lary gland after isoproterenol injection is at least twice the fall in Mg, sialic acid, or protein. The relationship of Ca to protein in rat saliva is linear. The data are consistent with the hypothesis that Ca is held at high concentrations in salivary glands by means of binding.

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Adenosine Phosphate of Rat Salivary and Lacrimal Glands *in vitro*.* (32424)

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The transfer of Ca *in vitro* in rat salivary and lacrimal glands is increased by both parasympathetic and sympathetic stimulants(1). This effect of stimulants occurs in relatively large pieces of gland and is only abolished by Na-lack, by high concentrations of metabolic inhibitors, or at 1°C. In the present study, the concentration of adenosine phosphates has been measured after incubation to determine the role of such substances in the transfer process.

Methods. Glands were removed under ether anesthesia from Wistar strain male rats maintained on Purina feed and water *ad lib* or from hooded male rats fed a mixed green diet. Glands were incubated for 2 hours with slow agitation in conical flasks gassed with 5% CO_2 in O_2 in an incubation medium made up in glass distilled water and containing the following (mM/l); 110 NaCl, 3 KCl, 0.1 $MgCl_2$, 1.0 $CaCl_2$, 0.5 KH_2PO_4, 25 $NaHCO_3$, 5 dextrose, pH 7.4. Experiments were ordinarily done in groups of 4 using one kind of gland. The experimental groups were usually divided as follows: immediately frozen gland, gland incubated in normal medium, and 2 groups of glands incubated with different concentrations of inhibitor or other altered conditions. Adenosine phosphates and inorganic phosphate (P_i) were separated by paper chromatography(2) and estimated by phosphate analysis(3,4) or by UV absorption(5).

Results and discussion. Table I gives the concentrations of adenosine phosphates and P_i in parotid, submaxillary or lacrimal glands for various conditions. After 2 hours incubation, glands had ATP concentrations ranging from 30-80% of that found in immediately frozen glands with the following exception. In glands cut perpendicularly to the greatest dimension the ATP concentration was only 13%. Pieces prepared in this way were approximately 4 mm thick whereas submaxillary gland cut perpendicularly to the smallest dimension or lacrimal and parotid glands as removed from the rat were approximately 2 mm thick. Thus, the system for maintaining ATP functioned as well in pieces up to 2 mm in thickness as in slices approximately 0.5 mm in thickness.

The addition of 5×10^{-5} M DNP reduced ATP concentration to approximately ⅔ whereas increasing DNP to 2×10^{-4} M reduced ATP to ⅕ of the level found after incubation without inhibitor. These results correlate well with effects of DNP on Ca^{45} activation by acetylcholine in these glands.

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TABLE I. Acid-soluble Phosphate Compounds in Frozen or Incubated Glands.

Gland	Condition*	P_i	ATP	ADP	AMP
		←——μM/g——→			
Submax	Frozen	2.65	1.24	.74	.58
"	"	3.98	1.46	.92	.42
Parotid	"	2.23	1.09	.46	.40
Lacrimal	"	3.19	1.49	.56	—
Submax	N, 35°C, †	5.81	.50	.30	.26
"	N, 35°C, ‡	3.45	.17	.28	.86
"	N, 1°C, †	3.64	.94	.28	.34
"	N, 35°C, §	5.10	.30	.19	.34
Parotid	N, 35°C	2.87	.30	.30	.18
"	N, 35°C	2.47	.79	.38	.35
"	N, 1°C	2.12	.53	.21	.29
Lacrimal	N, 35°C	6.45	.73	.26	.29
"	N, 1°C	2.45	1.13	.17	.24
Submax	.05 DNP, †	6.32	.45	.47	.56
Parotid	.05 DNP	3.31	.42	.21	.37
Lacrimal	.05 DNP	6.97	.43	.27	.30
Submax	.2 DNP, †	6.84	.09	.12	.33
Parotid	.2 DNP	5.19	.07	.14	.22
Lacrimal	.2 DNP	9.15	.17	.23	.38
Submax	1.0 CN, †	5.81	.02	.09	.18
Parotid	1.0 CN	3.30	.004	.06	.14
Lacrimal	1.0 CN	9.98	.02	.09	.53
Submax	.0 Na, †	4.69	.37	.23	.27
Parotid	.0 Na	2.22	.25	.22	.33
Lacrimal	.0 Na	3.36	.46	.18	.21

* Frozen = immediately frozen; all others incubated 2 hr. N, 35°C = normal medium, 35°C; N, 1°C = normal medium, 1°C; .05 DNP = .05 mM DNP, 35°C; .2 DNP — .2 mM DNP, 35°C; 1.0 CN = 1.0 mM CN^-, 35°C; .0 Na = Na replaced by Tris buffer, 35°C.

† = gland cut perpendicular to shortest dimension.

‡ = gland cut perpendicular to longest dimension.

§ = gland sliced approximately .5 mm thick; lacrimal and parotid glands incubated as removed from the rat.

DNP at 2×10^{-4} M inhibited Ca^{45} transfer activation almost completely whereas 5×10^{-5} M had only slight effect(1). Other procedures which also prevented the increase in Ca^{45} transfer by acetylcholine were tested for their effects on gland adenosine phosphates. Incubation in 10^{-3} M cyanide reduced ATP to 2% of the level after normal incubation whereas incubation in the absence of Na, at least in lacrimal glands, did not reduce the level of ATP below that found in the presence of 5×10^{-5} M DNP. Thus, it appeared that the inhibition of Ca^{45} transport activation produced by Na-lack was brought about by some mechanism other than interference with ATP formation. The effect of incubation at 1°C was also tested and found to have no appreciable effect on the level of ATP as compared to normal incubation at 35°C. Incubation at 1°C inhibits acetylcholine activation of Ca^{45} transfer completely. In this instance, lowered temperature could prevent the utilization of ATP in the transfer process.

Summary. Rat lacrimal and salivary gland pieces up to 2 mm in thickness are able to maintain functional concentrations of ATP *in vitro*. Reduction of ATP levels produced by chemical metabolic inhibitors agrees closely with effects of the same inhibitors on Ca^{45} transfer activation by acetylcholine.

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Iodine-131 Labeling of Purified Microbial Antigens by Microdiffusion. (32425)

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Although the capacity to bind antigen is the fundamental expression of antibody activity, most quantitative serological methods measure only secondary effects of such combination. Accordingly, these methods may provide inaccurate estimates of antigen binding capacity and inadequate tests of the significance of specific antibodies in a