Some Properties of Serum Lactic Dehydrogenase

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It was previously reported from this laboratory (2, 6, 7) that lactic dehydrogenase (LDH) activity is present in human blood serum. This activity was found in all sera examined but was elevated in many individuals with neoplastic disease and frequently in pregnancy. The present report deals with establishment of optimal conditions for measurement of the enzyme activity in serum and describes several applications of the procedure. A study was made of some of the properties of this enzyme, a comparison being made in several instances with crystalline LDH obtained from rabbit muscle.

MATERIALS AND METHODS

Reagents.—A 0.025 M solution of pyruvate was prepared daily in distilled water from reagent grade sodium pyruvate (Nutritional Biochemicals Corp.), and suitable dilutions were made from this stock solution. A commercial preparation of dihydrodiphosphopyridine nucleotide (DPNH) (Sigma Chemical Co.) of approximately 96 per cent purity was dissolved in distilled water to give a concentration of 1 mg/ml. Fresh solutions were prepared daily.

Crystalline LDH was a commercial preparation (Worthington Biochemical Corp.) from rabbit muscle and was obtained as a suspension containing 9 mg of crystals/ml of suspension. For preparation of a stock solution of LDH, 0.8 ml of the suspension was diluted to 2 ml with 0.1 M phosphate buffer (pH 7.4). After 10 minutes the solution was centrifuged at 1,500 r.p.m. for 10 minutes. For subsequent investigations 0.1 ml of the clear supernatant solution was diluted to 7 ml with 10-4 M glutathione (GSH) in 0.1 M phosphate buffer, pH 7.2. LDH diluted in a similar manner with buffer alone showed no variable activity which changed in an unpredictable manner. The addition of GSH, albumin, cysteine, 2,3-dimercaptopropanol (BAL), or human serum activated and stabilized the enzyme. The activity of the crystalline enzyme was increased frequently from two- to fourfold by the addition of glutathione, as little as 1 X 10-4 M GSH producing maximal activation. There was no significant loss in activity upon storage for at least a week at 4°C, although freezing and thawing decreased the activity.

One part of human serum, with no visible evidence of hemolysis, diluted with 9 parts of water was used for study of LDH activity of serum. Diluted serum decreased in activity if kept overnight, even at 4°C or frozen. However, undiluted serum retained its activity overnight at 4°C, and for at least a week in the frozen state. The addition of GSH had no effect on the LDH activity of serum.

Assay procedure.—All assays were carried out in 1-cm. cuvettes in Beckman spectrophotometers, Model B or DU. Each cuvette contained 0.1 ml of 0.025 M pyruvate, 2.5 ml of 0.1 M sodium phosphate buffer (pH 7.2). After 10 minutes the solution was centrifuged at 1,500 r.p.m. for 10 minutes. For subsequent investigations 0.1 ml of the clear supernatant solution was diluted to 7 ml with 10-4 M glutathione (GSH) in 0.1 M phosphate buffer, pH 7.2. LDH diluted in a similar manner with buffer alone showed no variable activity which changed in an unpredictable manner. The addition of GSH, albumin, cysteine, 2,3-dimercaptopropanol (BAL), or human serum activated and stabilized the enzyme. The activity of the crystalline enzyme was increased frequently from two- to fourfold by the addition of glutathione, as little as 1 X 10-4 M GSH producing maximal activation. There was no significant loss in activity upon storage for at least a week at 4°C, although freezing and thawing decreased the activity.

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Enzyme reaction was initiated by the addition of 0.02 ml of enzyme solution to 0.1 ml of a 1:10 dilution of serum, at which time a reading was taken before the addition of 0.02 ml of enzyme solution. Each cuvette contained 0.1 ml of 0.025 M pyruvate, 2.5 ml of 0.1 M sodium phosphate buffer (pH 7.2), and 0.4 ml of DPNH solution (1 mg/ml). The above components were generally premixed in the correct proportions. In the case of the serum enzyme the reaction was initiated by the addition of 0.01 ml of the solution to 0.1 ml of a 1:10 dilution of serum, at which time a reading was recorded. A reference cuvette containing 0.1 ml of water instead of serum was employed to adjust the spectrophotometer reading at 0.500 optical density. Decrease in optical density at 340 mµ was employed as a measure of enzyme activity. In the case of the crystalline enzyme the zero time reading was taken before the addition of 0.02 ml of enzyme solution, prepared as described previously, to a cuvette containing 3 ml of the assay mixture and 0.05 ml of water. The reactions were carried out for 15-30 minutes at 27°–37°C. For the serum enzyme and for 5–10 minutes at 27°C for the assay of crystalline LDH. In all cases it was established that the reactions were zero order for the period employed.

