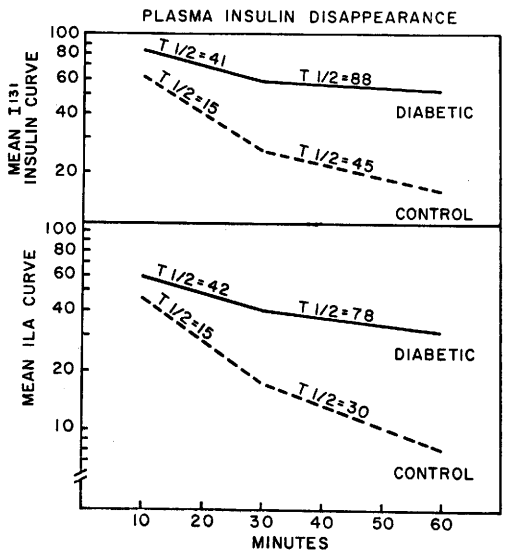

FIG. 1. Composite plasma disappearance curves for insulin measured simultaneously by assay of TCA precipitable radioactivity and by assay of ILA on the epididymal fat pad.

greater reproducibility of the insulin I^{131} curve renders this a more reliable measure of insulin dynamics in individual cases.

Summary. Plasma decay curves were determined in diabetics and normals after the injection of a mixture of insulin I^{131} and crystalline insulin. The general forms of the curves as determined by bioassay and assay of radioactivity were similar, consisting of an initial rapid component and a late slower component. Insulin I^{131} tended to overestimate the half life of disappearance determined by bioassay of insulin-like activity.

1. Welsh, G. W., Henley, E. D., Williams, R. H., Cox, R. W., *Am. J. Med.,* 1956, v21, 324.
2. Bolinger, R. E., Grady, H. J., *Ann. Int. Med.,* 1958, v48, 753.
3. Scott, G. W., Prout, T. E., Weaver, J. A., Asper, S. P., *Diabetes,* 1958, v7, 38.
4. Elgee, N. J., Williams, R. H., Lee, N. D., *J. Clin. Invest.,* 1954, v33, 1252.
5. Bolinger, R. E., Slinker, B. J., PROC. SOC. EXP. BIOL. AND MED., 1955, v88, 574.
6. Sheppard, C. W., *Ann. N. Y. Acad. Sci.,* 1963, v108, 29.
7. Renold, A. E., Martin, D. R., Dagenais, Y. M., Steinke, J., Nickerson, R. J., Sheps, M. C., *J. Clin. Invest.,* 1960, v39, 1487.
8. Berson, S. A., Yalow, R. S., Bauman, A., Rothschild, M. A., Newerly, K., *ibid.,* 1956, v35, 170.
9. Prout, T. E., Katim, R. B., *Diabetes,* 1959 v8, 425.

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Effects of Pilocarpine on Exchange of K^{42} in Slices of Submaxillary Gland.* (29382)

L. H. SCHNEYER AND C. A. SCHNEYER
Department of Physiology, University of Alabama Medical Center, Birmingham

Secretion of electrolytes by salivary gland appears to involve a complex series of separate processes which have generally proved difficult to localize and identify(1,2). In the case of potassium, however, evidence suggests that transfer from cells to secretory fluid involves the participation of a great proportion of the cells of the gland and that the content of potassium in the cells is the primary source of the secreted ion(1-4). On this basis it appeared of value to determine whether an *in vitro* preparation of salivary gland could prove useful for investigation of secretion of potassium. Recent work with slices of submaxillary gland as the *in vitro* preparation showed that during incubation when net accumulation of potassium is occurring, addition of a parasympathomimetic stimulating agent results in reduction in the net accumulation of this ion(5). Since net loss of potassium from the tissue marks the initiation of secretion of this ion *in vivo*(6-8), a resemblance between the effect of the parasympathomimetic agent *in vitro* and tissue events in secretion of potassium after parasympathomimetic stimulation *in vivo* was suggested(5).

