Table 1. Milk output and urine uptake by lactating females during a 24-hour period.

Species	No. in litter	Water (grams per young)			Milk recycled (%)	Water lost by young recycled (%)
		In milk	Total lost by young	Uptake of excreted		
M. musculus	3	0.89	0.43	0.34	38	79
N. alexis	3	1.46	0.80	0.46	32	58
Dingo	4	95.3	67.7	31.8	33	47

50 percent of the total water lost by the young is retrieved by the mother.

Since the kangaroos had only one pouch young each, the revised experimental procedure could not be employed. However, recycling of water is clearly indicated, as approximately 5 percent of the injected THO was present in the mother after 24 hours and 10 percent after 48 hours. Several conclusions may be drawn from our study.

1) In desert species in which it occurs, water recycling is likely to be important in the water economy of a lactating female under natural conditions. The osmotic concentration of the urine of suckling young is well below that of urine which the mother is able to produce (roughly one-fifth in *N. alexis* and one-third in dingoes). Consequently, the solutes contained in the urine of the young can be excreted by the mother in a smaller volume of water, leaving free water available to the mother. For example, lactating *N. alexis* with litters of four were found to consume no more water than nonlactating adults, and restricting the amount of drinking water of the lactating animals to half the amount taken when water was freely available did not significantly affect the growth rates of the young. In addition, one female *N. alexis* receiving only seed containing 10 percent free water and no drinking water successfully reared a single young while maintaining her own body weight. In the dingo, bitches that were lactating consumed only 25 percent more water than when they were pregnant (842 ml/day prepartum and 1056 ml/day postpartum), and this difference would not be sufficient to cover the increased water losses in milk secretion.

2) In animals that recycle water, milk production cannot be estimated by the THO procedure in the usual manner. At least one control young must be used so that the quantity of THO present in the injected young that is derived from milk can be determined.

3) Milk production in animals that recycle water cannot be determined by weighing the female and the young immediately before and immediately after a short period of lactation. This method has been used frequently in studies of the effects of hormones and drugs on lactation in laboratory animals (*7*), particularly in rats and rabbits, and may lead to serious errors of interpretation of results. We have found that lactating *Rattus norvegicus* do recycle significant amounts of water. However, lactating rabbits do not appear to do so. The absence of recycling in rabbits may be related to their reduced maternal behavior.

The appearance of water recycling in many species suggests that its function is not primarily that of water economy. The behavior pattern may have evolved for "nest" hygiene, or as a means of communication, by which the mother receives information about the physiological state (such as age) of the young via the composition of the urine. Thus, in desert species the saving of water by recycling during lactation is seen as probably a by-product of a behavior pattern that has evolved for some other purpose.

P. BAVERSTOCK
B. GREEN
Division of Animal Science, Institute of Medical and Veterinary Science, Adelaide, South Australia, 5000, and
Division of Wildlife Research, CSIRO, P.O. Box 84, Lyneham, Canberra, ACT, 2602

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Stroke in Rats Produced by Carotid Injection of Sodium Arachidonate

Abstract. *Unilateral cerebrovascular occlusion was produced in heparinized rats within 60 seconds after an injection of sodium arachidonate (in doses exceeding 0.45 milligram per kilogram) into the carotid artery. Electroencephalographic activity over the affected cerebral hemisphere was suppressed, and cerebral blood flow decreased by half. Microscopic examination revealed complete obstruction of the hemispheric microcirculation by platelet aggregates.*

Platelet aggregation is a fundamental component of thrombus formation in atherosclerotic cerebrovascular disease (*1*). Pathologic evidence indicates that platelets adhere to ulcerated atheromata because of local factors in the diseased artery (*2*); however, the degree to which intravascular humoral agents participate in the involvement by platelets remains to be precisely defined. Recent work has shown that arachidonic acid when injected into the ear veins of rabbits causes sudden death, associated with occlusion of the microcirculation in the lungs by platelet aggregates (*3*). Additionally, labile endoperoxide intermediates generated during the endogenous conversion of arachidonic acid into prostaglandins E_2 and $F_{2\alpha}$ have been shown to act as powerful biochemical triggers of platelet aggregation in vitro (*4*). These experimental findings suggested that intracarotid injection of arachidonate might produce acute cerebrovascular occlusion and thus serve as a model for investigating the pathogenesis and treatment of stroke.