RESULTS

Optimal pH.—The results in Chart 1 show the effect of pH on the activities of crystalline LDH and of samples of serum from a apparently healthy individual and from a patient with granulocytic leukemia. Measurements of pH at the end of the incubations showed no appreciable change from the initial value to have taken place during
the course of the reaction. In the case of sera, the optimal activity was attained at pH values between 6.6 and 7.2. This was confirmed in a large number of determinations. The optimal pH for the crystalline enzyme activity was between 6.4 and 6.8. Similar experiments performed after the addition of a sample of normal human serum of low LDH activity to a high level of crystalline LDH activity gave a pH-activity curve identical with that obtained with the crystalline enzyme alone, indicating that constituents of normal serum did not influence the pH optimum. Determinations made at pH 7.2, 7.84, and 7.8 in 0.1 M tris(hydroxy-
methyl)aminomethane buffer gave results identical with those obtained in phosphate. All subsequent experiments with crystalline LDH and serum were performed at pH 7.2 in 0.1 M sodium phosphate buffer, unless stated otherwise.

**Optimal pyruvate and DPNH concentrations.**—Chart 2 illustrates the effect of pyruvate concentration on the activity of serum and crystalline LDH. A final concentration of $8.1 \times 10^{-4}$ M pyruvate (0.1 ml. of 0.025 M pyruvate per determination) was chosen for subsequent experiments. Chart 3 shows the effect of DPNH concentration on serum and crystalline LDH activities. A final concentration of $1.9 \times 10^{-4}$ M DPNH (0.4 mg DPNH/assay) was chosen for subsequent experiments. Michaelis-Menten constants ($K_m$) for pyruvate were $8.2 \times 10^{-4}$ M for normal, $1.4 \times 10^{-4}$ M for leukemic serum, and $2.8 \times 10^{-4}$ M for crystalline LDH. The $K_m$ value for DPNH with leukemic serum was found to be $1.25 \times 10^{-4}$ M and $2.4 \times 10^{-4}$ M for crystalline LDH. The $K_m$ values for pyruvate and DPNH are in the range of those obtained by other investigators (Table 1).

**Time-activity curves.**—The time-activity curves (Chart 4) for serum and crystalline LDH show that activity is linear with time, the concentration of DPNH being the limiting factor. A period of 30 minutes was chosen as convenient for routine assays of serum, since the reaction rate was linear and the length of time of incubation permitted a number of samples to be set up consecutively.

**Serum concentration curves.**—The activity of serum was proportional to enzyme concentration (Chart 5). A similar finding was made with different dilutions of a solution of crystalline LDH. The concentration of serum was kept low in routine determinations to avoid complete oxidation of DPNH in less than 30 min. For subsequent investigations 0.01 ml. of serum (0.1 ml. of 1:10 diluted serum) was used for each assay.

**Summary of optimal conditions.**—For optimal serum activity 3 ml. of an assay mixture consisting of 0.1 ml. of 0.025 M pyruvate, 2.5 ml. of 0.1 M phosphate buffer (pH 7.2), and 0.4 ml. of DPNH solution (1 mg/ml) are incubated for 30 min. at 37°C. (water bath) with 0.1 ml. of 1:10 diluted serum. The assay carried out under optimal conditions gave somewhat higher values than obtained
with the procedure previously published (pH 7.95) (7), although either method is satisfactory for work requiring routine assays.

**LDH activity of normal human serum.**—Chart 6 shows a distribution curve for the serum activity of 104 apparently healthy adults. All values were determined by the assay method (pH 7.95) previously published (7). Chart 7 gives the relation of age to serum LDH activity (pH 7.95 method) for a number of apparently healthy children and adults. Although children have higher serum LDH activity than adults, the adult range of activity is approached at about 14 years of age.

