little to the already heightened regeneration when administered to pregnant rats. The depressant, nicotinamide (dietary level: 0.35%), normalized the extent of hepatic regeneration when fed to gravid animals but an inhibitory effect was apparent when the liver increment was compared to that of the pregnant group on the control diet.

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The Submaxillary Secretory Response in the Dog to a Series of Doses of Pilocarpine.* (32374)

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Although pilocarpine has been used widely in the laboratory(1,2,3) and has been given to man(4) to stimulate the secretion of saliva, its dose-response effects on salivary secretion have not been documented. Only the secretory effect of a single dose has been described (5). The present report is based on a study of the dose-response effects of pilocarpine on the dog submaxillary gland.

The administration of a series of increasing doses of pilocarpine was found to result in a flow-rate curve that resembled an inverted-V, indicating that saliva flow rate was suppressed by high doses of pilocarpine. Graham and Stavraky(6), who described a similar response to increasing doses of acetylcholine, showed that high doses of acetylcholine caused glandular vasoconstriction. As it was known that pilocarpine, also, causes vasoconstriction at high doses(7), the drop in response appeared to be due solely to the constrictive effect of the high doses. However, based on the observation that the viscosity of the saliva appeared to increase with dose, the hypothesis was tested that an increase in salivary viscosity at high doses of pilocarpine contributed also to the decline in flow rate.

* This work was done in partial fulfilment of the M. S. degree in pharmacology at Georgetown University.

Materials and methods. Thirty-two dogs of either sex, weighing 3.8 to 11.9 kg, and anesthetized with pentobarbital sodium (30 mg/kg intravenously), were used. A cannula (P.E. 50) was placed in the submaxillary duct at the chorda-lingual triangle(8) and tied in place to avoid loosening and leakage. The trachea and left femoral vein were cannulated. All doses were given intravenously; and each dose was followed by 2 ml 0.9% sodium chloride solution. Dogs 23-32, whose salivary viscosity was determined, were hydrated *via* the femoral cannula throughout the experiment with approximately 50 ml/hr normal saline. A Grass model S4G stimulator was used to stimulate the chorda tympani through a bipolar platinum electrode (Frequency: 20-50 f/s; strength: 5 volts).

The secretory responses of dogs 1-11, 31 and 32 were recorded by counting drops per unit time. The responses of dogs 12-30 were recorded using the sialogram, which is a combined tracing of salivary duct pressure and the rate of flow of saliva. The recording apparatus is demonstrated in Fig. 1.

The recording of flow rate with a transducer was made possible by the attachment of a flexible length of polyethylene cannula (Fig. 1, I) to the outflow end of a pressure transducer. As each falling drop caused the free end of the cannula to bend and rebound,

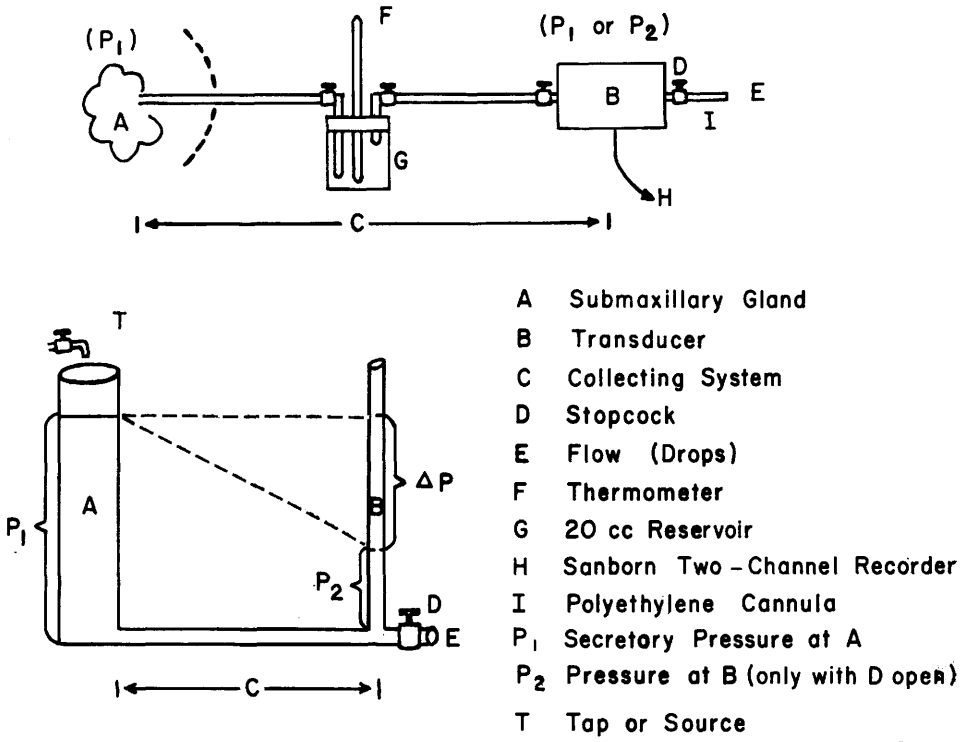

FIG. 1. Schematic diagram of apparatus for sialogram recording as compared to anlogous reservoir-tube system.

