restoration of the normal state in the vascular bed with cortical steroids. Works of Swingle and others(5,6) point to the role of the cortex in traumatic shock, while Huizenga, *et al.* (7) report on the ineffectiveness of cortical hormones in hemorrhagic shock. Remington (8) found that intact dogs given a large dose of dibenamine succumbed to minor hemorrhages, and that the cardiovascular changes in these animals resembled those of animals in terminal adrenal insufficiency or acute crises following bilateral adrenalectomy. Ramey, *et al.*(9) found that ACE potentiates the blood pressure response to norepinephrine in adrenalectomized dogs. We, however, were unable to confirm this in rats. Fritz and Levine(10) believe that cortical steroids are necessary for the efficient response of blood vessels to sympathin E.

Whatever may be the mechanism, the results obtained by us, together with the observations that adrenalin injection increases the resistance of adrenalectomized animals to histamine(11-13) suggest that at least under the conditions of the present experiments, the presence of the adrenal medulla is of primary importance.

Summary. 1. Administration of adrenalin prolongs survival time of adrenalectomized rats injected with KCl, formaldehyde, and sodium arsenite. 2. Under conditions of our experiments, adrenalin was more effective than cortisone in providing protection against these agents. 3. This protective effect is abolished by simultaneous administration of sympatholytic drugs. 4. The mechanism of protection appears to be through the prevention of vascular collapse. 5. It is suggested that the medullary secretion is an important factor in resisting traumatic shock.

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Studies on Platelets. XVI. Glutamic Oxaloacetic Transaminase Activity of Human Platelets.* (22275)

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Glutamic oxaloacetic transaminase (GOT) is a specific enzyme concerned with the synthesis of glutamic and oxaloacetic acids through the transfer of α-amino nitrogen of aspartic acid to α-ketoglutaric acid. Its presence has been demonstrated in serum of many animals and man, as well as in many animal tissues(1-4). It was observed by Karmen *et al.*(4) that human blood hemolysates

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contained GO-T activity greatly in excess of that of human serum. This observation suggested the presence of GO-T in formed elements of the blood. Due to our interest in platelets, a study was done to determine the GO-T activity of these bodies.

Methods. 1) *Determination of GO-T activity* was carried out as described by Karmen *et al.*(4). A minor modification was introduced consisting in the use of 0.03 ml of malic acid reagent instead of 0.1 ml as indicated in the original technic. This modification has given results quite comparable to those obtained with the original method(5). Experiments were run in triplicate. The reacting mixture was incubated for 10′ and readings were taken at 5′-10′-15′ after addition of the aspartic acid reagent. Values given in this study are, therefore, the average of nine figures obtained for each sample. 2) *Preparation of platelet-rich and platelet-poor plasma; and of platelet suspensions in saline* was carried out as described(6). Technics differed, however, in small details. Standard ACD solution (1/5 volume) was used as anticoagulant instead of EDTA-Na_2. Blood was freshly collected from healthy donors in plastic containers and processed without delay. Volume of saline used in washing platelets was identical to that of the platelet-rich plasma originally used. Platelet suspensions were adjusted to various concentrations by addition of suitable volume of saline solution. Platelets were counted directly(7) with the use of phase microscope. All preparations were assayed for enzymatic activity within 8 hours of blood collection. 3) *Preparation of water soluble platelet extract.* This original technic is being described(8). To packed, fresh, twice washed platelets from 50 ml of fresh human blood, 6 ml of distilled water and 5 ml of precooled ethyl ether were added. After centrifugation at 3,500 rpm/30′ at 4°C, three well differentiated layers were obtained: an upper layer, containing ethyl ether and lipoid substances; a middle one containing a precipitate, probably composed of protein material and stroma, and a bottom one containing a water-soluble protein. After separation of the supernant ether layer, the middle and lower layers were brought back with distilled water to the volume of platelet-rich plasma originally used. Centrifugation at 3,200 rpm/30′ at 4°C brought to sharp separation of a top layer containing insoluble protein material and stroma from a water-clear bottom layer. The bottom layer, representing approximately 19/20 of the total volume was transferred by suction to another container, leaving behind the top layer. Enough water was then added to full reconstitution of volume.

