expected beneficial result should be tempered by the knowledge that the rate of dialysis is slow and that the duration of treatment with the artificial kidney should be longer than the usual 6 hours used in treatment of renal insufficiency. This dialysis period could be shortened considerably if the existing artificial "kidneys" were enlarged to provide more dialyzing area. In the case of intoxication due to short-acting barbiturates, hemodialysis does not seem to be very helpful.

Hemodialysis may be life saving in those cases of acute barbiturate intoxication where the patient is comatose, areflexive with circulatory or respiratory difficulties and where the blood barbiturate level is elevated.

Summary. An artificial kidney of the Skegg-Leonards type was used successfully to lower blood barbiturate levels in dogs. Approximately 15 to 25% of the intravenously injected pentobarbital, 35% of intravenously injected amobarbital, and 40 to 70% of intraperitoneally injected phenobarbital were recovered in the respective dialysates. This therapy was life saving in the phenobarbital experiments, of some value in the amobarbital intoxication, but of questionable value in pentobarbital poisoning.

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Dehydrogenase Activities of Human Platelets.*† (21189)

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Although a good deal is known about the morphology and physical characteristics of platelets, little information is available concerning their biochemical properties. Platelets have been reported to contain certain enzymes such as esterases, acid phosphatase and β-glucuronidase(1), but, whereas according to some workers they have little or no intrinsic metabolism(2), others have found that they exhibit a very rapid metabolic activity(3). These activities, however, have usually been observed in freshly prepared platelets and always disappear completely following storage of the specimens for a period of 2 to 3 days (3). There is some indication that the methods used for the collection of blood and for the preparation and storage of platelets may exert an effect on the extent of their observable biochemical properties(4). It is

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of interest in this connection that platelets have been stored for as long as one year without significant decrease in cell numbers and that, although morphological changes and loss of clot-retracting ability occur during this time, thromboplastic activity apparently is maintained(2).

Materials and methods. Platelet suspensions were prepared from fresh, versene (ethylenediamine tetraacetate) collected human blood according to the method described by Minor and Burnett(5). Following their concentration the platelets were washed 3 times in physiological saline containing 2% Triton WR-1339‡ and made up in this medium to a standard photometric density (Coleman Junior spectrophotometer wavelength 420 mμ, saline-Triton blank) of 1.301 (5% transmission). This "standard platelet suspension" contained approximately 2.5 x 10^6 cells per mm^3. The reduction of 2,3,5-triphenyltetrazolium chloride was used to demonstrate the presence and estimate the levels of the various dehydrogenase systems studied(6). To 1.0 ml volumes of a freshly prepared (4-5 hours after collection) "standard platelet suspension" were added 0.5 ml of 0.20 M substrate, pH 7.0, and 0.5 ml of 0.5% freshly prepared 2,3,5-triphenyltetrazolium chloride in 0.10 M phosphate buffer, pH 7.0, containing 0.1% diphosphopyridine nucleotide. Control tubes were set up to contain instead of substrate either water or physiological saline and, instead of enzyme, saline containing 2% Triton WR-1339. Incubation was allowed to proceed at 37°C for 60 minutes and no measurable decrease in cell number was observed during this period of time. The reaction was stopped by the addition to each tube of 5.0 ml acetone, the mixtures were shaken vigorously and the precipitated cell proteins separated by 10 minutes centrifugation at 1500 r.p.m. The optical densities of the resulting clear supernatants were determined in a Coleman Junior spectrophotometer at a wavelength of 485 mμ and the various dehydrogenase activities expressed in terms of these optical densities. All determinations were carried out in triplicate and the results reported are the averages.

‡ Rohm and Haas.

Relationship Between Succinic Dehydrogenase Activity and No. of Intact Platelets Remaining After Successive Freezings and Thawings of Standard Platelet Suspension

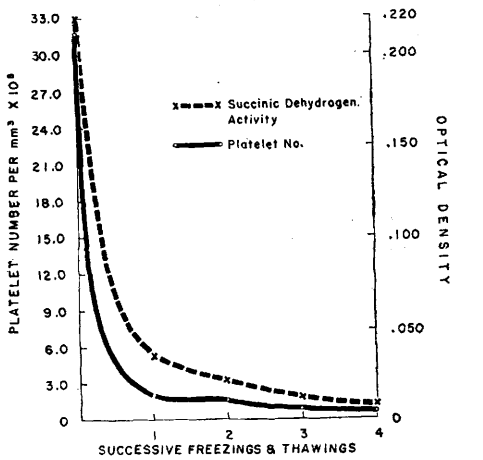

FIG. 1.

