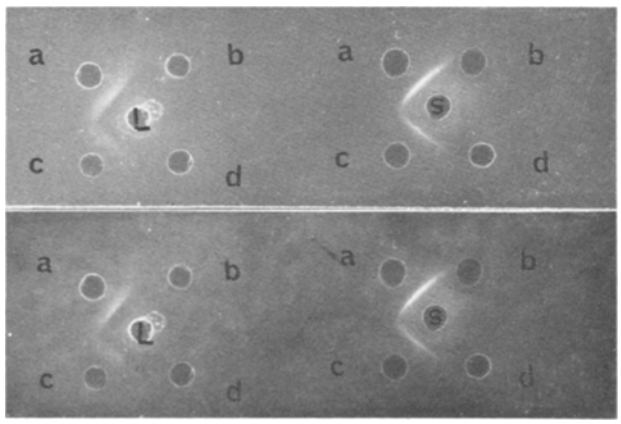
voided urine from the right kidney and the urine from the hydronephrotic left kidney were compared by the Ouchterlony technique with respect to the presence of the organ-specific antigens described above. No precipitation lines developed when urine from the hydronephrotic sac was reacted with antisera to the sediment and lipid fraction of the accessory genital glands. As expected, the spontaneously voided urine, passing through the urinary bladder and urethra, contained an antigen precipitating with the 2 antisera (figure). It is noteworthy that this reacting antigen is of high molecular weight, since it was excluded from a G-200 Sephadex column.

SELLERS[1] is of the opinion that proteinuria in the rat stems solely from the kidneys. THUNG[6] reports that in the mouse no proteins other than those of renal origin are demonstrable. RUEMKE and THUNG[7] believe that the mouse urine contains a protein produced in the liver, which is concentrated and excreted by the kidneys. The observations reported herein unequivocally demonstrate that the spontaneously voided urine of the male rat contains at least one proteinaceous component which is not excreted or secreted by the kidneys. This component has been found to be serologically organ-specific for the male accessory genital glands; its absence from the renal pelvic urine proves that non-renal tissue constituents are contributed to the proteinuria of the normal male rat[8].



Double immunodiffusion tests of antisera directed against the lipid fraction (L) and sediment (S) of the male accessory genital glands with the spontaneously voided urine (a and c) and renal pelvic urine (b and d). With the 2 urine specimens illustrated, a precipitation band developed only when the antisera were reacted with the voided urine.

Zusammenfassung. Es wird gezeigt, dass bei gesunden männlichen Ratten nur Urin, der die unteren Harnwege passiert, Bestandteile der Geschlechtsdrüsen enthält. Somit ist die normale Proteinurie zum Teil auf die Ausscheidung extrarenaler Gewebskomponenten zurückzuführen.

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Comparison of the Effects of Atropine Sulfate and Procaine Hydrochloride on Nerve Conduction

In interpreting the central action of cholinomimetic agents, atropine sulfate is frequently used as a blocking agent to determine whether the action is mediated by a nicotinic or muscarinic mechanism. The question arises whether it is acting as a muscarinic blocking agent or as a nonspecific local anesthetic, since the chemical structure of atropine has certain similarities to cocaine. Many atropine analogues[1,2] have been reported to have local anesthetic activity. In 1960, CURTIS and PHILLIS[3] demonstrated that atropine sulfate behaved similarly to procaine HCl in depressing the spike potentials of spinal neurons evoked by chemicals (acetylcholine, glutamate ions) and nerve stimulation (ventral-root).

In reviewing the literature, the only paper that quantitatively describes the local anesthetic effect of atropine sulfate was published in 1948 by DE ELIO[4]. Atropine was reported to have half the potency of procaine by the method of i.c. injection in guinea-pigs. Although this is a useful screening method for local anesthetics[5], it is possible that atropine may appear active in this test because of interference with pain receptor mechanisms. The local anesthetic activity of atropine has therefore been reinvestigated on the isolated frog sciatic nerve in order to obtain a more direct assessment of its properties.

Methods. Frog Ringer: The following solution was used throughout the experiments: NaCl, 110 mM; KCl, 2.7 mM; $CaCl_2$, 1.8 mM; *Tris*-(hydroxymethyl)-aminomethane, 1.0 mM and EDTA, 0.1 mM. The solution was adjusted to pH 7.4 with HCl. Each test solution was made up by dissolving the drug in the above solution and the pH was readjusted to 7.4 by adding dilute NaOH solution.

Nerve-bath: A small plastic rectangular tissue bath of $1.0 \times 2.0 \times 4.0$ cm^3 with a plastic cover was used. 9 platinum electrodes were arranged as shown in Figure 1, so that 2 of them (6 and 7) were immersed in the solution when it was partially filled (3 ml of the test solution). The distance between the platinum electrodes was 0.5 cm. During the experiment the nerve-bath was filled with 3 ml of the test solution. The nerve was threaded through the electrodes so that it lay above electrodes 1–5, below electrodes 6–7 and above electrodes 8–9, ensuring that a constant length of nerve was immersed in the test solution. The nerve was stimulated at its peripheral end through electrodes 1 and 2, by supramaximal square wave pulses (0.15 ms, 0.5/s) from a Grass S4B stimulator and General Radio 578A isolation transformer. Action potentials were recorded simultaneously between electrodes 4 and 5 and electrodes 8 and 9 using a Textronix 502 oscilloscope. Electrodes 3, 6 and 7 were connected to ground.