the resultant transient change in pressure within the transducer was recordable as a blip superimposed on the pressure tracing. The purpose of the reservoir (G) was to trap the heavier constituents of saliva and prevent them from passing through the transducer.

The theoretical system to which the apparatus was compared is also shown in Fig. 1. The reservoir, A, is analogous to the gland, the vertical tube, B, to the transducer, the horizontal resistance tube, C, to the collecting system, the valve, D, to the stopcock located on the outflow side of the transducer, and tap, T, to the secretory cells. When the stopcock, D, is closed, the pressure in the vertical tube will rise to that in the reservoir, A, *i.e.*, to P_1; when it is opened, flow will occur and P_2 can be measured at the vertical tube. It is known that in such a system $Q/t = \Delta P/R$. (Q/t = flow/unit time, ΔP = pressure gradient, R = resistance). Also, it is known that $R = nk$ (n = fluid viscosity, k = resistance constant), or by Poiseuille's law, $k = 8L/\pi r^4$ (L = length, r = radius). The assumption was made that the same laws and equations apply in both systems.

The total resistance of the apparatus may be expressed as $R_T = R_1 + R_2 + R_3 = n(k_1 + k_2 + k_3) = nk_T = nk$. ($R_1 = nk_1$ = resistance from gland to reservoir, $R_2 = nk_2$ = resistance of reservoir, $R_3 = nk_3$ = resistance from reservoir to end of system.) Using distilled water, whose temperature (°C) — viscosity (poise) relationship is known(9), and a mechanical pressure source for P_1, the resistance constant, k, for the apparatus was calculated with the equation: $(P_1 - P_2)/(Q/t) = R_T = n_{H_2O}\, k$. The viscosity of saliva, n_s, could be determined at a given temperature with the equation: $(P_1 - P_2)/(Q/t) = n_s k_1 + n_{H_2O}(k_2 + k_3)$. As k_2 and k_3, due to their larger diameter and shorter length, were small compared to k_1, then $k_1 \cong k_T$, and $n_{H_2O}(k_2 + k_3) \cong 0$, or negligible. Thus, $n_s = (P_1 - P_2)/(Q/t)(k_T) = (P_1 - P_2)/(Q/t)(k)$. This value was then compared to the viscosity of distilled water, at the same temperature, and

reported as viscosity units (V.U.): V.U. = $[n_s \text{ (at } \times \text{ °C)}]/[n_{H_2O} \text{ (at } \times \text{ °C)}]$.

Flow was recorded as drops/minute, the apparatus being similar in principle to the saliva outflow recorder described by Kerr (10). The saliva, which entered the bottom of the reservoir, displaced an equal weight of water out of the top of the reservoir, *i.e.*, the amount of saliva that was secreted by the gland was measured in terms of drops of water displaced from the reservoir. Inasmuch as the drops consisted of water alone, the drop size did not vary.

The system was filled with distilled water from the reservoir to the outflow end, the water being replaced at approximately 45 minute intervals and between the administration of different doses of the drug.

The method for determining P_1, P_2, and Q/t resembled that used by Langley and Brown(11) to study the flow-pressure relationship of the submaxillary and parotid glands.

The sialogram method for determining viscosity was compared to the Ostwald viscometer method as follows: after discarding the first 8 drops of saliva, 8 drops were collected in a test tube immersed in a 37°C water bath during the period of maximum secretory response to pilocarpine and/or chorda stimulation. The time for collection was recorded in order to determine the rate of flow. This sample was then diluted to 1 cc with distilled water, after which 1 cc of this dilution was immediately placed into the receiving cup of a calibrated Ostwald viscometer and the time for passage through the capillary tube was recorded. The time for passage obtained in this way was then compared to that obtained using 1 cc of distilled water to obtain the relative viscosity, n_{rel}.

