trophoretic detection. Furthermore, the properties of the –SH groups of myosin and enzyme would have to be closely similar to explain the experiments reported in Table II and Fig. 1.

The effect of arsenicals on the enzyme was studied as follows. Varying amounts of a 1×10^{-3} *M* solution of mapharsen (3-NH_2, 4-OH phenylarsine oxide)—standardized by KIO_3—were added to 1 cc of 0.97% myosin at pH 7.1, under the conditions outlined above. Five-tenths of a micromole of the arsenical gave no inhibition, and 10 micromoles of arsenoxide produced only 9% inhibition. (Five-tenths of a micromole of *p*-ClHg benzoate inhibited 80%.) Since in most other enzymes we have studied[8] such a marked difference in the effect of this arsenical and of *p*-ClHg benzoate was not found, the possibility arose that the substituents on the benzene nucleus of the compound interfered with the approach of the arsenical to the –SH groups of the enzyme. In agreement with this assumption, 0.5 micromole of the unsubstituted phenylarsine oxide per cc of myosin gave 22% inhibition. At higher concentrations the inhibition by phenylarsine oxide increased.

We are indebted to Dr. Harry Eagle and to Messrs. Parke, Davis and Co. for the generous samples of the arsenicals, and to Dr. L. Hellerman for the *p*-chloromercuribenzoic acid used in this study.

Summary and Conclusions. The inhibition of adenosinetriphosphatase by mercaptide forming compounds and by mild oxidizing agents, and the reactivation by glutathione can be considered as proof that adenosinetriphosphatase is a sulfhydryl enzyme. The close parallelism between the number of –SH groups of myosin attacked by *p*-ClHg benzoate and oxidizing agents and the degree of inhibition of adenosinetriphosphatase activity is evidence in favor of Engelhardt's assumption that myosin and adenosinetriphosphatase are identical.

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A New Method of Preventing Blood Coagulation.

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Many anticoagulant substances have been employed for preserving blood in the fluid state. Agents such as oxalates and fluorides act by combining chemically with the available calcium in the blood to form an insoluble calcium compound. Citrates have been employed as the sodium or potassium salt and act by forming calcium citrate, which does not ionize. Heparin has been used to preserve the blood in a fluid state; its action being that of an antithrombin which inhibits the prothrombin necessary for coagulation. Oxalates and fluorides may be employed effectively *in vitro* for maintaining the fluidity of the blood in order that it may be used for certain chemical determinations on either whole blood or plasma. The toxicity of these substances, however, precludes their use in transfusions.

Citrates, particularly sodium citrate, have long been used as anticoagulants in transfusion. Moreover, under present wartime conditions, their employment has increased manifold times, particularly in the preparation of plasma. No untoward effects have attended their clinical use. Heparin, although infrequently employed as anticoagulant in routine transfusions, may prove to be a more ideal agent, but this would be contingent on less expensive methods of manufacture. When blood was collected in paraffin-lined tubes it was found to remain fluid for some time due to the failure of the platelets to disintegrate and release thromboplastin. The same observation was subsequently made when methylmethacrylate tubes were employed.

A method was sought whereby the calcium could be removed by the physical phenomenon of adsorption. In a search for a suitable ion-

adsorbent, a phenol-formaldehyde resin with a polyhydric phenol base was investigated and found to be satisfactory. Several papers[1,2,3] have been published which review in detail the ion-exchange substances. This resin which was commercially available as Amberlite IR-100 had been employed on a large scale in the preparation of ion-free water. The action of this cation-exchange resin in the latter is illustrated as follows:

$$2NaR + CaSO_4 \rightarrow CaR_2 + Na_2SO_4$$
$$2NaR + MgCl_2 \rightarrow 2NaCl + MgR_2$$
$$2NaR + Ca(HCO_3)_2 \rightarrow CaR_2 + 2Na\,HCO_3$$

} Sodium cycle

$$2HR + CaSO_4 \rightarrow CaR_2 + H_2SO_4$$
$$2HR + MgCl_2 \rightarrow MgR + 2HCl$$
$$2HR + Ca(HCO_3)_2 \rightarrow CaR_2 + H_2O + CO_2\ (gas)$$
$$HR + NaCl \rightarrow NaR + HCl$$

} Hydrogen cycle

NaR = Sodium salt of cation-exchange resin.
HR = Hydrogen derivative of cation exchanger.

