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DEGENERATION SECRETION FROM PAROTID GLANDS AFTER SECTION OF THE AURICULO-TEMPORAL NERVES AT DIFFERENT LEVELS

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SUMMARY

1. In cats under ether or hexobarbitone anaesthesia the auriculo-temporal nerve was cut near the parotid gland on one side and 12–20 mm more proximally on the other. After 22–64½ hr the cats were anaesthetized with chloralose and the parotid ducts cannulated. Degeneration secretion of saliva which appears after post-ganglionic parasympathetic denervation was found to start 2–5½ hr later in the gland denervated proximally than in that denervated distally. It ceased, on the other hand, later in the former than in the latter gland.

2. Before degeneration secretion had started spontaneously it could be provoked by intravenous injection of acetylcholine, methacholine, carbachol or eserine and the effect was more pronounced on the gland denervated distally. When it had ceased spontaneously it could also be provoked, and the effect on the other gland was now more marked.

3. Earlier it has been assumed that while a nerve is degenerating there is a period when the nerve endings are unable to retain in a normal way acetylcholine still being synthesized. It is now suggested that this period starts later after proximal than after distal denervation because more of the material required for the normal function of the endings is available in a long piece than in a short piece of nerve.

INTRODUCTION

At a certain stage of degeneration of efferent nerve fibres the nerve endings seem unable to retain in a normal way the chemical transmitter still produced, and as a consequence the denervated effector may be activated temporarily. This was first observed as a 'degeneration secretion' in the parotid gland of the cat after section of the auriculotemporal nerve (Emmelin & Strömblad, 1958), and in other salivary glands it has been seen both after parasympathetic and sympathetic post-ganglionic denervation.

When the submaxillary and sublingual glands of the cat were (partly) denervated at the same time (by destruction of post-ganglionic parasympathetic neurones) it was noticed that secretion began earlier, and reached a maximum earlier, in the sublingual gland (Emmelin, 1962). Further comparison shows that these glands start secreting before the parotid gland, and the submaxillary gland of the cat starts earlier than that of the dog (Coats & Emmelin, 1962). To explain these differences in time of onset of the phenomenon it was suggested that degeneration secretion begins earlier the shorter the piece of nerve left in connexion with the gland (Emmelin & Malm, 1965). This hypothesis was tested experimentally on the parotid gland since it is supplied with fairly long parasympathetic post-ganglionic fibres, which can be cut at different levels. A preliminary account of this work has been given (Emmelin, 1967*a*).

METHODS

The experiments were made on twenty-three cats. The auriculotemporal nerve was first cut on one side according to the method of Burgen (1964); this section, medial to the lower jaw, was made as far proximally as possible. The auriculotemporal branches of the other side were then cut near the parotid gland using the method of Strömblad (1955). These operations were made under ether anaesthesia or ether followed by hexobarbitone given slowly intracardially (see Emmelin, 1964*a*). The two nerves were cut with a time interval of 10–15 min. The cats were anaesthetized 22–64½ hr later with chloralose (about 80 mg/kg intravenously after induction with ether). The parotid ducts were exposed and cannulated with fine glass cannulae. Drops of saliva falling from the cannulae were marked on a smoked drum by an electromagnetic signal, operated manually. Drugs were injected through a cannula in a femoral vein. In many experiments the auriculotemporal nerve on the side of proximal section was exposed and the peripheral part of it identified and stimulated electrically at supramaximal voltage, 20 shocks/sec and a duration of each shock of 2 msec. The acute experiment usually lasted for 15–17 hr and in the course of it additional doses of chloralose, 20–40 mg/kg, were given when required. At the end of the experiment the nerve cut distally was cut by the proximal approach also and the piece of nerve between the two sections was pulled out and measured. Its length varied between 12 and 20 mm.