**TABLE 1**

**SOME COMPARATIVE MICHAELIS-MENTEN CONSTANTS FOR LDH**

<table>
<thead>
<tr>
<th>Michaelis-Menten Constant (Km)</th>
<th>Source</th>
<th>Reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Pyruvate DPNH (mole/liter)</td>
<td></td>
<td></td>
</tr>
<tr>
<td>8.2x10^-4</td>
<td>Normal human serum</td>
<td>Present report</td>
</tr>
<tr>
<td>1.4x10^-4</td>
<td>Leukemic human serum</td>
<td>Present report</td>
</tr>
<tr>
<td>2.8x10^-4</td>
<td>Crystalline LDH (rabbit muscle)</td>
<td>Present report</td>
</tr>
<tr>
<td>9x10^-4</td>
<td>Crystalline LDH (rat muscle and Jensen sarcoma)</td>
<td>Kubowits and Ott (9)</td>
</tr>
<tr>
<td>5.2x10^-4</td>
<td>Beef heart</td>
<td>Meister (11)</td>
</tr>
<tr>
<td>Order of 10^-4</td>
<td>Heart</td>
<td>Neilands (12)</td>
</tr>
<tr>
<td>4.8x10^-4</td>
<td>Leukocytes: Normal</td>
<td>Beck (1)</td>
</tr>
<tr>
<td>5x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
</tr>
<tr>
<td>7x10^-4</td>
<td>Lymphocytic leukemia</td>
<td>Hess and Gehn (6)</td>
</tr>
<tr>
<td>5x10^-4</td>
<td>Human serum</td>
<td>Hess and Gehn (6)</td>
</tr>
<tr>
<td>4.2x10^-4</td>
<td>Beef heart</td>
<td>Meister (11)</td>
</tr>
<tr>
<td>2.4x10^-4</td>
<td>Crystalline LDH (rabbit muscle)</td>
<td>Present report</td>
</tr>
<tr>
<td>2.7x10^-4</td>
<td>Crystalline LDH (rat muscle and Jensen sarcoma)</td>
<td>Kubowits and Ott (9)</td>
</tr>
<tr>
<td>2.5x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
</tr>
<tr>
<td>1.3x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
</tr>
<tr>
<td>1.4x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
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<tr>
<td>5x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
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<tr>
<td>2.5x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
</tr>
<tr>
<td>2.4x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
</tr>
<tr>
<td>2.7x10^-4</td>
<td>Normal Myelocytic leukemia</td>
<td>Hess and Gehn (6)</td>
</tr>
</tbody>
</table>

**CHART 4.**—Activity of serum and crystalline LDH as a function of incubation time. The reaction mixture was the same as for Chart 1 with pH 7.2 phosphate buffer. The incubation temperature was 37° C. for serum; 30° C. for crystalline LDH. Serum (normal) was from an apparently healthy individual; serum (leuk.) from a patient with granulocytic leukemia.

**CHART 5.**—LDH activity as a function of serum concentration. The reaction conditions were the same as employed in experiments described in Chart 1 with pH 7.2 phosphate buffer. Incubation was performed at 37° C. for 30 minutes. Serum (normal) was from an apparently healthy individual; serum (leuk.) from a patient with granulocytic leukemia.

**CHART 6.**—Distribution curve for the serum LDH activity of apparently healthy adults. The sera of 104 adults over 20 years of age were assayed by the method (pH 7.95) previously published (7). The values obtained at pH 7.2 are approximately 1.8 times those obtained at pH 7.95. Results were calculated assuming a decrease in absorption of 0.200 to be equivalent to the oxidation of 0.1 mg. of DPNH.
Inhibition of LDH by mercuric chloride and p-chloromercuribenzoate (p-CMB).—LDH has been crystallized as an inactive mercury salt, which was decomposed by hydrogen sulfide or cysteine to regenerate the active enzyme (9). Neilands (18) found that LDH activity of heart was suppressed.