TABLE I. Glutamic Oxaloacetic Transaminase Activity in Various Preparations from Human Platelets.*

Preparation	No. of platelets/mm³ $\times 10^{-3}$	Units of GO-T activity
Normal plasma	235 ± 25	37.5 ± 7.5
Platelet-poor plasma	10 (approx.)	20.8 ± 2.0
Platelet suspension (saline)	2000 ±200	255.0 ±10.5
	470 ± 15	61.5 ± 3.5
	120 ± 24	43.0 ± 2.0
	40 ± 5	28.5 ± 1.5
Washings of sedimented platelets		10.5 ± 1.6
Water soluble fraction (see text)		104.2 ±10.0

* Values calculated on basis of 5 different experiments.

Results. (Table I). (a) Plasma containing an adequate number of platelets showed greater GO-T activity than platelet-poor plasma; (b) Slight GO-T activity was recovered from washing of platelets after their separation from plasma by high speed centrifugation. This may have represented GO-T activity of plasma which had remained absorbed into the platelets; (c) The transaminase activity of isolated platelet preparations containing discrete, separated bodies was in gross proportion to their number; (d) The water soluble protein obtained from platelets (bottom layer) appeared to possess high GO-T activity.

Discussion. There seems to be no question that platelets represent a source of GO-T in human blood. In view of the present uncertainty as to whether a factor discovered in platelets is an intimate constituent of these bodies or is more simply carried by them, it

became necessary to study the "location" of GO-T in human platelets. Low GO-T activity was found in washings from separated platelets. On the other hand, high activity was found in a water soluble preparation from platelets after treatment with ether. Ether appeared to destroy the physical integrity of platelets while removing most of their lipoid constituents. Both findings, therefore, suggested that GO-T was an intimate constituent of platelets, presumably a part of the protein, water-soluble fraction.

It appears premature to discuss the significance of the finding of GO-T in platelets as well as the possible relationship of the enzyme to the role of platelets in the hemostatic mechanism.

Summary. 1) Platelets are a source of glutamic oxaloacetic transaminase in human blood. 2) The enzyme is an intimate constituent of platelets, being found principally in a water soluble fraction of platelets after separation of lipoids by ethyl ether.

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Amperometric Titration of -SH Groups in Purified Clotting Factors By Improved Method. (22276)

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In studies on specific reactive groups of proteins involved in the coagulation of blood, it was observed that compounds commonly used to alter –S–S– bonds caused inhibition of clotting activity of purified bovine prothrombin, whereas those used to alter –SH groups did not(1). No –SH groups were detected in purified prothrombin, thrombin or fibrinogen, nor during the conversion of prothrombin to thrombin. In these experiments, quantitative –SH measurements were made by the argentimetric amperometric method using an ethanol-ammoniacal titration mixture(1). Benesch, Lardy and Benesch(2) recently have emphasized that this method, as applied to protein –SH groups, is open to criticism on the grounds that the high pH of the NH_4OH-NH_4NO_3 buffer used may denature proteins and accelerate the oxidation rate of –SH groups, that the ammonium ions may effect total disappearance of some –SH groups, and that the alcohol, usually necessary to obtain sharp end points, also denatures proteins. It therefore was possible, although unlikely, that the absence of detectable –SH groups in the purified clotting products could be attributed to these factors.

In this same paper, Benesch *et al.* introduced a modification of the amperometric method which overcame these objections. Instead of ammonia as the agent to form the silver complex, they employed tris-(hydroxymethylaminomethane) which made it possible to titrate protein –SH groups in aqueous, buffered solutions at pH 7.4. Such solutions had the significant additional advantage of exerting no demonstrable drastic effects on proteins, thereby allowing the study of –SH groups of proteins in the native state and a more precise determination of the relative ac-