RESULTS

The main observations are concerned with the beginning, the maximum and the end of the unprovoked parotid degeneration secretion appearing as a consequence of the parasympathetic denervation. In addition observations were made on provoked degeneration secretion. In the previous investigations it was noticed that at a time when degeneration secretion had not yet started but was expected to start soon, or when it had temporarily ceased as may occasionally happen in the parotid gland, it could be provoked by injecting acetylcholine intravenously. At that stage a small dose of acetylcholine evokes its ordinary quick secretory

response, followed, after a brief latency, by a flow of saliva which may last for a few minutes only or throughout a day's experiment. In the present experiments secretion was provoked using various secretory drugs and the responses of the two glands at different time intervals after denervation were compared. Attempts were also made to provoke secretion by electrical stimulation of the auriculotemporal nerve, but since it is difficult to find the fibres after distal section, this was only done on the side of proximal section.

Onset of degeneration secretion

In nine cats the experiment started 22–26 hr after denervation of the parotid glands. No secretion was then seen, but in the course of the following hours saliva started to flow, always first from the gland denervated distally. Secretion from this gland began 27–33 hr after denervation and the proximally denervated gland followed after an interval of about

TABLE 1. Beginning of degeneration secretion in hours after denervation

Expt. no.	1	2	3	4	5	6	7	8	9
Distal section	27	27½	28	28½	28½	29	30½	31	33
Prox. section	29	33	31	30½	32	31½	34	33½	36
Difference	2	5½	3	2	3½	2½	3½	2½	3

2–5½ hr (disregarding the fact that the proximal section was made 10–15 min before the distal section). Table 1 summarizes these observations, and Fig. 1 shows a typical experiment. It started 22 hr after denervation of the glands. No saliva was discharged until about 28 hr after denervation when a slow flow started from the gland denervated distally. It increased gradually in rate in the course of the following hours, reached a maximum of about one drop in 2 min and remained at this level until the end of the experiment. The contralateral gland started to secrete about 3 hr later than this gland and the rate of flow increased, reaching about the same level as that of the other gland towards the end of the experiment, about 38 hr after denervation. The flow from both glands had the characteristic paroxysmal appearance, with intervals of about 1 min, described previously (Emmelin & Strömblad, 1958).

In one case, when both glands were secreting at a rapid rate, the volumes of saliva were measured. In both glands fifty drops were found to correspond to 0·55 ml. saliva.

When extra doses of chloralose had to be administered the flow was often seen to diminish in rate or to stop completely for a period of about 1 hr; secretion was then resumed or could be started by provocation with a secretory drug (see below).

Maximum and end of degeneration secretion

In the experiment of Fig. 1 both glands secreted at the same rate about 38 hr after denervation, and this was obviously the maximal rate of degeneration secretion. In three other experiments of that series, lasting until 37–40 hr after denervation, equal rates were also reached by the two glands. In two cats the distally denervated gland still secreted more rapidly than the other gland at that stage. One experiment started 36 hr after denervation. Both glands secreted rapidly, producing about 1 drop/

