Evaluation of Blood Flow in the Submandibular Gland of the Dog by Fractional Uptake of Ionic and Particle Tracers[1] (35726)

MAURICE W. MEYER

Department of Physiology and School of Dentistry, Health Sciences Center, University of Minnesota, Minneapolis, Minnesota 55455

Several approaches have been used to study blood flow in salivary glands of experimental animals. The venous outflow method (1–3) requires some surgical intervention in the glandular region to make the measurements and assumes that the local physiological state is not modified. Indirect methods (4, 5) utilizing the isotope fractionation technique of Sapirstein (6) or external isotope monitoring technique (7) require specific indicators and certain assumptions. The assumptions may not be completely valid or remain valid if the circulation is altered. In the fractionation method, if a diffusible indicator is completely mixed in the blood before leaving the heart and entering each tissue, and if it is completely removed during the first transit time, then the fractional uptake of indicator would be equivalent to the fraction of the cardiac output going to that tissue. In 1967, the work by Rudolph and Heyman (8) strongly suggested that the fractional uptake of labeled particles is an index of blood flow to tissues. Subsequently, numerous investigators have utilized the particle distribution method to assess regional circulation to some tissues in experimental animals and these observations have been recently reviewed (9, 10).

In a recent study (11), blood flow to various oral tissues of dogs calculated from the fractional uptake of ytterbium-169 (^{169}Yb)-labeled microspheres and diffusible potassium-42 (^{42}K) or rubidium-86 (^{86}Rb) were compared. In animals showing higher uptakes in these tissues, the fractional uptake of the microspheres was correspondingly larger. This current study was directed at comparing the fractional uptake of a particle indicator and ^{42}K or ^{86}Rb by the submandibular salivary gland, a tissue in dogs which has been suggested by Henriques (12) to store K^+. Uptake of two differently labeled microspheres of similar dimension was also studied to examine the validity of utilizing labeled microspheres to assess the fraction of the cardiac output to the unstimulated salivary glands.

Method. The materials and methods utilized are similar to those presented previously (11, 13, 14). Briefly, dogs (7–15 kg) were anesthetized and maintained with sodium pentobarbital. Animals were divided into two groups, as shown in Table I. In group 1 (25 dogs) an ionic tracer, (^{42}K or ^{86}Rb)[2] was injected intravenously via the femoral vein, either prior or subsequent to left atrial or left ventricle injection of ^{169}Yb-labeled microspheres[3] (diameter of about 25 $\pm$ 5 μ SD). When the ionic tracer was injected first (in about 1 sec), the time delay between injection of the ionic and particle indicator was usually 25–30 sec. When the microspheres were given first, the delay time between completion of particle injection and injection of the ionic tracer was about 10–15 sec. Group 1 was subdivided as to time delay between injection of the ionic tracer and sacrifice of the animal to obtain tissue samples (40–60 sec, group 1A; 120–180 sec, group 1B). Animals acutely provided with a left atrial cannula to inject the microspheres were artifi-

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[2] Used as KCl and RbCl (purchased from Iso/Serve Corporation, Cambridge, Mass.).

[3] Purchased from Nuclear Products Division, 3M Center, St. Paul, Minn.

cially ventilated. For ventricular injection of spheres, the left ventricle was catheterized via the right brachial artery and animals were not artificially ventilated. Microspheres were suspended in dextran at a concentration of 0.5–1.0 $\times$ 10^6 spheres/ml. A known activity of microspheres (80–100 μCi/ml), determined by counting a standard solution prepared from an equivalent amount as injected into the animal, was flushed from a chamber either rapidly (10–20 sec) through the left atrial cannula or slowly (80–100 sec) via the left ventricular catheter. Following injection of a known activity of ^{42}K (60–80 μCi/ml) or ^{86}Rb (300–350 μCi/ml), aortic blood was continuously sampled for 15–25 sec to obtain an isotope dilution curve for calculating cardiac output. In group 2 (6 dogs), strontium (^{85}Sr)[3] and ^{169}Yb-labeled microspheres (60–80 μCi/ml each and diameters of about 25 μ) were injected sequentially via the left ventricular catheter. Animals in this group were artificially ventilated. The order of injecting the two differently labeled spheres was alternated in each dog. Indocyanine green dye was injected before and after each microsphere injection to determine cardiac output by the indicator dilution technique.

