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Influence of Steroids Upon Osmotic Pressure Changes in Isolated Frog Skin Pouches.*† (23975)

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It was reported(1) that various steroids, added to the interior of pouches prepared from skin of frog legs, suspended in and filled with frog Ringer's solution, were capable of raising the osmotic pressure on the inside of the pouch in experiments lasting up to 16 hours. Similar pouch preparations from the opposite leg of the same animal to which no steroid or just the solvent was added served as controls. The ability of surviving frog skin to transfer sodium across the membrane had been demonstrated before(2,3,4). It was assumed, from our experiments, that the further increase of osmotic pressure after addition of appropriate steroids was an expression of a direct action of such steroids upon electrolyte transfer at the cellular level. No measurements of electrolytes were performed in our previous investigations. Steroids which exhibited the most pronounced effect were: desoxycorticosterone, 9α-fluorohydrocortisone, hydrocortisone and, to a much lesser degree, estriol. Testosterone showed no effect. No significant changes in fluid volume inside the skin pouch were observed, despite the change of osmotic pressure.

Methods. In experiments to be reported here, the action of other steroids under similar experimental conditions was tested. All experiments were again performed on skin pouches prepared from hind legs of frogs (*Rana pipiens*), the epithelial layer remaining on the outside. Steroids in various concentrations were added to preparations from one leg, the other leg serving as control. Methods of dissolving steroids, determining freezing point depression in a Fiske osmometer etc., remained the same, as previously published(1). Most experiments were terminated after 8 hours. Sodium and potassium measurements were performed with the flame photometer. Data were computed in relation to total surface of skin pouch, rather than per cm^2. This is permissible, since in a sequence of 50 unselected preparations, the mean surface of the actively exposed skin was 16.37 cm^2 with standard deviation of only 0.58 and standard error of mean of 0.081. The steroids tested were: dl-aldosterone, 2-methyl-9α-fluorohydrocortone, prednisone and prednisolone. The first 2 were chosen because of their known strong effects upon electrolyte shifts, the latter because of their relative inertness.

Results. Table I illustrates the effects of these substances upon osmotic pressure. Dl-aldosterone and 2-methyl-9α-fluorohydrocortone both exhibited a striking effect resulting in a considerable increase in osmolarity of the fluid in the treated pouch as compared with control preparation. In general, the higher the steroid concentration, the more potent was the effect, with the exception of the ex-

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TABLE I. Osmotic Pressures (in Milliosmols/l) of Fluids inside Skin Pouches in 8 Hr Experiments, Expressed as Increases above Initial Concentration of about 200 Milliosmols/l.

Steroid	μg/cc	No. of animals	Control* Mean	Control* S.D.	Control* S.E.	Treated* Mean	Treated* S.D.	Treated* S.E.	Δ, mean
DL-aldosterone	.2	23	15.5	4.6	.9	94.2	49.3	10.3	+ 78.7
	1.0	15	22.5	5.6	1.5	126.5	74.6	19.2	+104.0
2-Methylfluorohydrocortone	.02	7	18.5	2.5	.9	37.1	11.1	4.2	+ 18.6
	.2	7	18.9	2.0	.8	181.3	17.6	6.7	+162.4
	.25	11	19.8	5.0	1.5	206.6	42.5	12.8	+186.8
	1.0	5	14.6	4.2	1.9	131.6	80.7	36.1	+117.0
Prednisone	2.0	10	14.2	3.6	1.1	15.8	5.6	1.5	N.S.
	20.0	10	24.7	4.5	1.4	21.2	6.0	1.9	"
Prednisolone	2.0	10	26.7	3.6	1.1	25.9	2.9	.9	"
	20.0	10	21.0	3.1	1.0	33.8	1.7	.5	+ 12.8

* Mean, stand. dev., and stand. error of mean are given. N.S. = Not significant.

periment in which the concentration of 2-methylfluorohydrocortone was highest (1 μg/cc). However, in this experiment, the number of observations was small and the standard deviation as well as standard error of the mean relatively high. Prednisone had no effect upon osmolarity in concentrations much higher than other steroids. Prednisolone exhibited only a slight increase when administered in a concentration as high as 20 μg/cc. The fluid volumes, inside the skin pouch measured gravimetrically, increased considerably under the influence of dl-aldosterone and the 2-methyl-9α-fluorohydrocortone (Table II). In some specimens the fluid content had nearly doubled at conclusion of the experiment. This could be readily observed by bulging of skin pouches compared to the controls. No significant difference in concentration of sodium, and possibly a slight increase in potassium concentration in most experiments was observed inside the skin pouch (Table III). However in view of increased volume of fluid, the total content of these electrolytes has risen considerably under the influence of the hormone.

