

recorder. Lateral nerves and connectives were stimulated by means of tightly fitting glass suction electrodes. The normal ringer used was that of KUFFLER and POTTER<sup>10</sup>. High  $Mg^{2+}$  ringer contained 20 mM  $Mg^{2+}$ , and the osmolality was maintained by removing an appropriate amount of sodium.

**Results and discussion.** The Figure is a reconstruction of a pair of RETZIUS cells in a segmental ganglion as seen in the cobalt-stained preparations. This technique showed that a major fibre of each RETZIUS cell is found in all the ipsilateral nerves, i.e. one in each lateral segmental nerve and one in both anterior and posterior ipsilateral connectives. Cobalt-staining shows no sign of the connection between the two cells reported by LENT<sup>6</sup>, but shows that there is an extensive dendritic tree within the neuropile. Thus the point of electrotonic coupling described by various authors<sup>2,3,11</sup> may be contained within this tree rather than at a clearly-defined connection.

Stimulation of any of the lateral nerves or connectives was followed by an excitatory event in both RETZIUS cells. These events showed the characteristics of antidromic stimulation<sup>12</sup>, i.e. they failed to fatigue, were blocked by hyperpolarization, followed the stimulation 1:1 with constant latency up to 20 Hz, and were composed of A and S spike components. These results therefore confirm that the RETZIUS cells are electrotonically coupled, and support the presence of axon branches in the lateral nerves and the anterior and posterior connectives.

The excitatory events elicited by stimulation of both lateral nerves and connectives were often blocked by high  $Mg^{2+}$  ringer, especially at low stimulus intensities. As the connection to the lateral nerves is certainly not synaptic<sup>7</sup> it seems that high  $Mg^{2+}$  ringer may, in addition to its effect on synaptic transmission<sup>13</sup>, also block nerve conduction. To test this possibility, a section of connective assumed to contain no synapses was stimulated using a suction electrode. The compound AP produced was recorded extracellularly a short distance away by a second suction electrode. This AP was also reduced in the presence of high  $Mg^{2+}$  ringer, indicating a block in

conduction probably associated with an effect on sodium conductance<sup>14</sup>.

The results of cobalt injection are in contrast to the results of Procion yellow studies performed by LENT<sup>6</sup>, who found no axon branches from the RETZIUS cells in the connectives. The electrophysiological confirmation of this depended on evidence obtained in high  $Mg^{2+}$  ringer. However, as magnesium appears to block nerve conduction as well as synaptic transmission, it is not an adequate criterion for the determination of the synaptic or antidromic nature of excitation.

**Résumé.** Les cellules de RETZIUS de la sangsue (*Hirudo medicinalis*) ont été teintes par injection intracellulaire de sulphide de cobalt. Chaque cellule fait émet une branche majeure dans tous les nerfs segmentaux connectifs (antérieur et postérieur) ipsilatéraux. Il y a aussi des ramifications dendritiques nombreuses dans la neuropile. Ces découvertes furent confirmées par des enregistrements électrophysiologiques en présence ou non de magnésium.

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## Cerebral Cortical Blood Flow in the Rat. Effect of Furosemide

An effect of furosemide on vascular smooth muscle is known to exist in the renal vascular bed<sup>1-4</sup> and the isolated portal vein<sup>5</sup>. Furthermore, the antihypertensive

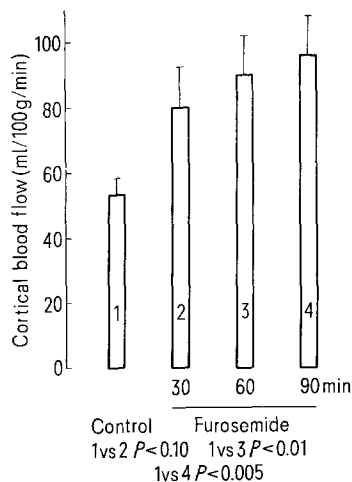


Fig. 1. Cortical blood flow before (1) and at different times after (2, 3, 4) topical application of furosemide (10 mg/ml).

effect of furosemide seems to depend on a systemic vascular action. Desensitization of vascular smooth muscle to noradrenaline by furosemide is also well known<sup>6</sup>. The effect of the drug on blood flow and vascular resistance of cerebral cortex was tested, after topical or systemic administration, on a preparation where blood flow was measured by means of the hydrogen clearance method in rats under urethane anaesthesia.