Animals in both groups were sacrificed by intravenously administering saturated KCl 10–14 sec after completing the microsphere injection or 40–60 sec after injecting the ionic tracer when microspheres were given first. Immediately after sacrifice, the submandibular gland was excised. Samples were placed in vials, weighed, and provided with the same counting geometry as used for counting the standard ionic or particle tracer. Samples were counted in a well-scintillation counter equipped with a dual pulse-analyzer to separate the radioactivity of each label in each sample in order to determine the radioactivity per unit weight or total gland. Fractional uptake per unit weight or per total gland of each tracer was calculated by dividing the radioactivity per unit weight or per total gland by the total radioactivity injected. The product of these fractional uptakes and the animal's cardiac output was considered to be a "blood flow" value per gram or per total gland for each tracer. These blood flow values were determined so that a comparison with values reported by other investigators could be made. A digital computer was utilized to facilitate calculation and analysis of the fractional uptake and "blood flow" values. The paired t test was used to examine differences in uptake of the two tracers, either the ionic and particle tracer or the two differently labeled particle tracers.

Results. The fractional uptake per gram of submandibular gland of the two isotopes in groups 1 and 2 animals are shown in Table I. The fractional uptake per gram of the ionic and particle tracer for animals in group 1A are not significantly different as indicated by the p value of 0.27. On the other hand, when

TABLE I. Fractional Uptake of Ionic and Particle Tracers by the Submandibular Salivary Glands in Dogs.

Group 1: sequential injection of ^{169}Yb-labeled microspheres and ^{42}K or ^{86}Rb.
Group 2: sequential injection of ^{85}Sr and ^{169}Yb-labeled microspheres.

Group I	Fractional uptake/g ($\times$ 10^{-4} $\pm$ SE) (^{42}K or ^{86}Rb)	(^{169}Yb)	p value (paired t test)
A[a] (11)	0.94 $\pm$ 0.13	0.84 $\pm$ 0.12	0.27
B[b] (14)	1.51 $\pm$ 0.14	0.95 $\pm$ 0.09	$<$ 0.001
	(^{85}Sr)	(^{169}Yb)	
Group 2 (6)	0.91 $\pm$ 0.14	1.05 $\pm$ 0.13	0.08

[a] Dogs sacrificed 40–60 sec after K or Rb injection; microspheres administered via left atrial cannula (8 dogs) or via left ventricular catheter (3 dogs).

[b] Sacrificed 120–180 sec after K or Rb injection; microspheres administered via left ventricular catheter.

TABLE II. Average "Blood Flow" in the Submandibular Salivary Gland and Cardiac Output in each Group.[a]

Group 1	Blood flow (ml/min/g ± SE) (^{42}K or ^{86}Rb)	Blood flow (ml/min/g ± SE) (^{169}Yb)	Cardiac output (ml/min/kg ± SE)
A	0.22 ± 0.03	0.20 ± 0.03	220 ± 9
	(1.01 ± 0.17)[b]	(0.91 ± 0.15)	
B	0.43 ± 0.04	0.27 ± 0.02	282 ± 9
	(1.84 ± 0.18)[b]	(1.14 ± 0.11)	
	(^{85}Sr)	(^{169}Yb)	
Group 2	0.16 ± 0.02	0.18 ± 0.02	159 ± 12
	(0.89 ± 0.10)[b]	(1.04 ± 0.10)	

[a] Grouped as in Table I.

[b] Total flow per gland (ml/min/gland).

the submandibular gland is sampled 120–180 sec after the injection of the ionic tracer, the fractional uptake of the ionic tracer appears to be significantly larger than that of the particle tracer (p value < 0.001). When comparing the fractional uptake of corresponding tracers in groups 1A and B, fractional uptake of the ionic tracer is significantly larger in 1B, whereas fractional uptake of the microspheres is not. For the 6 animals in group 2, the fractional uptake of the ^{169}Yb-labeled microspheres appears to be slightly larger than the fractional uptake of the ^{85}Sr-labeled microspheres, independent of the sequence of injection. Neither of these uptakes are significantly different from the uptake of microspheres observed in groups 1A and B. The "blood flow" values per gram or per total submandibular gland are shown in Table II. The average cardiac output per kilogram of the dogs in each group are also indicated. The paired fractional uptakes per gram of the ionic and particle tracers for animals in groups 1A and B are illustrated in Fig. 1 and the uptakes between groups 1A and B can be readily compared. The "least