Preparation of Resin. The cation-exchange resin acts either as a sodium or as a hydrogen exchanger. When employed in the removal of calcium from blood, the resin was placed in the sodium cycle. This was accomplished as follows:

The resin which was available as 50-mesh was placed in a cylindrical separatory funnel with a plug of adsorbent cotton above and below the material. A 5% solution of sodium chloride was then allowed to flow through the material at the rate of 200 cc per cu. inch per minute. Following the adsorption cycle, a back-wash was carried out for 10 minutes. Finally, the resin was rinsed with calcium and magnesium-free water. For the preparation of small portions it was only necessary to stir the resin with successive quantities of 5% sodium chloride solution employing a mechanical stirrer. The supernatant solution was decanted off or removed by filtration. Some of the resin employed was not of an analytical grade and was found to throw color when rinsed with the saline solution. This was eliminated by rinsing the resin with successive portions of boiling water. As successive cycles of regeneration were carried out the color diminished. Sterilization of the resin was accomplished by autoclaving without any deleterious effects.

Theoretically, 1 g of dry resin is sufficient to adsorb 2.2 milliequivalent of calcium. Since the average calcium content of the blood is approximately 5.0 m.e., 2.3 g of resin should have been sufficient to adsorb this quantity of calcium. However, since maximal conditions of adsorption have not thus far been devised, considerably more than this amount of resin was necessary to prevent coagulation of the blood (*cf.* below).

Several methods of collecting the blood were devised. For chemical or serological determinations the blood was mixed directly with the resin in a small bottle, tube, or flask and then obtained free of resin by filtering through an 80-mesh monel-metal or stainless steel screen. Another method adaptable for blood transfusion employed a 250 cc vacuum bottle (Baxter) containing 30 g of resin in the sodium cycle moistened with normal saline. This was autoclaved and the blood was collected in the usual manner. The needle was plunged directly into the vein with the beveled edge away from the vessel wall so that a free flow of blood was obtained. The bottle containing the resin was then inverted so that the blood passed through the resin bed facilitating suitable contact with the resin to assure maximum adsorption of the calcium. Occasional gentle agitation was necessary to avoid partial clotting.

Another method of removing the calcium consisted of filling a glass mantel (6½ x 11/16 inches) threaded on both ends and containing the resin in the sodium cycle. This container held approximately 20 g of the dry resin, which was sufficient to prevent clotting of 150 cc of blood, under the conditions employed. An 80-mesh monel-metal screen was placed at both ends between rubber washers and a glass cannula with a flange was held in place by a metal screw-cap. A 14-gauge needle was attached to one cannula by means of a short length of rubber tubing. A piece of rubber tubing was attached to the other cannula and led into a vacuum bottle through

[1] Myers, R. J., Eastes, J. W., and Myers, F. J., *Indust. Eng. Chem.*, 1941, **33**, 697.

[2] Myers, R. J., and Eastes, J. W., *Indust. Eng. Chem.*, 1941, **33**, 1203.

[3] Harrison, J. W. E., Myers, R. J., and Herr, D. S., *J. Am. Pharm. Assn.*, 1943, **32**, 121.

TABLE I.
Influence of Oxalate or Resin Treatment upon Properties of Blood.

