an important role in the inactivation of ACHE. Although it has been reported that the cell–TA complex tends to dissociate with increasing temperatures (5), we observed a very rapid rate of enzyme inactivation at temperatures above 37°C.

An inhibitory effect of TA on the reaction of several mammalian enzymes has been reported (17, 18) and by varying the concentration of TA and of the respective substrates it has been concluded that it was competitive in nature (18). Similar results have also been found with a tomato pectinesterase (19). In these studies as in ours, no significant effect was noted with the other phenolic compounds tested. Although TA has been used as a tool in the study of many properties of the red cell during the past 100 years (20), the work reported here represents the first instance in which an irreversible effect on a specific constituent of the human erythrocyte membrane could be demonstrated.

Summary. Treatment of human erythrocytes with tannic acid caused irreversible inactivation of the surface located acetylcholinesterase (ACHE). This effect was dependent on concentration, time, temperature, and pH. The ACHE inactivation was prevented by albumin but not by coating the cells with blood-group specific antibodies. The enzyme was not affected by the other phenolic compounds tested.

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Characterization of Physalaemin-Evoked Rat Saliva and Failure of Autonomic Blocking Agents to Modify Composition* (32920)

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It has recently been shown that physalaemin, a polypeptide extracted from the skin of a South American amphibian, can elicit secretion from salivary glands (1). Furthermore, although in flow rate and general consistency, the saliva resembles that usually evoked by parasympathomimetic stimulation, physalaemin-induced secretion is not blocked by cholinergic blocking agents (2); nor is it prevented when adrenergic blocking agents are administered (2, 3), or when the innerva-

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tion to the gland is severed (3). It has therefore been suggested that physalaemin causes secretion by some direct action on the glands which differs from that usually produced by autonomic transmitter substances (2, 3). However, if physalaemin were to act indiscriminately on all receptor sites, cholinergic as well as adrenergic, then separate cholinergic or adrenergic blockade would not prevent secretion. Moreover, flow rate generally may be a relatively insensitive reflector of selective elimination of autonomic pathways (4–6). On the other hand, the composition of the secretion with regard to the concentrations of Na, K, and protein, does appear to provide a sensitive indicator of such modifications (4–6). The present investigation was undertaken, therefore, to characterize the composition of the physalaemin-evoked saliva, and to test for alterations in the composition when cholinergic or adrenergic blocking agents, separately or together, are administered prior to the physalaemin, in order to clarify the mechanism of the secretagogue action of physalaemin.

Materials and Methods. Adult Long-Evans male rats, weighing approximately 350 gm, were anesthetized with 1% sodium pentobarbital, and the ducts from the parotid and submaxillary glands were then exposed and cut (7). Physalaemin was injected into the jugular or femoral vein in 0.1–1 μg quantities over a period of 6–10 sec, or infused at a rate of 1 μg/min per kg. Samples of saliva were collected directly from the cut ducts by micropipette a few minutes after injection of the physalaemin. In some experiments, 1–3 mg/kg of the α-adrenergic blocking agent, phenoxybenzamine (Dibenzyline), 3–6 mg/kg of the β-adrenergic blocking agent, propranolol (Inderal), and 0.5–2 mg/kg of the cholinergic blocking agent, atropine, were administered ip either alone, or in combination, 10–30 min before injection of the physalaemin. Salivary [Na] and [K] were determined using an IL flame photometer with lithium as the internal standard. Amylase activity was determined by the method of Myers *et al.* (8), and is expressed as milligrams of reducing substance (as glucose) formed per milligram of saliva (mg /mg). Flow rate was calculated by measuring the volume secreted per minute per milligram of gland (mg/min per mg).

Results and Discussion. Rapid intravenous injection of physalaemin (0.1–1 μg in 6–10 sec) elicited a copious flow of saliva from the parotid and submaxillary ducts following a latent period of approximately 25 sec. The flow, however, did not persist for more than a few minutes after cessation of physalaemin injection. During the period of injection,

TABLE I. Composition of Physalaemin-Evoked Rat Saliva and the Influence of Cholinergic and Adrenergic Blocking Agents on the Composition.[a]

	Submaxillary saliva			Parotid saliva			
Drugs administered	No. of rats	Na (meq/liter)	K	No. of rats	Na (meq/liter)	K	Amylase (mg/mg)
P	20	32 ± 5	46 ± 2	15	139 ± 4	20 ± 4	14 ± 2
IN + P	4	35 ± 9	46 ± 4	4	140 ±11	21 ± 5	14 ± 3
DI + P	4	41 ±11	46 ± 4	4	136 ± 4	21 ± 9	9 ± 1
AT + P	5	36 ± 7	48 ± 6	4	140 ± 9	26 ± 6	9 ± 2
IN + AT + P	4	47 ± 7	38 ± 5	4	139 ± 9	22 ± 6	14 ± 5
DI + AT + P	5	25 ± 6	45 ± 4	3	130 ±11	21 ± 5	8 ± 2
IN + DI + AT + P	7	35 ± 6	49 ± 4	7	143 ± 6	21 ± 3	18 ± 4

[a] Dosage of drugs administered was as follows: physalaemin (P), 0.1–1 μg/kg iv; atropine (AT), 0.5–2 mg/kg ip; dibenzyline (DI), 1–3 mg/kg ip; Inderal (IN), 3–6 mg/kg ip; sequence gives order of administration. 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 ± SE are given for amylase and electrolytes. The Na and K concentrations are expressed as meq/liter of saliva. Values with blocking agents are not significantly different from those without ($p > .01$).

salivary flow rate reached very high levels (0.1–0.2 mg/min per mg for submaxillary, and 0.06–0.1 for parotid), similar to those usually observed (9) with cholinergic nerve stimulation. When physalaemin was infused intravenously at a rate of approximately 1 μg/min per kg, flow rate of submaxillary saliva was maintained at high levels (0.1–0.2 mg /min per mg) during the period of infusion, while parotid flow rate tended to decrease, finally ceasing entirely.