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In the present investigation other features of exchange of potassium in slices of salivary gland have been examined for comparison with events in normal secretion. Thus, it had been reported by Burgen and Seeman(3) that stimulation of salivary gland *in situ* results in enhanced uptake and loss of K^{42} when glands are subjected to loading and unloading, respectively, with isotope. It has now been found that enhanced uptake and loss of K^{42} may also be observed in submaxillary slices when pilocarpine is introduced into the incubation medium under appropriate conditions and, further, that the effect of pilocarpine on unloading of K^{42} by slices can be inhibited by atropine. These findings further extend the aspects of apparent similarity between this *in vitro* system and the *in vivo* condition.

Methods. Submaxillary glands were obtained from Long-Evans male rats (260-300 g) which had been fasted 24 hours and killed by severing the cervical spinal cord. Slices were prepared(5) at 0.45 mm thickness by means of a Harvard sectioner, without wetting sectioner blade or platform, and were quickly blotted and weighed. Weights of individual slices were in the range 40 to 60 mg. Slices were then submitted to 2 periods of incubation(6,9). During the first period, the slices were individually immersed in 3 ml Krebs-Ringer-phosphate (KRP) solution, gassed with 100% N_2 (KRP-N_2), in Erlenmeyer flasks (25 ml size, with glass stoppers). Gassing with N_2 was continued for 10 minutes after addition of the slices to KRP (22°C), then the flasks were stoppered and transferred to a water-bath shaker at 37°C for 20 minutes. When washout of K^{42} was to be investigated, tissues were loaded with isotope by including K^{42} in the KRP-N_2. During the period of incubation in KRP-N_2, submaxillary slices become depleted of total K(9). After incubation in KRP-N_2, slices were blotted and transferred to fresh KRP, gassed with 100% O_2 (KRP-O_2) for the second period of incubation. During this period slices reaccumulate total K(8). This net reaccumulation, which was known(9) to reach appreciable values by 20 minutes in KRP-O_2, was found in preliminary experiments to be essentially completed by 6 to 10 minutes, after which steady state conditions were approximated. For this reason, loading with K^{42} was sometimes continued for 6 to 10 minutes during this phase of incubation before starting washout. Washout of K^{42} was followed, after blotting of grossly adherent fluid, by placing the sheet of tissue in a porous basket attached to the flat undersurface of a glass stopper, which interchangeably fit each of a series of Erlenmeyer flasks (25 ml size). This basket (Fig. 1) was constructed of a framework of dental sheet-wax, across which thin nylon-net (stocking nylon) was spread. The basket was attached to nichrome steel wire of appropriate length, and this was sealed by wax to the glass stopper. Glass-stoppered flasks used in a washout series were selected for uniformity of height so that the lower portion of the nylon mesh bas-

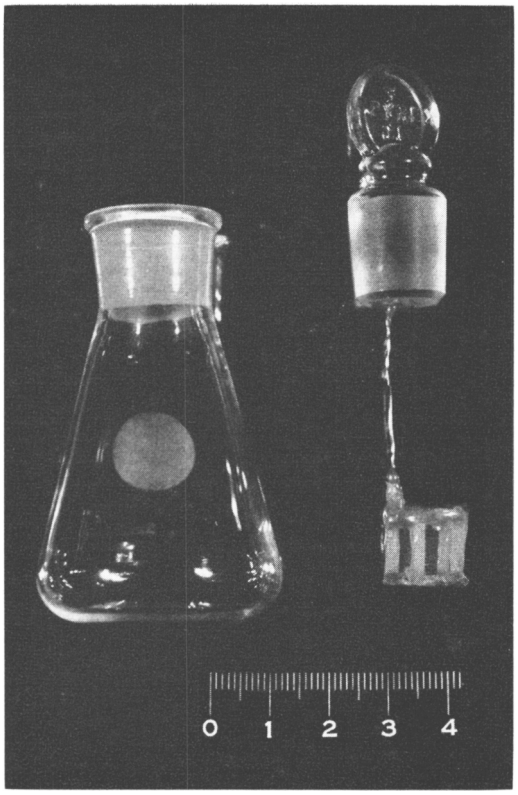


FIG. 1. Porous basket used to suspend and transfer tissue slice during washout of isotope. The slice was spread, without folding, on the nylon-net floor of the basket. Transfer was made by substituting the suspending glass stopper for another, in an array of matched flasks in the water-bath shaker.

