to improve giant axon stability also[5]. In the present work the longevity of lobster axon nodes has been studied as a function of sucrose solution composition. Normal isosmotic sucrose or isosmotic sucrose doped with the chloride salts of sodium, calcium or lanthanum, all matched for equal solution resistivity, were employed. Normal de-ionized sucrose had a resistivity of $1.5\times10^6$ $\Omega$ cm and gave a gap resistance in the absence of an axon of $2\times10^8$ $\Omega$. Doped sucrose had a resistivity of $6.5\times10^4$ $\Omega$ cm and gave a gap resistance of $10^7$ $\Omega$. The doping concentrations were NaCl $2.1\times10^{-4}$ M; $CaCl_2$ $1\times10^{-4}$ M; $LaCl_3$ $6.7\times10^{-5}$ M. Axons were the medial giants from the circumesophageal connective nerve in lobster *(Homarus americanus)*.

The results with sodium-doped sucrose show no improvement over unmodified sucrose and perhaps an exacerbation in the rate of rundown. With calcium there is a significant increase in longevity and a decrease in variation of resting potential. With lanthanum the improvement is even more dramatic. In 15 experiments employing lanthanum as the doping ion no node which was originally viable had to be terminated because of deterioration. This includes several experiments which ran for 60 min and one for 80 min. All were in good condition at the end. In addition, the stability during the experiments was significantly better than in normal sucrose. The figure shows 16 min records of peak sodium current at $-15$ mV and resting potential for 6 nodes from 2 axons in normal sucrose (panels a and c) and another 6 nodes from the same axons in lanthanum-doped sucrose (panels b and d). The smallest decline in resting potential with normal sucrose exceeded the largest decline with lanthanum-doped sucrose and only 1 of the nodes in normal sucrose survived the 16 min.

Lanthanum exerts strong pharmacological effects on active sodium and potassium conductances[6,7] but at the low concentrations used here there is no observable influence. When the artificial sea water bathing the node was switched to one containing $6.7\times10^{-5}$ M lanthanum, using normal sucrose in the gaps, no visual change in the pattern of clamp currents at 0 mV could be detected. Lanthanum has been described as a 'super calcium'[6], being about 20 times more effective in shifting the voltage dependence of active conductances than calcium. The artificial sea water contained $25\times10^{-3}$ M calcium and the addition of $6.7\times10^{-5}$ M lanthanum would be equivalent to raising calcium to $26.3\times10^{-3}$ M, a change which would be undetectable in terms of conductance vs voltage plots. On the other hand the addition of $6.7\times10^{-5}$ M lanthanum to the low ionic strength sucrose could bring about a major reduction in surface potential at the membrane under sucrose. To account for the influence of lanthanum I postulate that the membrane resistance under normal sucrose is very low, because the low ionic strength bathing medium and the absence of multivalent cations allow the existence of a large negative surface potential. According to this hypothesis the influence of lanthanum is to lower the surface potential and thereby raise the resistance back toward the nodal membrane resistance. This, in turn, decreases the perturbations in measured potential associated with changing properties of membrane under sucrose. The findings reported here make the double sucrose gap technique much more attractive as a means to study pharmacological modifications of channel properties because of the improvement in longevity and decrease in drift.

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## Demonstration of vascular redistribution after carotid clamping in rats

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*Summary.* In Long Evans rats, the vertebral arteries supply only the brain stem. Simultaneous application of clamps to both carotid arteries results in a considerable fall of cerebral blood flow and eventually death. If an interval of about 4 days is allowed between the clamping of the 2 carotids, redistribution of blood flow takes place. Owing to this mechanism, 50% of the rats survive with a satisfactory restoration of cerebral blood flow.