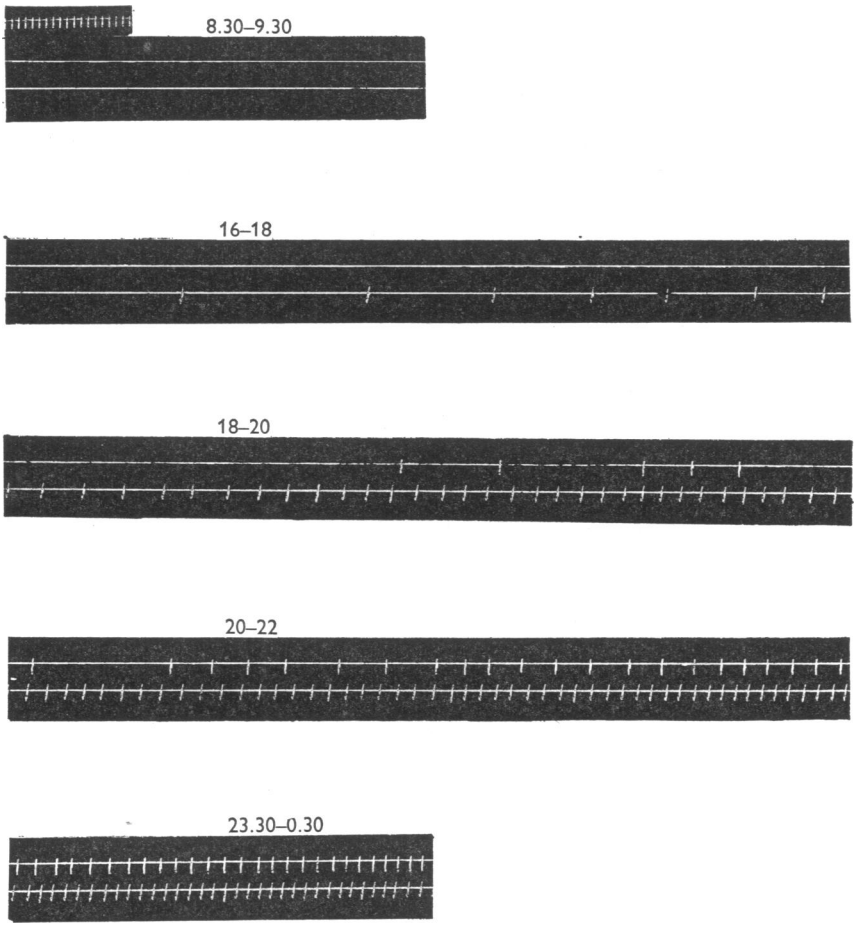

Fig. 1. Onset of degeneration secretion. The upper line in each section shows secretion from the proximally denervated gland, the lower line corresponds to the gland denervated distally. The figures above the sections show the time of the day. In the uppermost section min marks are shown. The auriculotemporal nerves had been cut at about 10.30 on the previous day.

min. The experiment lasted for 14 hr, and during that period the secretory rate decreased rapidly in the distally, but very little in the proximally denervated gland. In a series of six cats the experiments were begun 46–64½ hr after denervation (Table 2). In the last experiment, only the proximally denervated gland secreted, at a slow and decreasing rate for 1½ hr. In the experiment starting after 61 hr no degeneration secretion occurred. In the other four experiments both glands secreted in the beginning, but the proximally denervated gland much more quickly than the gland denervated distally. The secretory rate decreased in both glands, and the flow ceased first in the gland denervated distally. This is illustrated in Fig. 2 (Expt. no. 11 of Table 2).

TABLE 2. End of degeneration secretion in hours after denervation. The time after denervation (hr) at which the expt. started is also given

Expt. no.	11	12	13	14	15	16
Distal section	52	54	62½	56	—	—
Prox. section	58	58	> 64	61	—	66
Start of expt.	46	48	48	50	61	64½

Provoked degeneration secretion

Acetylcholine. The ability of acetylcholine to provoke degeneration secretion at an early stage after secretion of the auriculotemporal nerves is demonstrated in Fig. 3. No degeneration section had started 26 hr after denervation, when acetylcholine was given. It produced only a trace of saliva from each gland, but about 1 min later secretion was resumed on both sides. In the course of 3 or 4 min the gland denervated distally produced half a drop of saliva; from the other gland just a trace of saliva was obtained. When acetylcholine was again injected 27 and 29 hr after denervation the immediate secretory responses were the same as before, but the delayed effect was found to be increased. After 29 hr the provoked secretion of the distally denervated gland continued for the rest of the experiment at an increasing rate in the ordinary way. At this early stage it is thus possible to provoke degeneration secretion, and it is obvious that this is easier to do on the gland denervated distally, although the effectors of the two glands show the same sensitivity to acetylcholine. Similar observations were made in three other cats.