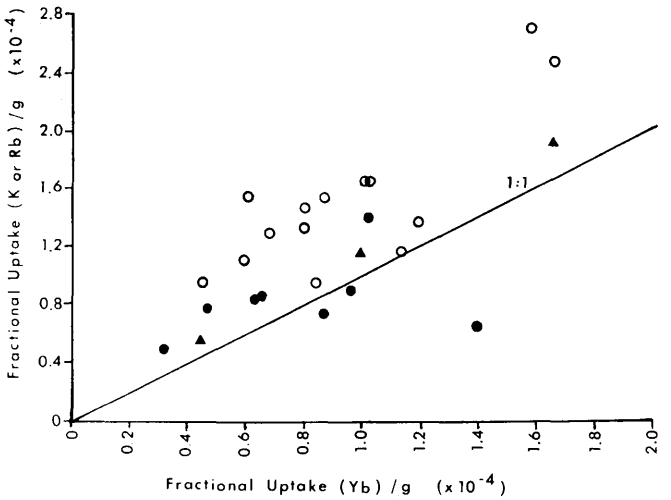

FIG. 1. The fractional uptake of an ionic tracer (^{42}K or ^{86}Rb) is related to that of the ^{169}Yb-labeled microspheres: (● and ▲) the paired values of group 1A animals, where three animals in which microspheres were injected prior to injecting the ionic tracer so that tissue samples could be taken 40–60 sec after injecting the ionic tracer. (○) the paired values of Group 1B. The perfect correlation (1:1) line is shown.

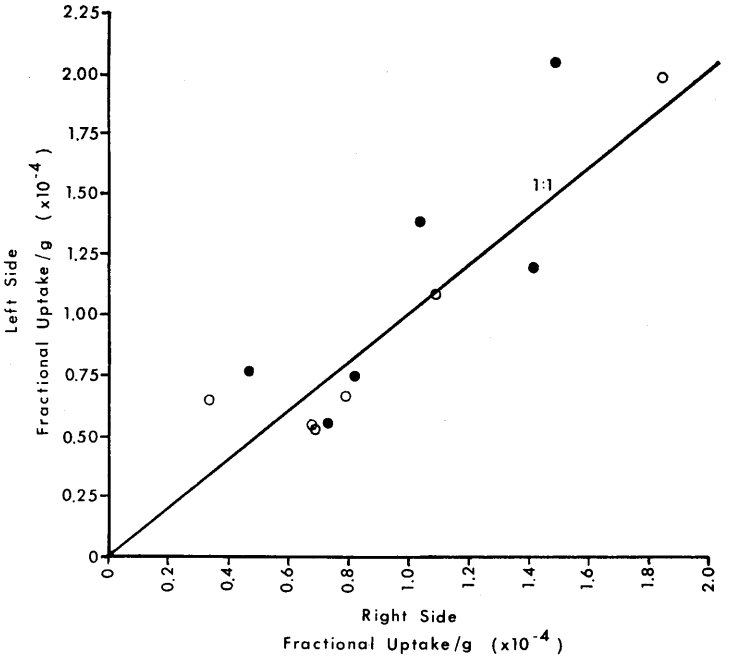

FIG. 2. The fractional uptake of ^{85}Sr (○) and ^{169}Yb (●) labeled microspheres in the left submandibular gland is related to that on the right gland for animals from group 2. The "least squares" correlation coefficient is 0.88. The perfect correlation (1:1) line is shown.

squares" regression analysis indicated significant correlations (correlation coefficients of 0.72 and 0.78, respectively). The intercepts on the ordinate for the best fit line for both groups 1A and B were positive. The fractional uptakes per gram of the ^{85}Sr- and ^{169}Yb-labeled spheres in the left side are compared to that in the right side in Fig. 2. The least squares regression analysis indicated a correlation coefficient of 0.88. The best fit line was not significantly different from the (1:1) line of perfect correlation (p value < 0.001).

Discussion. For diffusible tracers such as ^{42}K and ^{86}Rb, it has been pointed out (11) that the findings of others have indicated that the extraction ratio is inversely related to flow in some tissues. Furthermore, extraction ratio can vary considerably from tissue to tissue and is very low for the brain and spinal cord (6, 14). Some tissues may take up the recirculating label such that the amount taken up at the time the tissues are sampled may be equivalent to the amount that would be present with 100% extraction after one transit time. Under these conditions, and if both ionic and particle tracers are distributed as blood flow, then their fractional uptakes could be identical. Steiner and Mueller (4) found that fractional uptake of ^{86}Rb in the salivary gland remained the same 20–40 sec after injecting the tracer. Work by Sapirstein (6) indicated that in many tissues of dogs, the fractional uptake remained essentially the same up to 120 sec after injecting ^{42}K, but salivary glands were not sampled.

Analysis of the data in group 1A indicates that uptakes of K or Rb and microspheres were similar in the number of dogs used. From the distribution of these paired values shown in Fig. 1, we see that if the number of animals doubled and a similar proportion had higher uptake of ionic tracer, then a sign-test analysis would show uptake of the ionic tracer to be greater than spheres at a significant level of 0.025. The difference in the uptakes is more evident when glandular tissue is sampled at 120–180 sec as accomplished in group 1B. Since A-V shunts are present in the gland, the extraction ratio for ^{42}K or ^{86}Rb would not likely be close to unity during the first transit of these tracers.

