral lactate, pyruvate and adenosine triphosphate (ATP), and acid-base balance were measured in male and female spontaneously hypertensive rats (SHR) before, and 1, 3 and 5 hours after bilateral carotid occlusion.

In male SHR, cerebral lactate and the lactate/pyruvate (L/P) ratio progressively increased after occlusion, while cerebral ATP decreased. In female SHR, an increase in lactate and the L/P ratio was less marked than in male SHR. Cerebral ATP remained unchanged 5 hours after occlusion. These data suggest that bilateral carotid artery occlusion may cause more pronounced ischemic changes in the brain in male SHR than in female SHR, resulting in a greater increase in lactate with a concomitant decrease in ATP in male SHR. Results suggest that female SHR are more resistant to cerebral ischemia following bilateral carotid occlusion than male SHR. Blood pressure and gonads in the susceptibility to cerebral ischemia are discussed.

Methods

Adult SHR (Okamoto and Aoki®), aged 5–10 months, were used for the present study. Male SHR, weighing 300–450 g (339 ± 9 g: mean ± SEM), and female SHR, weighing 200–300 g (221 ± 6 g), were anesthetized with intraperitoneal amobarbital 10 mg/100 g body weight. Both common carotid arteries were exposed through a ventral midline incision in the neck, the arteries were separated from the vagosympathetic trunk, and ligated simultaneously. After the skin incision was closed with silk sutures, the animals were returned to their cages except for those subjected to a 1 h study of carotid artery occlusion.

All SH rats were reanesthetized with amobarbital (10 mg/100 g) at 1 h before sacrificing. One femoral artery was cannulated for blood pressure recording with an electromanometer, and for anaerobic sampling to determine pH, Pco2 and Po2 with an IL meter (Model 113). The rectal temperature was maintained close to 37°C, and the animals spontaneously breathed room air. Support of ventilation was not required since the animals tended to over- rather than underventilate.

After arterial blood sampling, the head was immediately frozen in situ with liquid nitrogen. Animals were sacrificed at 1, 3 and 5 h intervals following bilateral carotid artery occlusion. Liquid nitrogen was applied to the skull using a funnel. The frozen whole brain was chiselled out and separated grossly into the
Platelet thrombi in experimental cerebral infarction.

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