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THE EFFECTS OF ANTICHOLINESTERASES ON HUMORAL TRANSMISSION IN THE SUBMAXILLARY GLAND

BY

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The purpose of the investigation was to study the effect of anticholinesterases on the secretion of saliva by the submaxillary gland, and especially the spontaneous secretion provoked by those agents. The humoral transmitter of parasympathetic stimuli for salivary secretion has been shown to be acetylcholine (Beznák, 1932; Gibbs and Szelöczey, 1932), and that for sympathetic stimuli adrenaline (or noradrenaline) (Cattell, Wolff and Clark, 1934). There are definite but unexplained inter-relations between these two forms of nerve or humoral stimuli.

METHODS

Cats and dogs were used. Anaesthetics were pentobarbitone sodium ("Nembutal"), intraperitoneal or intravenous; chloralose, intravenous; urethane, intravenous; allobarbitone ("Dial")-urethane, intraperitoneal.

Both submaxillary ducts were usually cannulated, the left gland being used for the experimental modification and the right as a control. The saliva secreted was recorded by drop recorders, electronically operated, the jets delivering drops of 0.01 ml. for cats and 0.03 or 0.05 ml. for dogs. In order to obviate variation in drop size or viscosity, each saliva delivery tube was led to a bulb of about 200 ml. capacity filled with solution containing about 0.5% NaCl and 0.1% Na_2CO_3; the saliva entered at the bottom, and the saline solution was displaced through a tube at the top.

The chorda tympani and cervical sympathetic were prepared for stimulation when required; in the earlier experiments stimulation was effected by an Attree stimulator, at an appropriate voltage to give a submaximal stimulus, pulse width being usually 0.1 msec., and frequency 10 cycles/sec. (Wills, 1941). In the later experiments this stimulator was replaced by an inductorium having a mains transformer of 5V, in the primary. Electrodes were either a simple form of fluid electrode (Garry and Wishart, 1951), or plain double stainless steel electrodes applied to the nerve. Arterial pressures were recorded from the femoral artery with a mercury manometer.

Injections of chemical substances into the circulation were sometimes made intravenously into the femoral vein, but, more commonly, intra-arterially (at constant speed and volume) by a close arterial injection so as to be more or less localized to the district of one submaxillary gland. For this purpose the lingual artery was chosen, using a valved cannula of small capacity. In some of the earlier experiments we tried to limit the blood supply closely to the submaxillary gland by tying off all branches beyond the facial, but later this practice was often abandoned, and in consequence the close arterial injection did not affect the submaxillary gland alone; other regions affected were the parotid gland, the external ear, parts of the scalp, and some muscles of the face and pharynx. So far as we were able to judge, these areas of escape did not materially affect our results, except that larger doses were needed.

In some experiments the cholinesterase content of both glands was determined at the end, the right gland, which had not been injected, serving as a control: in other experiments the control gland was removed early in the experiment, and before any injections had been given. The glands were first frozen, cut up with a microtome, and then homogenized with a Potter-Elvehjem homogenizer. The activity was determined against acetylcholine, 0.015M, acetyl-β-methylcholine, 0.03M, and butyrylcholine, 0.03M, or benzoylcholine, 0.015M. Blood samples were also tested before and at the end of the experiment against acetylcholine, 0.015M.

In spite of every care in the homogenization of the tissue, there was some irregularity in the results, some of which were discarded.

As anticholinesterases we tried a variety of compounds, one of our aims being to inhibit, preferentially in one gland and not in the other, either the true or the pseudo-cholinesterase. In this we were more successful when using the pseudo-inhibitors than when using those which specifically inhibited the true cholinesterase, probably for the reason that the latter did not remain so firmly fixed at the sites of first presentation.

As specific inhibitors of true cholinesterase we used *N*-*p*-chlorophenyl-*N*-methyl carbamate of *m*-hydroxyphenyltrimethylammonium bromide (Nu 1250) (Hawkins and Mendel, 1949), and 1:5-bis(4-allyldimethylammoniumphenyl)-*n*-pentan-3-one dibromide

* The survival operations were performed by C.L.E.

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(BW 284C51) (Austin and Berry, 1953). We found the Nu 1250 the better, because of its slower reversibility.

For specific inhibition of the pseudo-enzyme we used tetra-*iso*propyl pyrophosphoramide ("Isoompa"), bis(mono*iso*propylamine)phosphinic fluoride ("Isopestox"; "Mipafox"), and di*iso*propyl phosphorofluoridate (DFP).

Inhibitors also used, and which acted on both types, were eserine, tetraethyl pyrophosphate (TEPP), *iso*propyl methylphosphonofluoridate ("Sarin"), ethyl *NN*-dimethylphosphoramidocyanidate ("Tabun"), cyclohexyl methylphosphonofluoridate (called "CHX" for short).

RESULTS

Response of the Submaxillary Gland to Transmitters

Close arterial injections of ACh evoke a secretion, the threshold dose varying considerably, according to the extent to which the substance is delivered to situations other than the glands concerned, to the rate of delivery of the injection into the blood stream, and to the state of the local circulation. When the arterial delivery area is restricted solely to the submaxillary (and sublingual) glands, doses as small as 0.01 μg. were found by Gibbs (1935) to be effective, and to be about as effective as (presumably maximal) chorda stimulation for 5 sec. In our experiments, in which the arterial supply was usually not restricted exclusively to the submaxillary and sublingual glands, we have used doses varying from 1 to 50 μg., and obtained responses usually over the threshold. The responses of the glands on the two sides were normally about equal, but there was a good deal of difference from one animal to another. Some glands seemed to be remarkably insensitive, and this seemed to be especially so with very large and old cats; no explanation is offered for this, but it was noted that in these instances the response to chorda stimulation and to adrenaline was normal.

Response to the less fugitive adrenaline could be obtained even by intravenous injection, and this method of administration has been regularly used by Emmelin (1953) in his recent studies. On arterial injection we usually got a small response to 5 to 10 μg. of adrenaline (Fig. 1). Noradrenaline was at most only about one-third as effective as adrenaline; this difference was reduced, and both responses increased, if cocaine was previously given. It may be, therefore, that the noradrenaline was less effective because of its more rapid destruction by amine oxidase; we did not pursue this point further. But it is in any case not due to the more intense vasoconstriction produced in the gland by the noradrenaline, because adrenaline injected at the peak of the rise of blood pressure caused by noradrenaline is as effective as when given alone (Fig. 1). Atropine in large doses to cats abolishes or reduces the response to adrenaline, a fact already well known.

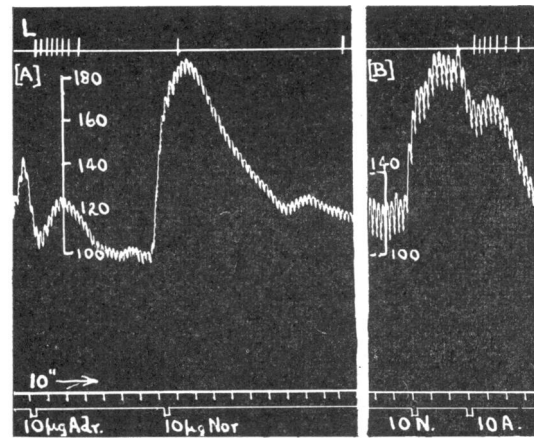

FIG. 1.—Cat. Allobarbitone-urethane. Tracings, in order from above down: top (L) drops of saliva from left Wharton's duct; second, femoral artery pressure; third, time, 10 sec.; bottom, signals. (A): 10 μg. adrenaline, then 10 μg. noradrenaline. (B): 10 μg. noradrenaline, and, at crest of blood pressure, 10 μg. adrenaline. In this and subsequent records, all injections are close arterial injections except where otherwise stated.

General Effect of Anticholinesterases

The effects of the intra-arterial injection of anticholinesterases differed according to whether the dose was small or large, and to the degree of reversibility of the effect on cholinesterase; there was also a broad difference in the effects of different anticholinesterases, according to their actions respectively on true and on pseudo-cholinesterase. Owing, however, to the fact that specific inhibitors of true cholinesterase are generally reversible, while those which specifically inhibit the pseudo enzyme tend to be irreversible, there was considerable overlapping in the types of action.

When the dose was small, but large enough to produce some effect, this consisted of potentiation of the action of ACh and of chorda stimulation. If the inhibition was reversible, these effects might also appear later on in the other gland, but, even with an easily reversible agent like eserine, the effect was often localized for a considerable time (15–30 min.). In that case, or when the reversibility was only slowly affected, as with TEPP or Nu 1250, the state of affairs seen in Fig. 2 appears. It is seen that the potentiation is confined to the initially poisoned side for at least 15 min., and that stimulation of the chorda tympani, or administration of ACh, on the poisoned side, causes secretion

(potentiated) on that side only. It was sometimes seen also that ACh administered to the unpoisoned side caused secretion from both glands, and that chorda stimulation or ACh applied to the poisoned side caused a fall of blood pressure much greater than that caused by similar treatment of the unpoisoned side.

With small doses of reversible inhibitors, the effects of potentiation of the action of ACh and of chorda stimulation were more clearly seen, but for the specific pseudo-inhibitors the doses necessary to produce effects were (taking the relative toxicities into account) sometimes rather large when compared with those of the reversible (true) inhibitors which produced similar effects.

As the dose of anticholinesterase was increased the effects described above were all intensified. The effects of ACh injection and of chorda stimulation were greatly augmented, and, especially after the dosage with a reversible inhibitor, were ultimately seen with both glands; ACh given intra-arterially to one gland, or stimulation of the chorda to one gland, were both often followed some seconds later by a fall of blood pressure, as was noted by Babkin, Gibbs and Wolff (1932), and by secretion of the other gland.

