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Action of Transmitters on the Responsiveness of Effector Cells

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A quarter of a century ago CANNON, in a lecture given in commemoration of the celebrated neurologist HUGHLINGS JACKSON, formulated 'the law of denervation'[1]. A number of scattered observations collected from the old literature and combined with a great many findings made in CANNON's own laboratory, indicated that a cell acquires a supersensitivity to chemical stimuli after denervation. Already BERNARD[2] (quoted from CANNON) expressed the view that 'the excitability of all tissues seems to augment when they are separated from the nervous influence which dominates them'. The law was described in the following way: 'When in a series of efferent neurones a unit is destroyed, an increased irritability to chemical agents develops in the isolated structure or structures, the effect being maximal in the part directly denervated'. Phenomena such as 'the paradoxical pupillary dilation' and 'the pseudomotor contractures' were now found to be related and due to chemical stimuli, administered to the organism or released within it and acting on cells rendered supersensitive by denervation.

It is obvious that the efferent nerve, in addition to its motor or secretory effect, exerts some action on the effector cell, the removal of which manifests itself in supersensitivity; in LOEWI's words: 'it may well be that what we call hypersensitivity in fact represents the genuine sensitivity of the effector organs which, however, is restrained as long as they are under the influence of their nerves. Hypersensitivity accordingly would just mean a state of desinhibition'[3].

The present paper deals with this particular restraining function of the efferent nerve, and in the experiments to be described a neuro-glandular preparation has been chosen as a model; most of the observations have been made on the submaxillary gland of the cat which has turned out to be a useful object for this type of study.

LUDWIG's classical work showed that the submaxillary gland is supplied with secretory parasympathetic and sympathetic nerve fibres[4]. Later some doubt was raised as to the existence of true sympathetic secretory fibres for the gland[5,6], but there is now considerable experimental evidence to show that there are such fibres and also, contrary to previous opinion, that the two types of fibres converge onto the same effector cells (see BURGEN and EMMELIN[7] and EMMELIN[8]). When BERNARD cut the chorda tympani, in which he traced the parasympathetic secretory fibres, he was surprised to find the submaxillary gland in a state of secretory activity some days after the operation[9]. This phenomenon, 'the paralytic secretion of saliva' was the starting-point of our experiments on supersensitivity some 15 years ago. It was found that the effect could be obtained under certain conditions only, for instance when morphine was used as an anaesthetic by the early investigators in this field[10–13]; and then the flow of saliva ceased if the suprarenals were removed or if an adrenergic blocking agent such as dihydroergotamine was given. Since morphine is known to release catecholamines from the adrenals[14] and section of the chorda to cause a supersensitivity of the submaxillary gland to these compounds[15], an explanation of the phenomenon of paralytic secretion could now be offered: in morphine anaesthesia the concentration of catecholamines in the blood is high enough to cause a flow of saliva from the gland cells, sensitized by previous section of the chorda[16–18]. Paralytic secretion can therefore be classed among phenomena such as 'the paradoxical pupillary dilation' and 'the pseudomotor contractures'. Common to all these cases is a supersensitivity, created in some way by section of an efferent nerve.

[1] W. B. CANNON, Am. J. med. Sci. *198*, 737 (1939).
[2] C. BERNARD, *Pathologie expérimentale* (1880). Quoted from W. B. CANNON and H. HAIMOVICI, Am. J. Physiol. *126*, 731 (1939).
[3] O. LOEWI, Confinia Neurol. *2*, 58 (1949).
[4] C. LUDWIG, Z. rat. Med. [N F] *1*, 255 (1851).
[5] A. KUNTZ and C. A. RICHINS, J. comp. Neurol. *85*, 21 (1946).
[6] C. A. RICHINS and A. KUNTZ, Am. J. Physiol. *173*, 471 (1953).
[7] A. S. V. BURGEN and N. G. EMMELIN, *Physiology of the Salivary Glands* (Monographs of the Physiol. Soc., Edward Arnold Ltd., 1961).
[8] N. EMMELIN, in *Salivary Glands and their Secretions*. Internat. series of monographs on oral biology (Ed. L. M. SREBNY and JULIA MEYER, Pergamon Press, 1964), p. 161.
[9] The paper usually quoted is C. BERNARD, J. Anat. Paris *1*, 507 (1864). It seems to have escaped notice that BERNARD published an account of the phenomenon two years earlier in C. R. Acad. Sci. *55*, 341 (1862).
[10] F. BIDDER, Arch. Anat. Physiol. *1867*, 1.
[11] R. HEIDENHAIN, Stud. physiol. Inst., Breslau *1868*, 73.
[12] J. N. LANGLEY, Proc. Roy. Soc. [B] *236*, 212 (1885).
[13] J. R. BRADFORD, J. Physiol. *9*, 287 (1888).
[14] T. R. ELLIOTT, J. Physiol. *44*, 374 (1912).
[15] A. J. FLEMING and F. C. MACINTOSH, Quart. J. exp. Physiol. *25*, 207 (1935).
[16] N. EMMELIN and A. MUREN, Acta physiol. scand. *21*, 302 (1950).
[17] N. EMMELIN, Acta physiol. scand. *26*, 232 (1952).
[18] N. EMMELIN, Physiol. Rev. *32*, 21 (1952).

(1) *Supersensitivity of denervated salivary glands.* The sensitization of salivary glands shows the general characteristics of a denervation supersensitivity as described by CANNON and ROSENBLUETH in their monograph 'The supersensitivity of denervated structures'[19].

