Brief Report

Preservation of the Lactic Dehydrogenase Activity of Platelets by Freezing in Dimethylsulfoxide and Plasma

By Philip H. Geisler, Ioulios A. Iossifides and Mary F. Eichman

A medium composed of 15 per cent dimethylsulfoxide (DMSO) in a 50 per cent solution of plasma has previously been reported to preserve the clot-retracting activity of frozen human platelets stored under liquid nitrogen at −175°C. In order to study further the maintenance of platelet “viability” by this technic, the activity of lactic dehydrogenase (LDH) was examined before and after freezing.

Lactic acid dehydrogenase was chosen as a representative platelet enzyme because it plays a crucial role in the glycolytic metabolism. Maupin and Gross have shown that platelets contain considerable amounts of glycogen and have an active anaerobic glycolytic metabolism. As demonstrated by Campbell and his associates, the activity of platelet lactic acid dehydrogenase is closely related to the rate of glucose consumption. Furthermore, the LDH activity parallels clot retracting activity and is lost rapidly when platelets are lysed chemically or mechanically.

PROCEDURES AND RESULTS

The technics used for separation, counting, freezing and thawing have been described previously.

Lactic acid dehydrogenase was determined on platelets by a modification of the technic for serum LDH published by Berger and Broida and by Cabaud and Wroblewski. Lactic dehydrogenase catalyzes the reaction:

\[
\text{Lactic acid + DPN} = \text{Pyruvic acid + DPNH}
\]

Pyruvic acid reacts with 2, 4, dinitrophenylhydrazine to form a colored hydrazine with a high optical density in the 400–500 nm wave length range. Since the rate of conversion of pyruvate to lactate is proportional to the lactic dehydrogenase activity present, it is possible to measure LDH activity by the decrease in optical density. In working with platelets, it was found convenient to double the incubation time recommended for the serum LDH determination. The adapted method is as follows: to a solution containing 1 mg of B-DPNH in 1 ml of pyruvate substrate is added 0.1 ml of 2,4 dinitrophenylhydrazine (Sigma color Reagent 505–2) in order to react with the residual pyruvic acid forming a colored hydrazone. After 20 minutes the reaction is stopped by 10 ml of 0.4 NaOH and the optical density of the hydrazone is measured by a Coleman Jr. spectrophotometer at a wave length...
Table 1

<table>
<thead>
<tr>
<th>Donor</th>
<th>LDH Activity Units Before Freezing</th>
<th>LDH Activity Units After Freezing</th>
<th>Donor</th>
<th>LDH Activity Units Before Freezing</th>
<th>LDH Activity Units After Freezing</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>M. S.</td>
<td>600</td>
<td>240</td>
<td>P. H.</td>
<td>400</td>
<td>640</td>
</tr>
<tr>
<td>R. G.</td>
<td>400</td>
<td>1250</td>
<td>D. S.</td>
<td>380</td>
<td>920</td>
</tr>
<tr>
<td>R. M.</td>
<td>360</td>
<td>680</td>
<td>M. P.</td>
<td>520</td>
<td>760</td>
</tr>
<tr>
<td>H. S.</td>
<td>760</td>
<td>380</td>
<td>P. G.</td>
<td>240</td>
<td>850</td>
</tr>
<tr>
<td>W. S.</td>
<td>840</td>
<td>1110</td>
<td>P. H. G.</td>
<td>280</td>
<td>200</td>
</tr>
<tr>
<td>R. B.</td>
<td>820</td>
<td>680</td>
<td>J. S.</td>
<td>1400</td>
<td>640</td>
</tr>
<tr>
<td>B. S.</td>
<td>1400</td>
<td>520</td>
<td>J. R.</td>
<td>1300</td>
<td>970</td>
</tr>
<tr>
<td>L. H.</td>
<td>800</td>
<td>220</td>
<td>M. M.</td>
<td>2000</td>
<td>920</td>
</tr>
<tr>
<td>M. E.</td>
<td>760</td>
<td>400</td>
<td>V. K.</td>
<td>280</td>
<td>1660</td>
</tr>
<tr>
<td>R. L.</td>
<td>420</td>
<td>840</td>
<td>N. G.</td>
<td>580</td>
<td>1740</td>
</tr>
</tbody>
</table>

The results are reported in terms of arbitrary units of activity (Berger-Broida units) based on a calibration curve constructed with pyruvate standard substrate.