When degeneration secretion ceased temporarily as a result of an extra dose of chloralose, it could be restarted by acetylcholine, as shown in the upper part of Fig. 4. Here both glands were secreting 43 hr after denervation. Chloralose stopped secretion in both glands, but the flow was resumed after intravenous injection of acetylcholine. In some experiments it was possible to compare the immediate secretory effects of acetylcholine before degeneration secretion had commenced and after it

had been interrupted by chloralose. The sensitivity of the gland cells was about the same, and it thus seems likely that chloralose in some way stops the release of acetylcholine from the nerve endings, rather than depresses

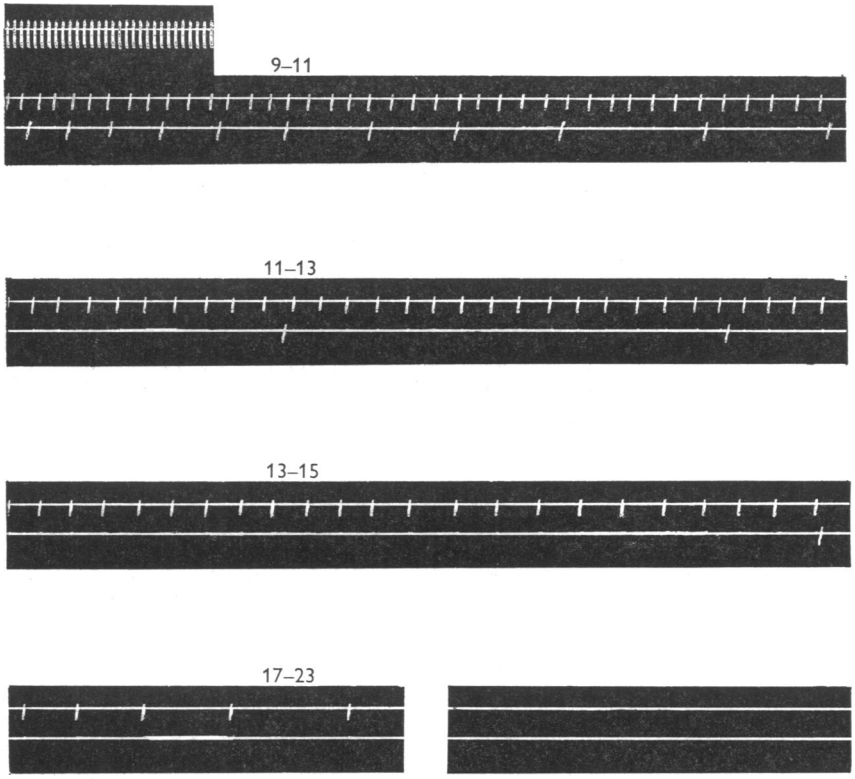

Fig. 2. End of degeneration secretion. Marks as in Fig. 1. The auriculotemporal nerves had been cut about 46 hr before the beginning of the experiment.

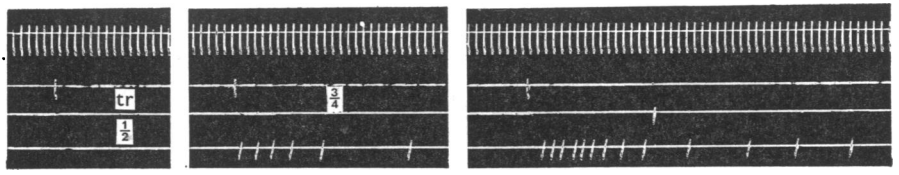

Fig. 3. Early secretion provoked by acetylcholine. The traces correspond to (from above downwards): min marks; signal; secretions from proximally and from distally denervated gland. The experiment started 24 hr after denervation, and there was no secretion. Acetylcholine 1 μg/kg was injected after 26 hr (left), 27 hr (middle) and 29 hr (right section). Each time both glands responded quickly with a trace of saliva (not marked), followed by a slow secretion about 1 min later.

the sensitivity of the glandular cells. Injected acetylcholine then restores the release.

