seems to be associated with lines or strains of virus. The differing hemagglutinative susceptibility of individual donors suggests that the mechanisms involved are rather complex.

The phenomenon of agglutination of mammalian erythrocytes has practical application in maintaining the identity of stock strains of virus and possibly also in determining probable intermingling of the viruses of live vaccines and wild strains in field outbreaks of Newcastle disease.

Received March 27, 1950. P.S.E.B.M., 1950 v74.

Effect of Mercurials on Kidney Adenosine Triphosphatase Activity.* (17846)

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Mercurial diuretics are capable of producing renal tubular damage. Preliminary experiments(1) have shown that kidney adenosine triphosphatase (ATP-ase) activity is inhibited by these compounds. Since very little is known about the nature of nephrotoxic action of drugs, it seemed of interest to study the possible relationship between the inhibitory effect of mercurials on kidney ATP-ase activity and the renal tubular injury produced by these drugs.

Material and methods. White rats of both sexes weighing approximately 200 g were used in this study. They were sacrificed by a blow on the head. The kidneys were homogenized at ice-water temperature in an all glass homogenizer. ATP-ase activity was determined by the method of DuBois and Potter(2). All enzyme determinations were done in quadruplicate. Inorganic phosphate was determined by the method of Fiske and Subbarow(3) using a Coleman spectrophotometer. ATP was obtained as the barium salt from the Nutritional Biochemicals Corporation, Cleveland, Ohio. The solution of the sodium salt was prepared just prior to the performance of the experiment. The mersalyl used was Salyrgan-Theophylline-Winthrop. The amount of theophylline in this preparation was found not to affect ATP-ase activity in the concentrations employed. In the *in vivo* experiments, one kidney of each rat was homogenized; the other was fixed in 10% formalin for microscopic studies. The ATP-ase activity of normal rat kidneys was determined simultaneously with the determinations done on kidneys of injected animals.

Results. Table I shows the effect of the addition of mersalyl and mercuric chloride on the ATP-ase activity of homogenized rat kidneys. Inhibition was observed consistently following the addition of these compounds in concentrations as low as 0.0001 M. The inorganic mercurial produced a greater inhibition at any given concentration than the organic compound.

TABLE I.
Effect of Mercurials on ATP-ase Activity of Homogenized Rat Kidneys.

	ATP-ase activity* per mg of kidney Final concentration of added drug		
Drug	0	0.0001M	0.00001M
Mersalyl	57	51	57
	58	50	55
Mercuric Chloride	57	35	54
	58	38	52

* μg of phosphorus liberated per hr.

* We are grateful to Dr. E. E. Muirhead for carrying out the microscopic studies

1. Goth, A., Holman, J., and O'Dell, V., *Fed. Proc.*, 1950, in press.

2. DuBois, K. P., and Potter, V. R., *J. Biol. Chem.*, 1943, v150, 185.

3. Fiske, C. H., and Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

TABLE II.
ATP-ase Activity of Rat Kidneys Following Intramuscular Injection of Mersalyl.

Exp. No.	Dose, mg/kg	Time after inj., hr	ATP-ase activity* per mg of kidney	ATP-ase activity* per total kidney, × 10⁻³	% difference from mean of controls† per mg of kidney	% difference from mean of controls† per total kidney	Microscopic tubular damage‡
1.	5	4	60	44	0	—19	0
			61	49	+ 2	—10	0
		24	57	43	— 5	—21	0
			56	43	— 7	—21	0
2.	10	4	54	59	—10	+ 8	0
			54	56	—10	+ 3	0
		24	62	57	+ 3	+ 5	+
			60	45	0	—17	+
		48	53	40	—12	—26	+
			54	43	—10	—21	+
		72	62	63	+ 3	+16	+
			55	57	— 8	+ 5	+
3.	50	4	62	50	+ 3	— 8	++++
			61	59	+ 2	+ 8	++++
		24	44	33	—27	—39	++++
			44	49	—27	—10	++++
		48	44	39	—27	—28	++++
			41	41	—32	—25	++++
		72	24	34	—60	—38	++++

* μg of phosphorus liberated per hour. ATP-ase activity per mg of kidney multiplied by the weight of the kidney in g gives total activity per kidney.

† The mean ATP-ase activity of 19 normal rat kidneys was 60 per mg of kidney and 54 per total kidney with the standard deviations of 8 and 10% respectively.