Cell-free dehydrogenase preparations were obtained (a) by incubating 1.0 ml volumes of a freshly prepared "standard platelet suspension" for 30 minutes at 37°C with 0.05 ml volumes of any one of the following: Toluene, 2% aqueous saponin or 10% aqueous phenol, or (b) by rapidly freezing and thawing a "standard platelet suspension" and repeating this process twice. Both types of treatment were found to result in the lysis of more than 95% of the originally present intact cells. Platelet counts were carried out according to the method described by Rees and Ecker(7). All glassware used in the collection of blood, in the preparation of platelets and during the study of metabolic activities was silicone-coated with Desicote (Beckman).

Results. The following substrates have been investigated: Succinate, l-malate, α-glycerophosphate, lactate, glutamate, β-hydroxybutyrate, glucose, and ethanol. The dehydrogenase systems concerned with the oxidation of all of these substances were found to be actively present in freshly prepared platelets. Omission of either substrate or enzyme (platelets) did not result in the reduction of the dye. Under the particular experimental conditions employed the most active dehydrogenases were those attacking succinate, l-malate, and α-glycerophosphate.

In an attempt to obtain cell-free enzyme

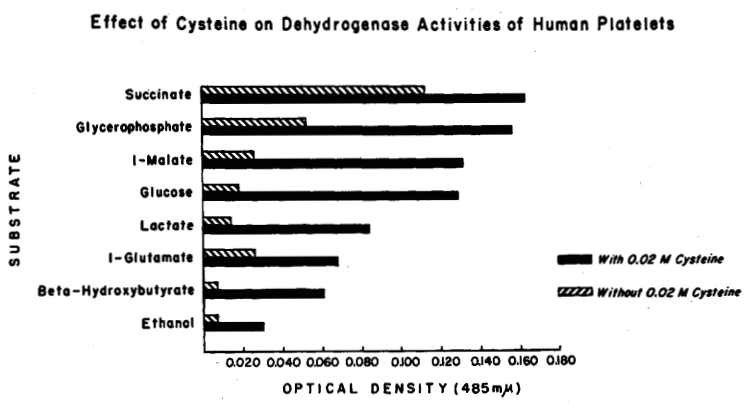

FIG. 2.

preparations it was found that platelets can be lysed quickly and readily by chemicals such as saponin, toluene, and phenol or by the physical means of repeated freezing and thawing. The resulting preparations were found to be homogeneous and opalescent, but completely lacking in dehydrogenase activity. That the activity of these enzymes appears to be closely related to the morphological integrity of the platelets is further substantiated by the data presented in Fig. 1. With increasing cell destruction, as observed following repeated freezing and thawing, the level of succinic dehydrogenase activity falls rapidly and closely parallels the extent of cell destruction. Essentially similar data have been obtained for some of the other dehydrogenase systems.

On the other hand, it was found that the addition to intact, freshly prepared platelets of cysteine in a final concentration of 0.02 M exerts a marked and apparently activating effect on enzyme activity (Fig. 2). The extent of activation varies for the different dehydrogenase systems studied, but appears to be greatest for those exhibiting lower activities in the absence of added cysteine.

Following this initial observation the relationship between cysteine concentration and its ability to activate platelet succinic dehydrogenase was studied. Control experiments were run with cysteine only and, in order to obtain the activation of succinic dehydrogenase proper, the optical density of reduced triphenyltetrazolium chloride as brought about by cysteine oxidation alone was subtracted from that observed in the presence of cysteine and succinate. The extent of activation was found to be most marked up to a cysteine concentration of 5×10^{-4} M, to reach a maximum of 100% at about 25×10^{-4} and to decrease at concentrations above 100×10^{-4} M. Similar observations were made on the relationship between malic and glycerophosphate dehydrogenase activities on the one hand and increasing cysteine concentrations on the other. Here again the rate of activation was found to be maximum up to a cysteine concentration of 5×10^{-4} M and peak levels of 500 and 300% respectively were reached at about 25×10^{-4} M, followed by a marked decrease in activation at concentrations above 100×10^{-4} M.

An essentially similar activating effect on the 3 dehydrogenase systems studied was produced by reduced glutathione, activation again increasing most markedly up to a concentration of about 10×10^{-4} M, reaching a maximum at 25×10^{-4} M and rapidly decreasing above 100×10^{-4} M. For a given dehydrogenase system the effects of low concentrations of cysteine and reduced glutathione are of approximately the same order of magnitude suggesting the possibility that it may be the cysteine moiety of the glutathione molecule which is responsible for the latter's activating effect. However, results obtained with various other sulfhydryl-containing substances appear to indicate that the presence of the cysteine moiety in a molecule is not essential in order for it to activate platelet dehydrogenase systems. As is apparent from the data pre-

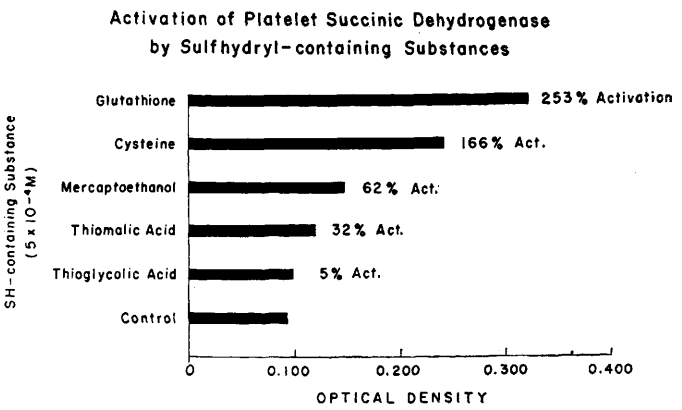

FIG. 3.

sented in Fig. 3 all of the other substances tested exhibit some activating capacity, although none of them even approaches the effect associated with either cysteine or reduced glutathione.

