spectrophotometric measurements, we found that rats excreted a large fraction of the administered dose as unmetabolized PA. The guinea pig, a species which is resistant to the nephrotoxic action of PA, reportedly excretes even more of its dose unchanged, while the rabbit, likewise resistant, metabolizes a major fraction of PA to a "substituted" product of unknown structure(9). The species susceptibility(3) and structural specificity(15) exhibited by PA are intriguing facets with mechanistic implications, and comparative metabolism studies may prove to be useful investigational probes for the elucidation of its mechanism of action.

Summary. The quantitative distribution and excretion of aminonucleoside-8-C^{14} was studied in normal and nephrotic rats. In the 8 hours following subcutaneous administration the normal rat excreted 65-70% of the dose in the urine, half of which was unchanged PA, and 15-26% in the feces. Despite being the target organ, the kidney contained less than 0.25% of the dose, while the liver contained considerably more. Nephrotic rats excreted much less of the radioactivity–total as well as in the form of unchanged PA. The decrease in excretion of total radioactivity was attributed to its retention in an expanded pool of body water characteristically seen in the nephrotic syndrome.

1. Frenk, S., Antonowicz, J. M., Craig, J. M., Metcoff, J., PROC. SOC. EXP. BIOL. AND MED., 1955, v89, 424.

2. Feigelson, E. B., Drake, J. W., Recant, L., *J. Lab. and Clin. Med.*, 1957, v50, 437.

3. Wilson, S. G. F., Hackel, D. B., Horwood, S., Nash, G., Heymann, W., *Pediatrics,* 1958, v21, 963.

4. Borowsky, B. A., Kessner, D. M., Hartroft, W. S., Recant, L., Koch, M. B., *J. Lab. and Clin. Med.,* 1960, v57, 512.

5. Michael, A. F., Jr., Venters, H., Jr., Good, R. A., *Fed. Proc.*, 1961, v20, 408.

6. Burchenal, J. H., *Current Research in Cancer Chemotherapy,* 1956, No. 4, p17.

7. Bartlett, P., Shelata, S., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 499.

8. Bartlett, P., *ibid.*, 1961, v108, 611.

9. Wilson, S. G. F., Heymann, W., Goldthwait, D. A., *Pediatrics,* 1960, v25, 228.

10. Passmann, J. M., Radin, N. S., Cooper, J. A. D., *Anal. Chem.*, 1956, v28, 484.

11. Berson, S. A., Yalow, R. S., *Science,* 1960, v131, 606.

12. Herberg, R. J., *Anal. Chem.*, 1960, v32, 42.

13. Aronof, S., *Techniques of Radiobiochemistry,* Iowa State Coll. Press, Ames, 1956, p35.

14. Baker, B. R., Joseph, J. P., Williams, J. H., *J. Am. Chem. Soc.,* v77, 1.

15. Borowsky, B. A., Kessner, D. M., Recant, L., PROC. SOC. EXP. BIOL. AND MED., 1958, v97, 857.

Received September 24, 1962. P.S.E.B.M., 1962, v111.

Effect of Pilocarpine on Acid-Soluble Phosphate Compounds of Rat Submaxillary Glands. (27843)

R. H. DREISBACH* AND E. GERLACH
Institute of Physiology, Freiburg i. Br., Germany

Recent work(1,2) indicates that the rate of transfer of Ca^{45} into rat salivary glands is relatively slow and is speeded by parasympathetic stimulants. For example, pilocarpine administration increases the rate of equilibration of Ca^{45} *in vivo* by 3-fold(1). Both uptake and loss of Ca^{45} by rat parotid glands are increased by pilocarpine or acetylcholine when studied *in vitro*(2). Rat salivary glands also contain more calcium than other soft tissues and approximately half of this calcium is lost when secretory activity is stimulated maximally by pilocarpine administration(3). A number of mechanisms can be postulated to explain some of these findings. For example, intracellular acid-soluble phosphate compounds could bind calcium(4) or furnish energy for the transfer process(5). Hence, changes in concentration or rates of incorporation of P^{32} are of interest.