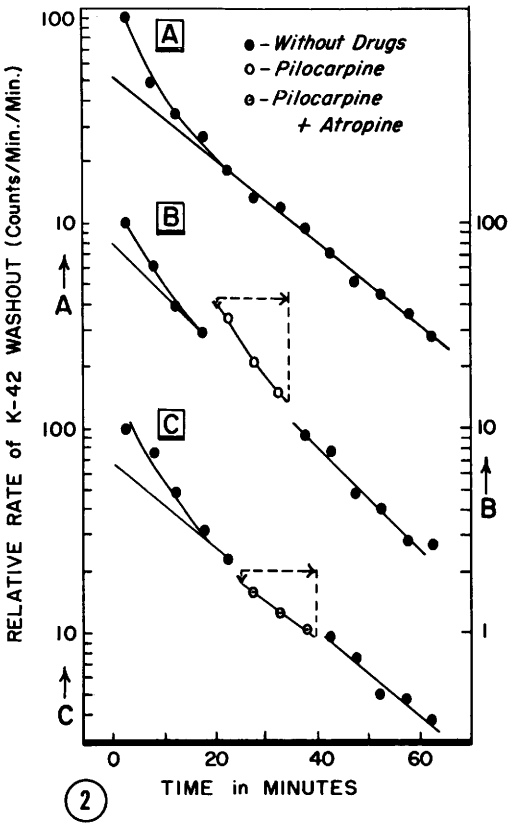
ket would be adequately submerged in 3.5 ml of fluid. To follow washout, the glass stopper, with the basket containing the sheet of tissue, was exchanged at pre-selected times for the stopper of another flask containing 3.5 ml KRP (without K^{42}), previously gassed with 100% O_2, in an array of such flasks in the waterbath shaker (37°C). Pilocarpine or atropine was included in the KRP when indicated. Uptake of K^{42}, in presence and absence of pilocarpine, was determined, after addition of isotope to the KRP used for the oxygenated phase of incubation only, by subsequent measurement of the specific activity of tissue K in relation to the specific activity of K of the incubation medium.

Krebs-Ringer-phosphate at pH 7.4 was prepared as reported previously(5). For Na, K, and Cl the concentrations (meq/l) were 149, 6.5, and 139, respectively. Pilocarpine was made, as the nitrate, to a final concentration of 1.2×10^{-5} M in KRP. Atropine sulfate was used in a final concentration of 2.4×10^{-5} M in KRP. K^{42} in HCl solution was obtained from Oak Ridge National Laboratory; solutions were titrated to neutrality with 0.1 N NaOH and appropriately diluted before use.

Electrolytes of tissue slices were extracted by de-ionized distilled water (tissue wet weight/water = 1/200) in a stoppered flask during overnight shaking(9). The aqueous extract was used for estimation of tissue radioactivity (scintillation well detector and scaler, Nuclear Chicago Corp.) and chemical content of Na and K (Coleman flame photometer). Incubation medium was similarly analyzed, after appropriate dilution, for radioactivity and total K. Concentrations in tissue were determined in relation to wet weight of tissue before incubation(9). Counts of radioactivity were generally made to at least 10^4 counts, using 3 ml of tissue extract or diluted medium and were corrected for radioactive decay from time of experiment.