(a) The threshold dose to chemical agents is lowered and a dose causing a submaximal response from the normal gland has a larger effect on the denervated one, the rate of flow being higher and the duration longer. The maximal rate of flow, on the other hand, is not increased; on the contrary, it is somewhat lowered after parasympathetic denervation, because there is some atrophy of the gland[20,21]. Extirpation of the superior cervical ganglion causes a slight hypertrophy of the submaxillary gland of the cat and, correspondingly, the maximal rate of secretion is slightly higher than that of a normal gland[21].

(b) The sensitization is unspecific. Various secretory agents, acetylcholine, pilocarpine, methacholine, adrenaline, noradrenaline, have a greater effect after denervation[20]. This is particularly obvious when the parasympathetic fibres have been cut. According to some statements in the literature, the secretory responses to acetylcholine and pilocarpine are not affected, or even lowered after section of the chorda, but this is apparently due to the fact that conventional doses of these drugs, used to evoke salivary secretion, are supramaximal already in the normally innervated gland. When sufficiently small doses are used, a supersensitivity can easily be demonstrated. There can be no doubt, however, that a subsensitivity to some drugs appears after denervation of salivary glands, for instance to cholinesterase inhibitors after parasympathetic[22], and to tyramine after sympathetic postganglionic denervation[23]; but these are indirectly acting agents, for the secretory effect of which the intact postganglionic fibre is a prerequisite.

(c) The supersensitivity develops gradually. This is best demonstrated using the following technique[24]: The experimental animal is anaesthetized with a short-acting barbiturate, given into the left ventricle after induction with ether, fine glass cannulae are inserted into the salivary ducts from the orifices in the oral mucosa and secretory agents are administered through the needle in the heart. The salivary responses to the drugs are recorded. The animal is then allowed to wake up. The experiment can be repeated again and again with intervals of a few days. Using this method it can be shown that, in a submaxillary gland of a cat, supersensitivity is just discernable 2–3 days after section of the chorda, increases gradually and reaches a maximum within 2–3 weeks.

(d) The supersensitivity is less marked after preganglionic section (decentralization) than after postganglionic severance ('denervation' in a restricted sense of the word), in agreement with the pronouncement of the law of denervation. Thus, the parotid gland becomes more sensitive to acetylcholine when the postganglionic parasympathetic fibres, accessible in the auriculo-temporal nerve, have been cut than after parasympathetic decentralization[25]. The postganglionic parasympathetic fibres of the submaxillary (and sublingual) glands are more difficult to reach, the synapses being situated near the gland and some of them even within the glandular tissue, but if the chorda fibres are traced, under the dissection microscope, in their course along the salivary ducts and cut as closely to the gland as possible, a partial postganglionic denervation is achieved[26]. It will then be found that secretory agents now cause larger responses than if only the preganglionic fibres in the chorda or the chorda-lingual trunk had been cut. Regarding the sympathetic innervation of the glands, it has been found that some supersensitivity develops following postganglionic section[20;27,28], whereas decentralization scarcely changes the sensitivity of the cells of the cat's submaxillary gland[28].

(2) *The role of the secretory impulse.* It is obvious that when the submaxillary gland has been disconnected from the central nervous system by section of the chorda tympani, some changes occur in the gland cells which appear gradually and manifest themselves in a successively rising sensitivity to secretory agents; 2–3 weeks are required for these changes to develop fully. The restraining effect of the central nervous system on the level of sensitivity will now be further analysed.

The specificity of the effect. The supersensitivity remains as long as the parasympathetic pathway is broken, but when the chorda fibres regenerate, the sensitivity declines. The process of reinnervation can in fact be followed using the level of sensitivity as an indicator. This suppressing effect on the sensitivity is not specific to the chorda. Its function can be taken over by other nerves, as shown in cross-suture experiments[29]. If the hypoglossal and chorda-lingual nerves are cut and the central end of the former is sewn to the peripheral end of the latter nerve, the sensitivity is

[19] W. B. CANNON and A. ROSENBLUETH, *The Supersensitivity of Denervated Structures* (Experimental Biology Monographs, MacMillan, New York 1949).
[20] N. EMMELIN and A. MUREN, Acta physiol. scand. *24*, 103 (1951).
[21] N. EMMELIN, L. MALM, and B. C. R. STRÖMBLAD, Quart. J. exp. Physiol. *45*, 349 (1960).
[22] N. EMMELIN and B. C. R. STRÖMBLAD, Brit. J. Pharmacol. *13*, 193 (1958).
[23] B. C. R. STRÖMBLAD, Acta physiol. scand. *36*, 154 (1956).
[24] N. EMMELIN, in *Salivary Glands and their Secretion.* Internat. series of monographs on oral biology (Ed. L. SREEBNY and JULIA MEYER, Pergamon Press, 1964), p. 301.
[25] R. STRÖMBLAD, Acta physiol. scand. *33*, 83 (1955).
[26] N. EMMELIN, Brit. J. Pharmacol. *15*, 356 (1960).
[27] F. A. SIMEONE and J. P. MAES, Am. J. Physiol. *125*, 674 (1939).
[28] N. EMMELIN and J. ENGSTRÖM, J. Physiol. *153*, 9 (1960).
[29] N. EMMELIN, A. MUREN, and B. C. R. STRÖMBLAD, Acta physiol. scand. *41*, 18 (1957).

found to rise, because of denervation, but then to start falling in the course of the following months. In an acute experiment it can finally be demonstrated that electrical stimulation of the hypoglossal nerve causes secretion and vasodilatation in the submaxillary gland. Obviously the hypoglossal nerve has been able to take over not only the immediate actions of the chorda but also the long-term action on the responsiveness to secretory agents. Injection of a ganglionic blocking agent, hexamethonium, is found to abolish the secretory and vasomotor effect of hypoglossal stimulation, and the regenerating nerve has thus not reached the gland cells but the intact postganglionic neurone. In the parotid gland, however, the hypoglossal nerve can be connected to the postganglionic parasympathetic fibres of the auriculo-temporal nerve and when the supersensitivity due to the denervation has declined, stimulation of the hypoglossal nerve causes a secretion, which is not abolished by hexamethonium but only by atropine[30]. Here, therefore, not even the normal parasympathetic postganglionic fibres are left in contact with the glandular cells; the hypoglossal nerve has replaced the whole parasympathetic pathway in its regulatory effect on the level of sensitivity.