To explore this possibility, 35 adult male albino rats of the Sprague-Dawley strain (150 to 175 g) were curarized (*5*) under light chloroform anesthesia and mechanically ventilated on room air via a tracheostomy. After intravenous injection of heparin (*6*), the left external carotid artery of each rat

was ligated under local anesthesia (*7*), and the left common carotid artery was catheterized (*8*). Each animal then received 50 μl of sodium arachidonate solution (*9*) ranging in concentration from 0.33 m*M* to 33 m*M*, injected in doses of .036 to 3.6 mg/kg into the left internal carotid artery within 0.5 second. Electroencephalographic evidence of acute cerebral injury regularly appeared in all animals ($N = 20$) after injection of sodium arachidonate solution in doses exceeding 0.45 mg/kg: within 15 seconds after an intra-arterial bolus, the amplitude of the background wave pattern became attenuated over the ipsilateral hemisphere and by 60 seconds pronounced suppression of electrical activity was evident. Over the same time period, the contralateral hemisphere showed polymorphous slow waves of progressively increasing amplitude together with some preserved fast activity (Fig. 1). Injection of the solvent alone in five rats or of sodium arachidonate in concentrations of less than 0.36 mg/kg in ten rats produced no more than minor transient alterations of the electroencephalogram. The electrocardiogram remained normal throughout the evolution of the irreversible cerebral insult (*10*).

Whether any change in blood flow through the brain took place following injection of arachidonate solution was assessed by means of an indicator-dilution technique using carboxyl-[^{14}C]inulin (*11*). Blood flow through both cerebral hemispheres averaged 84 ± 19 μl sec^{-1} in three rats immediately before injection and remained unchanged in two animals after injection of solvent alone. Five minutes after carotid injection of the arachidonate (1.2 mg/kg), cerebral blood flow fell to 36 ± 5 μl sec^{-1}, a decline of approximately 50 percent. Postmortem examination confirmed the suspicion that unilateral vaso-occlusion had occurred in the rats. The affected left cerebral hemisphere was grossly hyperemic, while the contralateral hemisphere, cerebellum, and lower brain stem appeared normal. The carotid arteries and dural sinuses were patent, and the cerebrospinal fluid was clear.

Light microscopic examination (*12*) of coronal sections of the cerebral hemisphere showed intense congestion of arteries and arterioles within the carotid territory ipsilateral to the side of the injection. Numerous capillaries were distended with amorphous material which stained strongly with periodic acid–Schiff reagent. Except for occasional hyperchromatic neurons, all neural elements appeared normal (Fig. 2A). Electron microscopic observations (*13*) localized the site of vascular obstruction to the arterial side of the microcirculation. Precapillary arterioles and larger capillaries were completely occluded with platelet aggregates which contained a few erythrocytes but no fibrin (Fig. 2B). The noninjected right hemisphere, cerebellum, and lower brain stem appeared normal, as did the lung, heart, kidney, and liver.

The present study has thus demonstrated that complete and irreversible occlusion of the microcirculation of a single cerebral hemisphere can be produced by an internal-carotid injection of sodium arachidonate. Ordinarily, arterial thrombosis is a tripartite process requiring (i) a preexisting arteriopathy with (ii) aggregation of platelets at the site of arterial injury climaxed by (iii) local formation of fibrin (*2*). In this model, the arteries are normal, and the clotting cascade has been interrupted with heparin. Hence, occlusion of the cerebral microcirculation has been induced by a mechanism mediated solely by platelets and triggered by a chemical stimulus.

Platelet aggregation undoubtedly plays a major role in the pathogenesis of many cases of ischemic cerebrovascular disease in man. Whether transient elevation of an endogenous compound like arachidonate acts as a metabolic trigger of thrombosis in human disease remains to be proved (*14*). Nevertheless, the model under discussion promises to contribute to better under-

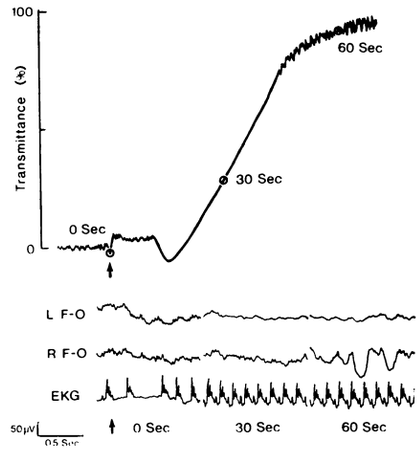

Fig. 1. (Top) In vitro effect of sodium arachidonate (40 μl of 3.3 m*M* solution) on heparinized rat platelet-rich plasma (360 μl) stirred at 37°C. The rise in transmittance measured by a Chronolog aggregometer indicates irreversible platelet aggregation within 60 seconds after adding sodium arachidonate (upper arrow). (Bottom) In vivo effect of 1.2 mg of sodium arachidonate per kilogram (50 μl of 11 m*M* solution) after injection into the left common carotid artery. Electroencephalographic recording shows progressive and irreversible suppression of electrical activity over the left cerebral hemisphere at 30 and 60 seconds, and high-amplitude slowing over the right hemisphere at 60 seconds. (L F-O and R F-O denote left and right fronto-occipital leads). The electrocardiogram shows transient carotid-sinus slowing immediately after injection (lower arrow) but is otherwise unaffected.