In many species the ponto-medullary areas are supplied with blood by the vertebral arteries, whereas the rest of the brain is supplied by the carotid arteries. However, in the rat there is evidence that the vertebral arteries do not supply the brain[2] or that they only supply the spinal bulb-pons area[3]. The latter was demonstrated indirectly by the injection of radioactive microspheres by heart puncture into the left ventricle or into the carotids and by comparing their distribution in the brain: these studies were carried out in healthy animals and it was therefore possible to show any redistribution of cerebral blood flow, which occurs in certain disease states. In our study a direct method was used to investigate the changes of cerebral blood flow after simultaneous clamping of both internal carotids. The redistribution of cerebral blood flow was analyzed allowing a time interval between the ligation of the 2 vessels.

*Material and methods.* 1. Ligation of the carotids. Male Long Evans rats weighing $200\pm20$ g were anesthetized with chloral hydrate (300 mg/kg i.p.). After careful dissection the internal carotid arteries were ligated just above the bifurcation. The ligation of the 2 arteries were performed simultaneously or at an interval of 15 min, 24 h, 48 h, 72 h,

5, 7 or 15 days. The rats were anesthetized once if the interval did not exceed 15 min, but otherwise they were anesthetized twice.

2. Mortality. The number of deaths was recorded each day for 60 days following the 2nd ligation and a graph showing the survival was plotted.

3. Cerebral blood flow. Two methods were used. a) Total cerebral blood flow was determined using a diffusible indicator technique; 2 μCi of iodine-125 labeled iodoantipyrine were injected into the vein of the penis 1 h after the 2nd ligation. Blood samples were then taken every 5 sec for 30 sec, after which the rats were killed. The brain was rapidly removed and the 2 hemispheres separated. The radioactivity was determined by $\gamma$-spectrometry and the blood flow calculated on the basis of Sapirstein's equation[4]: C.B.F. = cardiac output × brain activity (as percentage of the amount injected) × K. Cardiac output was determined by the changes in blood radioactivity and K is the diffusion coefficient of iodoantipyrine (1.76 under these conditions).

b) Distribution of cerebral blood flow in 8 different brain areas was determined by injection of cerium 141 labeled microspheres (15 μm) 60 days after the 2nd ligation. 120,000 of these microspheres were injected by heart puncture into the left ventricle. 1 min later, the animals were killed; the brains were removed as quickly as possible and the following structures were isolated, spinal bulb, pons, cerebellum, mid-brain, hypothalamus, striatum, hippocampus and cortex. The results were expressed either as a percentage of the injected radioactivity recovered from the isolated area of the brain or as a percentage of the total radioactivity in the brain. The results were compared with those determined in rats after simultaneous ligation of both carotids.

*Results and discussion.* Simultaneous clamping of both carotids in Long Evans rats produced 100% mortality. Most rats died within a few hours after clamping, although, some survived for several days. Figure 1, which shows the survival rate in relation to the time interval between the ligation of the 2 carotids, indicates that an interval of about 4 days was required to allow a 50% survival. When this interval was between 8 and 10 days, all the animals survived.

The investigation of the cerebral blood flow, measured using a diffusible indicator, showed that redistribution can occur (fig. 2). When the ligation of both vessels was performed simultaneously, the cerebral blood flow fell by 70%. These results confirm the findings of Siesjo[5], who recorded a considerable fall in the cerebral blood flow after such a procedure. The diminution of blood flow was inversely

Distribution of cerebral blood flow measured by injection of labeled microspheres into the left ventricle of the heart. The measurements were performed in control rats and in rats 1 h after simultaneous ligation of both carotid arteries

| Cerebral structure | Distribution of cerebral blood flow | |
|---|---|---|
| | Control animals | Animals after bilateral ligation of the carotid arteries |
| Bulb | 11.7±2.5 | 29.9±3.9** |
| Pons | 5.9±0.5 | 12.7±2.2** |
| Cerebellum | 22.9±1.3 | 8.2±0.5** |
| Mid-brain | 10.9±0.5 | 0.5±0.2** |
| Hypothalamus | 3.1±0.2 | 0.8±0.1** |
| Striatum | 10.9±0.5 | 1.3±0.2** |
| Hippocampus | 8.1±0.3 | 0.3±0.1** |
| Cortex | 81.6±1.9 | 5.1±0.5** |

Percentage of injected radioactivity × $10^{-2}$ (X ± SEM, n = 6). **p ≤ 0.01, comparison between control and operated animals.