* Fellow, Nat. Inst. Dental Research, U. S. Public Health Service. Present address: Stanford Univ. School of Med., Palo Alto, Calif.

TABLE I. Effect of Pilocarpine on Acid-Soluble Phosphorus Compounds in Rat Submaxillary Glands.

	Control		Pilocarpine	
	μM/g	%*	μM/g	%*
Orthophosphate	2.52 ± .26	11.9 ±1.0	2.65 ± .38	12.3 ±1.5
Creatine phosphate	.82 ± .08	3.9 ± .3	.90 ± .12	4.3 ± .5
ATP	1.59 ± .67	23.2 ± .8	1.53 ± .09	22.2 ±1.6
ADP	.49 ± .05	4.6 ± .3	.52 ± .06	4.8 ± .4
AMP	.48 ± .10	2.2 ± .4	.39 ± .03	1.8 ± .2

Pilocarpine or saline (control) given 1 hr prior to analysis.
Arithmetic mean ± S.E. for 5 rats in each group.
* % of total acid-soluble phosphorus.

Methods. Hooded male rats weighing from 200-250 g were used. Pilocarpine (10 mg/kg) and P^{32} (200 μC) were given subcutaneously in 0.15 M NaCl. At end of experiment, submaxillary glands with attached sublingual glands were carefully dissected under ether anesthesia and then frozen *in situ* by applying tongs having 0.3 × 3.0 × 3.0 cm blades cooled to temperature of liquid N_2. Concentrations of acid-soluble phosphate compounds in submaxillary glands removed by this procedure were not different from glands taken from rats frozen by immersion in liquid N_2 without prior dissection. For experiments with P^{32}, blood for specific activity determination of extracellular orthophosphate was drawn from the heart immediately prior to freezing of glands. The methods for preparing perchloric acid extracts, for chromatography, and for counting radioactivity have been described(6,7). Plasma acid-soluble inorganic P and, in some cases, tissue acid-soluble inorganic P were determined by the methods of Berenblum and Chain(8) or Martin and Doty(9).

Results. Table I gives the concentration of inorganic phosphate, creatine phosphate, and adenosine phosphates of submaxillary glands of control and treated rats. Administration of pilocarpine 60 minutes prior to removal had no apparent effect on the concentrations of any of these substances or on total acid-soluble phosphorus which averaged 652 μg/g wet weight in control rats and 680 μg/g wet weight in pilocarpine treated rats. Other phosphorus compounds, including the guanosine, uridine, and inosine nucleotides, and the hexose and triose phosphates, were also visible on the chromatograms but none showed evident differences between control and treated rats. These compounds were not analyzed.

The effect of pilocarpine on 30-minute incorporation of P^{32} into acid-soluble phosphorus compounds is shown in Table II. Although the relative specific activity of the various substances analyzed averaged 17-24% higher in rats given pilocarpine, these differences were not statistically significant. From 60-70% of total radioactivity in acid extract was recovered in fractions shown in the Table.

Specific activity of inorganic phosphate and of total acid-soluble phosphate was also determined 15 minutes after P^{32} subcutaneously (Table III). At this time, inorganic phosphate specific activity was 22% higher and organic phosphate specific activity was 35% higher in pilocarpine treated rats as compared to control. In these experiments, the amount of acid-soluble P^{32} which was not extractable into iso-butanol was the same (69%) in both control and pilocarpine treated rats, indicating that the rate of incorporation of P^{32} into organic compounds was the same in both groups.

TABLE II. Effect of Pilocarpine on 30-Min Incorporation of P^{32} into Acid-Soluble Phosphorus Compounds of Rat Submaxillary Glands.

	Relative specific activity*	
	Control	Pilocarpine
Orthophosphate	39.0 ± 6	46.0 ± 6
Creatine phosphate	32.0 ± 8	37.3 ± 6.8
ATP	20.5 ± 6.7	24.1 ± 5.4
ADP	16.6 ± 5.5	20.6 ± 4.6
AMP	7.3 ± 3.8	8.7 ± 3.3

* Ratio × 100 ± S.E. of P^{32} specific activity in fraction/specific activity in plasma inorganic phosphate 30 min after P^{32} for 3 rats in each group.
Pilocarpine or saline (control) given 5 min prior to P^{32}.