The cross-suture experiments, in which only the ganglion cells are reinnervated, emphasize the connection with the central nervous system as a decisive factor in the regulation of the responsiveness of the gland cells. They suggest that impulses from the hypoglossal nucleus are able to affect the sensitivity of the submaxillary gland. It seems reasonable to assume that normally the flow of secretory impulses from the salivary nucleus determines the level of sensitivity.

Section of afferent nerve fibres. This assumption can be tested experimentally in the following way. The traffic of secretory impulses to the gland is to a great extent dependent on the intensity of the inflow of afferent impulses to the salivary nuclei in the lower brain stem. By cutting afferent fibres of the secretory reflex arc it is therefore possible to reduce the bombardment of the gland with secretory impulses. When lingual fibres from the mouth are severed, without damaging secretory fibres to the submaxillary gland, a supersensitivity develops in this gland[31,32]. Unilateral section of the fibres is usually followed by supersensitivity in both submaxillary glands, but particularly in that on the side of operation. It is worth noting in this connection that afferent stimulation of lingual fibres is known to cause secretion from both submaxillary glands, and more from the homolateral than from the contralateral gland. Cutting the contralateral afferent lingual fibres as well increases the sensitivity further, and both glands now reach the same level. This level is far below that attained by section of the efferent chorda fibres – understandably, since other afferent pathways of the reflex are preserved, and the influence from higher regions of the central nervous system remains. Some supersensitivity can be superimposed by section of the afferent glossopharyngeal fibres. This operation will, however, particularly raise the responsiveness of the parotid gland – findings in general agreement with the fact that afferent glossopharyngeal stimulation causes some secretion from the submaxillary, but a much livelier flow from the parotid glands.

The observation that section of the afferent lingual fibres of one side can sensitize the contralateral submaxillary gland may, incidentally, throw some light on a puzzling phenomenon, discovered by HEIDENHAIN[31]. In his investigation on 'paralytic secretion', denervating one gland he noticed a slow salivation from the apparently normal contralateral submaxillary gland. This flow, called by LANGLEY[33] 'anti-paralytic' or 'antilytic' secretion', was much less pronounced than the paralytic secretion of the operated side. It is interesting to find that HEIDENHAIN (for technical reasons, as is often done) cut the chorda-lingual nerve and not the chorda, and therefore cut both efferent fibres of the gland and afferent fibres from the mouth. The experiments were carried out under morphine anaesthesia, and it is reasonable to suppose that the release of catecholamines from the adrenals was large enough to cause a marked homolateral paralytic secretion, and in addition a small antilytic secretion from the contralateral gland, slightly sensitized because some afferent fibres had been cut. Experiments show, in fact, that a submaxillary gland, thus moderately sensitized by section of contralateral afferent lingual fibres, is quiescent in cats under chloralose anaesthesia but starts secreting when perfused from the arterial side with blood from another cat, anaesthetized with morphine[34]. Be that as it may, the pertinent conclusion to be drawn in this connection is that supersensitivity of the gland cells can be created simply by reducing the flow of secretory impulses to the gland, and without interfering surgically with the efferent secretory pathway.

Section of the sympathetic trunk. Secretory impulses from the central nervous system reach the salivary glands mainly by way of the parasympathetic fibres. It is, in fact, very doubtful whether any impulses in digestive reflexes are mediated via the sympathetic pathway, the function of which is unknown – it may be that these fibres are activated in angry cats, spitting in rage. It seems, at any rate, safe to conclude that the traffic of secretory impulses in the sympathetic fibres to the glands is very low. It is now interesting to note

[30] N. EMMELIN, L. MALM, and B. C. R. STRÖMBLAD, Exper. *16*, 282 (1960).
[31] N. EMMELIN, XXth Int. Physiol. Cong. Brussels, Abstract of Communications (1956), p. 269.
[32] N. EMMELIN, J. Physiol. *157*, 402 (1961).
[33] J. N. LANGLEY, J. Physiol. *6*, 71 (1885).
[34] N. EMMELIN, J. Physiol. *157*, 410 (1961).