Results. The flow responses to 4 successively larger doses of pilocarpine, determined in 6 dogs are demonstrated in Table I. The greatest response (maximal rate of flow) occurred, on the average, during the first 4 minutes after the dose was given. Duration of the response was directly related to dose. The greatest volume of flow tended to occur with the greatest dose, due to the longer-lasting stimulatory period.

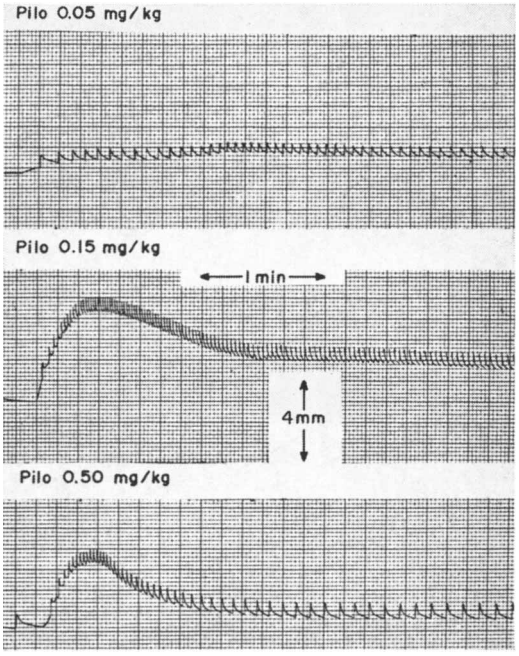

FIG. 2. Sialogram response to 3 doses of pilocarpine to demonstrate the drop in maximum rate of secretion to a high dose. (Dog 17).

The maximal rate of secretion of the submaxillary gland in response to various doses of pilocarpine was determined in 10 dogs. The dose that caused the maximal rate of secretion (optimal secretory dose) varied between 0.05 and 0.30, the mean being 0.16 ± 0.02 mg/kg. In each of these dogs there occurred, at doses higher than the optimal secretory dose, a fall in the maximal rate of flow. An example of this is shown in Fig. 2.

The viscosity of the saliva was determined

TABLE I. Average Rate of Flow (Drops/Min) in 6 Dogs in Response to 4 Doses of Pilocarpine.

Dose (mg/kg)	Min after injection 1	2	3	4	5	6	7	8	9	10	11	12	13	14	15	20	25	30	45	60	120	Mean total drops ± S.E.
.05	13	16	14	14	13	12	11	10	9	8	7	7	6	5	5	4	3	3	1	0	0	258 ± 55
.10	24	29	25	22	19	17	16	15	16	16	13	13	12	12	11	10	8	7	4	2	0	534 ± 110
.15	31	30	26	25	27	20	17	14	13	12	11	11	11	11	11	10	9	8	5	4	0	688 ± 81
.30	23	29	26	25	22	20	17	18	13	12	12	11	11	11	11	12	13	13	11	8	1	1007 ± 253

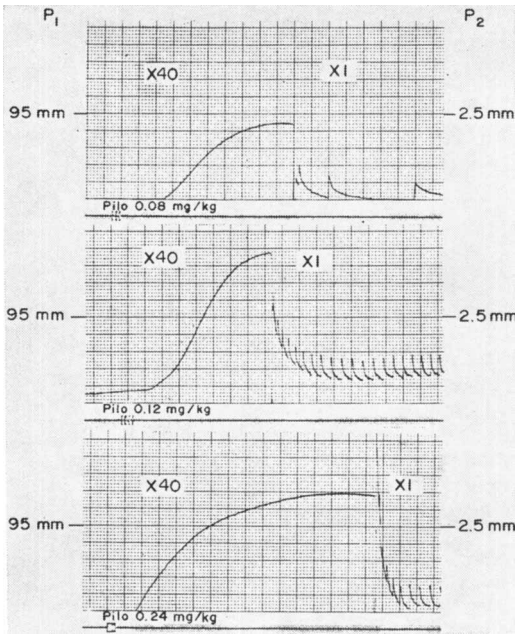

FIG. 3. Sialogram for viscosity calculation from Poiseuille's Law. The attenuator range settings (× 40 and × 1) provided a 40:1 ratio between P_1 on left and P_2 on right. When P_1 (left side of graph) of the gland, whose flow had been obstructed, reached a maximum in response to a dose of pilocarpine, the obstruction was removed and P_2 and flow were recorded (right side of graph). Salivary viscosity, n_s, was calculated with the formula, $n_s = P_1 - P_2 / k \cdot \text{Flow}$. k = resistance constant. (Dog 26).