On the other hand, agents capable of oxidizing sulfhydryl groups such as iodosobenzoate and oxidized glutathione as well as mercaptide-forming substances such as p-chloromercuribenzoate and phenylmercuric hydroxide were found to inhibit platelet dehydrogenase activities. However, depending upon their concentration and the length of incubation, these agents also bring about a more or less marked destruction of platelets. The nature of this destruction appears to be different in type from that produced by the previously mentioned lysing agents in that a heavy precipitate is formed leaving an almost transparent supernatant fluid. Whether or not the observed dehydrogenase inhibitions are a direct result of enzyme sulfhydryl inactivation proper or whether they are only a secondary effect due to prior platelet destruction can, therefore, not as yet be stated. What the results do seem to indicate, however, is the existence in the cells of protein sulfhydryl groups, enzymic or not, which are essential for the maintenance of platelet integrity.

Further support for this conclusion is provided by evidence of a different nature. Thus, it has been found that the rate of platelet destruction occurring during storage can be reduced by –SH-compounds or environmental conditions interfering with sulfhydryl oxidation(4). In addition, it has been observed that the yield of platelets obtainable from a sample of versene-collected blood is several times that obtainable from a citrate-collected sample of the same blood and prepared in the same manner(4). Versene may bind metal ions which catalyze the oxidation of –SH groups. Of interest in this connection is a recent report by Benesch *et al.* on the relation between erythrocyte integrity and sulfhydryl groups(8).

Discussion. The finding that lysed platelets are lacking in dehydrogenase activities is surprising since in most other tissue preparations these enzymes are not dependent upon an intact cell structure for their activity. Preliminary work with repeatedly frozen and thawed, densely packed platelets seems to indicate that the observed absence of dehydrogenase activity is due to an inactivation of the enzyme proper. However, not sufficient evidence has been obtained so far to definitely exclude the dilution and disorganization of the complete enzyme system as contributory factors. It is of interest that findings similar to those reported here have been described previously by Penrose and Quastel(9) for a number of dehydrogenase systems in whole and lysozyme-lysed cells of *Micrococcus lysodeikticus*.

The observed activation of platelet dehydrogenase systems by low concentrations of –SH-substances may be explained by assuming that although some enzyme sulfhydryl groups were inactivated (oxidized) during the preparation of the platelet concentrates they could readily be reactivated (reduced) by

cysteine or reduced glutathione. The rapid decrease in activation noted with increasing sulfhydryl concentrations above 100 x 10^{-4} M is probably due to the gradual build-up of cystine and oxidized glutathione both of which are dehydrogenase inhibitors(10,11).

The fact that platelet dehydrogenase systems can be activated strongly by suitable concentrations of –SH-compounds suggests the possibility that other types of –SH-requiring platelet enzymes may need similar reactivation in order to exhibit maximum activity. The findings that versene improves the stability of platelets and that conditions interfering with sulfhydryl oxidation slow down platelet destruction not only underline the importance of free, active sulfhydryl groups to both platelet integrity and function, but may well explain the discrepancy in the literature concerning the rate of platelet metabolism. It would appear that, in order to prepare platelets with the least possible damage to their structure, inherent biochemical properties and resulting biological activities, conditions of collection and concentration should be employed which minimize damage to essential –SH groups.

Summary. 1. The presence in versene-collected, freshly prepared human platelets of a number of active dehydrogenase systems has been demonstrated. 2. Platelets can be lysed readily by various chemical and physical means, the resulting preparations being homogeneous and opalescent, but completely lacking in dehydrogenase activity. Evidence has been obtained to indicate that platelets have to be morphologically intact for dehydrogenase systems to be active. 3. The dehydrogenase systems present in platelets are activated by sulfhydryl-containing substances, the most marked effects of those studied being produced by cysteine and reduced glutathione. 4. The addition to platelets of sulfhydryl-oxidizing or mercaptide-forming agents results in platelet destruction apparently different in nature from that brought about by lysing agents not reacting with sulfhydryl groups. 5. Preliminary indications are that platelet destruction occurring during storage can be reduced by sulfhydryl-containing substances or environmental conditions interfering with sulfhydryl oxidation. 6. Possible implications of some of these findings have been briefly discussed.

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