that disconnecting the gland from the central nervous system via the sympathetic path by cutting the preganglionic sympathetic trunk gives rise to no supersensitivity in the submaxillary gland[28], whereas the same operation sensitizes the nictitating membrane and the pupillary dilator, structures known to be bombarded with impulses. It is, likewise, interesting to find that the responsiveness of the gland can be affected if impulses are directed to the gland by way of the sympathetic fibres. This can be done in cross-suture experiments, similar to those already described. It may be recalled that in these experiments hypoglossal impulses were found to be able to supply a restraining influence on the responsiveness of the gland cells, which is normally exerted by the chorda. These hypoglossal impulses can, instead, be sent to the gland via the sympathetic fibres if the central end of the cut hypoglossal nerve is allowed to regenerate into the peripheral end of the preganglionic sympathetic fibres in the neck[35]. When the suture is made, the chorda is cut at the same time, in order to create a high level of sensitivity in the gland to start from. Some months later functional union between hypoglossal and sympathetic fibres is evidenced by the fact that swallowing movements, elicited by offering the cat cream or by pushing a rubber tube into its pharynx from the mouth, are accompanied by dilatation of the pupil and withdrawal of the nictiating membrane; and in the anaesthetized animal it can be shown that electrical stimulation of the hypoglossal nerve causes secretion from the submaxillary gland which can be abolished by dihydroergotamine and hexamethonium but not by atropine. These effects on the eye have been described earlier by DE CASTRO[36]. In the present investigation they have merely been used as signs of reinnervation. What is of interest for this discussion is that as soon as these signs appear, the supersensitivity of the gland caused by section of the chorda is found to have started declining[35]. The experiments suggest that normally the flow of impulses from the central nervous system via the sympathetic fibres is insignificant, and they are in accordance with the view that the level of sensitivity of the gland is dependent on the bombardment with secretory impulses. In addition, the fact that supersensitivity following section of the chorda can be influenced by impulses, artificially directed to the gland via the sympathetic fibres, supports the opinion expressed in the introduction that the fibres of the two divisions of the autonomic nervous system act on the same gland cells.

Pharmacological denervation. An organ can be disconnected from the central nervous system not only surgically but also by means of blocking agents, and if such an agent is made to act over a sufficiently long period of time, changes occur in the effector cells which in many respects resemble those appearing after section of the nerve. Supersensitivity to chemical stimuli develops, as first demonstrated in salivary glands[37–40]. The law of denervation can, in fact, be assumed to be valid for 'pharmacological denervation' also, ganglion blocking agents producing a 'pharmacological decentralization' and agents acting on peripheral nerve endings or the receptor area of the effectors creating a 'pharmacological denervation' (the word denervation now being used in its limited sense)[41].

After repeated injections of a ganglion blocking compound such as chlorisondamine, supersensitivity develops in the submaxillary gland[42]. It seems to be difficult to attain a complete and long-lasting block of the transmission of impulses across the parasympathetic submaxillary synapse and the level of sensitivity reached is lower than that produced by cutting the chorda. A more favourable preparation is, however, obtained if the hypoglossal nerve is allowed to replace the preganglionic parasympathetic fibres. It has been found that the new synapse formed is extremely susceptible to hexamethonium, and very small doses of this drug therefore produce full and prolonged block of ganglionic transmission[35]. If hexamethonium is injected repeatedly, once hypoglossal impulses have more or less abolished the supersensitivity caused by section of the chorda, a supersensitivity will reappear which is similar quantitatively and in the time course to that first seen when the chorda has been cut[42]. These observations after 'pharmacological decentralization' seem to speak in favour of the view that the secretory impulse from the central nervous system has a regulatory function with regard to the responsiveness of the gland cells.

It is well-known that the secretory impulse is transmitted from the postganglionic parasympathetic endings to the glandular cell by acetylcholine. A reasonable question to ask seems to be: Would the gland cells acquire a heightened responsiveness if deprived for a long time of any action from this acetylcholine? Two types of 'pharmacological denervation' experiments were carried out. In one of them the release of acetylcholine from the postganglionic parasympathetic endings was prevented by administration of botulinum toxin, in the other atropine abolished the action of the released acetylcholine on the gland cells.

When atropine was injected subcutaneously twice a day, the sensitivity of the normally innervated submaxillary glands gradually rose to a maximum within

[35] N. EMMELIN, A. MUREN, and B. C. R. STRÖMBLAD, Acta physiol. scand. *41*, 35 (1957).
[36] F. DE CASTRO, Arch. int. Physiol. *59*, 479 (1951).
[37] N. EMMELIN and A. MUREN, Nature *166*, 610 (1950).
[38] N. EMMELIN and A. MUREN, Acta physiol. scand. *24*, 103 (1951).
[39] N. EMMELIN, D. JACOBSOHN, and A. MUREN, Acta physiol. scand. *24*, 128 (1951).
[40] N. EMMELIN and A. MUREN, Acta physiol. scand. *26*, 221 (1952).
[41] N. EMMELIN, Pharmacol. Rev. *13*, 17 (1961).
[42] N. EMMELIN, Brit. J. Pharmacol. *14*, 229 (1959).

2–3 weeks[38,40]. Obviously the atropinized gland does not respond to acetylcholine and related drugs, and sympathomimetic agents such as adrenaline were used to estimate the sensitivity. A high level of sensitivity was reached; if the cat was anaesthetized with morphine a lively paralytic secretion ensued[43]. The changes in the gland responsible for the supersensitivity are obviously reversible, for when the treatment with atropine was discontinued the sensitivity fell and reached the original level within 3–4 days. Against the objection that the changes in the gland produced by atropine may not be of the same nature as those brought about by section of the chorda, the following observation may be quoted[44]. When the supersensitivity caused by atropine reached a high level, the treatment was interrupted and at the same time the chorda was cut. Three days later the sensitivity was again estimated and found to be high, in fact as high as if the chorda had been cut 3 weeks previously – in spite of the fact that supersensitivity caused by atropine treatment vanishes in 3–4 days, and that supersensitivity due to section of the chorda is just discernable after 3 days. This finding surely indicates that atropine treatment causes fundamentally the same type of changes in the gland as severance of the chorda. A quantitative difference does exist, however, as will be discussed later.

