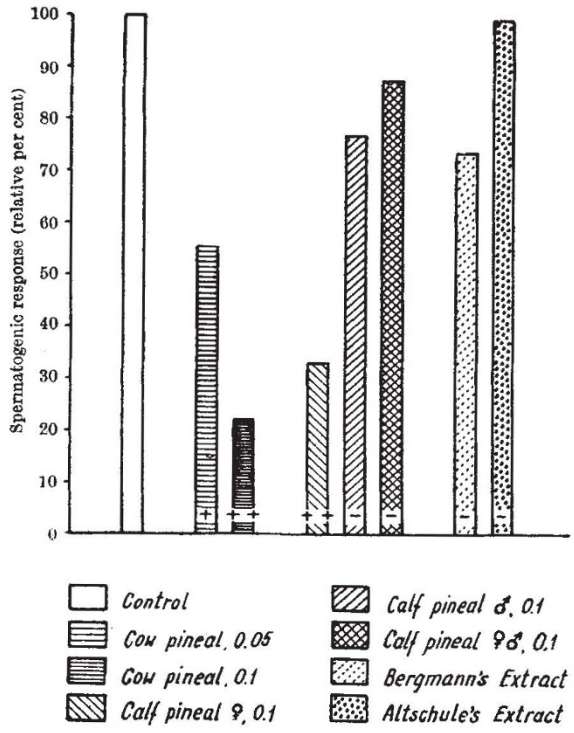

Fig. 1. Inhibition with pineal preparations of male frog spermatogenic reaction. ++, $P < 0.01$; +, $P < 0.05$; −, $P > 0.05$

connected with the sex of the animals used as pineal tissue donors. Moreover, it should be pointed out that after an application of 50 mg of cow pineal gland the inhibition of the frog reaction was nearly twice as weak as after a dose of 100 mg. It is to be expected that the described reaction of the inhibition of the frog spermatogenic test might be applied as a method of biological assay of the anti-œstrogenic properties of the pineal gland. Although several methods have already been suggested[1,2,10] for assaying the potency of pineal preparations, our method seems to have some advantages because of its simplicity, speed and cheapness.

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Excretion of Neostigmine labelled with Carbon-14 in Urine

Although neostigmine (prostigmin) has been used for about thirty years for the treatment of myasthenia gravis, and later for the post-operative reversal of tubocurarine, relatively little seems to be known about the mechanisms involved in its metabolism and excretion. The inhibition of plasma cholinesterase has been used as a convenient indicator of neostigmine concentration, and it has been shown that after administration to dogs and to patients with myasthenia gravis, elimination of neostigmine depends on the dose and method of administration[1]. Some of these conclusions have been confirmed in more recent work where a colorimetric method was used for the measurement of neostigmine in urine[2]. Thus when neostigmine methylsulphate is given by intramuscular injection to patients with myasthenia gravis, about 50 per cent of the dose is excreted unchanged, whereas after oral administration little or no unchanged drug can be detected in the urine[2,6].

Further work in this laboratory has shown that a high proportion of each dose by intramuscular injection is excreted by myasthenic patients within 2 h. Confirmation that neostigmine is rapidly excreted was obtained by administration of neostigmine labelled with carbon-14 to rats, when up to 50 per cent of the dose was detected in the urine within 60 min of intramuscular injection. This evidence suggested that the rapid excretion of neostigmine might be due to renal tubular secretion.

Using the technique for estimating renal tubular secretion in the hen described by Sperber[7,8], we have found that after intramuscular injection of labelled neostigmine considerably more of the dose was excreted by the ipsilateral kidney than by the contralateral kidney (Table 1). Similar results were obtained when the drug was infused at a constant rate into the saphenous vein.

Table 1. Renal Excretion of Neostigmine by the Hen after Intramuscular Injection of Neostigmine labelled with Carbon-14

Hen No.	Dose neostigmine (mg)	Percentage of dose recovered in urine from each kidney		Duration of collection (min)
		Ipsilateral	Contralateral	
1	0·667	39·7	5·7	90
2	0·333	65·2	16·1	90
3	0·333	59·8	7·7	120
4	0·333	57·4	6·5	135

The renal tubular transport of *N'*-methylnicotinamide (NMN) and of a number of other quaternary nitrogen compounds has been shown to be inhibited by the basic cyanine dye, 1'-ethyl-3,6-dimethyl-2-phenyl-4-pyrimido-2'-cyanine chloride ('Cyanine 863')[3–5]. Experiments in which labelled neostigmine was injected in single doses and by continuous infusion into the saphenous vein of the hen have shown that the apparent tubular excretion factor is substantially reduced by prior or concurrent administration of 'Cyanine 863'.

We conclude from this evidence that when neostigmine is injected a substantial proportion of the dose is eliminated by renal tubular secretion.

The urine collected in the experiments on hens has been examined by paper chromatography and electrophoresis; most of the carbon-14 is present as neostigmine but there is also evidence of *m*-hydroxyphenyltrimethylammonium, a metabolite of neostigmine previously reported by Scott *et al.*[6].

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