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UPTAKE OF INJECTED [^{14}C]ADRENALINE IN DENERVATED AND IN NORMALLY INNERVATED SUBMAXILLARY GLANDS OF THE CAT

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Radioactive adrenaline was injected into cats after one submaxillary gland had been denervated, either by section of the chorda-lingual nerve or by excision of the superior cervical ganglion. No indication was found that the supersensitivity caused by denervation was due to a greater uptake of adrenaline in the denervated gland. Stimulation of the chorda tympani greatly increased the amounts of radioactive adrenaline taken up by normally innervated submaxillary glands.

Denervation of an excitable structure leads to an increased sensitivity to chemical agents. It has been suggested that this is caused by the development of a greater affinity towards drugs. Thus this increased response could be due to the chemical agent being taken up to a greater extent in the denervated structure. This hypothesis can be put to experimental test by using a substance which can be detected in small amounts. In the present series of experiments adrenaline labelled with ^{14}C was injected into cats, and the amounts present in normal and previously denervated glands at the height of secretion were estimated.

METHODS

Animal Experiments.—Cats weighing 2.5 to 3.5 kg. were used. About three weeks before the acute experiment the right chorda-lingual nerve was cut or the right superior cervical ganglion was excised. For the acute experiments, the animals were first anaesthetized with ether and then chloralose (80 mg./kg.). Glass cannulae were introduced into the submaxillary ducts. The glands were isolated, being connected to the animal only by the artery, veins, and duct. Drugs were injected either intravenously through a cannula in the femoral vein or intra-arterially through a cannula in the lingual artery. The intra-arterial injections (0.1 ml.) were made during 10 sec. In the case of intra-arterial injections, the venous outflow from the gland was drained off by a cannula introduced into the jugular vein. (±)-Adrenaline was injected and the drops of saliva falling from the cannulae in the salivary ducts were recorded on a smoked drum. A suitable dose giving a submaximal response was chosen. The time lag between the injection and the maximal rate of flow of saliva was noted. Later, injections of (±)-[^{14}C]adrenaline were made. After an intravenous injection both glands were removed simultaneously after an interval determined earlier in the same experiment. After an intra-arterial injection, the gland was similarly removed after a period determined earlier with non-radioactive adrenaline. In the case of intra-arterial administration, one gland was injected and removed first. Later the venous outflow from the remaining gland was sampled and finally this gland was injected and removed. The interval was the same for both sides.

Estimation of Radioactivity.—The activity of the whole gland was estimated, disregarding the fact that the gland loses in weight after denervation. The glands were minced in 5% trichloroacetic acid, homogenized and the volume brought up to 10 ml. with 5% trichloroacetic acid. The homogenate was centrifuged at low speed for 1 hr. The supernatant was decanted and the residue resuspended in trichloroacetic acid and centrifuged. This was repeated a third time. The three supernatants were mixed and excess of trichloroacetic acid was removed with ether. Finally, the supernatant was evaporated and the residue plated. The radioactivity was estimated in a flow counter with a background of 22 to 25 counts/min. Blood samples of 1 ml. were treated exactly as were the glands. (±)-[^{14}C]Adrenaline was obtained from Tracerlab Inc., Waltham, Mass., U.S.A.

RESULTS

Intravenous Injections

Non-stimulated Normal and Denervated Glands.—In a series of four cats with the right chorda-

lingual nerve cut 20 to 26 days earlier 20 μg./kg. of [^{14}C]adrenaline was injected intravenously. In Table I, which summarizes these experiments, it can be seen that the right (denervated) gland showed a higher radioactivity.

TABLE I

INTRAVENOUS INJECTIONS OF (±)-[^{14}C]-ADRENALINE IN CATS WITH THE RIGHT CHORDA-LINGUAL NERVE CUT

In this and subsequent Tables, the drops of saliva were counted after a previous injection of the same amount of unlabelled adrenaline.

No. of Days Dener-vated	Dose (μg./kg.)	Right Gland		Left Gland		Ratio of Counts/ min. (Right/ Left)
		Drops of Saliva	Counts/ min.	Drops of Saliva	Counts/ min.	
20	20	8·5	45	Trace	27	1·78
24	20	9	69	0·5	46	1·50
26	20	23	32	5	24	1·34
26	20	28	46	4	36	1·38
Mean		17	48	2·4	33	1·50

As the extraction procedure was not specific to adrenaline, radioactive metabolites of adrenaline might therefore contribute to the radioactivity estimated. Since the glands were removed shortly after the adrenaline had reached them, there is reason to believe that the radioactive metabolites of adrenaline present contributed only to a small extent to the estimated activity. The radioactivity detected was therefore considered to be a measure of the amount of adrenaline present at the moment of removal of the gland, and the greater activity exhibited by the denervated gland was interpreted as indicating that a greater amount of adrenaline was present. The results of the experiments in which adrenaline was administered intravenously did not necessarily suggest a greater uptake by the gland because the difference between the denervated and the normal gland could also be accounted for by a greater amount of blood containing radioactive adrenaline present in the denervated gland. Greater secretory activity in the denervated gland would have been accompanied by a greater vasodilatation.