Preparation of nerve: Frogs (*Rana pipiens*) of 2 to 2.5 inch length were used throughout the experiments. The frogs were decapitated and the sciatic nerve removed in the usual way. The nerve was immersed in frog Ringer's solution and kept overnight in the refrigerator. It was transferred to fresh solution at room temperature for 30 min and then mounted in the bath in such a way that the same segment of nerve was always immersed in the solution. The experiments were carried out at room temperature (24 °C). The progress of the block was followed photographically (Dumont Type 297) or by observation of the oscilloscope until the action potential was reduced to about 30%, when the drug solution was aspirated and replaced by fresh solution. The nerve preparation was removed from the bath, washed repeatedly with fresh solution and placed in the refrigerator until the next trial, which was carried out after an interval of at least 5 h.

Results. The Table summarizes the results obtained. Although the rate of conduction block increased somewhat on repeated exposure to 0.2% procaine, this increase

Time required for procaine hydrochloride or atropine sulfate to produce a 50% reduction in the action potential of an isolated frog sciatic nerve

No. of experiments	Drug	Concentration %	Concentration m*M*	Order of test	Time to 50% block (min) Mean ± S.E.
12	Procaine HCl	0.2	7.4	1	7.5 ± 1.9
		0.2	7.4	2	6.5 ± 1.2
		0.2	7.4	3	5.0 ± 1.0
7	Procaine HCl	0.1	3.7	1	16.6 ± 3.5
		0.2	7.4	2	3.8 ± 0.7
		0.4	14.8	3	1.6 ± 0.3
7	Atropine SO_4	4.0	60.0	1	15.2 ± 2.2
		8.0	120.0	2	2.4 ± 0.3
9	Procaine HCl	0.1	3.7	1	14.5 ± 5.0
	Atropine SO_4	4.0	60.0	2	6.3 ± 2.1

In each group of experiments 2 or 3 sequential tests were carried out on the same nerve preparations in the order indicated in the third column.

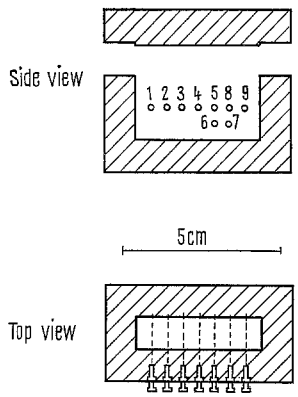

Fig. 1. The nerve bath.

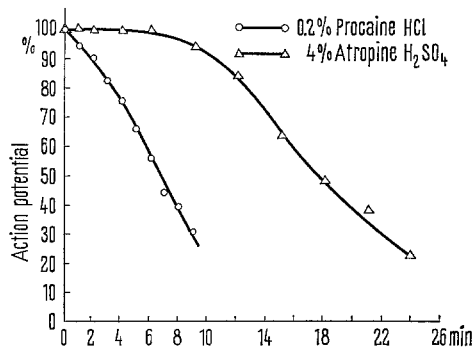

Fig. 2. The kinetics of conduction block for 4% atropine sulfate and 0.2% procaine HCl.

was barely significant ($0.05 > P > 0.02$ for paired *t*-test between first and last trials). The rate of block was very clearly dependent on concentration of both procaine and atropine, and comparison of the 2 agents indicates that procaine is 20–40 times more active on a weight basis when measured in this way. At a concentration of 2% atropine sulfate failed to produce a 50% block in 6 of 8 preparations within 60 min. Figure 2 compares the kinetics of conduction block for 4% atropine and 0.2% procaine in a representative nerve. The long latent period before the onset of atropine block was seen consistently and is in marked contrast to the immediate effect of procaine.

Conclusions. While a number of in vivo techniques are of value in the screening of compounds for potential use as local anesthetics, direct electrophysiological investigation of the conduction properties of an isolated frog sciatic nerve offers significant advantages in the precise definition of local anesthetic potency[6–8]. When atropine sulfate is compared to procaine hydrochloride in this way, its relative potency is less than 1:20 on the basis of weight or molarity. The concentration of atropine required to produce a consistent 50% block within 60 min was 4% or 60 m*M*, and the sulfate ion may have played a significant role, since the Ca^{++} concentration would be depressed and it is known that Ca^{++} is required for propagated action potentials.

From these results it is concluded that a local anesthetic action of atropine is unlikely to play a significant role in modifying the effects of other drugs or of nerve stimulation when it is injected intracerebrally in concentrations of the range of 1–5 mg/ml.

Zusammenfassung. Die lokalanästhetische Wirksamkeit des Atropinsulfats wurde mit der des Novokains mittels Messung der Kinetik des Nervenimpulsblocks am isolierten Nervus ischiadicus des Frosches verglichen. Die relative Wirksamkeit des Atropinsulfats ist weniger als 1:20.

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