and the surrounding connective tissue of the stomach and intestine; (6) the transformation of the miracidia into the mother sporocysts; and (7) all stages up to the emergence of the cercariæ.

The consistent evidence, stage by stage, of the hatching, penetration and metamorphosis phenomena within the snail indicates for the first time the exact locations and methods by which the snail becomes infected with these *Reniferine trematodes*. Information is now available indicating the method by which those families of trematodes whose eggs do not hatch *in vitro* are able to infect appropriate snails and to proceed with development within those molluscs.

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Choline-Esterase Content of Normal and Denervated Submaxillary Gland of the Cat.*

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The heightened sensitivity of the cat's submaxillary gland to sympathetic stimulation, following degenerative section of the chorda tympani, appears to be due to some spontaneous activity of the decentralized parasympathetic supply, the phenomenon thus being analogous to the well-known "augmented secretion".[1] Histological examination of the unstimulated paralytic gland, indeed, reveals changes characteristic of activity in the demilune cells,[2] which are normally controlled by the sympathetic.[3] An attractive hypothesis is that the latter elements are sensitized by acetylcholine which is liberated elsewhere in the gland through the spontaneous activity of the postganglionic neurones, and which reaches the demilune cells by diffusion. After degenerative section of the chorda tympani, how-

* During the preparation of this paper for publication, a paper by F. Th. v. Brücke has appeared (*J. Physiol.*, 1937, **89**, 429) in which it is shown that section of the cervical sympathetic nerve in the cat greatly diminishes the choline-esterase content of the superior cervical ganglion. This case is clearly not analogous to that of the submaxillary gland, in which doubtless only a small part of the total esterase content has a special relation to the terminations of the (preganglionic) fibres which are cut.

1 MacIntosh, F. C., and Rawlinson, H. E., *Quart. J. Exp. Physiol.*, 1935, **25**, 199.

2 Rawlinson, H. E., *J. Anat.*, 1935, **70**, 143.

3 Rawlinson, H. E., *Anat. Rec.*, 1933, **57**, 289.

ever, there is no increase, but a decrease, in the acetylcholine equivalent of the submaxillary gland.[4] Since the latter term includes both combined and free acetylcholine, it might still be possible that in the paralytic gland there is a higher concentration of the *free* ester. Thus it might be thought that denervation impairs the ability of the gland to inactivate acetylcholine which has been liberated within it, by reducing the gland's supply of choline-esterase.

Choline-esterase occurs in different tissues, as well as in the blood, in widely varying concentrations, the richest sources being, in general, those organs possessing a cholinergic innervation.[5] There has hitherto been no evidence to indicate whether or not the amount present in any organ is affected by section of the nerve supply to the organ. I have therefore made a series of determinations of the rate at which acetylcholine is destroyed by simple extracts of paralytic, as compared with normal, submaxillary glands.

The method used was a modification of that of Plattner and Hintner,[5] acetylcholine being assayed on the eserinized *rectus abdominis* of the frog, as described by Chang and Gaddum.[4] Aseptic section of the chorda-lingual trunk was performed on one side 12 to 20 days before the determinations. Under chloralose-urethane anesthesia the submaxillary glands were perfused with Ringer's solution at body temperature until blood-free, then rapidly dissected out of their capsules, dried superficially, and weighed. The glands were then ground with washed quartz sand in 3 volumes of M/3 phosphate buffer (pH 7.4). (The denervated gland weighed on the average 20% less than the normal, confirming Langley.[6]) At least 30 minutes' vigorous grinding was required for each gland; otherwise low values for the esterase content were obtained. The residue from the grinding, after twice washing and centrifuging, yielded very little esterase activity on repeated grinding; the great bulk of the enzyme was therefore presumably extracted. The extracts were covered and allowed to stand 3-4 hours at room temperature. Six volumes of water were added to make the final dilution of the tissue tenfold, and the mixture again centrifuged and decanted. For the assay, one cc. of the extract was added to one cc. of a 1:10,000 solution of acetylcholine chloride, and the mixture allowed to stand at room temperature for a definite time (30-300 seconds). One cc. of the mixture was then added to the bath (volume 100 cc.) containing the eserinized rectus, and the resulting contraction compared with

[4] Chang, H. C., and Gaddum, J. H., *J. Physiol.*, 1933, **79**, 255.

[5] Plattner, F., and Hintner, H., *Pflügers Arch.*, 1930, **229**, 19.

[6] Langley, J. N., *J. Physiol.*, 1885, **6**, 71.

that produced by a known dose of acetylcholine. Control experiments showed that the presence of gland materials did not affect the reaction of the rectus to acetylcholine simultaneously or subsequently applied to it. The enzymatic destruction of the acetylcholine was quickly halted by the eserine present in the bath: precipitation with trichloracetic acid, as in the method of Plattner and Hintner, was therefore unnecessary. At least 3 determinations were made with each extract.

From the values obtained a curve was plotted, from which was read the time required for 50% destruction of the added acetylcholine. The results obtained are given in Table I, the values for the gland from the same animal being grouped in pairs.

TABLE I.

Gland	Time required for 50% destruction of added acetylcholine (minutes)
Normal (left)	1¾
" (right)	1¾
" (right)	1¼
" (left)	2½
Paralytic (right)	2
Normal (left)	1¾
Paralytic (right)	2¼
Normal (Plattner and Hintner)	5

The acetylcholine-inactivating power of the cat's submaxillary gland is thus somewhat higher than that determined for the dog's submaxillary gland by Plattner and Hintner, the dilutions of the tissue being the same in both cases.

Since the catalytic destruction of acetylcholine is a monomolecular reaction, the assay can be checked by ascertaining the rate of inactivation at different times after the addition of the esterase solution. This, of course, is proportional to the concentration of the unhydrolyzed ester, *i. e.*,

$$k = \frac{1}{t}\log\left(\frac{a}{a-x}\right)$$

where k is constant and a and a-x are the concentrations of acetylcholine after 0 and t seconds respectively. Table II illustrates the results when this calculation is made. Considering the rather large error of the biological assay, the values for k determined for the same extract show tolerably good agreement.

TABLE II.

Gland	t	a—x(a = 100)	$k = \frac{1}{t}\log\left(\frac{a}{a-x}\right)$
Left (normal)	60	75	.00208
	120	57.5	.00200
	240	30	.00218
Right (paralytic)	50	75	.00250
	60	70	.00258
	75	67.5	.00228
	150	42.5	.00248

Solutions of acetylcholine subjected to prolonged treatment with gland extracts lost all perceptible activity.

Statistical analysis of a much larger series of determinations would be necessary to determine whether denervation of the gland has any definite influence on its esterase content. Nevertheless it is clear that this is of the same order of magnitude in the normal and denervated gland, and that no difference exists which can adequately explain the tremendously increased sensitivity of the paralytic gland to sympathetic stimulation. It is, however, still possible that degenerative section of the chorda stimulates the production of free acetylcholine within the gland. The *chorda tympani* fibres cut were, of course, preganglionic, and it is possible that degeneration of the postganglionic fibres might significantly affect the esterase content of the gland: unfortunately these are inaccessible to section.

The production of enzymes by other glandular tissues continues after section of the nerves which normally control these enzymes. A familiar example is provided by the Heidenhain pouch of the stomach, which has no extrinsic vagal innervation but continues indefinitely to elaborate pepsin. Nothing is known of the conditions under which choline-esterase is formed in the body. Its high concentration in the blood would suggest, indeed, that the intervention of the nervous system is unnecessary.