When the degeneration secretion had come to an end, a period of flow could still be provoked by injecting acetylcholine. This is shown in Fig. 5, which corresponds to experiment no. 12 of Table 2. The gland denervated distally had ceased to secrete 54 hr, the other gland 58 hr after denervation. In the figure acetylcholine given 60 hr after denervation caused a quick

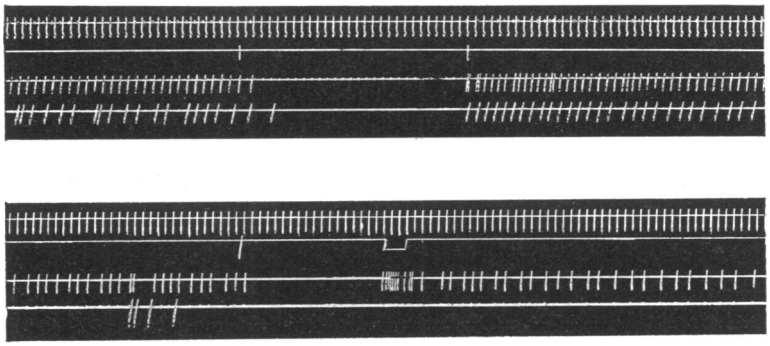

Fig. 4. Degeneration secretion stopped with chloralose and restarted, in two different cats. Records as in Fig. 3. Upper experiment: bilateral degeneration secretion 43 hr after denervation, ceased after chloralose, 30 mg/kg (first signal) and started again after acetylcholine, 2 μg/kg (second signal).

Lower experiment: Proximally denervated gland secreted rapidly after 48 hr. Chloralose, 40 mg/kg, stopped the flow (first signal); auriculotemporal stimulation for 3 min (second signal) caused a lively secretion and restarted the degeneration flow. The other gland showed slow, irregular secretion.

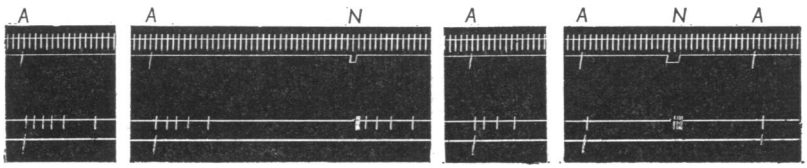

Fig. 5. Provoked secretion after the end of the unprovoked degeneration flow. Records as in Fig. 3. After 54 hr the flow had ceased from the distally and after 58 hr from the proximally denervated gland. The four sections show effects 60, 61, 62 and 64 hr after denervation. *A*: acetylcholine, 2 μg/kg. *N*: stimulation of the auriculotemporal nerve, cut proximally.

secretory response of one drop from each gland, but the proximally denervated gland continued to secrete for more than 10 min. The other gland, equally sensitive, showed no late secretion. During the following hours the acetylcholine sensitivity of the glands remained unchanged but the late secretion of the proximally denervated gland decreased and had ceased 64 hr after denervation. In some similar experiments acetylcholine provoked

secretion from both glands, but more from the gland denervated proximally than from the other gland. It is obvious that when the unprovoked secretion has ended, a period of degeneration secretion can still be provoked with acetylcholine, and the effect is now more marked on the gland that has been denervated proximally, in spite of the fact that the two glands show about the same sensitivity to acetylcholine.