In a first series of experiments using the above technic, it was determined that concentrations of 40,000 and 60,000 platelets per cu. ml. provided optical densities in the optimal mid-range of the Coleman Jr. spectrophotometer at 535 m$. These concentrations were used in subsequent studies of the effect of the storage on LDH activity.

A second series of experiments confirmed the findings of Koppel and Olwin. LDH activity was found irreversibly lost in either fresh or frozen platelets following their lysis by ultra-sonification.

Lactic dehydrogenase activity was measured in the blood platelets of 20 healthy adults between the ages of 19 and 45, before and after storage for 24 hours at $-175^\circ$C, in a suspending medium composed of 15 per cent DMSO and 85 per cent of a mixture containing equal parts of original plasma and saline. The results are summarized in table 1. There was marked variation in platelets LDH activity among the various individuals both before and after freezing. The range of activity before freezing was 240–2000 Berger-Broida units with a mean value of 781. Platelet LDH activity was decreased after the 24-hours storage in ten of the subjects, increased in the other ten. In every instance, however, a significant degree of lactic dehydrogenase activity was maintained by freezing and storage under the conditions described above.

**DISCUSSION**

There are several possible explanations for the variable decreases and increases in platelet LDH activity observed after freezing in different subjects.

1. Lactic dehydrogenase may be released from an inactivating bond or inhibitor on standing. Crowley has reported that lactic dehydrogenase levels rose progressively in serum and citrated plasma samples which were allowed to remain in contact with blood cells and platelets during storage, while the activity declined in serum and plasma samples separated immediately after collection. It has been proposed that an increase in the stored plasma LDH activity may be due either to rupture of a molecular bond releasing the en-
zyme from a bound, inactive form, or to the disappearance over a period of time of an inhibitor of LDH activity.10,11

2. Platelet LDH activity may be accelerated at low temperatures. Zucker and Borelli12 observed an increase in LDH activity when normal human platelets were allowed to stand overnight, either at 4 °C or -20 °C.

3. Campbell, et al.4 in their metabolic studies of human blood platelets found a wide range of variation in dehydrogenase activity as did DiFrancesco and his colleagues.13 Since LDH activity was measured only once before and once after freezing, individual variations in rate of activity per unit time may have been responsible for the apparent variations in activity observed in these studies.

It is concluded that a medium composed of 15 per cent Dimethylsulfoxide and 85 per cent of a mixture of equal parts native plasma and saline effectively preserves platelet lactic acid dehydrogenase activity against slow freezing, storage for 24 hours in temperatures of liquid N2 and fast thawing. Platelet lysis results in a complete loss of LDH activity.

**SUMMARIO IN INTERLINGUA**

Es reportate experimentos e investigationes que permitte le sequente conclusion: Un medio componite de 15 pro cento de dimethylsulfoxydo e de 85 pro cento de un mixtura de equal partes de plasma native e solution salin protege le activitate de dehydrogenase de acido lactic in plachettas contra omne damno per congelation lente, thesaurisation durante 24 horas in le temperatura de N2 liquide, e disgelation rapide. Le lyse de plachettas resulta in le complete perdita del activitate de dehydrogenase de acido lactic.

**REFERENCES**


The Late Philip H. Geisler, M.D., Associate Member Cardeza Foundation for Hematologic Research, Assistant Professor of Pathology, Jefferson Medical College, Philadelphia, Pa.

Ioulios A. Iossifides, M.D., Assistant Member Cardeza Foundation for Hematological Research, Assistant Professor of Pathology, Jefferson Medical College, Philadelphia, Pa. Present address, AFL-CIO Hospital, Langdon and Cheltenham Avenues, Philadelphia, Pa.

Mary F. Eichman, M.T. (ASCP), Immunohematologist, Temple University Hospital, Blood Bank, Philadelphia, Pa.
Brief Report: Preservation of the Lactic Dehydrogenase Activity of Platelets by Freezing in Dimethylsulfoxide and Plasma

PHILIP H. GEISLER, IOULIOS A. IOSSIFIDES and MARY F. EICHMAN