TABLE III. Effect of Pilocarpine on 15-Min Incorporation of P^{32} into Acid-Soluble Fraction of Rat Submaxillary Gland.

	Relative specific activity*	
	P_i	P_t
Control	20.3	7.6
"	23.9	7.9
"	25.8	12.2
"	20.0	9.7
Avg	22.5 ± 1.4	9.4 ± 1.2
Pilocarpine	30.1	12.7
"	24.8	8.3
"	30.1	18.0
"	24.8	11.7
Avg	27.4 ± 1.5	12.7 ± 2.3
P	.05	.15

* Ratio × 100 of specific activity in fraction/specific activity in plasma inorganic P. Each line is 1 rat. P calculated by one-tailed t test.

P_i = orthophosphate; P_t = total acid-soluble phosphorus.

Discussion. The effect of stimulation on salivary gland phosphate compounds has been studied by several investigators. For example, Northrup(10) reported a 63% fall in creatine phosphate in the anesthetized dog after electrical stimulation. Bergonzi(11) found a loss of phosphate from perfused dog glands on stimulation. The source of phosphate in these perfusion experiments was not determined. In the present experiments, anesthesia was limited to the time of removal of the gland. Thus, the salivary glands of the rats in the absence of anesthesia are able to maintain unchanged concentrations of creatine phosphate and ATP during the increase in activity brought about by pilocarpine stimulation. In the present experiments, the effects of anesthesia were not examined.

The effects of pilocarpine on incorporation of P^{32} into various components within the salivary glands 30 minutes after administration of the radio-isotope appear to be limited to an increase in rate of transfer of inorganic phosphate into the tissue. Increases in relative specific activity induced by pilocarpine, including inorganic phosphate, were approximately the same in all compounds which were analyzed. An average increase of 22% in relative specific activity of P^{32} in the gland was also found in experiments of 15-minute duration.

The rate at which orthophosphate in the gland reaches equilibrium with that in the plasma appeared to be somewhat faster than is true for Ca^{45}(1). In the present experiments, the specific activity of orthophosphate in the gland reached 39% of plasma in control rats and 46% of plasma in pilocarpine treated rats 30 minutes after administration of $P^{32}O_4$. In contrast, in control rats the specific activity of Ca^{45} in the gland was 20% of blood 30 minutes after Ca^{45} administration whereas in pilocarpine treated rats gland Ca^{45} specific activity was 51% of blood 30 minutes after Ca^{45} administration. Thus, the rate of equilibration of inorganic phosphate is faster than calcium in control rats but slower than calcium in pilocarpine treated rats. The rate of transfer of phosphate is apparently considerably higher than that of calcium since a large fraction of the P^{32} appears in organic combination.

The present experiments cannot be used to answer the question whether creatine phosphate or ATP breakdown play a part in the effects of pilocarpine on the gland. Incorporation of P^{32} into creatine phosphate appears to occur almost simultaneously with transfer of P^{32} into the gland since relative specific activities for intracellular inorganic phosphate and for creatine phosphate must be very nearly the same. The value of measured tissue orthophosphate specific activity is somewhat higher than true intracellular orthophosphate because inorganic phosphate present in the extracellular space of the tissue presumably has the same specific activity as plasma inorganic phosphate and thus introduces an error in the determination of the intracellular inorganic phosphate specific activity. Thus, the rate of incorporation of P^{32} into organic phosphates in the gland is limited by the rate of entry of P^{32} into the gland.

Summary. 1. Administration of pilocarpine increases the rate of entry of P^{32} into submaxillary glands of rats by approximately 20% but does not appear to have any additional effect on the rate of incorporation of P^{32} into creatine phosphate or adenosine phosphates. 2. Pilocarpine administration does not alter the concentrations of creatine phosphate or adenosine phosphates in rat submaxillary glands.