Normal Stimulated Glands.—To test the effect of extreme vasodilatation on the radioactivity of normal glands in the cat, the chorda tympani on one side was electrically stimulated continuously during injection and removal of the glands. The outcome of three experiments of this kind is shown in Table II. Stimulation increased the amount of adrenaline present. To get information about the radioactivity of the blood, [^{14}C]adrenaline (in doses of 10, 20, and 20 μg./kg.) was injected into three cats (weighing 2.8, 2.7, and 3.2 kg. respectively). Blood was withdrawn from the left side of the heart at the same time as the removal of glands had been carried out in the earlier experiments. Activities of 30, 40, and 59 counts/min./ml. blood were found. Thus we can say that 20 μg./kg. of adrenaline gave about 60 counts/min./ml. and 40 μg./kg. about 120 counts/min./ml. The volumes of blood present in the stimulated glands calculated on the basis of these values would be 0.7, 1.6, and 2.4 ml. (Table II). The two last estimates of blood volume in the gland are obviously absurd, for the gland has only a weight of about 1 g. Some explanation for the increased radioactivity other than increased blood volume must therefore be found. Similarly an increase in the amount of tissue fluid in the gland could not account for the highly increased activity, even if adrenaline were carried only in the blood plasma and therefore values for tissue fluid should be only approximately one half of those calculated for blood. There must be a selective transport or uptake of adrenaline in some part of the stimulated and secreting gland probably involving the secreting cells.

TABLE II

INTRAVENOUS INJECTIONS OF (±)-[^{14}C]-ADRENALINE IN CATS WITH NORMAL SUBMAXILLARY GLANDS ON STIMULATION OF THE RIGHT CHORDA-LINGUAL NERVE

See Table I for explanation of drops of saliva.

Dose (μg./kg.)	Right Gland		Left Gland		Ratio of Counts/ min. (Right/ Left)
	Drops of Saliva	Counts/ min.	Drops of Saliva	Counts/ min.	
20	Trace	56	Trace	15	4·0
40	1	234	2	45	5·2
20	1·5	182	2	39	4·7
Mean					4·6

Intra-arterial Injections

Section of the Chorda.—To minimize the error due to different amounts of radioactive blood present in the denervated and the contralateral gland, injections of adrenaline were made intra-arterially. In these experiments, the non-radioactive blood flowing during the time between the end of the injection and the removal of the gland could be supposed to wash out adrenaline not taken up by the tissues. To be able to compare the two sides, not simultaneously injected, the venous effluent from the gland first injected was drained off. The activity of blood emerging from the second gland was sampled before that gland was injected. When activity was found in this blood sample the experiment was discarded. The results obtained in cats with the right chorda-

TABLE III

INTRA-ARTERIAL INJECTIONS OF (±)-[^{14}C]-ADRENALINE IN CATS WITH THE RIGHT CHORDA-LINGUAL NERVE CUT

See Table I for explanation of drops of saliva.

No. of Days Denervated	Dose (μg.)	Right Gland		Left Gland		Ratio of Counts/min. (Right/Left)
		Drops of Saliva	Counts/min.	Drops of Saliva	Counts/min.	
24	0·5	8	48	1·5	46	1·04
24	0·5	5	11	Trace	20	0·55
30	0·5	9	27	1	27	1·00
21	0·5	19	57	1	68	0·84
18	0·5	5	51	3	104	0·49
17	0·5	12	136	1·5	73	1·86
15	0·5	7	83	1	73	1·14
19	0·5	16	82	2	107	0·77
Mean:		10	62	1·4	65	0·96

lingual nerve cut 15 to 30 days beforehand are shown in Table III, and it will be seen that the amounts of adrenaline taken up by the denervated and normally innervated gland were about the same. There was no indication that the denervated gland took up more active substance than the normally innervated one.