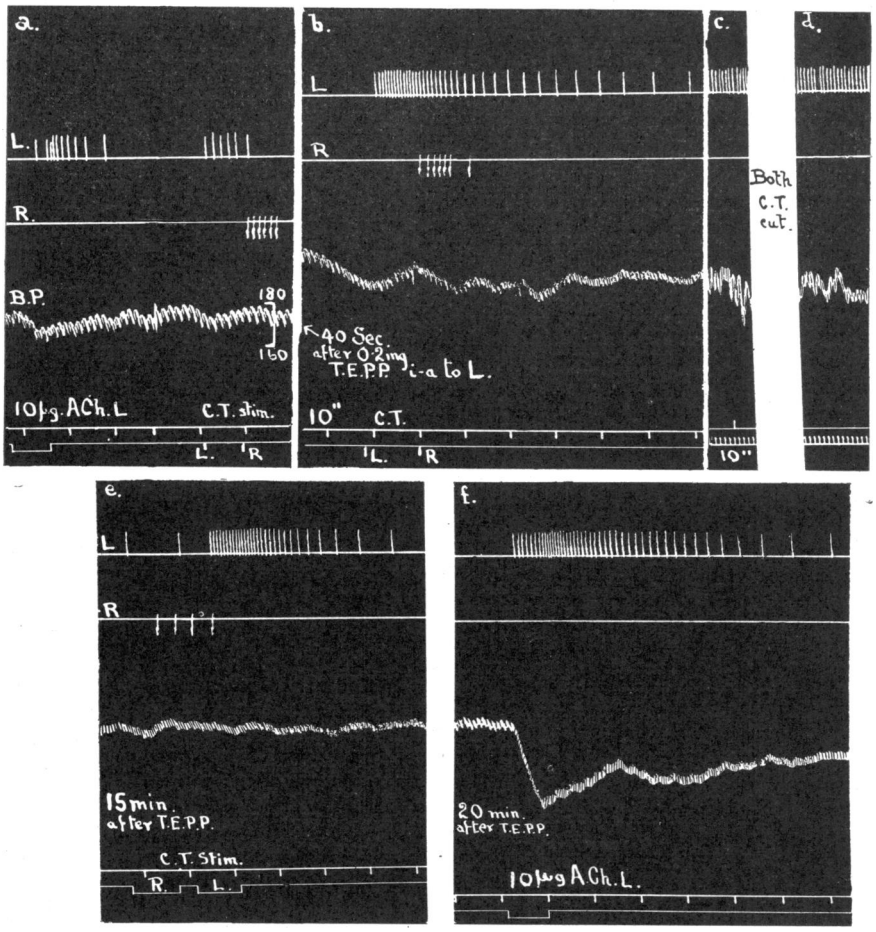

FIG. 2.—Dog. Allobarbitone-urethane. Records from above down, as before, except for (c) and (d) in which time trace and signal were transposed. Injections into left lingual artery. Stimuli 10/sec. 1 msec. (a): effect of 10 μg. ACh and of 10 sec. stimulation of left and of right chorda tympani. (b): begins 40 sec. after dose of 0.2 mg. TEPP i.a. to L; effect of 10 sec. stimulation of L and of R chorda. (c): 1 min. after (b) on slow drum to show steady flow from L gland. Between (c) and (d), also on slow drum, both chordas cut. (e): 15 min. after the initial dose of TEPP, potentiation of effect of 10 sec. stimulation of chorda is still only seen on L. (f): 5 min. later, spontaneous flow has stopped; 10 μg. ACh given.

At a certain further stage of poisoning it was found that stimulation of the gland, either by ACh or *via* the chorda, not only produced prompt and abundant flow of saliva, but also a steady and slowly dwindling after-flow (Fig. 2b). This after-flow was usually confined to the side stimulated, and might last for several minutes, the other gland remaining unaffected ; but sometimes, after a time-lag of about a minute, the second gland also began a slow rate of secretion which might even outlast that of the first affected gland. These phenomena of spread were most conspicuous with the reversible and true inhibitors.

With further dosage, and in every instance in which the dosage was carried to the point of producing symptoms of general poisoning, such as muscular fasciculations, a " spontaneous " secretion of saliva began in the initially poisoned gland ; sometimes only the initially poisoned gland gave a spontaneous flow, but often, especially with reversible inhibitors, the other gland followed ½–2 min. later (Fig. 3c). In some instances the gland remained quiescent until the chorda was stimulated or a dose of ACh was given, when the first brisk response started off a prolonged spontaneous secretion (Fig. 2). The spontaneous secretion, once established, sometimes continued indefinitely, and was very abundant, but at other times, where the dose was only marginal for this effect, it slowly faded out, but could be revived again by a further dose of the anticholinesterase. With eserine the spontaneous flow soon slackened off, and larger and larger doses were required to maintain it.

These results can probably be satisfactorily explained in terms of the ease of reversibility of the poisoning of the cholinesterase, and of the site and amount of the dose of the drug given. If the dose is small, not too rapidly injected, and the action irreversible, then the anti-ChE is fixed in the tissues of the injected field and in some of the blood which carried it there, but being immobilized will have but little if any effect in any other parts of the body. When the chorda of the injected side is stimulated, or when ACh is injected into that side, the stimulating effect will be intensified and prolonged because of the inactivation of the ChE in the gland or in its ganglia. For the same reason, and because the ACh set free by stimulation, or injected into the gland arteries, is not destroyed, it is passed on into the general circulation and causes a fall of arterial blood pressure. In our experience, the potentiation of the action of ACh and of chorda stimulation appear together, and in somewhat the same proportion, in which we are in agreement with Gibbs (1935).

With the reversible anticholinesterases, the drug, though fixed, is less firmly held in the tissues, and so is sooner or later washed out by the circulating blood and distributed all over the body. After an interval of time, therefore, if the dose be very small, the gland initially dosed will cease to show any effect, such as a potentiation of stimulation, nor, because of the smallness of the dose, will there be any effect on the other gland. Nevertheless we have been surprised by the relative tenacity with which even an easily reversible inhibitor like eserine is held in the gland. If the dose is a large enough one, however, the gland on the other side will sooner or later be affected, and will show the potentiation or even the spontaneous flow of saliva.

Causation of the Spontaneous Flow

The spontaneous flow of saliva which follows upon the administration of a sufficient dose of any of the anticholinesterases might be attributed to one or more of a number of causes, such as:

(i) To the presence of ACh, formed elsewhere, and accumulating in the circulating blood.

(ii) To a central action of the drug upon the salivary centres in the medulla.

(iii) To an action upon the ganglion cells on the course of the chorda fibres.

(iv) To an action upon cholinesterase at some site near to the secreting cells, e.g., in the parasympathetic nerve endings or in the gland cells themselves.

(v) To an action of the drug, other than as an anticholinesterase, upon the gland cells.

The first possibility is probably eliminated, at any rate as a main cause, in view of the fact, observed by Emmelin and Muren (1950), and which we have confirmed, that there is a continuous secretion of saliva from a gland perfused with eserinized blood or plasma ; moreover, the onset of secretion following the intra-arterial injection of a large dose of anticholinesterase would seem too prompt to admit of this explanation. But after large doses of anti-ChE it is possible that circulating ACh may later on be a minor contributory factor, because there is sometimes seen a slight build-up of secretion rate for some minutes after an initial injection.

That a central action of eserine affected blood flow and secretion of the glands was claimed by Heidenhain (1872), but this explanation is unquestionably inadmissible in these experiments, since the secretion rate is unaffected by section of all the nerves to the gland (Fig. 2d).

The third possibility, viz., that there might be some action on the relay ganglia on the course of

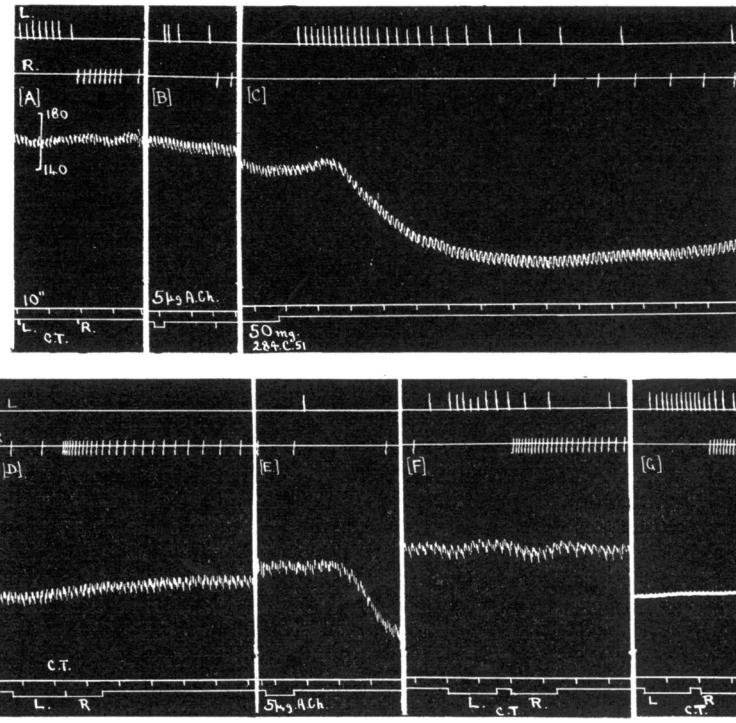

FIG. 3.—Dog. Allobarbitone-urethane. Injections into L lingual artery. (A): stimulation of L and R chorda tympani. (B): 5 μg. ACh. (C): 50 mg. 284C51. (D): stimulation of L chorda ineffective, that of R potentiated. (E): 5 μg. ACh also almost inactive. (F): slow spontaneous flow has returned to L, and was slightly potentiated by chorda stimulation, some 5 min. after (E). (G): 10 min. after F, full potentiation restored.

