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.

1. Taubenhaus, M., Fritz, I. B., Morton, J. V., *Endocrinology,* 1956, v59, 458.

2. Ussing, H. H., *Ann. Rev. Physiol.,* 1953, v15, 1.

3. Huf, E. G., *Electrolytes in Biological Systems,* A. M. Shanes, edit., p205, Am. Physiol. Soc., 1955, Washington, D. C.

4. Huf, E. G., Doss, N. S., Wills, J. P., *J. Gen. Physiol.,* 1957, v41, 397.

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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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TABLE I. Effect of Drum Shock on Incorporation of P^{32} Labelled Inorganic Phosphate into Adenosine-Triphosphate.

	O_2 uptake, μmoles	ATP, μmoles	ATP, counts per min.	ATP, counts per min. per μmole
		Heart		
Normal:				
No addition	.0	1.173	2,400	2,048
Succinate	4.07	1.372	32,100	23,420
Drum shock:				
No addition	.0	1.289	600	466
Succinate	3.50	1.415	11,700	8,270
		Brain		
Normal:				
No addition	.86	2.122	4,425	2,082
Glutamate	1.02	2.070	6,680	3,230
Drum shock:				
No addition	.70	2.610	5,100	1,954
Glutamate	1.02	3.095	3,000	969

was stopped after 15 minutes by addition of perchloric acid and ATP was isolated by the ion-exchange method of Cohn and Carter(7) and its radioactivity counted. For electrolyte analysis, heart mitochondria pooled from 6 normal and 6 shocked animals were prepared simultaneously in 0.32M sucrose. Kjeldahl nitrogen analyses were performed on mitochondrial suspensions and the results expressed as protein by multiplying by 6.25. Following this, the suspensions were digested with nitric acid, ashed, and the electrolyte analyses performed. Sodium and potassium were determined by flame photometry, phosphorus by the Ennor and Stocken method(8), magnesium by a modification of the Heagy method(9), and calcium by a modification of the Ashizawa method(10).

Results. Table I shows that a clear difference exists in incorporation of inorganic phosphate in ATP in heart and brain mitochondria from normal and drum shocked rats. In shock, the incorporation was decreased 73% in heart and completely suppressed in brain. In parallel studies, these findings were confirmed by showing that specific activity changes in inorganic phosphate were inversely related to the ATP changes. The rate of respiration in normal and shocked preparations do not appreciably differ. In other experiments, the concentration of respiratory enzymes of normal and shocked preparations is identical, which agrees with the similar rate of respiration observed.

Since some diminution in ATP turnover and hence phosphate incorporation could have been due to decreased adenosine-triphosphatase activity, this activity was subsequently examined in normal and drum shocked preparations (Table II). No significant difference in activity was found. Activity in both heart and brain preparations was promoted by magnesium and calcium and was relatively stable to storage. Thus differences in P^{32} incorporation suggested an impairment of ATP synthesis.

These findings were extended by employing heart mitochondria of normal animals and those in which 3 different types of shock were induced. Table III summarized data from the phosphorus to oxygen ratios obtained by the polarographic method(5) during respiration of alpha-ketoglutarate. The data show that the efficiency as well as net synthesis of ATP are lowered in heart preparations from the 3 types of shock employed.

Since profound electrolyte changes accompany the onset of shock *in vivo*(2), it seemed possible that a changed electrolyte distribu-

TABLE II. Adenosine-triphosphatase Activity of Heart and Brain Mitochondria.

	μmoles phosphate formed/hr/mg protein			
	Heart		Brain	
	Normal	Shock	Normal	Shock
No addition	.45	.35	7.37	6.62
$MgCl_2$ (5 mM)	3.34	3.47	27.41	14.71
$CaCl_2$ "	2.91	3.31	25.10	
$MgCl_2 + CaCl_2$ $+MgCl_2$ (5 mM)	3.91	4.97		
Fresh preparation	9.42	9.00	7.80	7.14
Aged 4 days at 2°C	7.20	7.14	3.42	5.22

TABLE III. Effect of Different Types of Shock on Oxidative Phosphorylation in Heart Mitochondria.