Excision of Superior Cervical Ganglion.—Not only section of the chorda but also excision of the superior cervical ganglion causes a supersensitivity in submaxillary glands. There is some indication that the mechanisms for the supersensitivity in glands denervated by these two procedures are different (Emmelin and Muren, 1951).

In four cats, the right superior cervical ganglion was removed; otherwise experimental conditions of the intra-arterial injection were the same as for the animals with the chorda cut. No indication of an increased uptake of radioactive adrenaline caused by the ganglionectomy was found (Table IV).

Dose/Activity Relation.—In work of this type it is of importance to use doses of adrenaline causing submaximal responses. For the animals with the right chorda cut, the dose given intra-arterially was 0.5 μg. of radioactive adrenaline, since higher doses caused almost maximal responses from the highly sensitized gland. After ganglionectomy, the dose chosen was in three out of four cats 1 μg. The mean of the uptake of the normal glands in the first series, with 0.5 μg. injected, was 65 counts/min., while the mean for the glands given 1 μg. was 73 counts/min. Into three normal glands 2 μg. was injected, giving activities of 107, 53, and 86 (mean 82) counts/min. In one cat, 10 μg. was injected, giving an activity of 126 counts/min. In Fig. 1, radioactivity and secretory responses have been plotted against the doses.

TABLE IV

INTRA-ARTERIAL INJECTIONS OF (±)-[^{14}]C-ADRENALINE IN CATS WITH THE RIGHT SUPERIOR CERVICAL GANGLION EXCISED

See Table I for explanation of drops of saliva.

No. of Days Denervated	Dose (μg.)	Right Gland		Left Gland		Ratio of Counts/min. (Right/Left)
		Drops of Saliva	Counts/min.	Drops of Saliva	Counts/min.	
24	1	3·5	46	2·5	107	0·43
21	1	1·5	67	Trace	58	1·15
23	0·5	5	23	1	36	0·64
20	1	5·5	94	3·5	54	1·75
Mean:						0·99

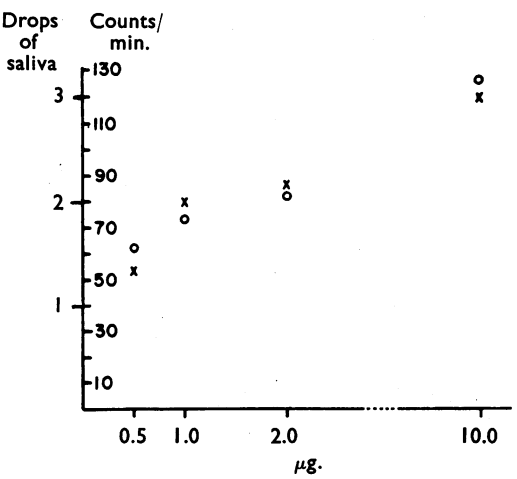

FIG. 1.—The number of drops of saliva produced from and the radioactivity in normally innervated submaxillary glands have been plotted against the dose of (±)-[^{14}C]adrenaline given intra-arterially. The values are means. **X**, Secretion; **O**, radioactivity.

DISCUSSION

When radioactive adrenaline was injected intravenously, it was found that the amount present in the glands, made supersensitive by previous section of the chorda, was greater than in the normally innervated glands. No such difference could be found when the injections were made intra-arterially. The difference after intravenous injections could be due to the greater amount of blood present in the denervated gland, this gland giving a greater secretory response. The difference in radioactivity shown in Table I could well be accounted for in this way. This conclusion was supported by the finding that, after intra-arterial injections, there was no increased radioactivity in the denervated gland

However, when an attempt was made to test this hypothesis further by comparing the activity of a resting and an electrically stimulated gland, considerably more radioactivity was found in the stimulated gland; this difference was larger than that to be expected from the presence of an increased amount of blood or extracellular tissue fluid. It was concluded that adrenaline moves into the gland cells when they are made to secrete by stimulation of the chorda tympani.

The radioactivity after intravenous injections was less than after intra-arterial injections even though the secretory response to adrenaline given was greater after intravenous administration (Tables I and II). This was probably due to the fact that the duration of action of the drug was longer after intravenous injection, the drug being continuously supplied to the gland.

No indication was found that sympathetically or parasympathetically denervated glands took up more adrenaline than normally innervated glands when the drug was given intra-arterially. It is possible, but unlikely, that there is much unspecific absorption and that a specific difference is obscured. It is of interest that in normally innervated glands when different doses were given intra-arterially and the response and uptake were plotted against the dose there seemed to be a parallelism between the secretory effect and the amount of adrenaline taken up (Fig. 1).

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Reference

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