tured cells despite a number of attempts.

It is of interest, however, that reovirus type 3 has been isolated from a case of Burkitt lymphoma(5). Thus the above findings suggest a further possibility as to a virus etiology of the Burkitt lymphoma.

On the other hand, these bodies may be a non-specific result of cell degeneration or modified cytoplasmic vesicles. Interpretation at present is difficult.

Particles resembling what Epstein *et al*(2) consider to be immature forms of virus have been seen in the nucleus of some of these lymphoblasts (Fig. 4). However, they are somewhat similar in appearance to tangentially sectioned nuclear membrane pores and therefore not absolutely convincing.

The linear nuclear projections have been described in electron micrographs of cultured Burkitt lymphoma cells(2). The presence of similar projections in the murine lymphoma cells suggests a possible association in the etiological mechanism.

The bundles of filaments associated with osmiophilic granules were a frequent finding. Filaments and tubules have been described in association with the development of the reovirus crystal formation(4). The function of these structures in these lymphoma cells may be related.

Summary. Spleen cells from a reovirus infected mouse produced a lymphoma when injected into neonatal mice. Electron microscopic studies showed the presence of virus like particles in the cytoplasm of the lymphoma cells. These did not resemble reovirus. A resemblance to Burkitt lymphoma is noted.

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Autonomic Pathways Involved in a Sympathetic-Like Action of Pilocarpine on Salivary Composition.* (30707)

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It has recently been shown that stimulation of the rat parotid gland by the parasympathomimetic agent, pilocarpine, causes elaboration of a secretion that contains markedly higher levels of total protein and amylase activity than that evoked by stimulation of the gland by way of its parasympathetic innervation(1). The high levels of amylase observed with pilocarpine stimulation were somewhat reminiscent of levels observed when sympathetic agents were used to evoke secretion (2). When the adrenergic α and β blocking agents were employed in conjunction with pilocarpine, it was found that the sympathetic-like effect of pilocarpine could be differentially modified(3). While it is apparent from those experiments that receptor sites ordinarily stimulated when pilocarpine is employed are inhibited when the adrenergic blocking agents are used, it is not clear whether this stimulation involves a direct action of pilocarpine on adrenergic receptor sites of the gland cells, or an indirect effect through sympathetic routes. This investigation was undertaken to delineate the site of the sympathetic-like action of pilocarpine on rat parotid gland.

Materials and methods. Long-Evans male rats, 5-6 months of age, were fasted for 24 hours (water *ad lib*) prior to experimenta-

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tion. After anesthetization by Nembutal (50 mg/kg, i.p.), the duct from the parotid gland was dissected free and cut. Flow of saliva was evoked by injection, in supramaximal dose, of pilocarpine (3 mg/300 g animal, i.p.) or epinephrine (total of 20-40 μg i.v.) or isoproterenol (15 mg/300 g, i.p.) or by electrical stimulation (Harvard stimulator, 10 to 60 c/s) of the sympathetic (superior cervical ganglion) or the parasympathetic (auriculo-temporal nerve) innervation. Saliva was collected by micro-pipette applied to the orifice of the duct. The adrenergic (1-isopropyl-amino-3-(1-naphthyloxy)-2-propanol HCl, Inderal†) and cholinergic (atropine) blocking agents were used alone or, in at least equimolar dose, together with chemical stimulating agents. Amylase of saliva was determined by the method of Myers, Free and Rosinski (4), using samples properly diluted with phosphate-buffered saline, and expressed as mg of reducing substance (as glucose) formed during the 15-minute digestion period per mg parotid saliva. [Na] and [K] were determined by Coleman flame photometer, using samples appropriately diluted with deionized, distilled water. Rate of flow of saliva was obtained by measuring the volume of saliva secreted per minute and relating this to the wet weight of gland producing the saliva. In the experiments on adrenalectomized rats, bilateral adrenalectomy was performed under ether anesthesia and rats were maintained with drinking water fortified by NaCl for 3-7 days before acute stimulation of the salivary glands. When experiments required surgical denervation, this was performed unilaterally immediately before proceeding with stimulation.

