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## Clearance of Plasma Enzymes in Normal and LDH Agent Infected Mice.* (29578)

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The presence of a viral-like agent in many transplanted mouse tumors which causes elevation of plasma lactic dehydrogenase (LDH)† is now well established(1-5). The agent can be transmitted through many generations in the absence of a tumor by injection of infectious plasma alone. This is followed by a 5- to 10-fold increase in the plasma LDH beginning in 30 hours and persisting through the lifetime of the animal. The mechanism of elevation of LDH enzyme is not known. Explanations which have been proposed but not yet substantiated include:

1) Destruction of some target organ or tissue releasing LDH to plasma; 2) Increased cellular permeability to enzyme; 3) Decreased clearance of enzyme from plasma; 4) Overproduction of enzyme.

1) and 2) above presumably would entail elevation of additional enzymes in plasma. However, we have pointed out previously(7) that without knowledge of the clearance rate of different enzymes, the degree of elevation could not be predicted. This paper describes experiments in which the clearance of LDH

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† Abbreviations: LDH—lactic dehydrogenase; MDH—malic dehydrogenase; PHI—phosphohexose isomerase; GOT—glutamic-oxalacetate-transaminase; GPT—glutamic-pyruvic-transaminase; and RBC, WBC—red and white blood cells, respectively.

and certain other plasma enzymes was measured in normal and infected mice to test certain of these proposals.

*Materials and methods.* Original source of the agent was CD/5 lymphoma carried in BALB/c mice and kindly supplied by Dr. Morris Barrett, National Cancer Institute. The agent was maintained in tumor-free Swiss Webster white mice by intraperitoneal injection of 0.01 ml infectious plasma. Mice were bled from the retro-orbital plexus by the method of Riley(12). Measurements of plasma LDH, MDH, GOT and GPT were performed by methods described previously (11). Infection was confirmed by a 5-10 fold rise in plasma LDH, 2 to 4 days following infection.

*Measurement of plasma clearance rate.* Packed blood cells from Swiss Webster mice, hemolyzed in 2 volumes of distilled water, were used as the source of enzyme. For intravenous injection through the tail vein, mice were anesthetized lightly with diethyl ether and held in a mouse warmer (Nuclear Ohio, Inc.). Between 0.1 and 0.3 ml of hemolyzed blood was injected using a #27 gauge needle. For intraperitoneal injection, the solution was diluted with 3 volumes of saline and between 0.2 and 1 ml used. Enzyme content of the hemolyzed RBC's used was as follows: LDH, 34,000; MDH, 46,000; and GOT, 16,700 units per ml of packed cells, respectively. Control animals received similar amounts of saline. Normal mice were stock Swiss Webster, white, 3-6 months old and of mixed sexes. Experimental mice had been infected from 5 days up to 3 weeks previously with .01 ml of plasma containing the CD/5 lymphoma LDH agent. Following administration of hemolyzed blood cell enzymes, mice were bled at frequent intervals for the first 8 hours and at daily intervals thereafter.

*Results.* Plasma enzymes increased rapidly following intravenous injection of hemolyzed RBC's, reaching maximum levels usually within 2 hours (Fig. 1, 2). When injection was made intraperitoneally, the rise in plasma enzymes was slower, but reached peak levels which did not differ considerably from those attained using the intravenous route (Fig. 1).