Maximal inhibition was obtained by pre-mixing the inhibitor with buffered enzyme 10 minutes prior to the addition of substrate. Charts 8 and 9 show the inhibitory effect on serum and crystalline LDH, respectively, of various concentrations of mercuric chloride, p-CMB, and an isomolar mixture of DPNH and mercuric chloride. The mixture of DPNH and mercuric chloride was tested, because these substances form a 1:1 complex, and it was of interest to determine whether the mercuric salt or the complex was the actual inhibitor of LDH. Since in each instance the mercuric chloride-DPNH complex was considerably less effective as an inhibitor than either mercuric chloride or p-CMB alone (Charts 8 and 9), it would appear unlikely that the complex is the inhibitory form of mercuric salts. Versene decomposed the mercuric chloride-DPNH complex but did not reactivate the serum enzyme inactivated with mercury. p-CMB was found to be a highly effective inhibitor of LDH but did not appear to form a DPNH complex. It appears likely from the above that the mercuric salts react directly with the enzyme protein. Fifty percent inhibition of the crystalline enzyme was achieved by 8.5 X 10^-7 M mercuric chloride and by 9 X 10^-7 M mercuric chloride in the case of the serum enzyme. The latter enzyme is probably less sensitive to mercuric salts than is the crystalline enzyme because of the protective action of the relatively large amount of protein present in the serum. The inhibited enzyme could be reactivated by the addition of a large excess of sulfydryl compounds such as glutathione, cysteine, or 1,2-dimercaptopropylsulfoxide, but not by versene (Charts 10 and 11). It was found that the inhibition of crystalline and serum
LDH by mercuric chloride and p-CMB was non-competitive with pyruvate.

The formation of a 1:1 complex of mercuric chloride and DPNH or DPN. — The classical procedure for the isolation of DPN from natural materials involves the precipitation of DPN as a salt of a heavy metal and decomposition of the complex with hydrogen sulfide (15). In the present study it has been shown that a 1:1 soluble complex of mercuric chloride and DPN or DPNH is formed in dilute solution and that this complex can be decomposed with sulfhydryl compounds. The results in Charts 12 and 18 show the spectral shifts produced in solutions of DPNH and DPN, respectively, upon the addition of mercuric chloride in approximately threefold excess and indicated that complexes are formed. The absorption curve of the DPNH-mercuric chloride complex was similar to that reported for DPNH-X, a compound formed from DPNH in a reaction catalyzed by triose phosphate dehydrogenase (10, and Rafter, cited in [15]). In plotting changes in optical density in DPNH solution at 290 and 340 mμ, two wave lengths at which particularly marked changes were noted on addition of mercuric chloride, a sharp break was noted at a ratio of mercuric chloride to DPNH of 1:1 (Chart 14) which showed that 1 mole of mercuric chloride can form a complex with 1 mole of DPNH. The small continued rise in optical density at 290 mμ after formation of the complex was probably attributable to the slight absorption of mercuric chloride at this wave length. The normal spectrum of DPNH could be regenerated by the addition of excess versene, the sulfhydryl compounds cysteine, glutathione, and 2,3-dimercaptopropanol, as well as by human

![Chart 10](image_url)

**Chart 10.** — Inactivation of serum and crystalline LDH with mercury; reactivation with glutathione (GSH). The reaction mixture contained 0.1 ml. of dilute serum (1:10) and a final concentration of 1.6 X 10⁻⁴ M mercuroic chloride, 8.1 X 10⁻⁴ M pyruvate, and 1.9 X 10⁻⁴ M DPNH with 0.1 M phosphate buffer (pH 7.2) to a total volume of 3.1 ml. GSH was added to give a final concentration of 6.4 X 10⁻⁴ M, and a second portion raised the concentration to 1.3 X 10⁻³ M. Versene was added in amounts equivalent to GSH. The incubation temperature was 27° C.

![Chart 11](image_url)

**Chart 11.** — Inactivation of serum and crystalline LDH with mercury; reactivation with glutathione. The reaction mixture was the same as for Chart 10, except that 0.54 μg. of crystalline LDH replaced the serum and the final concentration of mercuric chloride was 3.4 X 10⁻⁴ M. Incubation was performed at 27° C.

![Chart 12](image_url)

**Chart 12.** — Spectra of DPNH and a mixture of DPNH and mercuric chloride. Final concentrations of 9.4 X 10⁻⁴ M DPNH (purity about 90 per cent) and 3.3 X 10⁻⁴ M mercuric chloride in 3 ml. of 0.1 M phosphate buffer (pH 7.2) were employed.
serum or albumin, or crystalline LDH from rabbit muscle. No interaction between DPNH and p-CMB was detected by the above procedure, suggesting that a complex similar to that found with mercuric chloride was not formed with p-CMB.