‡ Microscopic tubular damage: 0 = none; + = occasional tubules necrotic; ++++ = majority of tubules necrotic.

Table II shows the effect of the injection of varying doses of mersalyl on the kidney ATP-ase activity of rats sacrificed at varying intervals following the injection. Regardless of the dose injected, there was no significant change in ATP-ase activity as early as 4 hours following the injection of the mercurial. Inhibition was evident in 24 hours and subsequently, especially in the group which received 50 mg per kg of mersalyl. In this group, marked renal tubular damage was present 4 hours following the injection. At that stage no significant changes in ATP-ase activity could be demonstrated.

The decreases in ATP-ase activity could not be due to dilution caused by the swelling of the kidneys, since the total ATP-ase activity of the kidney was decreased also. Furthermore, a number of kidney ATP-ase determinations in which enzyme activity was calculated on the basis of dry weight indicated also that there was an actual depletion of ATP-splitting enzyme.

Discussion. The results of this study show that mersalyl and mercuric chloride inhibit kidney ATP-ase activity *in vitro*. It is also evident from this study that the injection of mersalyl into rats leads to a decrease in kidney ATP-ase activity. Since microscopic studies demonstrated a marked renal tubular damage which preceded the enzymatic changes, it appears that the decrease in ATP-ase activity is a consequence and not a cause of tubular damage. It is possible that the inhibition of other enzymes may precede the development of kidney damage, but it is also possible that the "disorganization of the protoplasm which may or may not precede the inactivation of numerous enzymes" as suggested by Bernheim(4) represents the cause of renal injury in acute mercury poisoning.

Conclusions. 1. Mersalyl and mercuric chloride were found to inhibit ATP-ase activity of homogenized rat kidneys. 2. Following the intramuscular injection of various

4. Bernheim, F., The Interaction of Drugs and Cell Catalysts, Burgess Publishing Co., Minneapolis, 1948, p43.

doses of mersalyl into rats, renal ATP-ase activity was unchanged in 4 hours; it was decreased after 24 hours. 3. Simultaneous microscopic and enzymatic studies suggest that the decrease in ATP-ase activity is a result and not a cause of renal injury produced by mersalyl.

Received April 3, 1950. P.S.E.B.M., 1950, v74.

Acetylcholine Content of Tyrode Solution Perfused Through Muscles as Affected by Calcium and Procaine Hydrochloride.* (17847)

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There appear to be many similarities between muscle fibrillation caused by denervation and fibrillation produced by calcium lack(1,2,3,4,5). These experiments were performed in order to examine the possible rôle of acetylcholine in fibrillating dog muscle caused by these two conditions.

Method. A week to 10 days before an experiment a dog's left sciatic nerve was severed aseptically. On the morning of the experiment the animal was lightly anesthetized with pentothal sodium and prepared for cannulation of the right iliac artery and vein. The following solutions were in readiness: mammalian Tyrode solution containing sufficient heparin to provide the animal with a total of 10 mg/kg; Tyrode solution containing eserine in a concentration of 1:100,000. Two Ca^{++} free Tyrode solutions were also prepared and to one of them was added 1 millimol of procaine hydrochloride per liter. Provision was made for warming these solutions to body temperature by passage through a glass coil immersed in a tank maintained at about 40°C. Electromyograms were obtained from wire recordings made continuously throughout the experiment. We have recently described this technic and full details are readily available in another journal(6). The method is recommended as the wire recorder permits reproduction of the electrical activity of the muscle for photography at a later time when the experimenter is not preoccupied with operative and other technics. The standard procedure was as follows: the right sciatic nerve was cut, a concentric needle was inserted into the right gastrocnemius, and with the amplifier, wire recorder and loud speaker turned on, the right iliac artery was perfused with Tyrode solution containing heparin. Usually no electrical activity from the muscle was heard on the speaker. The iliac vein was then cannulated and the sanguinous perfusate collected. The muscle was then perfused with Tyrode solution containing eserine 1:100,000, calcium free Tyrode solution and lastly, calcium free Tyrode solution containing procaine hydrochloride 1 millimol per liter. Samples of perfusate were collected in separate flasks, additional eserine added, and the perfusates stored in the ice box for further use. A complete recording of the electrical activity of the perfused muscles was obtained. The whole procedure was then repeated on the denervated side and again separate eserinized samples of perfusate were

* These experiments were supported in part from a grant from the National Foundation for Infantile Paralysis.

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