

LD₅₀ of 968 mg/kg in mice(19).

The present results are of some interest in the light of the work of Richards and Kueter(20) who observed that the incidence of procaine convulsions in guinea pigs was reduced by the prior administration of either DEAE or PABA, or of both of these substances simultaneously. They presented evidence favoring competitive inhibition as the mechanism of this phenomenon. They observed however that PABA and DEAE had no inhibitory effect on the local anesthetic action of procaine, nor on procaine-induced convulsions in mice. Our results further fail to show in mice any evidence of competition between procaine and these substances for procainesterase, or for any other receptor

substance or structure which may be involved in the gross lethal action of intravenously administered procaine.

Summary. The subcutaneous LD₅₀s of 25% PABA, 5% DEAE, and 5% DMAE in mice were found to be 4.39, 1.61, and 2.08 g/kg respectively. Subcutaneous premedication by 1 g/kg of 7% PABA did not alter the LD₅₀ of intravenous procaine in mice, while subcutaneous premedication by 0.5 g/kg of either 5% DEAE or 5% DMAE lowered the LD₅₀ about 35%. Neither PABA, DEAE, nor DMAE changed the rate of disappearance of procaine from the whole body of the mouse. It is concluded that the influence observed to be exerted by DEAE and DMAE on the toxicity of intravenous procaine is probably due to their additive toxic effects on the cardio-vascular system, and not to the inhibition of procainesterase *in vivo*.

18. Richards, R. K., *Fed. Proc.*, 1942, v1, 71.

19. Ting, K. S., and Coon, J. M., *Arch. internat. de Pharmacodyn. et de Thérap.*, 1951, (in press).

20. Richards, R. K., and Kueter, K. E., *J. Pharm. and Exp. Therap.*, 1946, v87, 42.

Received February 27, 1951. P.S.E.B.M., 1951, v76.

Phosphocreatine and Adenosine Triphosphate Content of Rat Tissues after Adrenalectomy and Cortisone Treatment (18551)

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In studies on the metabolic effects of adrenalectomy and hormone treatment, the question frequently arises as to whether adrenalectomized animals lack an adequate supply of readily available energy. One index of their energy supply is the adenosine triphosphate (ATP) and phosphocreatine content of the tissue. Data on the ATP and phosphocreatine content of rat tissue from adrenalectomized animals are somewhat inconsistent(1). Part of this confusion is due to lack of correction for salt balance and part is probably due to earlier inadequate methods for measuring ATP. It was therefore of interest to determine the ATP and phosphocreatine content

of tissues of animals under various conditions of treatment. Using rats maintained on saline we have found no significant alteration in the phosphocreatine or ATP content of muscle after adrenalectomy or after treatment with cortisone. All data presented are on animals starved for a period of 24 hours before analysis, since this condition might be likely to result in altered phosphocreatine. No difference was found between well-fed or starved animals.

Methods. *A. Preparation of animals.* The rats, weighing approximately 150 g were adrenalectomized bilaterally in the laboratory of Dr. H. C. Stoerk to whom we are indebted for this material. After adrenalectomy, the animals were supplied an adequate diet and

1. Hartmann, F. A., and Brownell, K. A., 1949, *The Adrenal Gland*, p. 236, Philadelphia.

TABLE I. Phosphocreatine Content of Rat Muscle.

Treatment	Phospho- creatine, mg %	Inorganic phosphorus, mg %	Total phosphorus, mg %
Normal, untreated	257	45	—
	248	24	—
	305	45	—
	253	31	131
	244	30	134
	270	29	116
	284	34	135
	Avg	266 ± 20*	34 ± 7
Adrenalectomized, 10 days	248	33	118
	259	25	109
	301	25	125
	303	23	—
	271	13	—
	326	24	—
	310	35	—
	297	35	—
Normal, treated with cortisone, 10 days	Avg	289 ± 25	27 ± 7
	261	28	114
	326	28	125
	279	34	125
Adrenalectomized, treated with cortisone, 10 days	Avg	288 ± 27	30 ± 5
	303	32	135
	244	24	104
	260	27	117
	Avg	269 ± 24	28 ± 3

* Stand. dev. of the mean.

0.9% NaCl in place of drinking water. Treatment with cortisone consisted of the intramuscular injection of 1 mg Cortone* per day supplied as a single daily dose. Animals were starved by removing their food for 24 hours prior to analysis.