Results. The level of amylase activity in parotid saliva averaged 235 mg reducing sugar per mg of saliva (mg/mg) when pilocarpine was used to evoke secretion, and 38 mg/mg when secretion was obtained by electrical stimulation of the auriculo-temporal (parasympathetic) nerve (Table I, Groups A and B). When the sympathetic agent, isoproterenol, was used to evoke secretion, the mean amylase level was 772 mg/mg (Table I, Group C). In these cases, the innervation to the glands had been left intact and other drugs, except for Nembutal, had not been given. The average values for amylase activity in the saliva, for these 3 conditions of stimulation, were consistent with those found previously(1,3). Further, for comparison, samples of parotid saliva were obtained during electrical stimulation of the ipsilateral superior cervical ganglion, as well as during stimulation by intravenously injected epinephrine. Average levels for amylase activity under these two conditions were, respectively, 336 $\pm$ 24 and 271 $\pm$ 13 mg/mg (Table I, Groups D and E).

When one superior cervical ganglion was extirpated and pilocarpine was administered immediately thereafter, samples of parotid saliva could be obtained from innervated and denervated glands in the same animal. As shown in Table I, Groups A and F, the level of amylase activity in parotid saliva from the unoperated side, at 284 $\pm$ 49 mg/mg, did not differ significantly ($P>.05$) from that of non-denervated control animals. However, the level of amylase activity of saliva from the parotid gland of the operated side did differ markedly from control values for pilocarpine-stimulated saliva. After extirpation of the superior cervical ganglion, levels of amylase activity of pilocarpine-evoked saliva from the ipsilateral parotid gland dropped to values (27 $\pm$ 4 mg/mg) characteristic of those found in saliva evoked by stimulation of the auriculo-temporal nerve (Table I, Groups B and F). Similar values were observed for amylase of pilocarpine-stimulated saliva when the β-adrenergic blocking agent Inderal was used in conjunction with pilocarpine stimulation of normally-innervated glands (Table I, B, and(2)). Interruption of the preganglionic fibers to the superior cervical ganglion, however, had no effect on the amylase level of pilocarpine-evoked saliva, and salivas from the normally-innervated and denervated (preganglionic sympathetics) glands exhibited amylase levels of 202 $\pm$ 31 and 253 $\pm$ 46, respectively (Table I, G). It is also clear from the data that removal of one superior cervical ganglion had no effect on amylase levels of parotid saliva obtained

† Inderal was obtained through the courtesy of Dr. Sahagian-Edwards of Ayerst Laboratories, N. Y.

TABLE I. Comparison of Effects of Pilocarpine and Auriculo-Temporal Stimulation on Composition of Rat Parotid Saliva Following Acute Surgical Procedures or Chemical Treatment.

Group	Surgical or chemical treatment used preceding stimulation	Mode of stimulation	Amylase (mg/mg)	Na (meq/l)	K (meq/l)	Flow rate (mg/min)	Flow rate (mg/min/mg)
A(4)	None	Pilocarpine	235 ± 9*	140 ± 3	19 ± 1	13 ± 1	.053 ± .005
B(5)	"	Auriculo-temporal	38 ± 8	159 ± 9	17 ± 1	23 ± 2	.092 ± .004
	Inderal		38 ± 6	142 ± 4	18 ± 1	24 ± 3	.096 ± .011
	"	Pilocarpine	37 ± 3	137 ± 4	18 ± 1	12 ± 4	.035 ± .003
C(17)	None	Isoproterenol	772 ± 29	105 ± 4	54 ± 5		.015 ± .005
D(12)	"	Superior cervical ganglion	336 ± 24	127 ± 5	32 ± 2		.021 ± .006
E(12)	"	Epinephrine	271 ± 13	115 ± 8	49 ± 5		.011 ± .008
F(6)	"	Pilocarpine	284 ± 49	137 ± 4	13 ± 1	19 ± 4	.076
	Removal of s.c.g.		27 ± 4	154 ± 2	16 ± 1	22 ± 3	.088
G(4)	None	"	202 ± 31	144 ± 3	18 ± 2	13 ± 2	.052
	Cut preganglionic fibers to s.c.g.		253 ± 46	137 ± 5	19 ± 1	17 ± 5	.068
H(7)	None	Auriculo-temporal	25 ± 3	166 ± 7	21 ± 2	31 ± 6	.124
	Removal of s.c.g.		28 ± 5	163 ± 4	16 ± 2	28 ± 3	.113
I(4)	None	Pilocarpine	274 ± 20	136 ± 5	17 ± 2	24 ± 9	.088
	Cut auriculo-temporal		261 ± 36	140 ± 4	16 ± 2	21 ± 5	.084
J(5)	Inderal	"	35 ± 6	122 ± 6	19 ± 3	11 ± 2	.040
	Removal of s.c.g. plus Inderal		23 ± 4	126 ± 6	15 ± 1	8 ± 2	.036
K(5)	Bilateral adrenalectomy 3-7 days before	"	281 ± 25	144 ± 4	21 ± 2	13 ± 5	.049 ± .014