If the microspheres are distributed as blood flow and are too large to pass through these A-V shunts, then one would expect fractional uptake of microsphere to be consistently larger. Our earlier study (13) showed that overall body shunting was less than 1.5% of the total number injected for this sized microspheres. If A-V shunts in the gland are similar in size to those in other tissues having shunts, then some shunt blood flow would be measured with the microspheres as part of the total blood flow to the gland. The difference between the ionic and particle tracer uptake by the submandibular gland can best be explained by the capacity of the tissue to take up recirculating ^{42}K or ^{86}Rb continuously as observed by Henriques (12) for K^+. In the resting or unstimulated gland, the rate of salivary secretion is very low. At these rates, concentration of K^+ in the saliva is 6–7 times greater than in the venous plasma (15), thus, a gradient for further uptake of ^{42}K or ^{86}Rb could be created. At these low secretion rates, most of the ^{42}K or ^{86}Rb secreted could be included in the tissue sample. On this basis the differences observed between groups 1A and B, as illustrated in Fig. 1, could be expected. The fractional uptake of labeled microsphere may therefore be a better index of total blood flow to the gland than that of ^{42}K or ^{86}Rb.

The similar fractional uptake of microspheres per gram in the right and left submandibular gland supports the views of others (9, 11, 14) that uptake per unit weight in bilateral structures is similar. In groups 1A and B, right and left tissue samples were usually pooled; thus, tracer uptakes in the left and right side were not compared. In a concurrent study of dogs (unpublished data) in which only an ionic tracer was injected, the findings suggested that uptake per gram was similar in the right and left submandibular gland.

The average "blood flow" per gram as determined by the fractional uptake of the labeled microspheres is similar to that reported for dogs by Terroux *et al.* (2) using a venous outflow method. Steiner and Mueller (4) found that the blood flow in rats was 0.40 ml/min/g determined from the fractional uptake of ^{86}Rb, similar to that calculated for dogs in group 1B for K or Rb as shown in Table II. The blood flow per gram calculated from the uptake of the microsphere averaged largest in group 1B. This group also had the higher average cardiac output per unit weight. Differences in cardiac output per kilogram in the three groups can perhaps be best explained on the basis of method used and age and size of the animals. Earlier findings (14) have suggested that placing dogs on a positive pressure respirator reduces cardiac output per kilogram. Moreover, cardiac output per kilogram was lower in older animals. Primarily small young dogs were used in group 1, but those in 1A were artificially ventilated. Animals in group 2 were mainly older dogs and were ventilated with a positive pressure respirator.

Studies by others (1, 3, 5, 7, 16) have assessed blood flow to the submandibular gland more qualitatively to examine shunts, the effect of increased functional activity, and the role of vasoactive kinins. Earlier work by Hilton and Lewis (1) and more recent work by Hilton and Torres (16) suggested that functional dilatation of vessels in the submandibular gland is mediated by kinins. Arcidiacono *et al.* (5), examining the fractional uptake of ^{86}Rb, observed that bradykinin reduced blood flow to the submandibular gland in rats. Schachter (3) cited investigations for and against the vasodilatory role of kinin-releasing enzymes on the submandibular gland.

From our study, it appears obvious that comparing the uptake of ^{42}K and ^{86}Rb with labeled microspheres does not provide any evidence for the presence of shunts or indication of their functional significance in unstimulated glands. Fractional uptake of microspheres may be the better index of blood flow to the submandibular gland. Application of the particle distribution method involving the use of different sized spheres may clarify some of the contradictory evidence with respect to the functional significance of shunts and the role of kinins in the regulation of blood flow to the salivary glands.

Summary. This study examined the fractional uptake of ^{169}Yb-labeled microspheres

(about 25 μ in diam) and an ionic tracer (^{42}K or ^{86}Rb) by the same unstimulated submandibular gland as an index of its blood flow (total and/or functional). Cardiac outputs were measured to calculate a "blood flow" from these uptakes. To examine any changes in the uptake of the ^{42}K or ^{86}Rb with time, tissues were sampled 40–60 or 120–180 sec after injecting the ionic tracer for uptake of the ionic and particle tracers. For the 40–60-sec group of dogs, fractional uptake of the ionic tracer appeared to be similar to that of the microspheres. In the other group, uptake of the ionic tracer was significantly larger than that for the labeled microspheres. Injection of two differently labeled spheres (^{169}Yb and ^{85}Sr) sequentially demonstrated that uptakes of the spheres injected secondly were not different from those injected initially. Fractional uptakes of each type of sphere per unit weight were similar in the right and left gland, further supporting the particle distribution method as an approach for assessing blood flow to the salivary gland in experimental animals.

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