	Specimen	Resin-treated	*Direct or Oxalate
RBC, million/cu mm	1	4.85	4.78
	2	4.76	4.60
	3	3.73	3.52
	4	4.09	4.05
	5	5.21	5.00
Hemoglobin, g/100 cc	1	14.8	14.5
	2	13.5	13.2
	3	12.5	11.6
	4	14.5	14.2
	5	14.0	13.5
Sediment rate, mm/hour	1	3.5	9.2
	2	8.0	13.5
	3	15.0	22.0
Hematocrit, %	1	37.0	32.0
	2	33.0	28.5
WBC, per cu mm	1	6,250	5,200
	2	7,400	6,700
	3	10,500	8,800
Platelet count, per cu mm	1	310,000	260,000
	2	240,000	220,000
	3	375,000	320,000
	4	270,000	250,000
Prothrombin time, sec.	1	16.8	21.2
	2	24.2	25.6
	3	13.6	15.4
	4	15.4	17.2

*Direct refers to finger puncture.

a suction-control valve. The entire apparatus was wrapped and autoclaved. Employing any of the aforementioned methods, the blood was found to remain in a fluid state indefinitely.

The used resin was washed with a 2% sodium carbonate solution, then again washed and regenerated with saline as described previously. This regeneration could be accomplished repeatedly without exhausting the ion-exchange properties of the resin.

Properties of Resin-treated Blood. Blood collected in this manner compared favorably with heparinized blood. It was found satisfactory for hematological, serological, and biochemical examinations. The morphology of the cellular elements of the blood was minimally disturbed. Such tests as the Kolmer-Wassermann, Kahn, Mazzini, complete blood count, hemoglobin, specific gravity, hematocrit, sedimentation rate, total protein, sugar, urea, uric acid, N.P.N., etc., (*cf.* Tables I and II) were successfully performed. Prothrombin times were performed more accurately since no variation in recalcifying the blood was encountered. Blood collected under sterile conditions was satisfactorily employed for culture media in bacteriology.

Four rabbits were bled from the heart and the blood collected in this manner was treated with the resin to prevent coagulation. When the resin-treated blood was then replaced in the rabbits to restore the blood volume no untoward effects were observed.

Summary. 1. A new method of preventing coagulation of blood was devised by the employment of ion-exchange adsorbents. The principle is based on the replacement of the calcium ions in the blood with sodium ions. The most ideal of the ion-exchange agents was found to be a phenol-formaldehyde resin with a polyhydric phenol base. As a calcium-adsorbent the cation-exchange resin was employed in the sodium cycle. 2. Resin-treated blood was employed in hematological, sero-

TABLE II.
Serological and Biochemical Studies of Resin and Oxalate Treated Blood.

Determination	Resin treated*	Oxalated*†
Sugar, mg %	111.2	114.0
Urea nitrogen, %	14.3	13.8
Uric acid, %	4.1	4.4
Total protein, g	7.31	7.25
A/G ratio	2.03	2.16
Albumin, g	4.90	4.75
Globulin, g	2.41	2.20
Fibrinogen	0.268	0.324
Non-protein nitrogen, mg %	29.1	26.4
Inorganic phosphorus, mg %	2.6	2.2
Sugar (24 hours), mg %	22.5	45.0
Specific gravity—whole blood	1.0623	1.0608
" " plasma	1.0285	1.0278
Oxygen capacity, cc	22.73	21.99
Cephalin-cholesterol flocculation test	Negative	Negative
Creatinine, mg %	1.2	1.2
Cholesterol, mg %	182.0	194.0
†Serology:		
Wassermann	Negative	Negative
"	++++	++++
Kahn	Negative	Negative
"	++++	++++
Mazzini	Negative	Negative

* Average of five samples.
† Serum for Serology.

logical and biochemical studies. Minimal alterations in cellular morphology were observed. 3. The resin is inexpensive and can be regenerated innumerable times and re-employed.

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Vaso-Excitor and -Depressor Substances as "Toxic" Factors in Experimentally Induced Shock*

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In our observations on the visceral circulation of animals in tourniquet and traumatic shock, capillary changes, in addition to those produced by generalized vasoconstriction, were noted remote from the site of injury. This and the fact that similar changes occurred in irreversible hemorrhagic shock led us to look for the presence in the blood of abnormal substances which would account for the observed reactions. Descriptions of the vascular reactions after these shock treatments involving fluid loss are being published.[1] They indicate that the changes are of the order of an initial hyper-reactive condition, suggesting the presence of vaso-excitors, and a

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1 Zweifach, B. W., Lee, R. E., Hyman, C., and Chambers, R., *Ann. Surg.*, in press.