2. Hellstrom, K. E., Hellstrom, I., Bergheden, C., Nature, 1965, v208, 458.
3. Frenkel, E. P., Korst, D. R., Raccuglia, G., J. Lab. & Clin. Med., 1962, v59, 81.

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Partial Isolation and Perfusion of Rat Submaxillary Gland.* (32320)

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Salivary gland separated from the systemic circulation and perfused with substituted fluid has provided a useful system for study of electrolyte secretion in dog(1) and cat(2-4), even though maintenance of the perfused gland in a normal functional state is frequently difficult to achieve. Perfusion of salivary gland in rat provides even greater technical difficulties. However, because of the potential usefulness of a method for separate perfusion of rat salivary gland, the possibilities of accomplishing this were investigated, using submaxillary gland. It was found that, although maintenance of functional and structural integrity in the completely isolated gland perfused with artificial serum was not readily feasible, the gland could be well maintained during partial isolation and artificial perfusion. The method involves the addition of an infusate of fortified Ringer's solution to the normally existing arterial supply to the submaxillary gland and deflection of the venous effluent from its normal return to the systemic system. Glands thus perfused were maintained for long periods in apparently normal structural and functional state.

Materials and methods. Adult male rats of the Long-Evans strain, 300-380 g in weight, were anesthetized by the intraperitoneal injection of sodium pentobarbital (50 mg/kg body weight). The submaxillary gland was exposed through a midline neck incision and the external maxillary artery was located between the masseter and the anterior belly of the digastric muscles, distal to the origin of the glandular artery as schematically shown in Fig. 1. The vein emerging from the hilus of a gland was dissected free at the point where it unites with the anterior facial vein. The sublingual gland was eliminated from the preparation by ligation at its hilus. Just prior to cannulation of the artery, heparin (5 mg in 0.5 ml of isotonic NaCl) was administered intravenously through a cannula in the femoral vein. The external maxillary artery was then cannulated just distal to the origin of the glandular artery, using polyethylene tubing of 0.6 mm outside diameter (Clay-Adams PE 10). The glandular and femoral veins were cannulated with polyethylene tubing of 1.0 mm outside diameter. The infusate consisted of Krebs-Ringer bicarbonate solution containing 4% bovine albumin. A syringe-type infusion pump was used to deliver the infusate at a constant

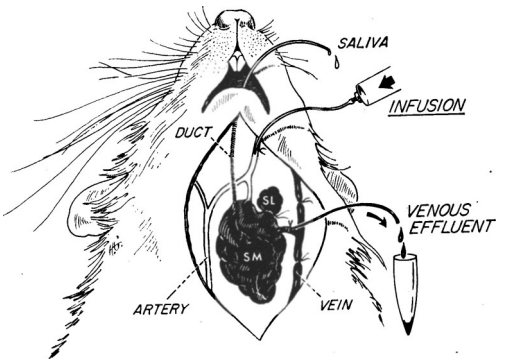

FIG. 1. Schematic representation of rat submaxillary gland (SM) prepared for infusion. Polyethylene tubing is inserted into the oral opening of the submaxillary duct for collection of saliva, into the external maxillary artery for infusion, and into the glandular vein for collection of venous effluent. The sublingual gland (SL) is ligated at the hilus.

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TABLE I. Flow Rate and Hematocrit of Venous Effluent from Rat Submaxillary Gland.

	Flow rate, ml/min	Hematocrit, %
Before infusion	.14	50
After " *	.32	33

* Samples were taken during the second half of a 60 min period of infusion of Krebs-Ringer bicarbonate solution (with 4% albumin) into the external maxillary artery at a rate of 0.11 ml/min (4 rats).

rate of approximately 0.11 ml/minute for a period of 1 hour. The venous effluent was collected. However, to maintain constancy of total blood volume, in these experiments a portion of the venous effluent was returned to the systemic circulation by way of the femoral vein. Saliva, evoked by stimulation of the chorda tympani nerve, was collected (10 μl samples) by micropipette from a short length of polyethylene tubing inserted into the oral opening of the submaxillary duct. At the end of the experiment, control and perfused glands were removed and weighed. The glands were dried overnight at 105°C and reweighed for determination of water content. Dried glands were ashed at 550°C and the ash was dissolved in water(5). These solutions and the diluted saliva samples were analyzed by flame photometry for Na and K. In some experiments, pieces of the excised control and experimentally perfused glands were placed in Bouin's fixative for preparation of histological sections stained by hematoxylin and eosin.

Results and discussion. Blood flow through the submaxillary gland before infusion was found to average 0.14 ml/min, as determined from timed collection of venous effluent. After infusion with Krebs-Ringer bicarbonate solution (containing 4% serum albumin) retrograde into the external maxillary artery for at least 30 minutes, the rate of flow of venous effluent was found to be increased. This is shown in Table I for experiments in which the infusion rate was 0.11 ml/min. Flow rate of the venous effluent averaged 0.32 ml/min under these conditions. Hence, at an infusion rate of 0.11 ml/min, approximately one-third of the fluid flowing through the vascular system of the gland could have been contributed by the infusate. Determination of the hematocrit of the venous effluent before and after the start of infusion did show a reduction which averaged 34%, as shown by data in Table I. Hence, it was clear that retrograde infusion of the modified Krebs solution into the external maxillary artery just distal to the origin of the glandular artery resulted in admixture with the blood entering the glandular artery.