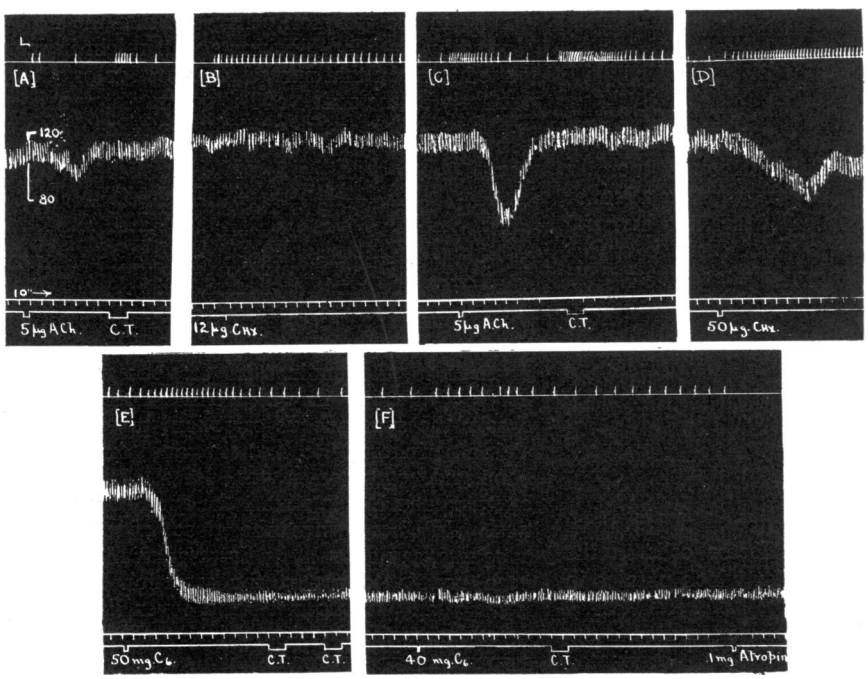

FIG. 4.—Dog. Allobarbitone-urethane. L, submaxillary duct; blood pressure; time 10 sec.; signals. (A): test dose of 5 μg. ACh to L and stimulation of L chorda 10/sec. (B): 12 μg. "CHX" to L artery. (C): 5 μg. ACh and stimulate L chorda. (D): 50 μg. "CHX" starts rapid spontaneous flow. (E): 50 mg. C6 slows but does not stop flow; stimulation of c.t. now ineffective. (F): 40 mg. C6 does not stop flow; 1 mg. atropine does so. Note C6 at first accelerates flow.

the chorda, is less easily appraised. It could, for instance, be claimed that in the perfused gland the scattered ganglion cells contained in it had also been affected by the drug. But, on the other hand, the administration of even large doses of ganglion-blocking agents, such as hexamethonium (C6), tubocurarine, M and B 2050, or nicotine, failed to do more than first to accelerate, and then, often only temporarily, slow down the flow (Fig. 4), except in cases where there was a catastrophic fall in blood pressure. The action of tubocurarine differed from that of the other agents, inasmuch as large doses given beforehand did prevent the spontaneous flow, whereas when given after the flow had been established only slowing occurred. Thus, in Fig. 13, the spontaneous flow due to 1.4 mg. of Nu 1250 to each gland was only slowed by a total of 40 mg. of tubocurarine, but in another experiment a preliminary dose of 12.5 mg. tubocurarine prevented spontaneous secretion when 7.5 mg. Nu 1250 was given to one gland alone. The dose of Nu 1250 usually needed to produce spontaneous secretion was from 0.05 to 0.5 mg. intra-arterially. After these heavy doses of blocking agents the chorda was always found to be blocked (Fig. 4), but ACh to be still effective. In one experiment the chorda and the hilum of the gland were painted with 10% nicotine in order to block the ganglia preferentially, but with no effect on the spontaneous flow, caused by 275 μg. of Nu 1250.

The fourth possibility, that the essential action is upon cholinesterase in or near to the secreting cells, is what would be expected. We should suppose this cholinesterase to be situated near to the parasympathetic nerve endings, though there is much uncertainty as to the structural relation of these to the secreting cells. Further, the humoral transmission hypothesis would lead us to expect that, in the normal functioning of the secretory cells in response to chorda impulses, ACh would be liberated at or near to the nerve endings. Both essential constituents are present, for the gland contains plenty of cholinesterase, and Chang and Gaddum (1933) have shown that the dog's submaxillary contains from 1.5 to 3.3 μg. ACh/g., while Emmelin and Muren (1950) found the cat's chorda to contain about 1.5 μg./g.

It seems likely that the relation between nerve endings, ganglion cells and effector cells in the salivary glands is similar to that which obtains in the small intestine. Feldberg and Lin (1949) showed that ACh is constantly being set free in the small intestine, and they inferred that it originated either in the nerve endings or in non-nervous structures. As regards the salivary glands, the recorded findings show a discrepancy, for although Beznák (1932) and Gibbs and Sze.öczey (1932) could find no ACh in the effluent from perfused glands prior to chorda stimulation, its presence was demonstrated by Henderson and Roepke (1933) and by Emmelin and Muren (1950), the amount being greatly increased by chorda stimulation. The last-named authors found that when curare was first added, chorda stimulation only released about one-third as much ACh, and this amount they thought might have come from preganglionic nerve endings.

If we accept the opinion that ACh is normally set free constantly from some structures (" nerve endings ") peripheral to the parasympathetic ganglion cells, and suppose that cholinesterase is present in the vicinity of liberation, so that under normal conditions the small amounts set free at the endings are not effective in producing secretion, though perhaps keeping the gland in a condition of constant subliminal excitation, then it would be reasonable to admit that after poisoning with an anticholinesterase the small amounts of ACh liberated will accumulate, and ultimately will reach a threshold concentration so that secretion will ensue. Since this flow is not stopped by the action of ganglion-blocking agents, it may be inferred that the source and site of action of the ACh cannot be wholly in the ganglion cells ; but that the flow is slowed by ganglion-blocking agents might be interpreted to mean that part of the secretion originated similarly by the action of ACh spared at ganglionic synapses. This seems unlikely, however, since, so far as we know, ACh is not liberated at synapses except on the arrival of impulses along the presynaptic fibres (Feldberg and Gaddum, 1934), and moreover it has been shown that eserine can rather have a paralysing action on ganglionic transmission (Feldberg and Vartianen, 1935).

We conclude, therefore, that the site of the liberation of the ACh is mainly if not entirely at, or near to, the endings of the parasympathetic nerves ; atropine in quite small doses at once checks the spontaneous secretion, presumably by acting peripherally to that site, and, once this has happened, even enormous doses of anticholinesterase may fail to restart the spontaneous secretion. In fact, when the dose of atropine is adequate, say over 20 μg. intra-arterially, we have been unable to restart the secretion by administering an anticholinesterase, even in doses 8 times larger than those originally needed to start a spontaneous secretion.

The Cholinesterases of the Submaxillary Gland

There is much confusion concerning the nomenclature of the cholinesterases, and we have no wish to add to it; we may admit the existence of two classes of enzyme, which differ not only in their relation to different substrates, but also in their responses to different inhibitors. Enzymes which resemble, say, the cholinesterase of human red cells in being able to hydrolyse ACh and acetyl-β-methylcholine more readily than other esters could reasonably be designated as true cholinesterases if they were also inhibited by those substances which inhibit red-cell cholinesterase. Similarly, enzymes which resemble human serum cholinesterase in rapidly hydrolysing benzoylcholine or butyrylcholine could be provisionally classified as pseudo-cholinesterases if they were found to be inhibited preferentially by the inhibitors of human plasma cholinesterase.

When these two types of enzyme occur together in the same tissue the estimation of their relative amounts is attended with a good deal of uncertainty. But we have made estimations on submaxillary gland tissue, using as substrates ACh, acetyl-β-methylcholine, benzoylcholine and butyrylcholine. The results are given in Table I, which shows that there is considerable variation

TABLE I

CHOLINESTERASE CONTENT OF SUBMAXILLARY GLAND OF CAT AND DOG EXPRESSED AS μl. CO_2/G./HR.

Species	Acetylcholine 0·015M	Benzoylcholine 0·015M	Acetyl-β-methylcholine 0·03M	Butyrylcholine 0·03M
Cat ..	645		282	518
	4,810		2,510	1,390
	6,550		3,200	3,200
	6,475		3,225	2,450
	5,715		2,695	1,428
Mean ..	4,839		2,382	1,797
Dog ..	1,680	—	1,000	1,360
	2,700	—	961	1,682
	1,560	948	1,130	2,415
	1,972	218	1,150	1,660
	3,100	787	2,100	3,580
	3,550	477	2,060	2,498
	477	—	445	111
	1,650	183	1,670	366
	2,840	187	1,917	102
	1,946	154	1,372	—
	842	243	823	—
	2,490	351	2,640	2,230
Mean ..	2,067	394	1,439	1,600

in the amount, but that on the average the cat's gland contains more of the true enzyme than the dog's, and that in both species there is plenty of true cholinesterase, but also some pseudo-cholinesterase.

Relation of True and Pseudo-enzymes to Function

In attempting to link together the types of enzyme with function, we have tried to find whether there was any discoverable relation between the degree of inhibition of one or the other class of enzyme and a clear physiological effect of that inhibition on salivary secretion. For this purpose we chose as indicative of effect the production of spontaneous salivation, and adopted two courses. In some experiments we removed the right gland for control at the start of the experiments, and then, when either secretion or symptoms of general poisoning had been produced by intra-arterial injection of the chosen anticholinesterase into the left gland, this was similary treated, and the change in the cholinesterase content during the experiment determined. In other experiments the injections of drug were made into the left gland, and both glands were removed for assay at the end of the experiment. We had expected that this procedure would be suitable, at all events when the irreversible inhibitors were used, since, from the physiological results, we believe these to produce effects only at the site injected.