The increase in milliosmols/liter was evidently not explained by changes in sodium or potassium concentrations, which represented the major components of Ringer solution. One must assume, therefore, that elevation of osmotic pressure under the influence of the steroids must have resulted from a discharge of osmotically active molecules from the frog skin itself, since not enough excess of electro-

TABLE II. Volumes (cc) of Fluids inside Skin Pouches in 8 Hr Experiments, Expressed as Increases above Initial Volume of 4 cc.

Steroid	μg/cc	No. of animals	Control* Mean	Control* S.D.	Control* S.E.	Treated* Mean	Treated* S.D.	Treated* S.E.	Δ, mean
DL-aldosterone	1.0	15	.33	.28	.07	2.03	.54	.14	+1.70
2-Methylfluorohydrocortone	.02	7	.41	.11	.04	.64	.10	.04	+ .23
	.2	7	.48	.10	.04	3.40	.31	.12	+2.92
	.25	11	.45	.21	.06	3.98	.61	.18	+3.53

* Mean, stand. dev., and stand. error of mean are given.

TABLE III. Sodium and Potassium (ME/l) in Fluids inside Skin Pouches in 8 Hr Experiments. Each figure represents values obtained in pooled specimens from 5 skin pouches.

Steroids	Conc., μg/cc	Control Na	Control K	Treated Na	Treated K
2-Methylfluorohydrocortone	1	114.1	1.08	108.1	1.08
	1	99.2	1.13	101.6	1.28
	1	102.4	1.16	100.8	1.25
	1	100.0	1.15	99.8	1.32
DL-aldosterone	1	101.6	1.32	100.8	1.77

lytes was present in the solution inside the skin bag to account for this phenomenon. No significant differences were found in concentration of total protein, amino acids, urea, hexosamines or uronic acid. Further investigations are being performed to characterize the contents of the fluids. That the increase in osmotic pressure is directly related to a specific action of certain corticoids is strongly suggested by the observation that other steroids tested had no such effect. The nature of the material released by the active steroids might offer some insight into the mechanism of steroid-promoted water and electrolyte transfer.

Summary. The influence of dl-aldosterone, 2-methyl-9α-fluorohydrocortone, prednisone and prednisolone upon osmotic pressure of fluids inside of isolated frog skin pouches in a Ringer bath was tested. A considerable increase of osmolarity and increase in total fluid volume as the result of the action of the first 2 steroids was observed. The sodium and potassium concentrations remained unchanged. The significance of this observation was briefly discussed in terms of appearance of osmotically active material from the skin under the action of certain steroids.

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Effect of Shock on Rat Heart and Brain Mitochondria. (23976)

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From earlier work, it is known that changes in metabolic pattern and concentration of metabolites of brain and heart occur under the influence of anoxia and fatigue(1). Similarly, changes induced by shock in various organs and tissues may be caused by anoxia and electrolyte imbalance which is part of the shock syndrome(2). Since this suggests a disruption of the enzymatic pattern in the cell, it was of interest to study the effect of shock on mitochondria. The efficiency of phosphorylation processes was investigated because failure of energy metabolism has been implicated in irreversibility of shock.

Methods. Irreversible shock was produced in 200-250 g male rats of Sprague-Dawley strain by the tourniquet, Noble-Collip drum and hemorrhagic technics. A degree of shock which resulted in a mortality of 80-90% in 24 hours, was employed. The animals were sacrificed 1-2 hours after induction of shock and mitochondria were prepared from heart(3) and brain(4). Mitochondria were pooled from 6 control and 6 experimental animals, except for hemorrhagic shock when only one animal was available for each experiment. Polarographic assay for oxidative phosphorylation in heart mitochondria was carried out as previously described(5). Adenosine-triphosphatase activity was assayed according to Zetterstrom and Ernster(6). The incorporation of P^{32} labelled inorganic phosphate into adenosine-triphosphate was done in Warburg apparatus at 18°C. The reaction mixture (3 ml) contained phosphate buffer (10 mM at pH 7.6), $MgCl_2$ (1mM), adenosine diphosphate (3.3mM), sucrose (0.30M), P^{32} inorganic phosphate (750,000 counts/minute), succinate (20mM) where indicated, and heart mitochondria. For brain experiments, malonate (10mM) and NaF (10mM) were also added, sucrose was 0.25M, and glutamate (20mM) where indicated. The experiment

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