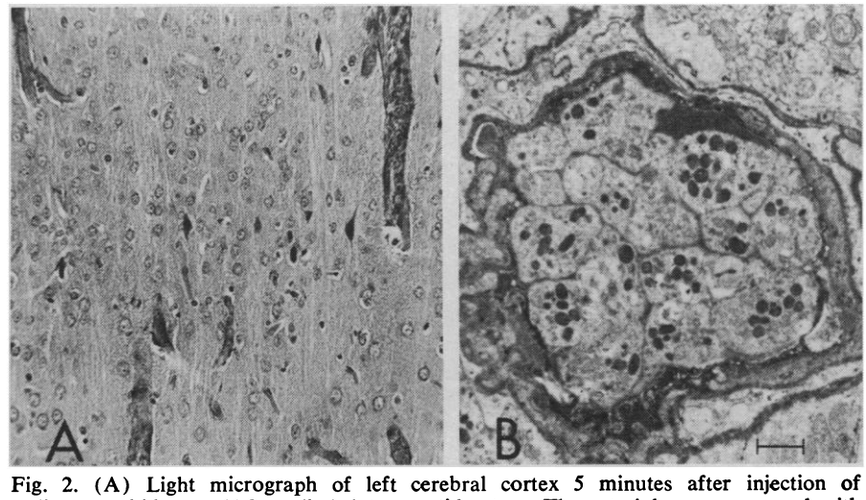

Fig. 2. (A) Light micrograph of left cerebral cortex 5 minutes after injection of sodium arachidonate (1.2 mg/kg) into carotid artery. The arterioles are engorged with erythrocytes, and the capillaries are distended with amorphous material. Neural elements appear normal. (Periodic acid–Schiff–hematoxylin, × 150). (B) Electron micrograph of occluded precapillary arteriole in cerebral cortex 30 seconds after injection of sodium arachidonate. The vascular lumen is obstructed by closely aggregated platelets. No fibrin is present (× 3160).

standing of the biochemical mechanisms of ischemic cerebrovascular disease and perhaps lead to more effective preventative treatment.

THOMAS W. FURLOW, JR.
NORMAN H. BASS

Department of Neurology, University of Virginia School of Medicine, Charlottesville 22901

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5. Tubocurarine chloride injection, U.S.P. (20 units per milliliter, E. R. Squibb, Princeton, N.J.) was injected intramuscularly in a dose of 10 units.
6. Sodium heparin injection, U.S.P. (1000 units per milliliter, Upjohn, Kalamazoo, Mich.) was injected into the right external jugular vein in a dose of 100 units.
7. Liberal infiltration of the anterior cervical region was carried out with xylocaine HCl, 1 percent (Lidocaine, Astra, Worcester, Mass.).
8. A piece of PE-10 tubing (Clay-Adams, Parsippany, N.J.) was inserted into the common carotid artery, and its tip was positioned at the bifurcation of the carotid.
9. Arachidonic acid (>99 percent pure, Sigma, St. Louis, Mo.) was dissolved in a nitrogen atmosphere at 0°C in a clear physiologic-saline solution containing 65.6 m*M* Na_2CO_3 and 10 percent ethanol. Aliquots of the resulting 33 m*M* sodium arachidonate solution were pipetted into glass ampules, frozen in liquid nitrogen, and sealed under vacuum. All ampules were stored in the dark at — 60°C until use. Subsequent dilutions were made with sterile physiologic saline just prior to use.
10. Several rats received carotid injections of arachidonate under ether anesthesia and were allowed to awaken. The details of the unusual clinical picture of irreversible neurologic deficits that resulted are in preparation for publication.
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12. The brain and other organs were removed immediately after death and fixed in 10 percent neutral formalin. The paraffin blocks were cut at 5 μm and stained with hematoxylin and eosin and with periodic acid–Schiff reagent.
13. Small blocks of cerebral cortex were excised from barbiturate-anesthetized rats 30 seconds after injection of the arachidonate solution. The tissue was fixed by immersion in 2 percent glutaraldehyde in 0.1*M* cacodylate buffered to *p*H 7.3, post-fixed in osmium tetroxide, dehydrated in alcohol, and embedded in epoxy. Sections were cut 0.8 μm in thickness and stained with toluidine blue.
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Basal Forebrain and Hypothalamic Connections to Frontal and Parietal Cortex in the Rhesus Monkey