In order to test this hypothesis we made an experiment with a radioactive inhibitor (Sarin labelled with ^{32}P), but, although this showed a greater fixation in the gland injected than in the control, most of the radioactivity was in the blood. However, it was disappointingly clear that, despite the clarity of the physiological results, even a supposedly irreversible inhibitor is by no means retained only at the first site, and that much of the inhibitor or its products is carried away by the blood, probably without ever having been fixed in the tissues at all, and although there might be no demonstrable effects produced on other parts of the body.

Table II gives the results of 17 experiments with various anticholinesterases, and is divided into two parts according to whether spontaneous salivation occurred or not. It will be seen that, in all experiments in which no secretion followed, the inhibitor was one which is known to attack the pseudo-enzyme preferentially (and the dose was not large), whereas in all those where the spontaneous secretion was seen the inhibitors were either of the class which attacks both types, such as TEPP, or were specific inhibitors for the true type. It is understood that the degree of specificity *in vivo* is not extremely high, and that if enough of any inhibitor is given both types will be affected, and salivation will ensue. We tried to strike a dose which would inhibit one type very much more

TABLE II
SPONTANEOUS FLOW IN RELATION TO CHOLINESTERASE INHIBITION

Expt.	Species	Drug	Control Out at	% Inhibition of Gland				Blood ACh	Spontaneous Flow
				ACh	MCh	BuCh	BCh		
9	Dog	DFP	End	88	88	—	69	—	No
11	,,	IO	,,	7	10	64	67	—	,,*
15	,,	IP	,,	28	56	87	—	—	,,
16	,,	DFP	Start	42	42	91	81	67	,,
24	Cat	DFP	,,	90	75	98	—	63	,,
26	,,	IO	,,	29	22	99	—	23	,,*
28	,,	IO	,,	20	1	99	—	43	Slow†
30	,,	IO	,,	60	45	73	—	25	Slow‡
			Means	45	42	87	72	44	
10	Dog	284C51	End	28	24	—	36	—	Yes
12	,,	TEPP	,,	74	76	82	66	59	,,
13	,,	TEPP	,,	93	90	86	81	—	,,
14	,,	284C51	,,	62	—	37	—	40	,,
17	,,	CHX	Start	63	77	60	54	85	,,
18	,,	5158	,,	90	85	86	83	100	,,
19	,,	Nu 1250	,,	—	70	35	—	—	,,
20	,,	Tabun	,,	84	77	74	—	46	,,
21	,,	Nu 1250	,,	63	70	26	—	51	,,
			Means	70	71	61	64	63	

IO=Iso-ompa. IP=Isopestox. * No potentiation of ACh or chorda. † 5 mg. cocaine i.v. previously. No potentiation of chorda after IO. ‡ 2 mg. cocaine i.v. previously.

than the other, but it will be seen that this effort was only partly successful. When the right gland was left *in situ* until the end of the experiment, the actual per cent inhibitions of ChE in the left gland must have been greater than appears from our results, especially in the instances in which reversible inhibitors were used—that is, in most of the instances in which the inhibitors acted predominantly on the true enzyme. The figures for whole blood, which were only assayed with ACh, are but little guide in assessing the degree of general poisoning; but if they show anything at all they indicate that this was more general and intense with the reversible inhibitors, which is what would have been expected.

Despite these various shortcomings, however, the mean values have interest, since they suggest that in order to result in a spontaneous flow there must be inhibition of something over 60% of true cholinesterase (with pseudo inhibited to a slightly less extent), whereas an inhibition of 70 to 80% of pseudo-cholinesterase will not result in salivation even if the true esterase is also some 40% inhibited. The generally accepted view that it is the true enzyme which has the more important physiological function is thereby substantiated. But some of the experiments do point to the possibility that the pseudo-enzyme may play a subsidiary part (Table II, Expt. 28), and it is well known that intense salivation is a conspicuous phenomenon in general poisoning with inhibitors such as DFP which preferentially inhibit pseudo-cholinesterase; it is possible, however, that this might be due to circulating ACh. The results of Koelle, Koelle and Friedenwald (1950) and of Burn, Kordik and Mole (1952) on the intestine are of interest in this connexion; they find that a fall in the pseudo-cholinesterase content of the intestine increases the tone of the small intestine, and sensitizes it to ACh. The small intestine in our view resembles the salivary glands in being the site of constant production of ACh, though from what particular sites is again uncertain; but it may well be that the pseudo-cholinesterase in both situations has a general mopping-up function of a subsidiary kind, preventing ACh concentrations from passing a certain limit. That it can only be subsidiary is shown by the fact that relatively large doses of pseudo-inhibitors are necessary even to produce a potentiation of the action of ACh or of chorda tympani stimulation, and in some of the experiments in Table II (11 and 26) despite considerable inhibition of the pseudo-enzyme there was no potentiation. In experiments 28 and 30 small doses of cocaine had been given previous to the iso-ompa, so that the cause of the slow spontaneous secretion is uncertain.

Block Phenomena in the Submaxillary Gland

It is well known that there is an optimal rate of stimulation of the chorda tympani (Willis, 1941) and that very rapid stimulation gives a slower rate of secretion than a slow one. This is an example of the Wedensky inhibition, which is well seen in neuromuscular conduction, where it has been shown (Evans, 1951) to be greatly exaggerated in poisoning by anticholinesterases.

What may be the same phenomenon can also be discerned in the salivary gland (Graham and Stavraky, 1951), though not with the same clarity and precision as at the neuromuscular junction. Fig. 5A is an example, and shows block by ACh during spontaneous flow due to Nu 1250. Sometimes the phenomenon may be seen even in the unpoisoned gland. In Fig. 5B it is seen that immediately following on a dose of 20 μg. ACh to the gland, stimulation of the chorda is much less effective than it was immediately before or about 1½ min. after.

We have seen no instance of "block" being produced by the administration of an anticholinesterase alone, however large the dose. We may interpret this to indicate that the amounts of ACh which are being constantly liberated in the gland are insufficient to cause block, even when the cholinesterase in the gland has been very greatly reduced; no doubt diffusion would account for its

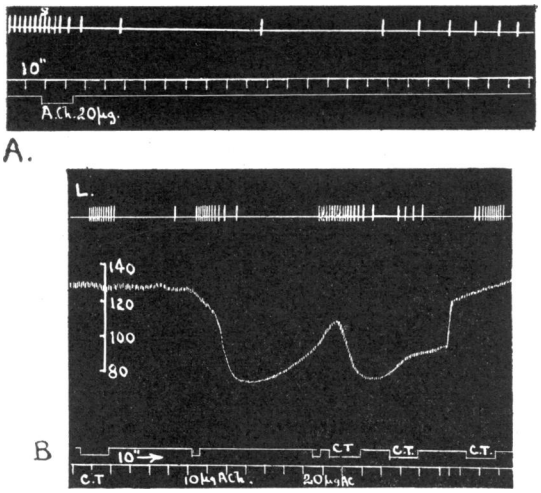

FIG. 5.—Cat. (A): Pentobarbitone. (Chorda cut 10 days before.) Steady flow due to Nu 1250 0.3 mg., 20 min. before. Block by 20 μg. ACh. (B): Allobarbitone-urethane. Second stimulation of chorda blocked and third stimulation partly blocked by a preceding dose of 20 μg. ACh. By the fourth stimulation the effectiveness of the chorda was restored.

failure to accumulate to that extent; but any further access of ACh either from the blood stream when it has been injected, or at tissue sites when the chorda is stimulated, may raise the amount to a level at which block occurs if its destruction is inhibited.

Phenomena of "Augmented Secretion"

It was first shown by Bradford (1888) that the secretory response to stimulation of either the chorda or the sympathetic was augmented if the chorda had been stimulated a few seconds beforehand. (The name "augmented secretion" was given to the phenomenon by Langley in 1889.) Subsequent investigations showed that instances could be found in which stimulation of either nerve disposed the gland to give an augmented secretion when the other nerve was subsequently stimulated (Babkin, 1950).

We have at various times obtained instances of these augmented secretions, and have also found that a dose of ACh could replace a chorda stimulation, and a dose of adrenaline replace sympathetic stimulation; that is to say that sympathetic stimulation or adrenaline gave a larger response when preceded by chorda stimulation (or by a dose of ACh), and *mutatis mutandis* for the other permutations of modes of stimulation. The phenomenon is not always demonstrable, and sometimes it is one permutation which shows it best and sometimes another. Fig. 6 illustrates the augmentation of ACh by adrenaline.

A satisfactory explanation of the phenomenon has never been given, and we only mention it here because it bears on the question of the effect of anticholinesterases on the response to sympathetic stimulation or to adrenaline. In many instances it was found that the response to both these forms of sympathetic stimulation was augmented after poisoning by anticholinesterase. A similar observation was made by Secker (1934), who inferred that ACh acted as the immediate transmitter of sympathetic impulses as well as of the parasympathetic impulses. Feldberg and Guimarāis (1935), however, considered it as merely another instance of the augmented secretion, and this is our explanation. We may suppose that after the poisoning by anti-ChE there is, as after chorda stimulation, sufficient ACh in or near to the gland cells to give an enhanced response when the adrenaline was given, or when the sympathetic was stimulated.

Phenomena of Diminished Secretion

The converse phenomenon of the application of one mode of stimulation resulting in a reduction of the rate of secretion has also been met. One instance of this is that of the occurrence of "block" already referred to.