Controls in the experimental groups are from contralateral glands in the same animals (No. in parentheses refer to No. of rats). Dosage of drugs administered was as follows: Inderal, 3.5 mg/rat, i.p.; pilocarpine, 2.2 mg/rat, i.p.; epinephrine, 20-40 μg/rat, i.v.; isoproterenol, 15 mg/rat, i.p. Supra-maximal electrical stimuli at frequencies >10 c/s were used to stimulate the auriculo-temporal nerve and superior cervical ganglion (s.c.g.).

* Amylase is expressed as No. of milligrams of reducing substance, as glucose, formed in a 15-min digestion period at 37°C per milligram of saliva, and means ± standard errors are given for amylase, electrolytes, and flow. Flow rate is expressed as mg/min, and also as mg/min/mg of gland. Where flow rate is expressed as mg/min/mg and no standard errors of the mean presented, the mean flow rate was calculated from a mean gland weight of 250 mg; where standard errors are also presented, mean flow was based on the weight of the specific glands involved in the experiment.

by subsequent stimulation of the auriculo-temporal nerve (Table I, H). Severing of the auriculo-temporal nerve also did not affect the amylase level of saliva obtained by pilocarpine stimulation of the gland 3-10 minutes after denervation (Table I, I).

In one group of animals, the superior cervical ganglion was removed from one side, Inderal administered to the animals, and pilocarpine was then used to evoke secretion. The amylase level of the secretion from the denervated side showed no statistically significant difference ($P > .05$) from that of the innervated gland in the same animal (Table I, J). Furthermore, when the mean levels obtained with Inderal after removal of the superior cervical ganglion (Table I, J) were compared with mean levels obtained with the sympathectomy alone (Table I, F), the levels were indistinguishable, at 23 ± 4 and 27 ± 4 mg/mg, respectively.

Bilateral adrenalectomy was performed on another group of 5 rats. Three to 7 days after adrenalectomy saliva was obtained from the parotid gland by pilocarpine stimulation. Amylase levels were not decreased below levels usually obtained with pilocarpine stimulation of control glands (Table I, K).

It is apparent from the data in Table I that flow rate of saliva obtained from sym-

pathetic stimulation was generally much lower than that observed with parasympathetic stimulation. However, flow rate was not appreciably modified, with stimulation either by pilocarpine or through the auriculotemporal nerve, by adrenalectomy, cervical ganglionectomy, severance of preganglionic sympathetic fibers or use of Inderal. None of these procedures, itself, evoked flow of parotid saliva.

The concentration of sodium in the saliva was not notably affected by the experimental procedures. Where lower values for [Na] were evident (Table I), they seemed to be accompanied by some depression of flow rate. The concentration of potassium was much higher in sympathetically than in parasympathetically evoked saliva (Table I), as noted previously(2). However, [K] in pilocarpine-evoked saliva did not differ from that of saliva secreted during stimulation of the auriculo-temporal nerve, nor did adrenalectomy, sympathetic denervation or administration of Inderal effect any change in [K] of the saliva.

Finally, it may be mentioned that the cholinergic blocking agent, atropine, completely prevented secretion by subsequently injected pilocarpine.

Discussion. Recent work(1) with rat parotid gland has shown that saliva produced by electrical stimulation of the parasympathetic innervation (auriculo-temporal nerve) contains low levels of amylase activity and total protein, in comparison with saliva evoked by administration of pilocarpine. Administration of the sympathetic agent, isoproterenol, to stimulate secretion, also results in salivary levels of amylase activity which are very much higher than those of the saliva provided by stimulation of the parasympathetic innervation(2). Moreover, the use of the β-adrenergic blocking agent, Inderal(5), results in reduction of the amylase levels of the secretion to levels characteristic for auriculo-temporal-stimulated saliva(3). The present work confirms and extends those observations. For example, in this work, observations were made of amylase levels of salivas obtained by stimulation of the sympathetic innervation (superior cervical ganglion) and by stimulation with intravenously administered epinephrine. Amylase levels, under these conditions, were as high as, or higher than, those found when pilocarpine was used to stimulate flow of saliva, thus confirming the impression from the use of isoproterenol that levels of amylase after pilocarpine are generally suggestive of sympathetic rather than parasympathetic stimulation.

