ceased. The subconvulsive dose of strychnine causes a small increase of the frequency (27.5±2.1 per min) and sometimes of the intensity of the diaphragmatic discharges. After the injection of the subconvulsive dose of strychnine, the threshold of the respiratory reflex was 4 or 5 times less. The respiratory frequency increases till 36±1.7 per min and persists after the cessation of nerve stimulation for about 15–60 sec. The effect of strychnine lasts 15–20 min. Inhalation of hypercapnic mixture as usual evokes an increase of the intensity and frequency of the respiratory discharges (figure 1). After injection of strychnine, inhalation of the same gas mixture increases the respiratory discharges more intensively and activity of new motor units appears (figure 2). Thus the inhibitory synapses play an important role in preventing an excessive hyperventilation. From the times of Sherrington[4], it has been known that each stimulus exerts simultaneously an excitatory and an inhibitory effect: the latter restricts excessive reflexes. Probably the stimuli influencing respiration obey the same rule.

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Staining of isolated rabbit neurons and neuroglial clumps

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Summary. Isolated rabbit neurons stained just as intensely as neuroglial clumps did with Mallory's phosphotungstic acid haematoxylin, Weil and Davenport's, Marsland, Glees and Erikson's and with Gallyas's silver stain.

Isolated neurons and groups of neuroglial cells around them (neuroglial clumps) have been the subject of many studies[2–4]. During an examination of the histology of neurons and neuroglial clumps[5], it was noticed that some of the neurons were stained by the 4 techniques mentioned below[6–9], which are generally described as stains for neuroglia or their processes[10–16]. Therefore, it was decided to investigate the specificity of these stains by seeing if they would also react with neurons.

Neurons and also the adjacent neuroglial clumps (as control samples for the staining) were isolated from the cranial nerve nuclei by hand dissection using stainless steel wires by the techniques of Hydén[17]. They were placed in sucrose solution (0.25 M) in cavity slides to which they were made to adhere to the floor of the cavity. In this series of experiments, groups of at least 150 single neurons or neuroglial clumps were isolated for each staining procedure; about 15 neurons or neuroglial clumps were placed on each slide. The sucrose solution was drawn off with a Pasteur pipette, and the tissues were fixed in 10% formol saline, dehydrated with 50%, 80% and 100% ethanol (3 times), cleared with xylol and embedded in paraffin wax. The neurons or neuroglial clumps were then rehydrated and prepared with the following staining systems: Mallory's phosphotungstic acid haematoxylin[6]; Weil and Davenport's silver stain[7]; Marsland, Glees and Erikson's silver

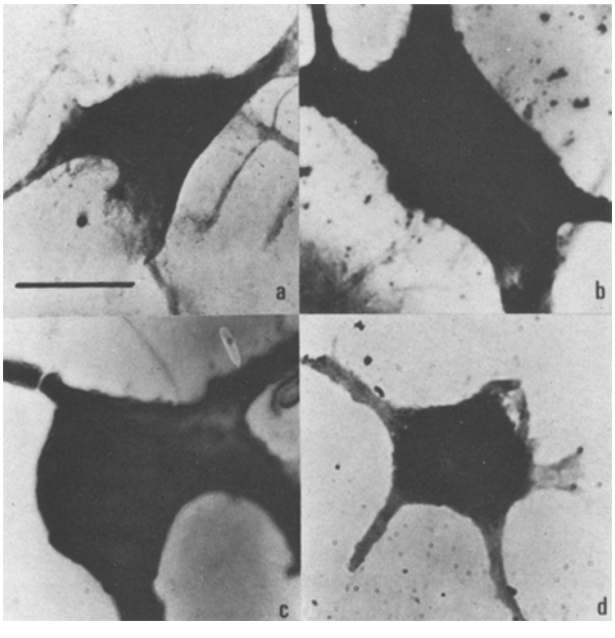

Fig. 1. Isolated rabbit neurons stained with the following systems, a: Mallory's, b: Weil and Davenport's, c: Marsland, Glees and Erikson's, d: Gallyas's. The bars in both figures are 20 µm.

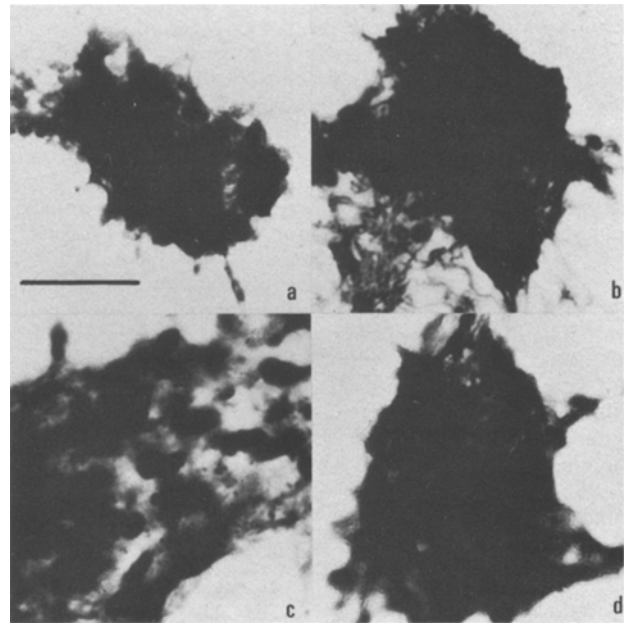

Fig. 2. Isolated neuroglial clumps, stained as were the neurons in figure 1, a–d.