Results and discussion. Unloading of K^{42} was measured during incubation of slices in oxygenated medium, with and without pilocarpine, after slices had been loaded with tracer during incubation in KRP-N_2, without pilocarpine. In most instances (8 out of 12), contact with K^{42} in the medium was continued for an initial 10 minutes of incubation in O_2-gassed medium (following KRP-N_2) before the tissue was transferred to the mesh basket for washout of tracer. In every case (12 experiments), washout of K^{42} was increased during periods when pilocarpine was present, whether or not an approach to steady state conditions, by incubation with isotope in KRP-O_2, had been made before washout was started. Representative data are shown in Fig. 2. Analysis of paired slices from the same glands from which the data on washout were obtained showed that after the complete period of loading with K^{42} approximately 70% of total K had exchanged. When the time-course of washout of K^{42} into O_2-gassed medium without added drugs was subsequently followed, it was found that, after a relatively brief period, rate of washout (as counts/minute, per minute of washout period) followed a logarithmic course ($t_{1/2}$ = 15 minutes; Fig. 2, Exp. A). Introduction of 1.2×10^{-5} M pilocarpine into flasks of the washout series for a limited period, after the logarithmic phase of washout had been achieved, resulted in a burst of K^{42} appearance in the medium, after which washout again approached a logarithmic course which was greater in rate than previously. Continuation of washout without pilocarpine resulted in return to the logarithmic rate observed for washout before introduction of the pilocarpine (Exp. B, Fig. 2). The effect of pilocarpine (1.2×10^{-5} M) in increasing washout of K^{42} was completely suppressed by the simultaneous presence of 2.4×10^{-5} M atropine (2 experiments), as shown in Fig. 2 (Exp. C). Atropine (2.4×10^{-5} M) alone was without significant effect on rate of washout of K^{42}. The possible slight decrease in rate of washout with atropine and pilocarpine present, suggested by the data of Fig. 2, Exp. C, may have been related to small endogenous production of acetylcholine, in this case, since in submaxillary gland postganglionic parasympathetic fibers arise from ganglion cells within the gland parenchyma.

Loading of K^{42} was measured after timed



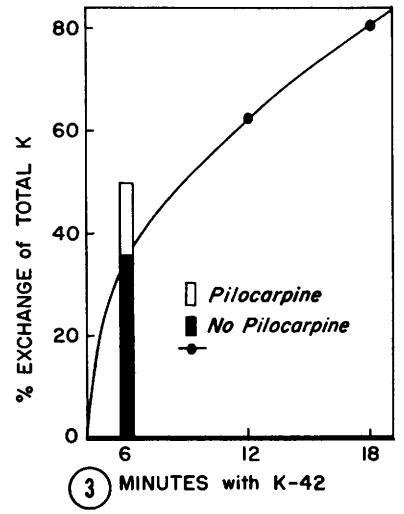


FIG. 2. Influence of pilocarpine on washout of K^{42}, in individual representative experiments. Slices were exposed to K^{42} in KRP-N_2 and then, for 10 minutes, in KRP-O_2, without pilocarpine. Slices were next subjected to washout in KRP-O_2 without added isotope. In Exp. A, no drug was present at any time of washout. In Exp. B and C, 1.2×10^{-5} M pilocarpine (with 2.4×10^{-5} M atropine in C only) was present for indicated periods.

FIG. 3. Percent exchange of total K (100 × specific activity of tissue K/specific activity of K of medium) in relation to time of exposure to K^{42}: influence of pilocarpine during the 6-min exposure period. Single slices were separately incubated in KRP-N_2, then for 6, 12, or 18 min in KRP-O_2 with K^{42}. In another instance, when exposure to K^{42} was for 6 min, 1.2×10^{-5} M pilocarpine was also present. Greater exchange of total K occurred during the 6-min period when pilocarpine was present.

exposure of slices to isotope in oxygenated medium, with and without pilocarpine. As usual, slices had previously been incubated in KRP-N_2 at 37°C, for 20 minutes, before transfer to KRP-O_2. In some instances, exposure to K^{42} was delayed for 6-10 minutes after start of incubation in KRP-O_2 to minimize effects of unsteady-state conditions, but this modification proved to be without appreciable effect.

Data from a representative experiment to determine the influence of pilocarpine (1.2×10^{-5} M) on extent of loading with K^{42} are shown in Fig. 3. When exposure of gland slices to K^{42}, during O_2-gassing, was limited to 6 minutes, a distinct increase was observed in percent of total-K which was exchanged (from 36 to 50% in experiment of Fig. 3) in the presence of pilocarpine. Analysis of comparable data from paired slices of glands of 7 animals confirmed that pilocarpine significantly ($P<.01$) increased uptake of K^{42} during a 6 to 8 minute period of exposure to the tracer.