The central question in this study concerns the locus of the sympathetic-like action of pilocarpine. The possibility was considered that this action of pilocarpine could have resulted from release of sympathetic amines from the adrenal medulla. Stimulation of the adrenal medulla by pilocarpine(6-8), as well as inhibition of such stimulation by atropine (9) is well known. Since bilateral adrenalectomy at least did not decrease amylase levels of subsequently collected samples of pilocarpine-evoked saliva, it is concluded that the sympathetic-like action is not mediated through the adrenal medulla. It was also considered possible that stimulation of the superior cervical ganglion by pilocarpine could have been involved in mediating these effects, since it is well established that pilocarpine can stimulate the superior cervical ganglion(6-8,10-12). This was indeed found to be the case since removal of the superior cervical ganglion resulted in alteration of amylase levels in subsequent samples of pilocarpine-evoked saliva to values characteristic of auriculo-temporal stimulation. On the other hand, severance of preganglionic sympathetic fibers to the superior cervical ganglion caused no change in amylase levels when pilocarpine was given to evoke secretion. Furthermore, there was little if any further modification of amylase levels when Inderal was used in conjunction with ganglionectomy, suggesting that pilocarpine has little if any direct effect on gland adrenergic receptor sites. Further support for this conclusion is provided by the use of atropine. It has been shown by a number of investigators including Marrazzi(10), using rabbit, and Ambache(7) and Trendelenburg(8,11,13), on cat, that the action of pilocarpine on the superior cervical ganglion can be abolished by atropine. In the present experiments, admin-

istration of atropine completely abolished the secretion of saliva in response to pilocarpine. It is clear then that the sympathetic-like character of pilocarpine-evoked saliva can be ascribed to an indirect effect mediated through the superior cervical ganglion, rather than a direct effect of pilocarpine on adrenergic receptor sites in the gland(3); furthermore, the preganglionic fibers to the superior cervical ganglion are not involved in mediating this effect.

The modified levels of amylase observed with denervation and subsequent pilocarpine stimulation as well as those observed when adrenergic blocking agents are used in conjunction with pilocarpine stimulation cannot be attributed to changes in flow rate. On the other hand, flow rate is high in all cases of denervation, and the flow rate from the denervated and normally innervated sides is similar. Nonetheless, the amylase under these circumstances can be quite dissimilar, levels from the normally innervated gland being 6-10 times as high as those from the denervated side when pilocarpine stimulation is used to evoke secretion. With Inderal, there is a small reduction in flow rate. However, reduction in flow rate cannot account for these changes in amylase since under conditions where flow rates are very low (as with sympathetic stimulation), amylase levels are very high.

Generally, levels of Na and K were not appreciably altered with any of the experimental procedures here employed. The few instances in which a change in Na levels was observed can be attributed to changes in flow rate(1). It is thus clear that pilocarpine is an adequate substitute for parasympathetic nerve stimulation when Na and K are considered but is not an adequate substitute when proteins(1) or possibly some other species of ions(14) are considered.

Summary. When stimulation of the rat parotid gland was effected by pilocarpine administration immediately following removal of one superior cervical ganglion, the amylase levels in the secretion obtained from the denervated gland were approximately 1/10 the levels obtained from the normally innervated gland, and were very similar to levels obtained as the result of stimulation of the auriculo-temporal nerve, or following administration of the β-adrenergic blocking agent Inderal prior to pilocarpine stimulation. On the other hand, amylase levels of pilocarpine-evoked saliva were not modified either by severing of the preganglionic fibers to the superior cervical ganglion or by removal of both adrenal glands. It is concluded that the sympathetic-like character of pilocarpine-evoked saliva results from an indirect stimulation of adrenergic receptors in the gland that is mediated through the superior cervical ganglion and the sympathetic postganglionic fibers.

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