Type of rat	P/O	μmoles ATP synthesized/sec./mg protein/l	No. of exps.
Normal	3.59	4.4	25
Tourniquet shock	3.08	3.4	9
Drum "	3.07	3.9	5
Hemorrhagic "	2.45	2.7	14

TABLE IV. Electrolyte Analysis of Rat Heart Muscle Mitochondria.

	μmoles/mg protein		Significance*
	Normal	Shock	"P"
Calcium	.114	.090	.10
Phosphorus	.552	.650	.05
Magnesium	.039	.045	.10
Sodium	.101	.069	.10
Potassium	.128	.154	.10

* Calculated by method of paired comparisons. Each value represents avg obtained from 30 hearts.

tion might be evident in mitochondria of shocked rats. This had further interest because of the well-known role which electrolytes have in phosphorylation reactions. The electrolyte content of normal and drum shocked heart preparations is reported in Table IV. The greatest difference was a 15% increase in total phosphorus content in shock. Also, a change in concentration of sodium and potassium was found in shock mitochondria, sodium having decreased and potassium increased. The magnesium content was little changed and some decrease in calcium was found in shock preparations.

Discussion. It appears certain that phosphorylation processes in mitochondria isolated from animals in shock are impaired. The data reported here are in close agreement with those of Strawitz and Hift(11,12) who observed lower P/O ratios in dog heart mitochondria obtained from animals in hemorrhagic shock. In agreement with these investigators we also found no differences between mitochondria of normal and shocked animals with respect to respiration rate and content of respiratory enzymes. Strawitz and Hift obtained photomicrographs which indicated that heart mitochondria from shocked animals differed in size and shape from normal mitochondria. It is well known that swelling and shrinking of mitochondria causes changes in metabolic activity(13,14). Since structural and functional changes in mitochondria are influenced by the electrolyte environment(13), we undertook a study of electrolyte distribution in normal and shocked preparations.

Although Slater and Cleland(15) have shown that calcium ions cause a decline in phosphorylative activity in rat heart mitochondria, this mechanism would not account for the lower P/O ratios of shocked preparations since increased concentration of these ions was not found. However, Hunter and Ford(14) reported a decreased phosphorylation in the presence of higher phosphate concentrations, and it may be that the increased phosphate content in shocked preparations is the cause of the lower P/O values. The elevated mitochondrial phosphate content may be related in increases in serum phosphate observed in shock(2). The altered distribution of sodium and potassium in these preparations also may be responsible for changes in phosphorylation processes. The changed pattern of electrolyte distribution is consistent with the structural changes reported by Strawitz and Hift(11,12). Thus, the electrolyte changes appear responsible for the structural and functional changes in mitochondria of animals in shock.

Summary. Phosphorylation processes were studied in heart and brain mitochondria of rats subjected to severe shock by the Noble-Collip drum, tourniquet and hemorrhage technics. A decreased synthesis of adenosine-triphosphate was found in drum shock. Lowered P/O ratios were obtained with heart mitochondria of all 3 types of shock when compared to normal preparations, and the adenosine-triphosphatase activity was unchanged. After shock, the electrolyte composition of heart mitochondria was altered; the total phosphorus and potassium increased while sodium decreased and magnesium and calcium showed only slight differences. The relationship of these findings to the structure and function of mitochondria in shock are discussed.

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1. Stone, W. E., Marshall, C., Nims, L. F., *Am. J. Physiol.*, 1941, v132, 770.