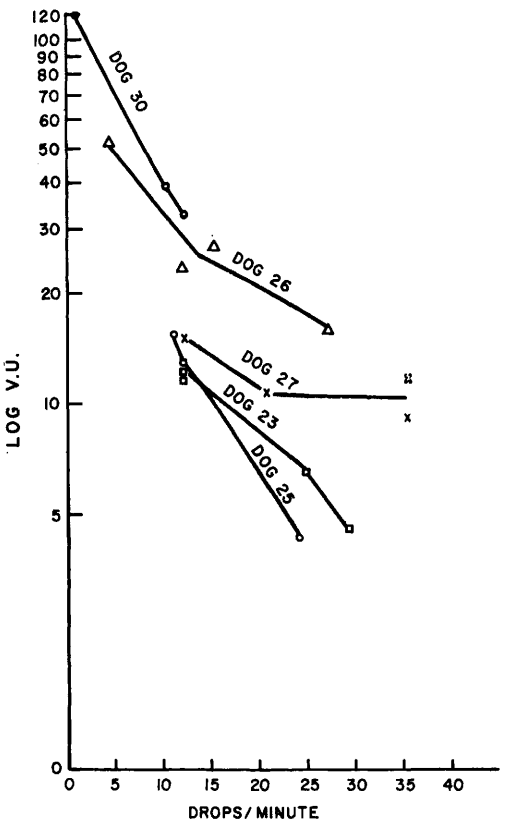

FIG. 4. Graphic representation of inverse relationship between salivary viscosity (V. U.) and rate of flow (Drops/Min.) in 5 dogs.

at various doses in 7 dogs. It was calculated in 5 dogs from a sialogram tracing (Fig. 3). When the stopcock on the outflow side of the transducer (Fig. 1) had been closed, the temperature recorded, and the drug administered, P_1 was permitted to rise to its maximal level (to obtain a maximum glandular pressure), then the stopcock was opened to permit the recording of flow and P_2 at the transducer at a new amplifier attenuation. Viscosity was calculated by means of Poiseuille's Law.

In each of the 5 dogs the calculated viscosity of the saliva changed with dose, and there was an inverse relationship between flow rate and viscosity. A basically similar slope of the curves is shown in Fig. 4.

To verify the assumption that the sialogram method for determining viscosity was valid, the viscosity of the saliva was determined also with the Ostwald viscometer in 2 dogs (dogs 31 and 32). These results are shown in Table II. The inverse relationship between viscosity and flow rate, indicated by the fall in the viscosity:flow ratio as flow rate rose, was found to be similar to that observed by the sialogram method (compare

TABLE II. Relationship of Relative Viscosity (RV) as Determined with the Ostwald Viscometer to Rate of Flow of Saliva (RF) in Response to Various Doses of Pilocarpine and Chorda Stimulation in Two Dogs.

Dose (mg/kg)	Dog No. 31 RV	RF	RV/RF	Dog No. 32 RV	RF	RV/RF
.05				3.4	16	.21
.07	4.4	4.4	1.00			
.13				2.4	48	.05
.15	3.8	32	.12			
.30	3.0	44	.07	5.7	23	.25
Chorda stimulation	1.5	44	.03	2.2	48	.04

Table II and Fig. 4). This finding supported the theoretical assumption that the sialogram may be used to determine salivary viscosity.

Discussion. The changing secretory pattern in response to varying the dose of pilocarpine is not a simple one. An understanding of it may be useful to the investigator who wishes to use pilocarpine therapeutically or to obtain saliva for analysis.

The knowledge that the osmolarity(12), and sodium content(13) of secreted saliva are proportional to salivary flow rate, suggests that the secretory effects of pilocarpine are related to variations in the release into saliva of protein, sodium, and other agents that have an osmotic effect on the ductal cells. Also, the similarity of the pilocarpine flow response to the protein and potassium output patterns of the parotid gland at a given rate of stimulation, *i.e.*, an early peak followed by a gradual decline(14), appears to relate these two constituents to the observed flow responses to pilocarpine. However, it would be interesting at this point to know if sodium, potassium, or protein output by the gland can be blocked independently of its water output.

The decline in gland responsiveness to high doses of pilocarpine paralleled the findings of Graham and Stavraky(6) who found this to occur using acetylcholine. A consideration of why this occurred led to the conclusion that it was due to both vasoconstriction that occurs in the gland at high doses of acetylcholine(6) and pilocarpine and an increase in the salivary viscosity. Since the decline in flow rate appears, then, to have been dependent on both of these factors, it seems logical to suggest that they may be interdependent, *i.e.*, vasoconstriction may be expected to have caused the observed rise in salivary viscosity; and by similar reasoning, vasodilation (which is known to occur when the secretory rate is increased(6)) can be expected to cause a fall in viscosity. If this be so, then in the light of the finding of Hilton and Lewis(15) that vasodilation of the submaxillary gland is mediated through the release of bradykinin, it may be suggested that both the vasoconstriction and the rise in salivary viscosity that occur in response to high doses of pilocarpine may be related to an inhibition by pilocarpine of bradykinin release.