At least 90% of the total potassium of slices of submaxillary gland was found to be exchangeable. Of this, somewhat more than half could be exchanged rapidly, *i.e.*, usually within 12 minutes. Exchange of the remainder was sometimes much slower and more erratic in rate, even when slices of glands from the same animal were compared. Apparently for this reason, effects of pilocarpine on uptake of K^{42} were not consistent when exchange of total K exceeded 60 to 70%. Investigation of uptake of K^{42} was, therefore, essentially limited to the 8-minute period when a consistent effect of the presence of pilocarpine on uptake of the isotope was observed.

The thickness(10) of the tissue slice and the complexity of the exchange-process for K

beyond the 50 to 70% found readily exchangeable, as well as the heterogeneity of the salivary tissue with regard to cell type, make compartmental analysis difficult with this system. In the absence of more extensive analysis it is not possible to estimate the magnitude of the potassium compartment in the slice which is affected by pilocarpine. Hence, no quantitative comparison of effects of pilocarpine on uptake and unloading of K in the slice and *in vivo* is appropriate. Nor can the mechanism of action of pilocarpine on the cell membrane be reliably deduced, although an increase in permeability would not be inconsistent with present data (11), especially if membrane potentials were not appreciably altered. Nonetheless, the data do give clear evidence that pilocarpine enhances uptake and loss of K^{42} in slices of salivary gland tissue and that the effect of pilocarpine on loss of K^{42} is inhibited by atropine. Thus, similarities are suggested in effects of stimulating agents on K-exchange *in vivo*(3) and in slices.

Summary. The presence of pilocarpine led to an increase in loss and uptake of K^{42} in slices of rat submaxillary gland incubated under selected conditions. The influence of pilocarpine on the loss of K^{42} was found to be inhibited in the presence of atropine. These effects on the exchange of K^{42} in slices bear at least a qualitative resemblance to effects known to occur during stimulation of salivary glands *in vivo*.

1. Burgen, A. S. V., Emmelin, N. G., *Physiology of the Salivary Glands,* Edward Arnold Publishers, Ltd., London, 1961.

2. Schneyer, L. H., Schneyer, C. A., *Advances in Oral Biol.,* 1964, v1, 1.

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

4. Burgen, A. S. V., Terroux, K. G., *J. Physiol.,* 1962, v161, 399.

5. Schneyer, L. H., Schneyer, C. A., *Am. J. Physiol.,* 1963, v205, 1058.

6. Wills, J. H., *ibid.,* 1941, v135, 164.

7. Burgen, A. S. V., *J. Physiol.,* 1956, v132, 20.

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

9. ———, *ibid.,* 1962, v203, 567.

10. Burg, M. B., Orloff, J., *ibid.,* 1963, v205, 139.

11. Durbin, R. P., Jenkinson, D. H., *J. Physiol.,* 1961, v157, 74.

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Bacterial Infection and Wasting in Neonatally Thymectomized Rats.* (29383)

HENRY A. AZAR (Introduced by Herbert C. Stoerk)
Department of Pathology, Columbia University College of Physicians and Surgeons and Francis Delafield Hospital, New York City

It is generally accepted that in several strains of mice and in other mammals thymectomy in early life is associated with lymphopenia, wasting, and certain defective immunologic reactions(1-4). It is not clear whether these changes are specifically due to ablation of the thymus at a critical period of development of lymphoid organs or whether they represent secondary phenomena exaggerated by neonatal sepsis and, in some situations, selective breeding(5,6). Post-thymectomy wasting has been likened to runt disease or graft-versus-host reaction and several hypotheses have been formulated to explain its pathogenesis. It is the purpose of this paper to evaluate the significance of bacterial infection and wasting among adult survivors of neonatally operated rats.

Methods. Sprague-Dawley rats, all born between September 1963 and January 1964, were operated upon within 48 hours after birth. Anesthesia was achieved by cooling animals on an ice block covered with moistened paper towels. Four types of operations

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