2. Engel, F. L., *Ann. N. Y. Acad. Sci.*, 1952, v55, 381.

3. Cleland, K. W., Slater, E. C., *Biochem. J.*, 1953, v53, 547.

4. Brody, T. M., Bain, J. A., *J. Biol. Chem.*, 1952, v195, 685.

5. Packer, L., *Arch. Biochem. Biophys.*, 1957, v70, 290.

6. Zetterstrom, R., Ernster, L., *Nature*, 1956, v178, 1335.

7. Cohn, W. E., Carter, C. E., *J. Am. Chem. Soc.*, 1950, v72, 4273.

8. Ennor, A. B., Stocken, L. A., *Aust. J. Biol. Med. Sci.*, 1950, v28, 647.

9. Heagy, F. C., *Can. J. Res.*, 1948, v28, 295.

10. Ashizawa, T., *Rpts. Balneol. Lab. Okayama Univ.*, 1953, v6, 15.

11. Strawitz, J. G., Hift, H., PROC. SOC. EXP. BIOL. AND MED., 1956, v91, 641.

12. Hift, H., Strawitz, J. G., *Fed. Proc.*, 1957, v16, 196.

13. Price, C. A., Fonnesu, A., Davies, R. E., *Biochem. J.*, 1956, v64, 754.

14. Hunter, F. E., Ford, L., *J. Biol. Chem.*, 1956, v216, 357.

15. Slater, N. C., Cleland, K. W., *Biochem. J.*, 1953, v55, 566.

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Antiparasitic Drugs. II. Anticoccidial Activity of 4,5 imidazoledicarboxamide and Related Compounds. (23977)

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Coccidiosis is an important protozoan disease of poultry which "costs poultry raisers millions of dollars each year because of unthriftiness, poor utilization of feed, loss of weight, and death of infected birds"(1). During the past 2 decades several effective chemotherapeutic agents have been discovered for prevention and control of coccidiosis in poultry and other domestic animals. Nevertheless, there has been a constant search for more effective and better tolerated drugs for use as coccidiostats. We recently found that 4, 5-imidazoledicarboxamide(2) (Fig. 1) is a potent and well-tolerated coccidiostat. The compound has been assigned the generic name of glycarbylamide.* This paper reports the results of chemical and biological studies on glycarbylamide and related compounds.

Materials and methods. Chemical. 4,5-Bis (N-acetylcarbamyl)-imidazole(V). Two g of 4,5-imidazoledicarboxamide was refluxed for 15 hours in a mixture of 100 ml of acetic anhydride and 10 ml of acetic acid. The reaction mixture was then concentrated to dryness *in vacuo* at 100°. The residue thus obtained was slurried with water, the slurry allowed to stand at room temperature for about an hour and then filtered. Two g of product was obtained. After recrystallization from methanol it melted at 259-60°. *Anal.* Calcd. for $C_9H_{10}O_4N_4$: C, 45.38; H, 4.23; N, 23.53; N.E. 238. Found: C, 45.42; H, 4.26; N, 23.77; N.E. 241. *4,5-Bis (N-propionylcarbamyl)-imidazole (VI).* This compound, prepared by the same general method as V, melted at 239-40° after recrystallization from ethanol. *Anal.* Calcd. for $C_{11}H_{14}O_4N_4$: C, 49.61; H, 5.30. Found: C, 49.36; H, 5.27. *4-Acetylcarbamyl-5-imidazolecarboxamide (IV).* Two and four-tenths g of 4,5-bis (N-acetylcarbamyl)-imidazole was suspended in 25 ml of water and 10 ml of N sodium hydroxide was added with vigorous shaking. The clear solution obtained stood at room temperature for about 60 hours during which time the product slowly precipitated. When

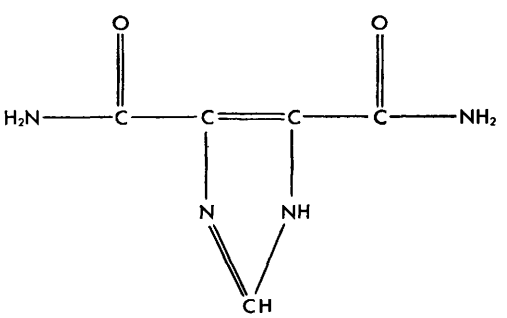

FIG. 1. 4,5-Imidazoledicarboxamide (Glycarbylamide).

* 'GLYCAMIDE' is the trademark of Merck & Co., for this compound.