In order to prevent the release of acetylcholine in the gland with botulinum toxin without poisoning the animal with a large dose, a small amount of the toxin was injected through the submaxillary duct towards the gland[45]. This method of restricting an action to the gland was found to be useful for many drugs[46]. To promote the fixation of the toxin, salivary secretion was temporarily suppressed with a small dose of a parasympatholytic agent. The sensitivity started to rise after a single injection of 1 μg botulinum toxin, type A (kindly supplied by Dr. THESLEFF, who had previously found the toxin to cause a supersensitivity in skeletal muscle[47]). The sensitivity rose to a high level within 2–3 weeks, remained there for a long time and gradually declined in the course of several months. The remarkably long duration of action of botulinum toxin can, in fact, be easily demonstrated in this type of preparation.

Action of secretory agents on the sensitivity. In the experiments described so far the gland was deprived in various ways of some action of acetylcholine, surgically by section of afferent fibres of the secretory reflex arc or pre- or postganglionic parasympathetic fibres, pharmacologically by treatment with ganglion blocking agents, botulinum toxin or atropine. In all these instances a supersensitivity ensued. The reverse experiment would be to study the effect of added acetylcholine or related agents on the responsiveness of the gland. The following experiment was made[37–40]. The chorda tympani of one side was cut, and, when supersensitivity had fully developed, treatment with pilocarpine was instituted, the drug being injected subcutaneously twice a day. The sensitivity of the decentralized gland decreased and reached the level of the contralateral gland within 3 days. Even the sensitivity of this latter gland was often somewhat depressed. On discontinuation of the treatment, the sensitivity again rose and after about 2 weeks the original conditions were re-established. Pilocarpine could be replaced by carbachol or even by adrenaline, if this drug was injected frequently. Acetylcholine is far too short-acting to be of any use in this type of experiment, but an experiment can be designed in which treatment with endogenous acetylcholine is given[48]. For that purpose eserine is injected twice a day, the effect being that the gland is exposed to excess of acetylcholine, preserved by the cholinesterase inhibitor. Very likely this acetylcholine derives from the postganglionic (and perhaps also the preganglionic) parasympathetic endings in the gland, but it cannot be excluded that some of it originates from extraglandular sources, brought to the gland by way of the blood stream[49]. What is interesting, at any rate, is the outcome of this experiment: the sensitivity of a normally innervated gland decreases and supersensitivity brought about by section of the chorda vanishes temporarily within 3 days.

The conclusion from these experiments, and from those of the previous section, is that the amount of acetylcholine in contact with the effector cells determines the responsiveness of the gland cells to secretory stimuli. Commenting on the fact that the transmitter disappears when nerves degenerate, DALE wrote in 1934: 'We may note, in passing, the probability that the exaggerated sensitiveness of the denervated effector cells, to the artificial application of the chemical transmitter, may be conditioned by this disappearance of its depot and failure of its normal release'[50]. Even the so-called normal level of sensitivity is obviously variable and depends on the amount of acetylcholine acting on the cells. Lack of acetylcholine induces slow changes in the gland which reach a maximum within 2–3 weeks whether due to surgical denervation or treatment of a normal gland with ganglionic blockers, botulinum toxin or atropine, or to discontinuation of eserine administration in a previously decentralized gland. These changes can be quickly reversed, within 3–4 days, as seen when atropine treatment of a nor-

[43] N. EMMELIN and A. MUREN, Acta physiol. scand. *22*, 278 (1951).
[44] N. EMMELIN and F. C. MACINTOSH, unpublished observation.
[45] N. EMMELIN, J. Physiol. *156*, 121 (1961).
[46] N. EMMELIN, A. MUREN, and R. STRÖMBLAD, Acta physiol. scand. *32*, 325 (1954).
[47] S. THESLEFF, J. Physiol. *151*, 598 (1960).
[48] N. EMMELIN, Exper. *20*, 275 (1964).
[49] N. EMMELIN, J. Physiol. *171*, 132 (1964).
[50] H. H. DALE, Proc. Roy. Soc. Med. *28*, 319 (1934).

mally innervated gland is stopped, or pilocarpine or eserine treatment of a previously decentralized gland started. It is reasonable that the return to normal conditions in a sensitized gland is more retarded when due to outgrowth of nerve fibres, chorda or hypoglossal fibres, or to the gradual elimination of the long-acting botulinum toxin. Similarly, the supersensitivity disappears slowly when caused by treatment with parasympatholytic agents with a longer duration of action than atropine[26].

(3) *The role of leaking transmitter.* Even between meals the salivary glands are reflexly activated, moistening the oral mucosa, and the responsiveness of their cells is therefore more or less continuously subjected to a restraining influence from the central nervous system. It can be shown, however, that transmitter liberated by the secretory impulse cannot be the sole factor responsible for the low sensitivity of normal gland cells to stimulating agents. From the postganglionic nerve endings there seems to be a leakage of transmitters which contributes to this effect.

Leakage of acetylcholine. Two series of experiments, referred to earlier in this paper, suggest in fact that the postganglionic parasympathetic nerve endings give off acetylcholine even when not invaded by secretory impulses from the central nervous system.