1. Dreisbach, R. H., *J. Pharm. Exp. Therap.,* 1961, v131, 257.
2. ———, *Am. J. Physiol.,* in press.
3. ———, *ibid.,* 1959, v196, 645.
4. Neuman, W. F., Neuman, M. W., *Chemical Dynamics of Bone Mineral,* Univ. of Chicago Press, 1958, 11.
5. Järnefeldt, J., *Biochem. Biophys. Res. Comm.,* 1961, v6, 285.
6. Gerlach, E., Bader, M., Schwoerer, W., *Pflügers Arch. Ges. Physiol.,* 1961, v272, 807.
7. Janke, J., Gerlach, E., Fleckenstein, A., *ibid.,* 1960, v270, 286.
8. Berenblum, I., Chain, E., *Biochem. J.,* 1938, v32, 295.
9. Martin, J. B., Doty, D. M., *Anal. Chem.,* 1949, v21, 965.
10. Northrup, D., *Am. J. Physiol.,* 1935, v114, 46.
11. Bergonzi, M., *Arch. di Sci. Biol.,* 1931, v16, 235.

Received September 24, 1962. P.S.E.B.M., 1962, v111.

Effect of Deoxycorticosterone on Soluble Beef Heart Mitochondrial ATPase.* (27844)

MELVIN BLECHER
Department of Biochemistry, Schools of Medicine and Dentistry, Georgetown University, Washington, D. C.

We have previously reported(1) that the latent ATPase activity of fresh, untreated rat lymphosarcoma and liver mitochondria is released by addition of deoxycorticosterone (DOC) *in vitro* or *in vivo* and that, in contrast, ATPase activities of particulate and solubilized fractions obtained by sonication of lymphosarcoma and liver mitochondria are inhibited by this steroid(2).

In an effort to determine whether or not DOC inhibition of solubilized, mitochondrial ATPase is a general phenomenon, we have examined the effect of DOC upon a preparation of soluble ATPase isolated by Pullman *et al.*(3) from beef heart mitochondria and have found that the activity of this enzyme, as determined by 2 different assay methods, was inhibited by low concentrations of DOC.

Experimental. Doubly glass-distilled water was used for preparation of solutions. Phosphoenolpyruvate (PEP), rabbit muscle lactic dehydrogenase (LDH) and rabbit muscle pyruvic kinase (PK) were products of Boehringer and Soehne, Mannheim, Germany. ATP, ADP, potassium pyruvate, and NADH were purchased from the Sigma Chemical Co., St. Louis; DOC was a gift of Dr. P. Perlman, Schering Corp., Bloomfield.

LDH was assayed spectrophotometrically in a Beckman DU spectrophotometer at 30° using 1.5 ml capacity silica cells of 10 mm light path. Incubation mixtures contained, in μmoles, 0.65 KCl, 50 Tris-acetate buffer (pH 7.4), 0.1 NADH, 3.0 pyruvate, and water and LDH in a total volume of 1.0 ml. When present, 0.025 ml of either propylene glycol (PG) or a 0.02M solution of DOC in PG were substituted for a portion of the water. Reaction was initiated by addition of LDH and the decrease in optical density at 340 mμ followed for 4 minutes. LDH assayed at 453 units per mg protein, as calculated from the straight-line portion (first 2 minutes) of the curve.

PK was assayed spectrophotometrically in a system containing, in μmoles, 70 KCl, 50 Tris-acetate buffer (pH 7.4), 10 $MgCl_2$, 0.1 NADH, 1.0 PEP, 0.24 ADP, and water, 0.05 ml of LDH (14.1 units per ml) and PK in a total volume of 1.0 ml; PG and DOC, where present, were added as described above. Reaction was initiated by addition of LDH and, after the optical density at 340 mμ had fallen to an equilibrium value, 0.05 ml of PK was added and the optical density decrease fol-

* This work was supported in part by grant from Nat. Inst. Health, U.S.P.H.S., and in part by N.I.H. grant to Dr. Abraham White while the author was a member of the Faculty of Albert Einstein College of Med., N. Y.