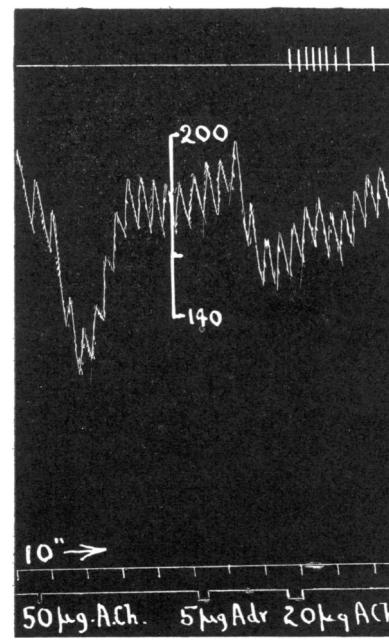

FIG. 6.—Dog. Allobarbitone-urethane. Showing that a gland which was relatively insensitive to ACh (50 μg.) was rendered more sensitive (to 20 μg.) by a dose of adrenaline (5 μg.) which itself had no effect on secretion.

We have, however, from time to time met with another phenomenon, namely, the inhibition by adrenaline or by sympathetic stimulation of a secretion caused by an anticholinesterase. This effect is strikingly seen when a rather large dose (50 μg.) of adrenaline is given intra-arterially during the spontaneous secretion evoked by an anticholinesterase, by pilocarpine, or during chorda stimulation, and is illustrated by Figs. 7, 8, 9 and 10A. Langley (1878) observed that sympathetic stimulation would check the pilocarpine secretion, and a similar result was noticed by Feldberg and Guimarāis (1935) in cats after dosage with eserine, followed by adrenaline. Langley attributed the phenomenon to ischaemia of the glands due to vasoconstriction. But the vascular responses of the gland to adrenaline or to sympathetic stimulation are now believed to be complex. Although Bunch (1900) found a shrinkage in volume of the gland under sympathetic stimulation, he observed that it was followed by a swelling. Cattell *et al.* (1934) found the blood flow to be reduced; but Carlson (1907), Carlson and McLean (1908), and Barcroft and Piper (1912) were all agreed that the constriction was transitory and was followed by a long-lasting and considerable dilatation, so that the blood flow through the gland was mainly increased.

Reduction of the blood supply to the gland has long been known to slow down the rate of secretion, and Carlson and McLean (1908) found that in the dog's parotid it reduced the rate of secretion from pilocarpine, as did also stimulation of the sympathetic. We found that stoppage of the blood supply by clipping off the carotid artery slowed down the spontaneous secretion, but not nearly so dramatically as did an injection of adrenaline (Fig. 8). When a large dose of "Ronicol" (Roche) (β-pyridyl carbinol) was given to produce arterial relaxation, the effect of adrenaline was more transitory (Fig. 9), and, after giving dihydroergotamine, adrenaline caused neither secretion nor slowing of the spontaneous flow; but stoppage of the arterial blood supply soon after this did speedily stop the secretion. This sudden arrest of secretion when the circulation is stopped just after the administration of adrenaline, as compared with the mere slowing which occurred after arrest of circulation in the normal gland, could, we think, be attributed to the fact that adrenaline produces a considerable increase in the oxygen usage of the gland (Barcroft and Piper, 1912), so that the anoxia resulting from ischaemia is much more sudden in onset. In short, we consider that the arrest of secretion caused by adrenaline is due to anoxia, as Langley supposed. When a condition of acute anoxia is suddenly produced by an intra-arterial injection of about 1 mg. KCN to an actively secreting gland, an arrest of secretion of very similar appearance is produced (Fig. 10B).

When the blood flow through the gland was observed, it was found (Fig. 11) that, in fact, the administration of anticholinesterase was accompanied by a considerably increased blood flow; ultimately a spontaneous secretion resulted, and the intra-arterial injection of adrenaline caused

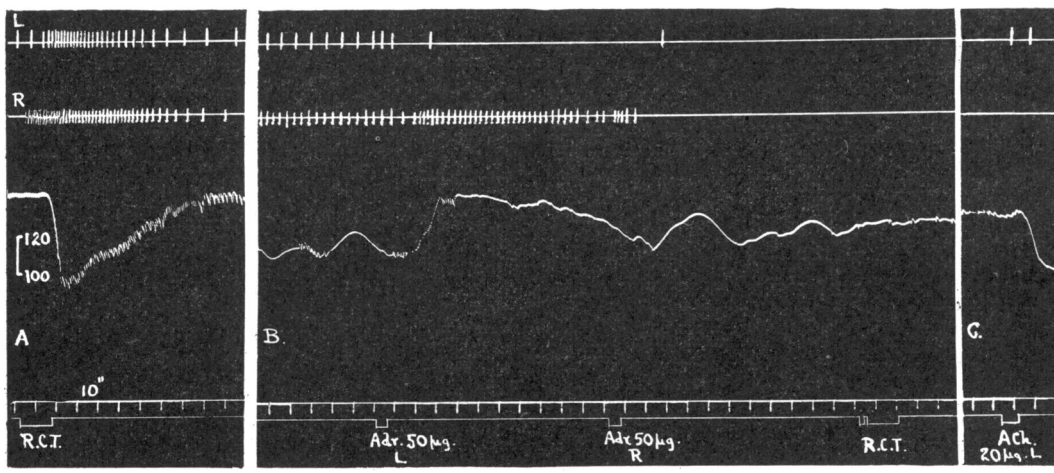

FIG. 7.—Cat. Pentobarbitone. (A): steady flow from both glands, following dose of 0.3 mg. Nu 1250 i.a. to each, 20 min. earlier was accelerated on both sides by stimulation of R chorda. (B): adrenaline, 50 μg. to L gland stops flow from L, and accelerates that on R; then 50 μg. to R gland checks its flow; the R chorda is then ineffective. (C): ACh 20 μg. to L produces small response 3 min. later; the initial response to 20 μg. ACh was 15–20 drops. (This cat had had L chorda divided 10 days before expt.)

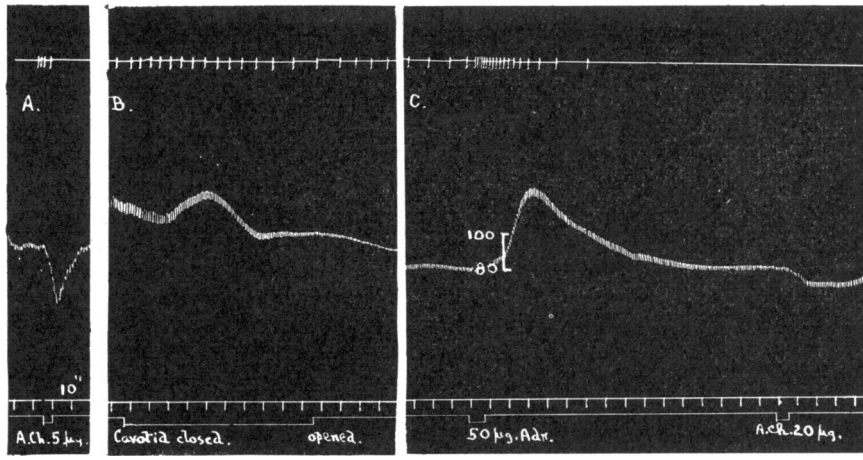

FIG. 8.—Cat. Pentobarbitone. (A): test dose of 5 µg. ACh. (B): spontaneous flow due to 1 mg. TEPP to gland 10 min. previously; effect of clipping of carotid artery. (C): effect of 50 µg. adrenaline in accelerating and then stopping spontaneous flow; during arrest 20 µg. ACh to gland gave no response. The arrest lasted 4 min.

both blood flow and secretion to be almost stopped. What we did not expect, however, was that even in the normal previously non-secreting gland intra-arterial adrenaline also caused considerable slowing or even prolonged arrest of the blood flow, and not, as commonly stated, a brief check followed by an increase. The explanation of this discrepancy was found when adrenaline was given intravenously; this caused a brief check followed by considerable increase in the blood flow through the gland. It would seem probable that the rise of general arterial pressure, as well as the production of products of metabolism, are together responsible for the increased blood flow, the vasoconstrictor action of the adrenaline being passively overcome by the raised arterial pressure.

One of the consequences of the ischaemia and anoxia of the gland which follow when adrenaline is given after the production of a spontaneous flow is that the gland temporarily fails to respond to chorda stimulation (Fig. 7)

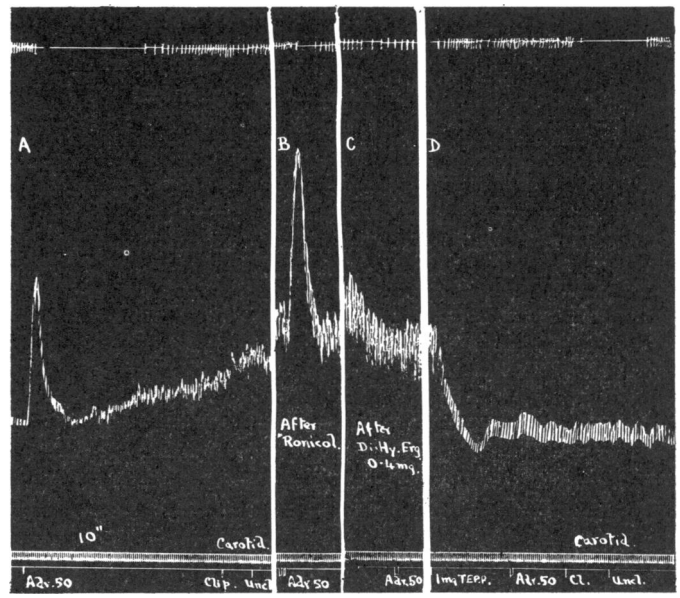

FIG. 9.—Same expt. as Fig. 8. Flow was increased by 1 mg. pilocarpine to gland. (A): adrenaline 50 µg. caused arrest; clipping off of carotid only slowing. (B): after 20 mg. "Ronicol," adrenaline only causes a stop of a minute or two. (C): after 0.4 mg. dihydroergotamine, adrenaline causes no slowing. (D): flow stimulated by 1 mg. TEPP is not arrested by 50 µg. adrenaline; closure of carotid artery now arrests the flow promptly.