(a) The first hint that there is such a release of acetylcholine came from the experiments with prolonged atropinization. The supersensitivity ensuing was compared to that of postganglionic denervation. If such a comparison is justified one would expect this pharmacological denervation to be more pronounced than that following section of the preganglionic fibres of the chorda. It was, however, found to be very difficult to ascertain which was really the highest level of supersensitivity attainable by treatment with atropine, the reason being the pronounced tolerance which develops towards this drug. The synthetic parasympatholytic drug α,α-diphenyl-γ-piperidinobutyramide (Hoechst 9980)[51] was found to be more useful than atropine in these experiments[52]. Very little tolerance developed towards it, and when it was injected over long periods a supersensitivity appeared in the submaxillary gland which definitely surpassed that reached after section of the chorda[53]. This drug was found to be remarkably specific as a parasympatholytic agent. It could therefore be inferred that when it caused supersensitivity, it did so exclusively by virtue of its parasympatholytic property. Since it could raise the sensitivity above the level attained after section of the chorda, this must be taken to show that it had abolished some action of a cholinergic mechanism, present and active even when the chorda fibres had degenerated. The cholinergic mechanism left is the postganglionic parasympathetic neurone. The conclusion reached is thus that this neurone even when disconnected from the central nervous system releases acetylcholine which normally serves to suppress the sensitivity of the gland cells to stimuli. Such an action must be exerted in spite of the fact that the amounts of acetylcholine thus released are subthreshold, as far as secretion is concerned; for when the chorda has degenerated there is no secretion going on, except under the highly abnormal conditions prevailing during paralytic secretion. In further support of the view that the postganglionic parasympathetic neurone releases acetylcholine independently of impulses from the central nervous system and that this acetylcholine affects the responsiveness, the following experiments may be quoted. Three other parasympatholytic drugs, lachesine, isopropamide and methscopolamine, likewise increase the sensitivity above the level reached after section of the chorda; when administered on different occasions to the same cat they all raise the sensitivity to the same level – and to the level reached after treatment with Hoechst 9980[26].

(b) It was mentioned above that eserine, injected for some days, lowers the sensitivity of a submaxillary gland, and that this occurs even if the chorda has degenerated. The effect is attributed to acetylcholine, preserved by the cholinesterase inhibitor. This acetylcholine could, admittedly, originate from extraglandular sources; but, if so, certainly not more than a fraction of it, as evidenced by the following finding. When, in addition to the chorda fibres, a great many postganglionic parasympathetic fibres have been cut and degenerated, treatment with eserine lowers the sensitivity much less than when the preganglionic fibres alone have degenerated[45]. Some acetylcholine, at least, must therefore originate from the postganglionic fibres.

Three more observations may be briefly mentioned, which speak in favour of the opinion that acetylcholine is continuously liberated from the postganglionic parasympathetic neurone in the salivary glands.

(c) When the submaxillary gland is perfused with eserinized plasma, traces of acetylcholine can be detected in the perfusate even when the chorda has been cut[54].

(d) When the sympathetic secretory fibres are stimulated at a low rate to produce a slow flow of saliva, intravenous injection of a specific parasympatholytic drug such as Hoechst 9980 is found to cause a small reduction of the secretion. Raising the dose of Hoechst 9980 even to high levels does not cause any further diminution of the flow[55]. Similarly, the secre-

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tory effect of a small dose of adrenaline is diminished by parasympatholytic agents[55,56]. A small dose of eserine slightly increases the secretory effect of pilocarpine[55]. All these observations can be made even when the chorda has been cut. They are easily explained on the assumption that subthreshold amounts of acetylcholine are permanently released, facilitating the action of other secretory agents.

(e) Eserine, preferably injected into the salivary gland through the duct, will cause a secretion if given in sufficient amount. Other cholinesterase inhibitors have a similar secretory effect[22]. A dose of a local anaesthetic given through the duct which abolishes the secretory effect of chorda stimulation but not that of intravenously injected acetylcholine, and therefore can be assumed to act on nerves and not on the gland cells, is found to abolish this secretion due to eserine. Intact nerve fibres in the gland are thus a condition necessary for the secretion to appear after eserine. The fibres in question are the postganglionic parasympathetic nerve fibres, as shown by the fact that eserine loses its secretory effect on the parotid gland when the auriculo-temporal nerves have been cut and given time to degenerate. These findings are in agreement with the supposition that acetylcholine, normally in subthreshold amounts, is released even from the decentralized postganglionic parasympathetic neurone. If, however, the auriculo-temporal fibres are cut acutely, eserine retains its full secretory effect, which indicates that the activity responsible for this release of acetylcholine does not originate in the cell body of the neurone[22]. The most reasonable explanation seems to be that the transmitter is continuously leaking from the nerve endings, as originally shown for somatomotor nerves[57] and more recently for preganglionic sympathetic fibres[58,59].

Leakage of transmitter in other areas has been demonstrated by the appearance of spontaneous miniature potentials. LUNDBERG[60] inserted microelectrodes in salivary gland cells and sometimes observed small changes in the resting potential when nerves were not excited nor secretory drugs injected, but he interpreted these potentials as artifacts and there is so far no electrophysiological investigation to quote, indicating a leakage of acetylcholine in salivary glands.

Summarizing the evidence given so far in this section, it may be stated that acetylcholine is continuously leaking from the postganglionic parasympathetic endings in contact with the glandular cells. Normally, the amounts given off are subthreshold with regard to secretion, but the subliminal action can be shown acutely in its additive effect to other secretory agents and, when preserved by cholinesterase inhibitors, amounts surpassing the threshold accumulate. Although normally subliminal, as far as secretion is concerned, even if the gland cells have been sensitized by previous decentralization, this leaking acetylcholine exerts some long-term action on the gland cells, the removal of which by prolonged atropinization manifests itself in supersensitivity. Similarly, the loss of this action after degeneration of postganglionic parasympathetic fibres, leads to a supersensitivity, higher than that due to the mere loss of transmitter released by the secretory impulse from the central nervous system. The restraining effect of leaking acetylcholine can be enhanced by treatment with eserine for some days.