The composition of the physalaemin-evoked secretions is shown by the data in Table I. The [Na] of parotid saliva, even when flow rate changed rapidly, did not vary to any appreciable extent (ranging generally between 128–156 meq/liter); the [K] was generally in the range 10–27 meq/liter, and amylase concentration was usually between 4–20 mg/mg of saliva. It is thus apparent that the composition of this secretion very closely resembles that of saliva evoked when cholinergic postganglionic nerve fibers are stimulated (9). It is also clear that physalaemin, unlike pilocarpine, does not have an effect mediated through the superior cervical ganglion, since amylase concentrations were very low and therefore did not reflect an adrenergic component (4, 9). The [K] of the physalaemin-evoked submaxillary saliva was virtually identical (usually ranging from 42–56 meq/liter) to that evoked by cholinergic stimulation (Table I) (9). Sodium of submaxillary saliva, however, although usually in the range characteristic of cholinergic stimulation, exhibited wide variations in concentration (ranging from 8–75 meq/liter) that were not apparently dependent on flow rate or duration of flow. At a fairly constant rate of physalaemin-infusion, and high flow rate, [Na] of saliva samples collected at 30–60 sec intervals, varied from 15 to 50 meq/liter. Mean Na, as a result of this wide variation within a single animal, (as well as from animal to animal), thus tended to be somewhat higher (32 ± 5) (Table I) than that usually observed with cholinergic stimulation (10–20 meq/liter) (9). The reasons for the variation are however not clear. It is possible that transitory vascular effects could contribute to this variability (1, 2); salivary flow rate and duration of flow probably do not. However, further investigation of both of these points is required to support these views since neither flow rate variations nor vascular effects (10) could be examined adequately under the conditions here employed.

The present studies confirm the fact that physalaemin-evoked secretion is not prevented by either cholinergic or adrenergic blocking agents, administered separately (1–3); physalaemin thus does not cause secretion by an action on either adrenergic or cholinergic receptors. However, the present study also shows that salivary flow is not prevented (nor appreciably modified) when cholinergic and adrenergic blocking agents are administered together prior to physalaemin injection. Under these conditions, flow rate ranged from 0.04–0.1 mg/min per mg. Failure to prevent secretion when all blocking agents are present strongly suggests that physalaemin does not evoke secretion by indiscriminately acting on all receptors simultaneously.

It is also clear from the data in Table I that Na, K, and amylase concentrations of the secretions were not altered ($p < .01$ in all cases) when cholinergic and adrenergic blocking agents were administered together, or separately, prior to physalaemin injection. Since composition of the saliva has been shown to be a more sensitive indicator than flow rate of selective elimination of autonomic pathways (4, 6), it may be concluded from the present data that physalaemin has no direct effect on either cholinergic or adrenergic receptors. These findings thus confirm and provide additional evidence for the conclusion that physalaemin does not act in a way analogous to that usually involved with autonomic transmitter substances (1–3). A possible mode of action may involve an indirect effect mediated through the hypothalamus. A potent sialogogue, described as a neurohormone, has recently been isolated from bovine and rat hypothalamus (11). It, like physalaemin, is a polypeptide which evokes a secretion that is not blocked by either parasympatholytic or sympatholytic agents. The similarities in action of these two secretagogues thus raises the possibility that this hypothalamic hormone

may mediate the action of physalaemin.

Summary. Electrolytes and amylase concentrations of rat saliva which is evoked by physalaemin, are virtually identical to those found in saliva evoked by stimulation of postganglionic cholinergic nerve fibers. Mean Na concentrations of parotid and submaxillary saliva were found to be respectively, 139 $\pm$ 4 and 32 $\pm$ 5, and K, 20 $\pm$ 4 and 46 $\pm$ 2; submaxillary flow rate was higher than flow rate from parotid, but both exhibited the high rates characteristic of cholinergic nerve stimulation; amylase concentration of parotid saliva was about 20 mg/mg. The secretion was, however, neither blocked nor modified in any way when the cholinergic blocking agent atropine was present. Furthermore, it was not modified by the presence of either α or β adrenergic blocking agents, or all three blocking agents simultaneously. An indiscriminate action on all receptor sites is thus ruled out. These data provide further evidence for the conclusion previously indicated (1–3) that this agent causes secretion by a direct action on gland cells that differs from that usually produced by autonomic transmitter substances.

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Relation of Local Circulation between Ovaries and Uterus to Lifespan of Corpora Lutea in Rats* (32921)

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The finding by Loeb (1) that removal of the uterus results in persistence of the corpora lutea in the guinea pig was confirmed in many other species (2). The part of the uterus which appears to influence the lifespan of the corpora lutea is the endometrium (3–5). Partial hysterectomy prolongs maintenance of corpora lutea in direct proportion to the amount of uterine tissue removed (4–6). Uterine transplants in hysterectomized rats were reported to shorten the duration of pseudopregnancy (7).

A substance apparently is produced by the endometrium which exerts a local luteolytic effect on the corpora lutea of the ipsilateral side, as recently demonstrated in swine (6), guinea pigs (8), sheep (9), and pseudopregnant rats (10). The pathway involved in this local control mechanism has not been well elucidated. Inskeep and Butcher (9) recently reported that ligature of the blood vessels supplying the anterior portion of the uterine horn increased luteal lifespan in sheep. The present

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