**Comparative LDH activities: extractability of LDH from human erythrocytes and ascites tumor cells.**—High LDH activity was found in the ascitic fluid of rats bearing the Yoshida sarcoma and the AA sarcoma, and in the ascitic fluid of mice with Ehrlich ascites tumor. In animals with the Yoshida sarcoma, LDH activity was found in the supernatant after removal of cells by centrifugation, while a homogenate of the tumor cells showed a much higher activity (Table 2). The activity of the ascitic fluid was found to increase with storage on contact with cells. Fluid obtained by centrifugation immediately after removal of a tumor sample from the animal catalyzed the oxidation of DPNH at a rate of 0.23 mg DPNH/min/ml, but when kept in contact with cells at 4°C for 3, 5, and 24 hours oxidized DPNH at a rate of 1.5, 2.5, and 3.5 mg/min/ml, respectively. Plasma in contact with erythrocytes from either normal or leukemic individuals did not show a significant rise in activity even after 24 hours at 4°C, although homogenates or lysates of erythrocytes and Yoshida sarcoma cells showed activity in the same range (Table 2). The high LDH activity found in erythrocytes as contrasted to relatively low levels detected in serum emphasizes the importance of avoiding hemolysis in the determination of serum LDH activity. Yoshida sarcoma cells and erythrocytes also acted differently upon successive extraction with isotonic saline. Table 3 shows that washes with isotonic saline of Yoshida sarcoma cells showed a high level of activity, yet washes of erythrocytes did not show significant activity. After as many as ten extractions some of the tumor cells were still intact and viable, as judged by cytological criteria, and still possessed a high level of LDH activity. However, in one instance, after sixteen extractions the cells were lysed, and little

**TABLE 2**

<table>
<thead>
<tr>
<th>Activity</th>
<th>No. determinations</th>
<th>Av.</th>
<th>Range</th>
</tr>
</thead>
<tbody>
<tr>
<td>Serum (apparently healthy adults)</td>
<td>30</td>
<td>0.35</td>
<td>0.17–0.30</td>
</tr>
<tr>
<td>Serum (leukemic adults)</td>
<td>40</td>
<td>0.77</td>
<td>0.38–1.50</td>
</tr>
<tr>
<td>Erythrocytes</td>
<td>9</td>
<td>55.6</td>
<td>42.5–73.5</td>
</tr>
<tr>
<td>Yoshida sarcoma ascitic fluid</td>
<td>12</td>
<td>1.29</td>
<td>0.23–3.34</td>
</tr>
<tr>
<td>Yoshida sarcoma cells</td>
<td>5</td>
<td>66.3</td>
<td>40.8–96.0</td>
</tr>
<tr>
<td>Cryst. LDH</td>
<td>4</td>
<td>162*</td>
<td>155–170*</td>
</tr>
</tbody>
</table>

* DPNH oxidized/min/mg dry weight of crystalline enzyme.
LDH activity was found in the debris. It is not certain whether the greater activity of the saline extracts of the tumor cells as compared with the erythrocytes is attributable to a greater permeability of the former cells for LDH or to a greater fragility of the tumor cells under the conditions employed.

Erythrocytes varied in LDH activity in individual samples (Table 2), but there was no correlation of the activity with the presence of neoplastic disease.

**DISCUSSION**

LDH activity has been found in human (2, 5, 6, 7) and mouse sera (8). The source of serum LDH is not known either in normal or in pathological states in which the enzyme activity is elevated. High levels of LDH are found in various tissues and in erythrocytes and leukocytes (1), and a small leakage from these sources could account for the LDH detectable in serum. An increased rate of destruction of erythrocytes in leukemia might account for the generally elevated levels found with this disease (2). The ease of extraction of LDH activity from Yoshida sarcoma cells, demonstrated in the present study, suggested that the malignant cells themselves might be a source of the serum enzyme.

It is of interest that generally higher levels of LDH activity were found in the sera of normal children than in healthy adults and that the activity reached the adult level during adolescence.