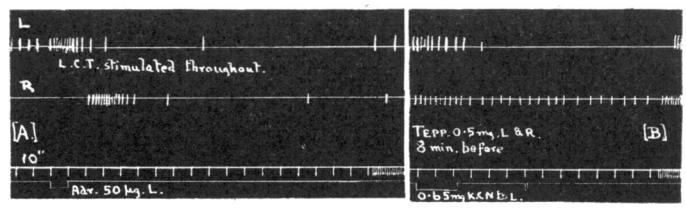

FIG. 10.—Cat. Pentobarbitone. (A): the L chorda was stimulated throughout. Intra-arterial injection of 50 µg. adrenaline to L gland first augmented and then stopped the flow; escaping adrenaline caused secretion of R gland. (B): flow started by TEPP, 0.5 mg. to each gland 8 min. before; injection of 0.65 mg. KCN into L gland stops flow abruptly.

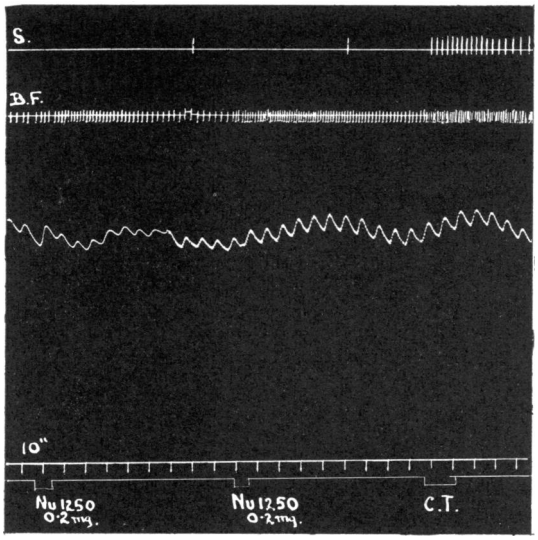

FIG. 11.—Cat. Pentobarbitone, heparin. S, drops of saliva from L gland. B.F., blood flow in drops from L gland. Two doses of Nu 1250 cause acceleration of blood flow, further accelerated by chorda stimulation; the flow of saliva was only slow.

and to ACh (Fig. 8); the latter evidently enters very slowly, as is shown by the slight effect which it produces on the arterial pressure, despite the dose of anticholinesterase (compare A and C, Fig. 8).

Graham and Stavraky (1953) also found that, whereas small doses of adrenaline intra-arterially cause mainly vasodilatation, large doses cause vasoconstriction, but with secretion also. But Stavraky (1942) finds, as we do, that when the gland is secreting spontaneously, under the influence of pilocarpine or eserine, small amounts of adrenaline increase, whereas large amounts diminish, the rate of secretion.

The Influence of Denervation

The left chorda tympani was sectioned or the left superior cervical ganglion removed under aseptic conditions, and the acute final experiments performed after the lapse of some days or weeks.

Section of the Chorda Tympani.—At the operation as great a length as possible of the chorda was removed, viz., from the point where the nerve left the chordo-lingual nerve up to or just beyond the point at which it ran alongside the duct, usually about 5–7 mm. In no instance was there a considerable loss of weight of the denervated gland at the time of experiment. It must be remembered that section of the chorda is only a decentralization, since, although some of the ganglion relay cells are scattered along its course, they are mainly aggregated near the hilum of the gland, and cannot be extirpated surgically. It was found by Chang and Gaddum (1933) that after section of the chorda the ACh content of the submaxillary gland fell considerably after 24 hr. and did not recover, but that sympathectomy had no effect on the ACh content. MacIntosh (1937) found no change in the cholinesterase activity of the gland after chorda section.

Incidentally, the relative sensitivity of the operated and intact glands to ACh and to adrenaline was tested. Opinions differ as to the effects of ACh after chorda section; some claim that the sensitivity of the gland is increased (Wills, 1941; Graham and Stavraky, 1951), others that it is diminished (Fleming and MacIntosh, 1935; Pierce and Gregersen, 1937).

Our experiments were inconclusive as regards the effect of chorda section on the sensitivity to ACh. Out of 11 cats the sensitivity to doses of ACh of the order of 1 to 10 μg. was increased in 3, unchanged in 4, and reduced in 4; in one dog it was reduced. The time after operation varied from 5 to 19 days, but within these limits did not seem to affect the results.

We concluded at the time that any change which was found after chorda section was due to some unknown incidental factors, and that there was no clear evidence that the sensitivity was necessarily altered by the operation in the sense that Cannon's law would demand. The important contribution of Graham and Stavraky (1953), which appeared while this paper was in course of preparation, does, however, seem to explain the discrepancies—including those found in our experiments—and will be referred to later. One peculiarity which we noticed was that, even when the total response of the two sides to ACh was the same, the rates of secretion differed, that on the denervated side always being more sluggish than that on the normal side (Figs. 12 and 14).

In testing the action of anticholinesterases some days or weeks after chorda section, we found that the gland on the denervated side was sometimes earlier and sometimes later in showing the characteristic effects of potentiation of ACh action and of the yielding of spontaneous secretion, but that the effects were complicated by the fact that it seemed to be more easily damaged or fatigued than the normal gland. This was especially noticeable when sarin was used, which was unfortunately the case in four of the experiments. This substance seemed to have a damaging effect on the denervated side, lowering all responses, and this led to the paradox of a reversal of the effects of

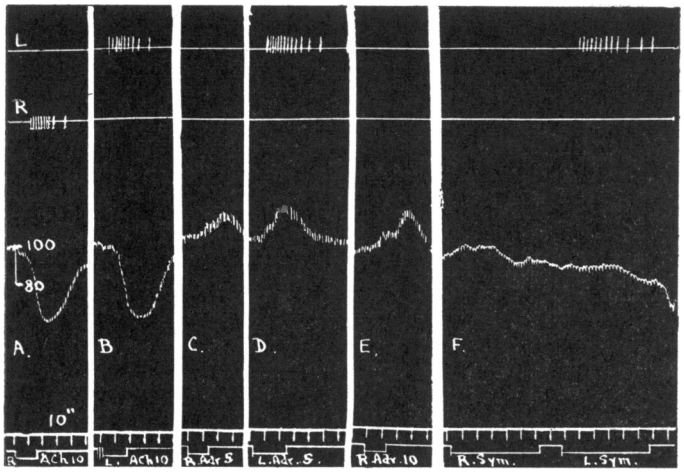

FIG. 12.—Cat. Pentobarbitone. Left chorda divided 7 days before. (A), (B): response of L and R glands to 10 μg. ACh. (C) and (D): responses of R and L sides to 5 μg. adrenaline. (E): response of R side to 10 μg. adrenaline. (F): responses to stimulation of R and L cervical sympathetic.

ACh on the two sides. Before the dose of sarin, the denervated side (L) was the more sensitive to both ACh and adrenaline; after the sarin, the response on the L side to both was greatly reduced, owing, we believe, to damage, whereas the response to both ACh and to adrenaline, following the usual course, was potentiated on the normal (R) side after the anticholinesterase.

There were no very striking differences in the action of TEPP or of Nu 1250 on the two sides. Small doses gave potentiation of the effects of ACh on both sides, and generally the threshold required on the denervated side was somewhat reduced, as compared with the other side. When larger doses were given, the spontaneous flow sometimes started sooner on the denervated side (Figs. 13 and 14), but was slower than that on the normal side; also, it tended to die out, as though from fatigue, leaving the normally innervated side the more affected.

In his studies on the pupil after decentralization or removal of the ciliary ganglion, Anderson found that pilocarpine was more, and eserine less, effective than on the normal side, eserine being in fact without action on the denervated pupil (Anderson, 1905b). It may be, therefore, that the varying results we got could be attributed to the removal of varying amounts of ganglia on the chorda.

In one experiment we tried the response of the two sides to pilocarpine, since Langley (1885) and Fleming and MacIntosh (1935) stated that it gave a smaller secretion on the denervated side, while Pierce and Gregersen (1937) and Emmelin and Muren (1951) found a greater one. Our results (Fig. 15) agreed with the latter finding, but the first rapid secretion from the denervated side was eventually outlasted by that from the normal, which developed more slowly.

The sensitivity of the chorda-denervated side to adrenaline, and to stimulation of the cervical sympathetic, was found to be clearly enhanced in most experiments (Fig. 12); where it was diminished, the response to ACh was also diminished, and it is perhaps worth mentioning that in 3 such cases out of 4 the cats were abnormally large and old; we agree with the findings of Fleming and MacIntosh (1935) and Emmelin that after chorda section the gland is more sensitive to adrenaline.

Sympathetic Denervation.—This was effected by surgical removal of the superior cervical ganglion; the acute part of the experiment was carried out a few weeks later. The denervated side, as expected, proved to be clearly more sensitive to adrenaline than the normal side; to ACh there was no difference. The action of the anticholinesterase appeared to differ in no way from that on the normally innervated gland.

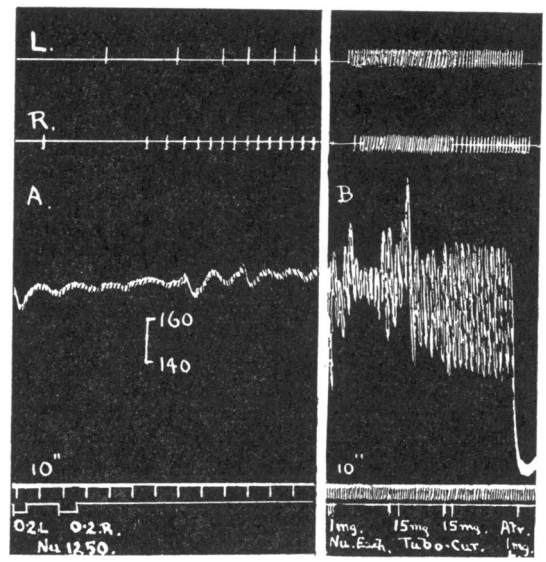

FIG. 13.—Cat. Pentobarbitone. L chorda cut 7 days previously. (A): Nu 1250 0.2 mg. to L and R glands. (B): 30 min. after A, flow has slackened; 1 mg. Nu 1250 (making a total of 1.4 mg. to each) given to each gland restarts it. Two doses of 15 mg. tubocurarine (making a total of 40 mg. to the two glands) fail to arrest flow; atropine 1 mg. arrests it instantly.