Recent investigations suggest that while the postganglionic parasympathetic fibres are degenerating there is a period in which the leak of acetylcholine is greater than normal. After some days this will affect the responsiveness of the gland cells, as shown in the next section.

Degeneration secretion. One day after cutting the auriculotemporal fibres, the parotid gland of the cat starts secreting, and the flow continues, intermittently, for about two days[61,62]. Partial postganglionic parasympathetic denervation of the submaxillary and sublingual glands causes a similar, but more continuous 'degeneration secretion' from these two glands[63–65]. It starts somewhat earlier than in the parotid gland, reaches a maximum 25–30 h after the denervation and ceases after about two days. Degeneration secretion from salivary glands has also been observed in dogs[66] and rabbits[67] after parasympathetic denervation.

This temporary secretion during a period when the nerve fibres are degenerating seems to be due to acetylcholine, for it is abolished by parasympatholytic agents. Further analysis of the phenomenon[64,65,68] discloses that during this period the leakage of acetylcholine is temporarily increased so that the threshold for secretion is surpassed. Of interest for the present discussion is the finding that when the secretion has continued for two days and ceased, the responsiveness of the gland cells to stimulating agents is markedly diminished. This is best shown in the following way[65]. The preganglionic fibres of the chorda are cut, and when the supersensitivity has reached a plateau, postganglionic parasympathetic fibres are sectioned. When the sensitivity is estimated three days later it is found

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to be considerably lowered, and to start rising in the course of the following days, reaching a higher level than before, in accord with the law of denervation. Eserine treatment, begun after the postganglionic denervation and continued for two days, will increase this dip in the sensitivity curve, a single injection of a small dose of atropine will prevent it. These observations on degeneration secretion emphasize the role of acetylcholine, leaking from the nerve ending, as a regulator of the responsiveness of salivary gland cells.

Leakage of sympathine. As mentioned earlier, section of the preganglionic sympathetic fibres does not increase the sensitivity of the submaxillary gland. This was attributed to the fact that the traffic of secretory impulses in the sympathetic pathway is small. It is well known, on the other hand, that extirpation of the superior cervical ganglion, i.e. postganglionic sympathetic denervation, gives rise to a moderate supersensitivity[27,28]. This can be superimposed upon that caused by chorda section[38], or even complete parasympathetic 'pharmacological denervation'[53]. Preventing the release of sympathine for some time by treatment with guanethidine or bretylium causes a supersensitivity similar to that following removal of the sympathetic ganglion[69]. It seems reasonable to assume that from the postganglionic sympathetic fibres there is a continuous leakage of sympathine, subthreshold as far as secretion is concerned but with some long-term restraining influence on the responsiveness of the gland cells. In support of the view that a leakage of sympathine exists, the following observation may be quoted. A slow flow of saliva, evoked by electrical stimulation of the chorda at a low rate of excitation, is found to be reduced by intravenous injection of a small dose of a sympatholytic agent such as dihydroergotamine; this effect is not obtained when the postganglionic sympathetic fibres have degenerated[70,71]. The fact that a special type of degeneration secretion, abolishable with dihydroergotamine, can be seen two days after excision of the superior cervical ganglion may also suggest that, normally there is a leakage of sympathine which can temporarily increase while the sympathetic fibres are degenerating. It may further be pointed out that in smooth muscle, supplied with sympathetic fibres, miniature potentials have been observed to appear without accompanying changes in muscle tension[72].

(4) *The mode of action of the transmitters.* The possibility that supersensitivity following section of efferent nerves is in some way secondary to the reduced activity imposed on the organ has been discussed (see CANNON and ROSENBLUETH[19]; LOEWI[3]). In the case of salivary glands this appears rather unlikely in view of the following facts:

(a) Unlike the submaxillary, the sublingual gland of the cat is secreting continuously, even when not stimulated by extraneous stimuli acting on the receptors. Its cells seem to be endowed with the inherent ability to secrete permanently[73] a type of secretion described by BABKIN[74] as 'spontaneous'. In spite of this incessant secretion the normal sublingual cells are just as sensitive to secretory agents as are the submaxillary cells. When the chorda is cut this continuous secretion cannot prevent the development of a supersensitivity as marked as that appearing in the submaxillary gland, whereas injection of pilocarpine repeated over a few days and causing a slow flow of saliva prevent any supersensitivity[73].

(b) The leaking transmitters seem to exert a restraining influence on the responsiveness of the gland cells although they are normally insufficient to evoke secretion.

These two facts centre interest on the receptor region as the area in which transmitters released by the secretory impulse or leaking from the nerve endings regulate the responsiveness of the gland cells. Little is known about the changes appearing there when some long-term action of the transmitters is lost. These changes appear gradually and require 2-3 weeks to develop fully, but they can be reversed within a few days. The unspecificity of the sensitization is a remarkable and unexplained fact, for instance the pronounced supersensitivity towards adrenaline obtained after treatment with atropine. As another example may be mentioned that the submaxillary gland of the rat seems to be supplied both with α- and β-receptors for sympathomimetic agents and that supersensitivity apparently develops both to agents stimulating receptors of the α-type and such acting on β-receptors, and furthermore that this occurs both after parasympathetic decentralization and sympathetic denervation[75]. As to the nature of the changes responsible for the supersensitivity, several findings suggest that surgical or pharmacological decentralization or denervation may reduce the activity of enzymes such as cholinesterase[76], but for the moment it seems unlikely that the so-called enzyme hypothesis could afford a general explanation of the phenomenon of supersensitivity following denervation of salivary glands. The approach used by AXELSSON and THESLEFF[77] in skeletal muscle to study the nature of the supersensitivity and resulting in the finding that denervation is followed by an extension of the receptor

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area seems to be technically difficult to apply to salivary glands.