Three glycolytic enzymes, LDH (2, 6, 7), phosphohexose isomerase (3, 4), and aldolase (14), have now been reported to be elevated in the sera of some patients with neoplastic disease. The relationship of the high glycolytic rate found in many tumors to the elevated levels of the above enzymes in serum has not yet been defined.

**SUMMARY**

1. Optimal conditions have been established for the measurement of LDH activity in human serum. Under these conditions the reaction is linear with time and proportional to enzyme concentration, the concentration of DPNH being the limiting component in the incubation mixture.
2. Higher levels of LDH activity were found in the sera of normal children than in healthy adults.
3. Serum LDH activity and crystalline LDH were inhibited by mercuric chloride, p-chloromercuribenzoate, and an isomolar mixture of mercuric chloride and DPNH. The inactivated enzyme can be reactivated by sulfhydryl compounds but not by versene.
4. Ascitic fluid and cells from rats bearing the Yoshida sarcoma and AA sarcoma and from mice with Ehrlich ascites tumor exhibited high levels of LDH activity, the cells having much higher activity than the fluid. Erythrocytes suspended in isotonic saline failed to cause an appearance of LDH activity in the salt solution, whereas a suspension of Yoshida sarcoma cells under similar conditions resulted in the appearance of considerable LDH activity in the saline.

**ACKNOWLEDGMENTS**

The author wishes to express his appreciation to Dr. Eugene Roberts for his generous support and for his aid and suggestions in the preparation of the manuscript; to Dr. Howard Bierman and the Medical Staff for providing blood from patients with neoplastic disease; to Dr. Robin Smith, Canoga Park, California, for blood samples from apparently healthy children.

**REFERENCES**

HILL—Serum Lactic Dehydrogenase


Announcements

Publication of Cancer Chemotherapy Screening Data

An Editorial Committee is now preparing another Supplement to Cancer Research devoted to screening data similar to Supplements 1 and 2 published previously. Investigators are invited to submit data. The Editorial Committee calls attention to the fact that data are solicited on all biological and biochemical systems which are designed to uncover agents useful in cancer chemotherapy. These include effects on animal tumors, tissue culture, microbiological systems, biochemical systems, growth of tumors in heterologous hosts, etc. Data on clinical experience are also solicited.

The next issue will include a cumulative empirical formula index including entries from Supplements 1 and 2. It is hoped that the inclusion of a cumulative index in the next and succeeding issues will make the specific information in these supplements more readily accessible.

The same format as in the previous supplements will be used in the forthcoming issue. It is strongly suggested that the actual evaluation measures employed in the procedure, viz., the ratio of tumor sizes or survival times (treated/control) be included whenever feasible, even though the materials tested are considered to be negative. Investigators are requested to follow the instructions outlined in the previous announcement (Cancer Research, 14:409, 1954).

Since one of the objectives for publishing these supplements is to provide a rapid exchange of information in this area and to provide a broader basis for eliciting structure-activity relationships, the Committee invites data on chemical agents of varying degrees of activity as well as on strictly negative agents. This should not prejudice the publication of such data in more detailed form in regular issues of other scientific publications. The Committee also solicits data on combination therapy where multiple agents are employed.

Data for publication in the next issue will be accepted until October 1, 1956. The Committee reserves the right to omit any data which provide inadequate information. The material may be submitted directly to the Editor of the forthcoming issue, Joseph Leiter, Cancer Chemotherapy National Service Center, National Cancer Institute, Bethesda 14, Maryland, or to any one of the Associate Editors listed below.

RALPH K. BARCLAY Sloan-Kettering Institute for Cancer Research 410 East 68th Street New York 21, New York ARTHUR FRIEDMAN Department of Pharmacology and Therapeutics Stanford University School of Medicine San Francisco 15, California

PUBLIC HEALTH SERVICE NOTICE

The Public Health Service has announced a new procedure to expedite the processing of research grant applications for those requests which do not exceed $2,000 plus indirect costs and which do not ask support for more than one year. Such applications will be accepted and processed on receipt and are not therefore subject to the usual deadlines for submission prior to review.

Council recommendations can be expected on these applications within 1—4 months from the time of submission. These procedures do not apply for requests for supplements to existing grants.

Address all applications as well as requests for forms or additional information to the Division of Research Grants, National Institutes of Health, Bethesda 14, Maryland.
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