Assessment of the functional and structural state of the perfused gland was made using a constant infusion rate of 0.11 ml/min, since the resulting one-third replacement of normal blood by infusate in the supply to the gland seemed adequate for future experimental use. The functional status of the perfused gland was evaluated with regard to content of Na, K and total H_2O, and with regard to the capacity of the gland to secrete in response to stimulation of its parasympathetic innervation. As shown by data in Table II, analysis of tissue from control and experimentally perfused submaxillary glands of the same animal showed no significant changes in glandular concentration of Na or K, or content of total water. Glands maintained on perfusate of which approximately one-third was a blood substitute also responded to stimulation of the chorda tympani nerve by elaboration of saliva of normal Na, K composition and rate of flow (Table III).

Microscopic examination of the perfused gland and its contralateral control indicated that no significant morphological changes occurred during a one-hour period of perfusion. It can be seen in Fig. 2 that there is no apparent increase in size of individual cells

TABLE II. Effect of Infusion on Content of Sodium, Potassium and Water in Rat Submaxillary Gland.

Gland*	Na, mEq/kg†	K, mEq/kg†	H_2O, %
Control	39.5 ± 1.5	87.3 ± 1.5	75.6 ± .2
Infused	42.8 ± 1.6	86.4 ± 1.1	76.1 ± .3

* Infused glands received Krebs-Ringer bicarbonate (with 4% serum albumin) at 0.11 ml/min for 1 hr; contralateral glands, not infused, are controls.

† Values (means ± standard errors) are based on wet weights of the glands from 9 rats.

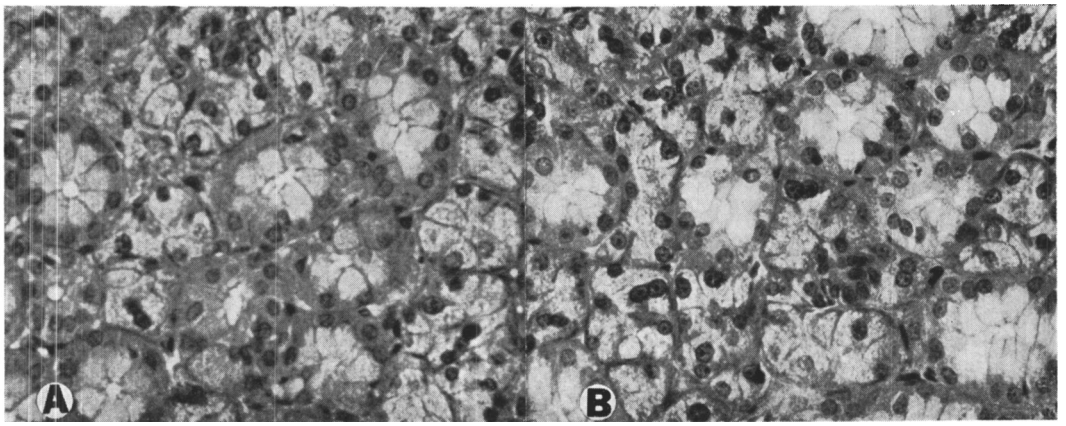

FIG. 2. (A) Control and (B) perfused submaxillary glands (×287) removed from a rat 1 hr after start of perfusion.

or of the interstitial space. Thus, the results of histological examination and of electrolyte and water analysis of perfused gland suggest that edematous changes during perfusion do not occur to any significant extent.

These results indicate that rat submaxillary gland can be maintained in a normal functional state for a significant length of time during perfusion by blood mixed with an infusate of Krebs-Ringer solution. This procedure has potential usefulness in studies with rat submaxillary gland by permitting a change in the environment within the gland without appreciably changing the environment of the whole animal; hence use of this procedure may facilitate investigation of factors which act at the gland level, independent of those which act at the systemic level.

TABLE III. Effect of Infusion on the Flow Rate and Electrolyte Composition of Rat Submaxillary Saliva.

	Flow,* μl/min/mg	Na, mEq/l	K, mEq/l
Before infusion	.14 ± .02	10.6 ± 3.4	37.0 ± 3.3
After "	.12 ± .02	9.6 ± 2.1	36.8 ± 2.9

* Saliva was evoked by electrical stimulation of the chorda tympani nerve before and after infusion (Krebs-Ringer bicarbonate with 4% albumin) at a rate of 0.11 ml/min into the external maxillary artery. Saliva samples taken after infusion are from the second half of a 60 min infusion period. Values (means ± standard errors) are from 9 rats.

Summary. An infusion-perfusion method has been devised for rat submaxillary gland which permits replacement by modified Krebs-Ringer bicarbonate solution of at least 30% of the blood supply to the gland. Glands maintained by this procedure for periods of 30 to 60 minutes secrete saliva of normal Na, K composition and show no significant change in tissue Na, K or total water, or in histological appearance.

1. Burgen, A. S. V., Seeman, P., Can. J. Biochem. Physiol., 1958, v36, 119.

2. Amberson, W. R., Hober, R., J. Cell Comp. Physiol., 1932, v2, 201.

3. Emmelin, N., Muren, A., Acta Physiol. Scand., 1950, v20, 13.

4. Lundberg, A., ibid., 1957, v40, 101.

5. Schneyer, L. H., Schneyer, C. A., Am. J. Physiol., 1959, v196, 365.

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