Methacholine and carbachol. Similar observations were made with these drugs as with acetylcholine. An effect of methacholine 60 hr after denervation, after the end of the unprovoked secretion, can be seen in Fig. 7. Particular attention was paid to carbamylcholine (carbachol), since this drug has been found to have an essentially indirect effect on the superior cervical ganglion, acting by way of released acetylcholine (Volle & Koelle,

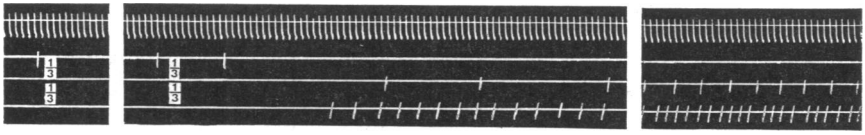

Fig. 6. Secretion after eserine. Records as in Fig. 3. No degeneration secretion had started 28 hr after denervation, when acetylcholine 5 μg/kg was given (first section). It was repeated after 32 hr followed by eserine sulphate 0·1 mg/kg (second section). The continuous flow that ensued is shown after 36 hr also (third section).

1961; McKinstry & Koelle, 1967). Control experiments showed, however, that 2–3 weeks after section of the auriculotemporal nerve carbachol produced a very lively secretion from the parotid gland, even considerably more than from the normal gland (owing to denervation supersensitivity). It can thus be concluded that in the parotid gland the dominating effect of carbachol is exerted directly on the glandular receptors.

Eserine. In the experiment of Fig. 6 no degeneration secretion occurred when the experiment started 24 hr after denervation, and when it had not begun 4 hr later acetylcholine was given. The sensitivity was found to be low; 5 μg/kg had to be given to produce a small response, and no delayed secretion was seen. When no degeneration secretion had started 32 hr after denervation and acetylcholine had a similar effect as before, eserine was injected. After a latency of about 15 min both glands started to secrete, the distally denervated gland much more rapidly than the contralateral gland; the flow continued for hours, increasing in rate, like the ordinary unprovoked secretion.

The effect of eserine when the unprovoked secretion had ceased is shown in Fig. 7 (Expt. no. 11, Table 2). The proximally denervated gland had stopped secreting 58 hr, the other 52 hr after denervation. Some secretion could be provoked with methacholine after 60 hr, particularly from the

proximally denervated gland; the sensitivity of the two glands was the same. Eserine given 30 min later caused secretion, which was more marked on the side where the nerve had been cut proximally.

Auriculotemporal stimulation. In five experiments the auriculotemporal nerve, cut proximally, was stimulated electrically as late as 60, 61, 62, 67 and 76 hr after denervation. Flow of saliva was always obtained, although it was probably less rapid than when the nerve had been more recently cut. The secretion seemed, however, too lively to be caused by the spread of the stimulus to intact sympathetic fibres in the vicinity, and this

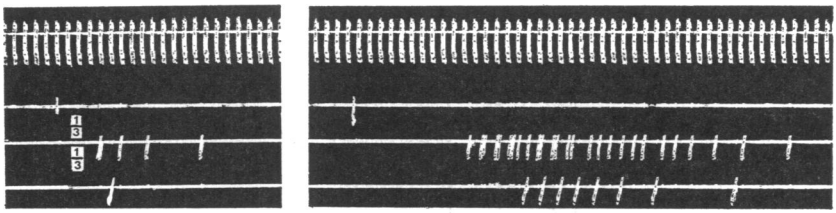

Fig. 7. Secretion after methacholine and eserine. Unprovoked secretion had ceased 58 hr (proximal) and 52 hr (distal) after denervation. First section: effect of methacholine 0·3 μg/kg 60 hr after denervation. Second section: eserine sulphate 0·1 mg/kg 30 min later. Records as in Fig. 3.

possibility was excluded by the finding that the flow was not affected by the injection of dihydroergotamine (0·3 mg/kg) but ceased after atropine (0·2 mg/kg). Stimulation of the nerve could provoke degeneration secretion which had been stopped by chloralose (Fig. 4, lower experiment). In the experiment of Fig. 5, where unprovoked secretion had come to an end, it caused a similar delayed response as acetylcholine 61 hr after denervation, but 3 hr later it had, like acetylcholine, only an immediate effect.