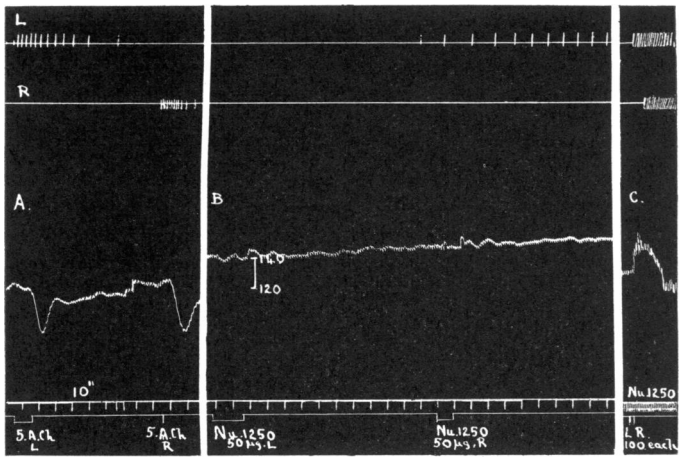

FIG. 14.—Cat. Allobarbitone-urethane. L chorda cut 12 days before. (A): equal but differently spaced responses to 5 µg. ACh on two sides. (B): Nu 1250 50 µg. first to L and then to R gland starts off flow from L, not from R. (C): Nu 1250 to L and R glands (100 µg. each) starts off flow first from L.

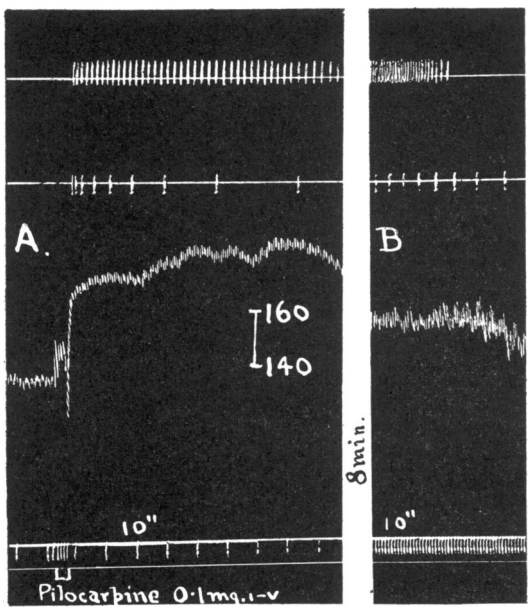

FIG. 15.—Cat. Allobarbitone-urethane. L chorda cut 19 days before. (A): pilocarpine 0.1 mg. i.v. starts off rapid flow from L gland, slow one from R gland. (B): 8 min. later, flow from L gland stops suddenly, that from R continues for some minutes.

DISCUSSION

All the anticholinesterases we have tried, when given in suitable doses, caused the potentiation of the effects of ACh in small amounts or of stimulation of the chorda tympani, and all of them when given in sufficiently large doses caused a spontaneous secretion of saliva. No evidence has been encountered which precluded the hypothesis that these effects were due to the action of these agents in inhibiting cholinesterase, except the general toxic effects of large doses, seen especially with sarin, and which may mean that some other enzyme systems are then made ineffective. When the drugs were given by close arterial injection the most pronounced effects tended to be confined to the sites of administration, despite some evidence of general dispersal of the anticholinesterase or of its decomposition products. The explanation of the potentiated action of small doses of ACh or of chorda stimulation seems obvious enough on the basis of cholinesterase inhibition. As regards the production of the spontaneous flow, and granted that the action is peripheral, there could be two explanations. The first would be that the anticholinesterases directly stimulate the secreting cells in addition to inhibiting cholinesterase; as a variant of this it could be supposed that they cause liberation of ACh as well as delaying its destruction.

The other, and more likely, explanation would be that there is normally, and independently of any arrival of nervous impulses, a constant liberation of ACh in the tissues of the gland, but that the rate of liberation is too slow to allow of the concentration, faced with the dual losses due to diffusion and to the action of cholinesterase, reaching a threshold value. The anticholinesterase, by checking one of these sources of loss, allows the concentration of ACh to mount up to a level controlled by diffusion alone, and adequate to cause a continuous secretion from the gland. There is evidence in the literature, to which reference has already been made, of such a liberation of ACh by the submaxillary gland, though it is not unanimous, and we have not fully confirmed it. In order to do so, and to exclude the possibility that the anticholinesterase might itself liberate ACh, it would be necessary to demonstrate the presence of ACh in venous blood or in saline perfusion effluent from the submaxillary gland without the addition of eserine or other anticholinesterase. But it is not improbable that a slow liberation of ACh is a universal phenomenon, with only quantitative differences from one tissue to another. We have, for instance, the example of the intestine, to which reference has already been made, and of the production of the miniature end-plate potentials in striated muscle by liberation of ACh (Fatt and Katz, 1952). In

the salivary glands of ruminant animals, moreover, there is a state of constant secretion, which could probably be attributed to such a liberation normally reaching values over the threshold. The constant state of secretion of the sublingual gland in the cat (Emmelin, 1953) probably does not fall into this category, since it is not stopped by atropine.

Unless the spontaneous flow is maximal, it can be accelerated by injection of small doses of ACh or by chorda stimulation, though larger doses of ACh or more prolonged or powerful stimulation of the chorda can produce "block"—a phenomenon which can also be demonstrated on the unpoisoned gland.

Small doses of atropine check the spontaneous flow at once, and thereafter the administration of even huge doses of anticholinesterase fails to restart it. This is understandable in view of what has been said. Atropine is regarded as raising a barrier to the action of ACh; when the barrier is adequate to keep back that concentration of ACh, which accumulates when all the cholinesterase has been inactivated, and if a state of equilibrium has been established between rate of ACh production and the rate of its dissemination by diffusion, any further dose of anticholinesterase, assuming it does not increase the rate of ACh production, could not overcome the atropine barrier. With pilocarpine or with ACh matters are different, since these probably act similarly and at similar sites; hence it is possible, in a series of administrations alternatively of pilocarpine and atropine, to start and stop the spontaneous secretion, as was shown by Langley (1880) and as we have verified.

Ganglion-blocking agents given by close arterial injection, or nicotine painted on the ganglia, do not arrest, though they may slow down, the spontaneous flow; the slowing is probably due to the considerable fall in arterial pressure which these agents cause, and not, we think, to interference with an excitatory state in the ganglia. The action causing spontaneous flow is therefore a peripheral one.

The spontaneous flow is accelerated by adrenaline or by sympathetic stimulation, and this would seem to be a phenomenon akin to the familiar accelerated or "augmented" secretion. If, as we suppose, the spontaneous flow is due to the ACh liberation having been rendered effective by inactivation of the cholinesterase, it would be reasonable to regard the gland as being virtually in a condition of parasympathetic excitation, and so to be liable to be highly sensitive to adrenaline.

The striking arrest of the spontaneous flow which is caused by the close arterial injection of large doses (e.g., 50 μg.) of adrenaline, and which has been noted in the literature without having been explained, may be attributed to the combination of raised glandular metabolism with a long-lasting ischaemia of the gland. It occurs also when the flow of saliva has been produced by chorda stimulation or by pilocarpine, and is not therefore peculiar to the state of anticholinesterase poisoning. Our experiments on the blood flow through the gland have shown that such large doses of adrenaline intra-arterially do cause prolonged ischaemia both in the normal and in the spontaneously secreting gland.

One of the main objects of our study was to find whether any correlation could be found between the type of cholinesterase inactivated and the production of such physiological effects as the spontaneous flow of saliva. Although the results were not so clear-cut as we hoped for, they did show that, for equal degrees of inactivation, that of the true cholinesterase was the more effective. In order to result in the production of a spontaneous flow it would appear that at least some 60% of the true cholinesterase must be put out of action, whereas the inactivation of some 80% of the pseudo-cholinesterase did not lead to a spontaneous flow, though it might produce some potentiation of the action of ACh or of chorda stimulation. In general agreement with these observations, Riker and Wescoe (1949) found that the maximal decrease of threshold of chorda stimulation occurred with DFP dosage when the total ChE content of the gland had been reduced to about 10% of its original value; and that at about the same level the spontaneous flow began. Reduction to 50% they found to have no physiological effects.

As regards the effects of chorda section, we were unable to demonstrate any regular alteration in the sensitivity of the gland to ACh, such as might resolve the existing discrepancy in the literature. One feature was invariable, viz., that the denervated gland always secreted more slowly than the normal one; but we can offer no explanation for this. It was found by MacIntosh (1937) that the cholinesterase content of the gland was unaltered after chorda section.

The work of Graham and Stavraky (1953), which appeared during the preparation of this paper, seems to have given the explanation. They have shown that doses of ACh above a certain level cause vasoconstriction in the gland, with reduced secretion. This is no doubt the same effect which

we have attributed to block from excess ACh. Moreover, they find that this effect is considerably enhanced in the denervated gland. It is not possible directly to compare the doses we used with those given by Graham and Stavraky. We did not confine the arterial supply to the gland alone as they did, so that to produce results similar to theirs our intra-arterial doses were much larger. Our doses ranged from 1 to 10 μg., or occasionally more, whereas theirs were from 10^{-4} to 10 μg. on the small-dose range—though from 50–1,000 μg. in the large dose-range. It is probable that, since the effect is enhanced by denervation, the doses we used were of an order that tended to slow down the effect on the denervated gland and not on the normal one. We believe that in accordance with the recent work of Graham and Stavraky it may be accepted that the gland is more sensitive to ACh after chorda section, provided that small doses are involved. This would explain our finding that, on the balance, the operated gland was found to be more sensitive to anticholinesterases. We also found it more sensitive to pilocarpine.