stain[8]; Gallyas's silver stain[9]. However, these 4 procedures stained every one of the isolated neurons as well as the neuroglial clumps (figures 1 and 2), and, therefore, they are not specific for isolated neuroglia. In the present experiments the neurons and neuroglia have not been sectioned but have been subjected otherwise to all the other techniques of the procedures cited[6-9]. However, in a previous paper neuroglial clumps *in sections* were also stained with these reagents[5]. In view of the present results, we are investigating whether identified neuronal cell bodies in cerebral sections also stain with neuroglial stains.

1 We wish to thank the Handicapped Children's Aid Committee, London, for generous support and a Unity Research Fellowship to K.D.

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Activation of the electrogenic Na-pump of cardiac muscle fibres by ACh in K-free solutions[1]

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Summary. The ionic mechanism of the membrane outward current (ACh-current) of bullfrog atrium muscle, induced by acetylcholine in K-free solution, was analyzed by a voltage-clamp experiment. The results suggested that the ACh-current was induced not only by an increase in K-conductance but also by an activation of the electrogenic Na-pump.

It has been known for long that the membrane of cardiac muscle fibres is hyperpolarized by the action of acetylcholine (ACh)[3-6]. This hyperpolarization has been suggested to be caused by an increase in K-permeability of the membrane on the basis of experimental evidence that the membrane conductance[7,8] and K^+ outflux[9] increase during the hyperpolarization. The present communication reports a new experimental finding that the membrane hyperpolarization induced by ACh in the K-free solution seems to be caused not only by an increase in K-permeability but also by an activation of the electrogenic Na-pump.

Material and methods. Muscle strips (300–500 μm in diameter) isolated from atrium of bullfrog *(Rana catesbeiana)* were used. Recordings of membrane potentials and currents were made by use of an experimental arrangement designed for a voltage clamp experiment[10]. Ionic compositions (mM) of the Ringer solution were 112 NaCl, 2.0 KCl, 0.1 $CaCl_2$, 6 $MgCl_2$, 2.4 $NaHCO_3$ and 2.5 glucose; a lower calcium level was used to reduce contraction and facilitate the prolonged maintenance of the microelectrode in individual cells. The K-free solution was prepared by omitting KCl from the Ringer solution. The sucrose solution used for sucrose-gap contained 230 mM sucrose. The muscle fibres were first equilibrated for 1–2 h in the K-free solution and the experiment started thereafter in this solution at room temperature. The resting membrane potential at this stage was -44 ± 2 mV (SE), n=22.

Results and discussion. The muscle fibres were markedly hyperpolarized by ACh (figure 1, a) in K-free solution; the mean amplitude of the hyperpolarization was 28 ± 4 mV, n=7, at a concentration of 10^{-5} M. The minimal effective concentration to produce the hyperpolarization was 10^{-7} M. During an application of ACh, a large membrane outward current (ACh-current) was produced when the membrane was held at the resting potential level (figure 1, b); the mean amplitude of the outward current induced by 10^{-5} M ACh was 1.6 ± 0.1 μA, n=20.

An interesting finding was that the ACh-current recorded in the K-free solution was sensitive to ouabain. It reduced to $14\pm4\%$, n=7, of its control value within 10 min of an application of 10^{-6} M ouabain (figure 1, c). A small part of ACh-current was, however, resistant to the action of ouabain; it was observed for more than 1 h in the presence of 10^{-5} M ouabain. These results suggest that ACh-current in K-free solution consist of 2 different current components, namely ouabain-sensitive and ouabain-insensitive ones. Both current components were completely blocked by an application of 10^{-5} M atropine to the perfusate.

The membrane conductance was increased by ACh to 1.8 ± 0.1, n=14, times larger than its control value. This

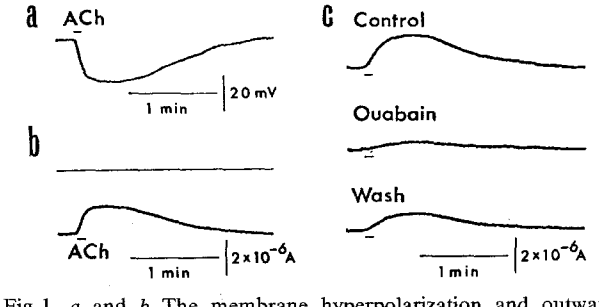

Fig. 1. *a* and *b* The membrane hyperpolarization and outward current by 10^{-5} M ACh, respectively; upper trace in *b* is for voltage recording. *c* The effect of 10^{-6} M ouabain on the ACh-current; the upper and middle records are before and 5 min after application of ouabain, respectively, and the bottom record is 10 min after removal of ouabain. Short horizontal bars in *a*, *b* and *c* indicate periods of 10^{-5} M ACh application.