The successive steps through which a high dose of pilocarpine acts to cause a decline in salivary flow rate may be postulated to be as follows: 1. Inhibition of bradykinin (or other similar agents) release. 2. Vasoconstriction. 3. Increased salivary viscosity. 4. Decreased flow rate.

Summary. The dose-response pattern of the submaxillary gland of the dog to pilocarpine was studied using the sialogram. Following the administration of each dose of pilocarpine, salivary flow rate rose to a peak in one to four minutes and then declined. Duration of the secretory response was directly related to dose. Rate of flow, however, was directly related to dose up to an optimal secretory dose. Doses higher than this caused the flow rate to decline. A study of the salivary flow and pressure changes showed that the decline is related to an increase in salivary viscosity.

The author wishes to thank Dr. Theodore Koppanyi, Georgetown University, for his kind assistance during this work. The assistance of Colonel George W. Burnett of U. S. Army Institute of Dental Research is also acknowledged with appreciation.

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Inhibition of Erythropoietic Effects of Hormones by Erythropoietin Antisera in Mildly Plethoric Mice.* (32375)

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Hormonal agents from the pituitary(1,2,3), thyroid(4,5,6,7), adrenal(8,9) and testis(3,6, 8,10,11,12) have been reported to stimulate erythropoiesis in the endocrine deficient as well as the normal animal. However, the mechanism by which these humoral substances stimulate erythropoiesis is not clearly understood. In a previous study(3) we demonstrated that the erythropoietic effects of ACTH, thyroid hormones and adrenocortical steroids in hypophysectomized rats were associated with an increase in oxygen consumption. Shalet *et al*(7) recently reported that 3,5,3′-triiodothyronine (T_3) did not stimulate erythropoiesis directly and suggested that T_3 exerts its effect by stimulating erythropoietin (ESF) production. Fried *et al*(13) and Gurney *et al*(6) have shown that plasma levels of ESF were elevated following testosterone injections and that mildly plethoric mice were more sensitive to the erythropoietic effects of androgens than polycythemic mice. Antiserum from rabbits immunized with ESF has also been found to block the action of ESF in polycythemic mice(14) and this effect is considered to be the result of an antigen-antibody combination. It has also been reported(15,16) that the erythropoietic effects of testosterone were completely abolished by ESF antisera. ACTH, 3,5,3′-triiodothyronine, and testosterone were studied in mildly plethoric mice in combination with erythropoietin antisera (Anti-ESF) in order to determine whether these hormones stimulate erythropoiesis by increasing erythropoietin production. The erythropoietic effects of these hormones were also compared in mildly plethoric and polycythemic mice.

Materials and methods. Mice were made polycythemic *via* a modification of the method of DeGowin *et al*(17). Male Swiss-Webster strain mice were injected with 1 mg of iron dextran prior to being placed at 0.45 atmosphere for 3 weeks. On the day of removal from the low pressure tank each mouse was injected intraperitoneally with 1.0 ml donor mouse blood at hematocrit 70. Either saline, sheep erythropoietin, or the humoral agents were injected subcutaneously into the mice each day for a period of 5 days following the transfusion. Each mouse was injected with 0.5 μc Fe^{59} i.p. on the 6th post-hypoxia day, exsanguinated on the 9th day and Fe^{59} incorporation in RBC determined.

The mildly plethoric mice were prepared according to the technique described by Fried *et al*(6). Female Swiss-Webster mice were injected i.p. with 0.2 ml donor mouse blood at hematocrit 80 on days 1 and 2 (total 0.4 ml) and injected with saline, sheep erythropoietin or the hormones each day for the first 5 days following transfusion. Fe^{59} was injected on the 6th day and 72 hour Fe^{59} incorporation in RBC determined on the 9th day as described above for the polycythemic mice. The hormones were injected subcutaneously and when they were administered at the same time as anti-ESF the injections were made at separate sites.

The erythropoietin antiserum was prepared by immunizing Race III strain albino rabbits with †Step III sheep erythropoietin

* Supported by USPHS Grant AM-02973.

† Step III sheep plasma erythropoietin from phenylhydrazine anemic sheep was supplied by USPHS Hematology Study Section.