We have also confirmed the now well-established fact that after chorda section there is an increased sensitivity of the gland to adrenaline. This augmentation of sensitivity to a close relative of the sympathetic transmitter, after decentralization of the parasympathetic supply, is apparently not an isolated phenomenon. The converse of it, an increase of sensitivity to ACh after sympathetic denervation, was found by Graham and Stavraky (1953). It is also, in a way, paralleled by the phenomenon of " paradoxical pupil constriction " seen after section of the third nerve or removal of the ciliary ganglion when the animal is anaesthetized and partially asphyxiated, i.e., under conditions in which adrenaline is liberated (Anderson, 1905a). The effect of adrenaline on the denervated pupil is paradoxical inasmuch as it resembles the action of the parasympathetic, though differing from it, as does the potentiation of the effect of adrenaline on the (chorda-) decentralized salivary gland, in being unaffected by small doses of atropine.

The effects of anticholinesterases on sympathetically denervated or parasympathetically decentralized glands did not differ in any essential respect from their effects on normal glands. The slightly more prompt response on the side with chorda divided might have been due to small differences in blood flow, or to the state of subliminal excitation due to an actual or incipient paralytic secretion. MacIntosh (1937) found no change in the cholinesterase content of the gland as a result of chorda section ; in any case, the paralytic secretion is not to be attributed to an increased release of ACh, since, as we have verified, it is not stopped by atropine, and moreover Chang and Gaddum (1933) found the ACh content of the gland to be reduced after chorda division.

The experiments, however, do show that the action of anticholinesterases on the salivary glands is almost wholly, if not entirely, due to a peripheral effect, exercised, it may be supposed, on the nerve endings of the chorda. These endings are inconspicuous, and the spatial relation of the cholinesterase sites and of the site of production or liberation of ACh to them is obscure ; it is not even known with any certainty whether the endings are inside the secreting cells or not, though there is some evidence that they are to be found only in the mucous cells, the serous cells being supplied by the sympathetic (Babkin, 1950). Whatever their site and relations, however, it seems reasonable to believe that there is a constant liberation of ACh from some place near to or within the secreting cells, that the cholinesterase is near to that place, so that this slowly liberated ACh never reaches such a local concentration in the resulting gland cells as to cause them to secrete. Stimulation of the chorda, by liberating ACh at a greatly accelerated rate, or anticholinesterases, by retarding the rate of its destruction, enable enough of it to reach the cells to cause them to secrete ; the potentiation of the action of ACh or chorda stimulation by anticholinesterases is self-explanatory.

The phenomena of the augmented secretion have been a puzzle to physiologists for the past half century or more, and enter here into our problem. Explanations have usually amounted to little more than a re-statement of the facts of experiment. It certainly seems odd that the activity of one type of nerve should augment that of the other, especially since the functions of the sympathetic and parasympathetic supply to many organs are apparently opposed, and seem to have little in common, their chemical transmitters being different. Looked at from the standpoint of modern theories of excitation, however, they have probably one thing in common—that the state of excitation is always the result of a depolarization of a polarized membrane. Even if we accept the view that the sympathetic and parasympathetic fibres are supplied to cells of different types, we might admit that some membrane in the mechanism of the acinus supplied by the sympathetic fibre was depolarized by adrenaline, while that of the other type was depolarized by ACh. These cells are in close histological proximity to each

other, they may be bounded on one side by a common membrane—the basement membrane ; and it does not seem beyond the bounds of possibility that in such adjacent cells, especially if they are of slow rates of accommodation, the depolarization of the bounding membrane of a cell of one type might induce a facilitated state in a contiguous membrane bounding an adjoining cell. Until some means of putting this explanation to the test of experiment can be devised, it must remain a speculation.

Summary

1. When an anticholinesterase is administered by close arterial injection to the gland much of it is retained by the gland tissues. The effect is, first, to potentiate doses of ACh or a stimulus to the chorda tympani, then, as further doses are given, to cause a spontaneous secretion of saliva.

2. The less readily reversible anticholinesterases, when given by close arterial injection, stay firmly and for long periods in the gland first dosed, while the more reversible ones may pass on and even affect the opposite gland.

3. The spontaneous flow of saliva is caused by a purely peripheral action, and is unaffected by section, and but slightly affected by degeneration, of the nerve supply. It is slowed, but not stopped, by ganglion-blocking agents.

4. The spontaneous flow of saliva produced by an anticholinesterase is hastened by ACh or by chorda stimulation, except when the flow is already maximal. It is also hastened by adrenaline ; this phenomenon is a variant of Langley's phenomenon of " augmented secretion." Large doses of adrenaline, however, when injected intra-arterially, stop the spontaneous flow for long periods ; this effect is due to local vasoconstriction and anoxia, enhanced by raised metabolism.

5. In order to produce spontaneous flow, a large fraction of the true cholinesterase must be inhibited ; inhibition of a larger amount of the pseudo-enzyme, if not accompanied by considerable inhibition of the true enzyme, does not produce a spontaneous flow, though potentiation of the action of ACh may be shown.

6. The production of the spontaneous flow is explained as being due to the revealing, by inhibition of cholinesterase, of a constant production of ACh, normally insufficient to cause secretion, by the gland.

7. Block phenomena attributable to the presence of excess ACh can be demonstrated in the salivary gland ; the explanation of this is similar to that for neuromuscular block. No block is produced by anticholinesterases unless ACh is also given or produced by chorda stimulation.

8. The phenomenon of augmented secretion may perhaps be explained on the modern theories of excitation.

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References

Anderson, H. K. (1905a). *J. Physiol.*, **33**, 156.
—— (1905b). Ibid., **33**, 414.
Austin, L., and Berry, W. K. (1953). *Biochem. J.*, **54**, 695.
Babkin, B. P. (1950). *Secretory Mechanisms of the Digestive Glands.* New York: Hoeber.
—— Gibbs, O. S., and Wolff, H. G. (1932). *Arch. exp. Path. Pharmak.*, **168**, 32.
Barcroft, J., and Piper, H. (1912). *J. Physiol.*, **44**, 359.
Beznák, A. von (1932). *Pflüg. Arch. ges. Physiol.*, **229**, 719.
Bradford, J. R. (1888). *J. Physiol.*, **9**, 287.
Bunch, J. L. (1900). Ibid., **26**, 1.
Burn, J. H., Kordik, P., and Mole, R. H. (1952). *Brit. J. Pharmacol.*, **7**, 58.
Carlson, A. J. (1907). *Amer. J. Physiol.*, **19**, 408.
—— and McLean, F. C. (1908). Ibid., **20**, 457.
Cattell, M., Wolff, H. G., and Clark, D. (1934). Ibid., **109**, 375.
Chang, H. C., and Gaddum, J. H. (1933). *J. Physiol.*, **79**, 255.
Emmelin, N. (1951). *Physiol. Rev.*, **32**, 21.
—— (1953). *Acta physiol. scand.*, **30**, *Suppl. III*, 34.
—— and Muren, A. (1950). Ibid., **20**, 13 ; **21**, 362.
—— —— (1951). Ibid., **24**, 103.
Evans, C. Lovatt (1951). *J. Physiol.*, **114**, 6P.
Fatt, P., and Katz, B. (1952). Ibid., **117**, 109.
Feldberg, W., and Gaddum, J. H. (1934). Ibid., **81**, 305.
—— and Guimarãis, J. A. (1935). Ibid., **85**, 15.
—— and Lin, R. C. Y. (1949). Ibid., **109**, 475.
—— and Vartianen, A. (1935). Ibid., **83**, 103.

Fleming, A. J., and MacIntosh, F. C. (1935). *Quart. J. exp. Physiol.*, **25**, 207.
Garry, R. C., and Wishart, M. (1951). *J. Physiol.*, **115**, 61P.
Gibbs, O. S. (1935). Ibid., **84**, 33.
—— and Szelöczey, J. (1932). *Arch. exp. Path. Pharmak.*, **168**, 64.
Graham, A. R., and Stavraky, G. W. (1951). *Fed. Proc., N.Y.*, **10**, 301.
—— —— (1953). *Rev. canad. Biol.*, **11**, 446.
Hawkins, R. D., and Mendel, B. (1949). *Biochem. J.*, **44**, 260.
Heidenhain, R. (1872). *Pflüg. Arch. ges. Physiol.*, **5**, 309.
Henderson, V. E., and Roepke, M. H. (1933). *Arch. exp. Path. Pharmak.*, **172**, 314.
Koelle, G. B., Koelle, E. S., and Friedenwald, J. S. (1950). *J. Pharmacol.*, **100**, 180.
Langley, J. N. (1878). *J. Physiol.*, **1**, 364.
—— (1880). Ibid., **3**, 11.
—— (1885). Ibid., **6**, 71.
—— (1889). Ibid., **10**, 291.
MacIntosh, F. C. (1937). *Proc. Soc. exp. Biol., N.Y.*, **37**, 248.
Pierce, F. R., and Gregersen, I. (1937). *Amer. J. Physiol.*, **120**, 246.
Riker, W. F., and Wescoe, J. (1949). *J. Pharmacol.*, **95**, 515.
Secker, J. (1934). *J. Physiol.*, **81**, 81; **82**, 293.
Stavraky, G. W. (1942). *Rev. canad. Biol.*, **1**, 64.
Wills, J. H. (1941). *Amer. J. Physiol.*, **135**, 164.