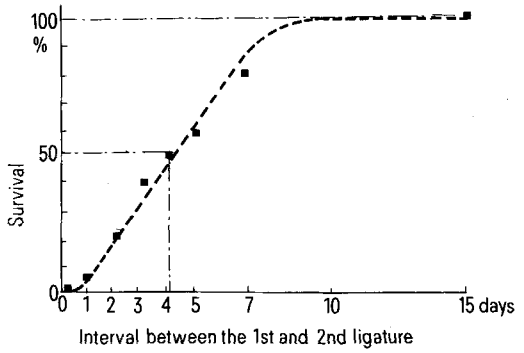


Figure 1. Survival of Long Evans rats after ligation of both carotids. (n = 10 per day).

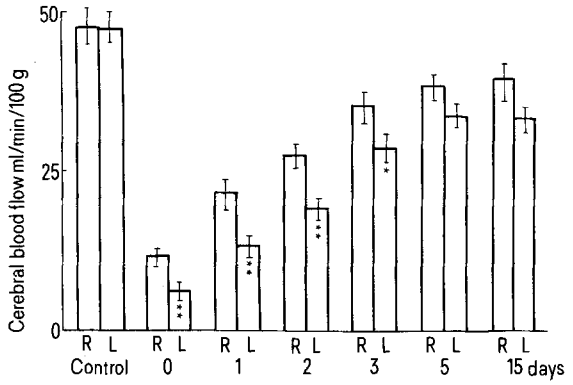


Figure 2. Cerebral blood flow in the right (R) and left (L) brain halves as a function of the time interval between the clamping of the 2 carotids. The 1st ligature was made on the right side, n = 5, X ± SEM, * ≤ 0.05, **p ≤ 0.01. Comparison between the right and left sides.

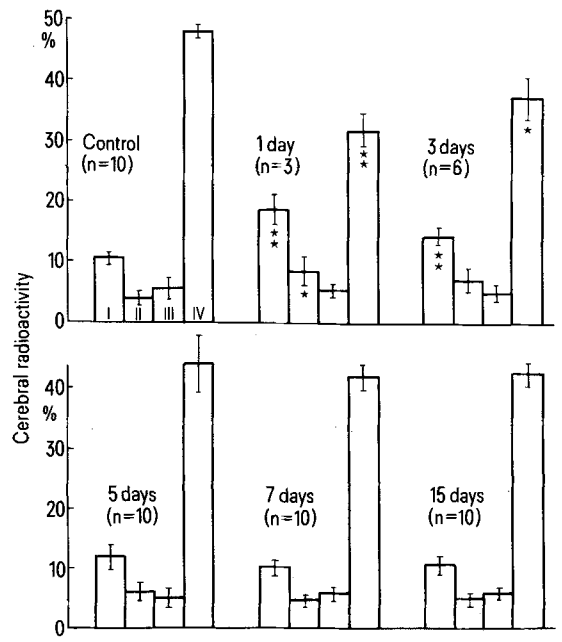


Figure 3. Regional distribution of cerebral blood flow 60 days after the 2nd carotid ligature. The results are expressed as the percentage of the whole brain activity after injection of labeled microspheres. The distribution depends on the delay between the 2 ligatures (1 day, 3 days, 5 days). The studied structures were: spinal bulb pons (I), mid brain (II), striatum (III) and cortex (IV). Mean ± SEM, *p ≤ 0.05; **p ≤ 0.01. Comparison between control and ligated rats.

related to the time-interval between the 2 ligations; the greater the interval the smaller the difference between the right and the left sides of the brain. These results demonstrate an almost complete redistribution of blood flow between the right and the left hemispheres if sufficient time is allowed between the 2 ligations (4–5 days). Initially, there was a marked reduction in blood flow in those structures supplied by the carotids e.g. the cortex, striatum, hippocampus, mid-brain and hypothalamus. In contrast, there was an increased blood flow to the brain stem (table). The blood supply to the cerebellum was greatly reduced, but not as dramatically as in other areas of the brain; this suggests that the supply to the cerebellum is not entirely carotid-dependent.