Abstract. *Horseradish peroxidase was injected in different parts of the frontal and parietal cortex in 17 rhesus monkeys. In all cases the enzyme was transported retrogradely to neurons in the substantia innominata and hypothalamus as well as in the thalamus. These new findings demonstrate that these cortical areas receive direct afferent fibers from limbic basal forebrain areas concerned with emotion and motivation.*

As part of the analysis of the motor system of the brain, an attempt was made to determine in the monkey the precise location of the subcortical cell populations which send their fibers to the different parts of the frontal lobe. For this purpose the retrograde transport of horseradish peroxidase (HRP) (*1*, *1a*) from fiber terminals to parent cell body was utilized, since with this technique cells of origin of the subcortical afferents to restricted cortical areas can be demonstrated very effectively (*1a*, *2*). The findings in this study showed that the afferents to the frontal and parietal lobes are not exclusively derived from thalamic cell groups but also come from the substantia innominata and the hypothalamus, thus linking regions involved in the control of mood and motivation to neocortical areas guiding somatic motor and sensory activities. Anatomical evidence for such a link between these functionally different brain structures has heretofore been rather elusive.

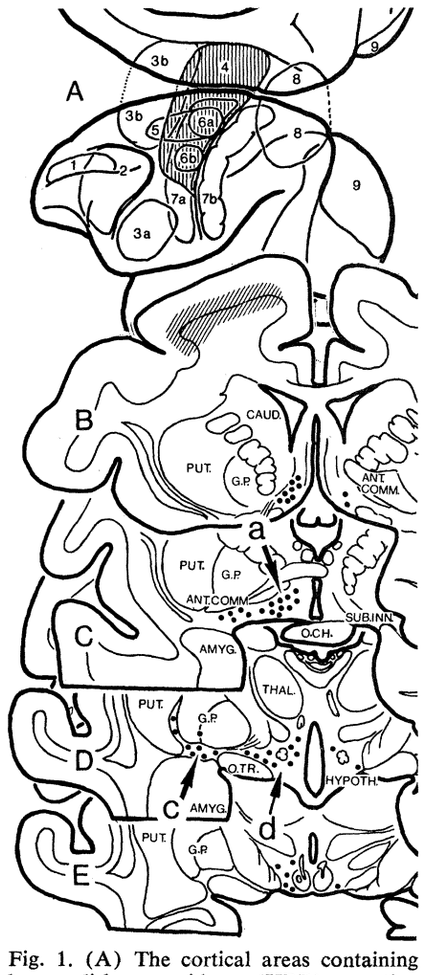

Fig. 1. (A) The cortical areas containing horseradish peroxidase (HRP) reaction products after injection of enzyme in the different lobes of the cases discussed in the text. In cases 3, 6, and 7, injections were made in both the left (*a*) and right (*b*) hemispheres. (B–E) The distribution of HRP-positive neurons in basal forebrain areas of case 4, that is, in the substantia innominata, especially the nucleus basalis, in the medullary laminae of the globus pallidus, and in the hypothalamus.

In 15 rhesus monkeys 6 to 25 closely spaced injections of 0.6 μl of 10 percent HRP (Sigma VI) in distilled water were made in different parts of the precentral gyrus and rostrally adjoining frontal areas, either unilaterally or bilaterally. In three other monkeys, the enzyme was injected in the postcentral gyrus, parietal lobules, and occipital lobe, respectively. In one control animal 30 injections of 0.6 μl of 0.0075 percent saline (approximately the same molarity as the 10 percent HRP) were made in the precentral gyrus. After 3 days the animals were anesthetized and perfused with 6 percent dextran followed by a 0.5 percent paraformaldehyde–2.5 percent glutaraldehyde mixture. The brains were kept in cacodylate buffer with 30 percent sucrose for 3 days, and cut transversally in 40-μm frozen sections which were incubated according to the method of Graham and Karnovsky (*1*), dehydrated, and covered. Some were lightly counterstained with cresyl violet. The material was studied with the microscope under bright-field and dark-field illumination.

In the control animal no retrogradely labeled HRP-positive neurons were found (Fig. 2B). In other animals HRP-positive neurons were present in the thalamus but also in the substantia innominata and hypothalamus, which will be referred to as basal forebrain areas. In some cases labeled neurons