B. *Analytic procedures.* The animals were injected intraperitoneally with 5 mg/100 g of pentobarbital. At the end of 10 minutes, when they were in surgical anesthesia, the skin was freed around the left gastrocnemius muscle. The muscle was dissected from the underlying fascia and the skin replaced over the muscle. At the end of 15 minutes the muscle was once more exposed, cut at its origin and at its insertion, and quickly dropped into liquid air; the removal of the muscle was completely within 10 seconds. This system of preparation of tissue for analysis gives

results comparable to those obtained by freezing the whole animal(2).

The tissue was fragmented without allowing it to thaw and transferred to tared centrifuge tubes containing 5 cc of cold trichloroacetic acid. The supernatant fluids were collected by centrifugation and the muscle residues extracted once with 2 cc of 10% trichloroacetic acid and then with 2 cc of 5% trichloroacetic. The combined supernatants were made to a volume of 10 cc. Samples were immediately withdrawn for determination of true inorganic phosphorus and total inorganic phosphorus according to the method described in Umbreit *et al.*(3). Determinations of the total phosphorus were also carried out on some samples. The remainder of the trichloroacetic acid extract was neutralized and precipitated with

* Cortone (Merck), consisted of cortisone acetate formulated in suspending agents and containing 1.5% benzyl alcohol as a preservative.

2. LePage, G. A., *Am. J. Physiol.*, 1946, v146, 267.

3. Umbreit, W. W., Burris, R. H., and Stauffer, J. F., 1949, *Manometric Technics and Tissue Metabolism*, Minneapolis.

barium acetate in the presence of 3 volumes of alcohol. The barium alcohol insoluble precipitate was collected, freed of barium and assayed for adenylic acid, adenosine diphosphate and adenosine triphosphate according to the method described by Albaum and Lipshitz(4). The method briefly consists in enzymatically converting adenosine diphosphate and adenosine triphosphate into adenylic acid and determining the latter as inosinic acid in the spectrophotometer.

Results. Data on the muscle phosphocreatine content of starved animals under various treatments are given in Table I. It is evident that adrenalectomy or cortisone treatment did not significantly alter the phosphocreatine content of the muscle; nor was there any effect of starvation. Well fed animals had the following phosphocreatine content in the muscle: Normal, 258 mg %, adrenalectomized, 281 mg %. It seems apparent that the adrenalectomized animal had as much phosphocreatine content in the muscle as the normal or cortisone treated animal and that, therefore, lack of phosphocreatine was not the cause of muscle fatigue or of increased sensitivity to stress. While the amounts of phosphocreatine found are very low (approximately 20 mg %) we were also able to find no change in the phosphocreatine content of

4. Albaum, H. G., and Lipshitz, R., *Arch. Biochem.*, 1950, v27, 102.

TABLE II. Adenosine Triphosphate Content of Rat Muscle.

Treatment	ATP, mg %	Avg
Normal, no treatment	262	242 ± 15
	224	
	242	
Adrenalectomized	265	254 ± 14
	235	
	263	
Normal, treated with cortisone, 10 days	200	213 ± 10
	223	
	215	
Adrenalectomized, treated with cortisone, 10 days	262	233 ± 24
	236	
	202	

kidney or liver upon adrenalectomy, with or without starvation.

Since phosphocreatine is not altered one would expect no change in the ATP and since phosphocreatine is relatively high, one would further expect to find little adenosine-diphosphate or adenylic acid in the muscle. None of the muscle samples contained adenosine-diphosphate or adenylic acid. Their ATP content is given in Table II from which it is evident that there is no significant change in ATP content.

Conclusion. There was no significant effect of adrenalectomy or cortisone treatment upon the phosphocreatine or ATP content of rat muscle.

Received February 21, 1951. P.S.E.B.M., 1951, v76.

Diasone and Promin as Therapeutic Agents in Experimental Toxoplasmosis.* (18552)

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Since this paper is concerned with the problem of therapy in toxoplasmosis, other pertinent information with respect to this

protozoan disease should be sought elsewhere (1-4). 2(para amino benzene sulfonamide) pyridine (Sulfapyridine) and 2(p-acetyl-

* These studies were aided by a contract between the Office of Naval Research, Department of the Navy and the University of Texas.

† Acknowledgement is made of the technical assistance of Bernard Schnitzer and Eugene Winograd.

1. Wolf, A., Cowen, D., and Paige, B. H., *Science*, 1939, v89, 226.

2. Binkhorst, C. D., *Toxoplasmosis*, 1948; H. E. Stenfort Kroese's Uitgevers-Maatschappij N. V., Leiden, Holland.