Our results show the role of the carotid and vertebral arteries in supplying blood to the brain. In Long Evans rats the circle of Willis cannot ensure immediate redistribution of the blood flow to all areas of the brain after bilateral ligation of the carotid arteries. However, when a time interval is allowed between the clampings, an apparent redistribution occurs via the circle of Willis and probably via anastomoses of other arteries of the vertebrobasilar axis.

Two months after ligation of the carotids, the surviving rats still showed differences in the distribution of the cerebral blood flow. In particular, it was noted that animals surviving carotid ligatures made at an interval of 24 h had a low blood flow to the cortical areas but a good supply to the brain stem (fig. 3). A similar but less marked result was obtained for animals in which ligatures were made at an interval of 3 days. The distribution of blood flow in rats where the interval between ligatures was 5, 7 and 15 days was very similar to that found in control rats. It is suggested that in some animals rapid redistribution can occur, leading to a new equilibrium which is then maintained.

The results here show that in Long Evans rats the cerebral trunk is supplied by blood from the vertebral arteries. In some rats this system can compensate for carotid failure. However, it is necessary for a certain time to elapse before there is optimal function of the system.

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## Aortic fibrous components in exercised rats

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*Summary.* In exercised female rats, the elastin content of the thoracic and abdominal aorta decreased by 4–8% ($p < 0.05$). The collagen content in the thoracic aorta, was unchanged but in the abdominal aorta was reduced by 5.2% ($p < 0.05$). These results are discussed in connection with physical training.

The metabolism of collagen and elastin in mammalian arteries is affected by various factors. Collagen production in rabbit aorta is enhanced by simultaneous administration of epinephrine and thyroxine[2] and by hypoxia[3]. In addition, collagen and/or elastin synthesis in the rat aorta and/or mesenteric artery is augmented by hypertension[4,5]. Thus, sympathetic activation and augmented secretory activity of the adrenal medulla during exercise appear to have an effect on the metabolism of arterial fibrous protein.

We estimated the amounts of collagen and elastin in rat aorta after forcing the rats to run on a treadmill, and the role of physical exercise in the metabolism of these components was considered.

5-week-old female Wistar rats weighing approximately 100 g before training were housed in 2 groups (C and R) and maintained on laboratory chow (Oriental Yeast Co. MF., Japan) and water ad libitum. The room temperature was kept at 19±1 °C. Group C (27 animals) served as the control (sedentary rats), while group R (24 animals) was forced to run on a treadmill. In the initial stage of the experiment, the rats ran at 10–26 m/min, on a slope of 10 °C for 1 h/day, 6 days/week. After 4 weeks, the same rats were made to run continuously at 30 m/min for 1 h/day. The entire experiment lasted 12 weeks. The animals were decapitated 24 h after the last exercise and the thoracic and abdominal aortae were immediately removed and perfused with a chilled solution of physiological saline (4 °C, pH 7.2–7.4). After blotting to remove the adherent fluid, the aortae were frozen in dry ice-acetone and stored at −80 °C until assay. The aortic materials were dried for 48 h at 100 °C and then immersed in acetone for 24 h and in ether for 18 h. Subsequently, they were dried for 24 h at 100 °C to prepare the dry defatted tissue. The separation of collagen and elastin in the dry defatted tissue and hydroly-

Effects of daily forced treadmill running on the contents of collagen and elastin in the rat aorta

| | Collagen (%) | Elastin (%) | |
|---|---|---|---|
| Thoracic aorta | | | |
| C | 28.6±0.7 | 54.9±0.6 | n=27 |
| R | 28.7±0.4 | 52.4±0.6* | n=24 |
| Abdominal aorta | | | |
| C | 42.2±0.7 | 38.1±0.4 | n=27 |
| R | 40.1±0.7* | 35.4±0.5* | n=24 |

C, sedentary rats; R, trained rats. Average of 24–27 tests. ±SE. *Statistical significance ($p < 0.05$).