(5) *Conclusions.* The supersensitivity to secretory agents which develops in the salivary glands after preganglionic parasympathetic denervation is attributed to the loss of some action of acetylcholine released by the secretory impulse. Treatment with ganglion blocking agents produces the same effect. The glands are more or less continuously bombarded with secretory impulses, even between meals, and the important role of the secretory impulse as a regulator of the responsiveness is reflected in the pronounced supersensitivity following surgical or pharmacological decentralization. Even a slight reduction of the flow of secretory impulses, as seen in a submaxillary gland after section of some contralateral afferent fibres of the secretory reflex arc, causes a noticeable supersensitivity. Sympathetic decentralization, on the other hand, produces no supersensitivity, corresponding to the fact that secretory impulses scarcely seem to be conveyed via the sympathetic secretory pathway.

Parasympathetic denervation brought about surgically or by treatment with botulinum toxin or parasympatholytic agents, likewise, deprives the gland of some action of acetylcholine, normally released by the secretory impulse. In addition, however, the gland loses the restraining action of acetylcholine, leaking from the postganglionic parasympathetic nerve endings, and the supersensitivity that ensues is therefore more marked than that following decentralization.

Even after these procedures some restraining influence on the responsiveness remains, exerted by sympathine leaking from the postganglionic sympathetic endings. The supersensitivity following parasympathetic denervation can therefore be further enhanced by sympathetic denervation, surgically by removal of the superior cervical ganglion or pharmacologically by treatment with guanethidine or bretylium.

In the introduction it was pointed out that salivary glands are useful objects for investigations on supersensitivity following denervation. Their advantages in this respect may be thus summarized: The glands are easily accessible and a method is available to study the sensitivity over long periods in one and the same animal. They are paired, and one gland can be used as control. They are not vitally important, and their function can be interfered with, without serious damage to the experimental animal. They are very susceptible to various blocking agents, as witnessed by the fact that dryness of the mouth is a common side effect of many therapeutic agents; such blocking agents can therefore be administered for instance by subcutaneous injections in doses which particularly affect the salivary glands; the action of drugs can also be restricted to the gland by injection through the duct. Afferent and efferent nerves of the secretory reflex arc are easily reached. The existence of a peripheral synapse makes it possible to cut pre- and postganglionically, thus separating actions of transmitter released by the impulse from the central nervous system from those due to transmitter leaking from the nerve endings. The double innervation of the gland cells from both divisions of the central nervous system is a complication, but a useful one: it makes it possible to use blocking agents such as atropine and still to excite the gland, for instance using adrenaline as an indicator, to test the sensitivity. Finally, the glands are normally activated to secretion exclusively by impulses from the central nervous system; there are, so far as is known, no local reflexes or hormonal mechanisms, as in lower parts of the digestive tract, to complicate the situation.

Zusammenfassung. Die Regulation der Empfindlichkeit der Effektorzellen ist in der vorliegenden Übersicht an Speicheldrüsen, hauptsächlich an der Submaxillarisdrüse als Modell untersucht worden. Es entwickelt sich eine gesteigerte Empfindlichkeit, wenn man die Verbindung mit dem zentralen Nervensystem durch chirurgische Dezentralisierung oder durch Behandlung mit ganglienblockierenden Substanzen unterbricht. Eine gewisse Sensibilisierung kann auch erhalten werden, indem man die afferenten Nervenfasern im sekretorischen Reflexbogen durchschneidet. Die Versuche deuten darauf hin, dass der sekretorische Impuls eine Rolle bei der Regulation des Empfindlichkeitsniveaus spielt. Dass dieser Effekt ebenso wie der sekretorische durch Acetylcholin vermittelt wird, geht daraus hervor, dass Sensibilisierung eintritt, wenn das Versuchstier mit Botulinustoxin behandelt wird, da dieses die Freisetzung von Acetylcholin verhindert, oder durch Atropinsubstanzen, die die Wirkung des Acetylcholins auf die Drüsenzellen verhindern. Andererseits kann eine durch parasympathische Dezentralisierung hervorgerufene Sensibilisierung durch tägliche Injektionen von Pilokarpin, Mecholyl oder Physostigmin aufgehoben werden; auch das normale Empfindlichkeitsniveau kann durch eine solche Behandlung gesenkt werden. Behandlung mit Botulinustoxin oder mit Atropinsubstanzen oder postganglionäre parasympathische Denervierung ergeben eine Sensibilisierung, die ausgesprochener ist als diejenige, welche auf parasympathische Dezentralisierung folgt. Diese und andere Beobachtungen deuten darauf hin, dass Acetylcholin ständig von den postganglionären Nervenendigungen abgegeben wird und dass dieses Acetylcholin, wie auch das von den Nervenimpulsen freigesetzte, die Empfindlichkeit der Effektorzellen kontrolliert. Entsprechend scheint Sympathin von den postganglionären sympathischen Nervenendigungen abgegeben zu werden und zur Regulation der Empfindlichkeit beizutragen; postganglionäre sympathische Denervierung ruft eine gewisse Sensibilisierung hervor, und eine Behandlung mit Bretylium oder Guanethidin hat den gleichen Effekt.