**Material and methods.** Rats were anaesthetized with i.p. urethane (1.5 g/kg) tracheostomized and fixed to a nose clamp. The left frontoparietal cortex was exposed and a platinized-platinum electrode inserted there. The platinum electrode, and an indifferent silver-silver

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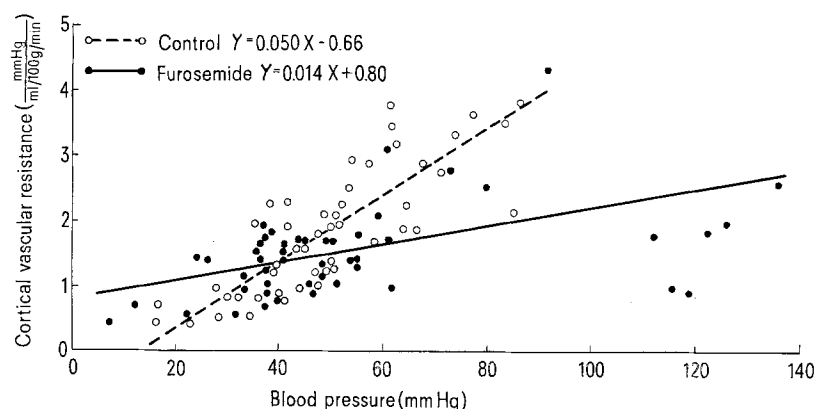


Fig. 2. Cortical vascular resistance as a function of blood pressure in untreated animals (broken line) and in animals given 10 mg of furosemide i.p. (full line).

chloride electrode at the neck were connected to the input of a Keithley 414 A Picoammeter, which output led to a D.C. channel of a Nihon-Kohden polygraph to record permanently the electrocorticogram and the change in hydrogen current. Hydrogen was admitted to the tracheal cannula and the gas flow maintained constant until the current from the tissue electrode showed a steady state. Hydrogen flow to the tracheal cannula was then stopped and the desaturation curve recorded. The half time for washout was determined from the record and local blood flow calculated from it as described elsewhere<sup>7</sup>. To allow the topical application of the drug, a ring, of 3.5 mm internal diameter and 2 mm height, was positioned over the exposed cerebral cortex by means of a micro-manipulator. The rest of the cortex not covered by the ring was sealed with vaseline. The ring was provided with inlet and outlet tubing connected to syringes to allow renewal of artificial C.S.F. in which furosemide was dissolved. In a group of animals, a femoral artery was cannulated in addition and blood pressure permanently recorded by means of Statham P 23 BB transducer connected to a Beckman polygraph.

**Results and discussion.** Topical application of furosemide at the concentration of 10 mg/ml induced a significant increase in cortical blood flow (Figure 1). The change in blood flow did not reach a maximum in the first determination after application but grew steadily as time ensued, a fact probably dependent on diffusion of the drug into the cortex. In the experiments in which the drug was administered systemically, cortical vascular resistance (CVR) was calculated from flow and pressure data. A positive correlation was found between blood

pressure and CVR representative of autorregulation, both in controls and treated animals. The slope of the regression line, however, was significantly lower in the group receiving the drug (Figure 2). From these results it is concluded that systemic furosemide is effective in decreasing cortical vascular resistance, although a correlation between CVR and blood pressure (autorregulation) is still demonstrable under the drug.

The mechanism by which furosemide decreases resistance of cortical vessels cannot be discovered by the present experiments but it probably depends on a direct action on vascular smooth muscle.

**Resumen.** Se estudió el efecto de furosemida administrada tópicamente o sistémicamente sobre el flujo sanguíneo cortical en la rata, medido por el método de la desaturación de hidrógeno. Se encontró que por ambas vías de administración la droga produce una disminución significativa de resistencia vascular cortical.

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## Influence du DDT sur la population germinale des gonades indifférenciées de jeunes embryons de Poulet et de Caille

Dans des travaux antérieurs<sup>1,2</sup>, nous avons mis en évidence un fort déficit en cellules germinales primordiales dans les gonades embryonnaires d'oiseau, sous l'effet d'un pesticide organochloré: le DDT. C'est ainsi, qu'aux stades 29 pour le poulet<sup>3</sup> et 20 pour la caille<sup>4</sup>, l'index gonocytaire des embryons issus d'œufs traités au DDT, à la concentration de 5 g par l d'eau, n'est que de 37% chez le poulet et 43% chez la caille (celui des embryons témoins est de 100%). Le dénombrement des gonocytes en voie de division et de ceux en dégénérescence (cellules hypertrophiées) indique que le DDT n'inhibe que faiblement le pouvoir mitotique mais provoque, par contre, la pycnose de nom-

breuses cellules. Il ne semble pas toutefois que ces deux faits puissent motiver entièrement le fort déficit des gonades en cellules germinales. Pour essayer de déterminer à quel moment le pesticide agit sur la lignée germinale, nous dénombrons les gonocytes dans les ébauches gonadiques à un stade plus précoce du développement, à la fin de leur phase de migration.

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