DISCUSSION

Several factors may contribute to produce the phenomenon of degeneration secretion after post-ganglionic parasympathetic denervation of salivary glands (see Emmelin, 1967*b*). The main cause, however, is that the leakage of acetylcholine from the post-ganglionic nerve endings which occurs normally but in quantities too small to evoke secretion (Emmelin, 1960, 1965; Assarson & Emmelin, 1964), temporarily increases above the secretory threshold. At one stage of degeneration the nerve endings can still synthesize acetylcholine (Nordenfelt, 1964) but are unable to retain it in a normal way. The present experiments show that this happens later if the piece of nerve left connected to the gland is long than if it is short. For instance, if the length of the piece is increased by 1–2 cm the onset of

the phenomenon is delayed by 2–5½ hr. Generally, this may be said to be the result of the degeneration proceeding from the point of lesion to the periphery. It is tempting, however, to assume that more material necessary for the normal function of the endings, and in particular for the storage capacity of the transmitter, is available in the longer than in the shorter piece of nerve. The transport along the axon in proximo-distal direction demonstrated by Weiss and co-workers (Weiss & Hiscoe, 1948; Weiss, 1961, 1963) has been supposed to be concerned with at least some of the components engaged in the transmission procedure at the endings, acetylcholinesterase and choline acetyltransferase (Feldberg & Vogt, 1948; Sawyer, 1946; Hebb & Waites, 1956; Lubińska, Niemierko & Oderfeld, 1961; Hebb & Silver, 1961; Lubińska, Niemierko & Zelená, 1963). It is also known that denervation supersensitivity, assumed to be due to loss of an action of acetylcholine, appears earlier (Luco & Eyzaguirre, 1955; Emmelin & Malm, 1965) and that the ability to synthesize acetylcholine decreases earlier (Emmelin, Nordenfelt & Perec, 1966) the nearer the effector a cholinergic nerve has been cut. If the delay of the degeneration secretion caused by denervation at a high level is dependent on axonal transport, such a process must be assumed to take place even in the piece of nerve disconnected from the cell body of the neurone; this seems to be in keeping with the views expressed by Weiss (1963) regarding the mechanism of transport.

Already before the degeneration secretion has started, and for some time after it has ceased, the mechanisms responsible for the retention of the transmitter are obviously in a labile state, as shown by the fact that secretion can be triggered off by a small dose of injected acetylcholine. The time at which this phenomenon appears and disappears is also dependent on the length of the axons left connected to the gland. The way in which this provoked secretion is brought about is unknown. Koelle (1962) has attributed a presynaptic action to acetylcholine, released by the nerve impulse, promoting further release of transmitter, and it may be that in the experiments described here injected acetylcholine has a similar effect under the present labile conditions. Attempts to support this hypothesis in experiments with carbachol failed because this substance was found to act preponderantly on the post-junctional receptors of the gland. In any case, impulses in the cut auriculotemporal nerve were found to be able to provoke degeneration secretion, and this may have been due to the acetylcholine released. In this connexion it is of interest to note that for the whole period during which degeneration secretion occurs or can be provoked by injected agents, impulses in the degenerating nerve can liberate acetylcholine in amounts sufficient for secretion.

Eserine was originally given in order to allow leaking acetylcholine to

accumulate to a secretory concentration in some experiments in which the sensitivity of the gland to injected acetylcholine was so low that it seemed unlikely that degeneration secretion should appear. Observations such as those shown in Figs. 6 and 7 suggest, however, that eserine can also provoke degeneration secretion, an effect probably mediated by preserved acetylcholine.

No attempts were made to study systematically whether the sensitivity of the gland cells changed in the course of the period following denervation or whether the two glands behaved differently in this respect. Since increased leakage of acetylcholine may temporarily depress the sensitivity of salivary glands (Emmelin, 1964*b*) it could be expected that during the observation period of the present experiments the sensitivity might decrease, and earlier in the gland denervated distally than in the other gland. There were certainly no marked differences in sensitivity between the two glands; minor differences would be difficult to detect because of the slow secretory response following the immediate response to injected drugs.

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