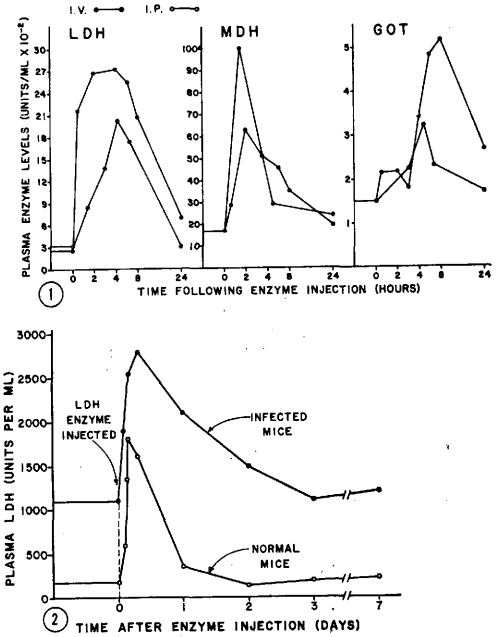


FIG. 1. Plasma enzyme levels in normal mice following intravenous or intraperitoneal injection of hemolysed red blood cells. Mice (Swiss Webster strain) were injected with isologous hemolysed mouse RBC's either intravenously (i.v.) through the tail vein, or intraperitoneally (i.p.) using a #27 gauge needle. Mice were bled at intervals from the orbital plexus and plasma enzymes were determined as described in Methods. Curves represent average values for the following numbers of mice: LDH i.v. (7), i.p. (4); MDH i.v. (3), i.p. (2); and GOT i.v. (4) and i.p. (4).

FIG. 2. Clearance of administered lactic dehydrogenase in normal and LDH agent infected mice. Enzyme (source was diluted hemolysed RBC's from a Swiss Webster mouse) was injected intravenously into the tail vein of 6 normal and 6 infected Swiss Webster mice. Mice were bled at intervals and plasma LDH level was measured as described in text.

*Clearance of LDH in infected mice.* Plasma LDH in normal mice rose from an initial value of about 180 units/ml to a peak of about 1800 units at 3½ hours, then was cleared rapidly, falling to 350 units/ml in 24 hours (Fig. 2). Initial values in infected mice were 1050 units per ml, rising rapidly after injection to a peak value of 2800 units at 7 hours. The enzyme was cleared more slowly in infected mice, not returning to the original (elevated) level of 1100 units for 2-3 days. The average clearance rate in infected mice was only 32 units/ml/hr, compared with 64

TABLE I. Plasma Enzyme Clearance Rates in Normal and LDH Agent Infected Mice.

| Enzyme | Normal | | | | Infected | | | |
|---|---|---|---|---|---|---|---|---|
| | LDH | MDH | GOT | GPT | LDH | MDH | GOT | GPT |
| No. of mice | 11 | 66 | 49 | 18 | 14 | 43 | 38 | 16 |
| Plasma level (units/ml)* | 185 ±25 | 1760 ±95 | 240 ±14 | 60.0 ±8.9 | 1070 ±84 | 2670 ±110 | 208 ±18 | 65.1 ±7 |
| No. of mice | 11 | 8 | 6 | — | 14 | 10 | 10 | 10 |
| Clearance rate (units/ml/hr)* | 64 ±8 | 98 ±27 | 10.6 ±2.9 | — | 32 ±6 | 58 ±10 | 5.1 ±.3 | 1.4 ±.1 |

* ± Standard error.

Numbers of control or LDH agent-infected Swiss Webster mice were injected either i.p. or i.v. with hemolysed RBC's (from Swiss Webster donor mice) as an enzyme source. Plasma enzyme levels were measured at intervals as described in text. Clearance rate was calculated where possible over the range 2-3 fold elevated falling to normal levels.

TABLE II. Plasma LDH Rise in Swiss Webster Mice Following Intraperitoneal Injection of Hemolysed RBC's from Different Species.

| RBC donor relationship | Mean plasma LDH, units/ml | | Mean plasma "hemoglobin" | |
|---|---|---|---|---|
| | Before inj | After inj | Before | After |
| Swiss Webster mouse (autologous) | 150 | 449* | 12 | 14 |
| " " " (isologous) | 180 | 461* | 14 | 16 |
| DBA/2 mouse | 145 | 405 | 15 | 64 |
| Rabbit | 208 | 431 | 17 | 45 |
| Human | 141 | 347 | 14 | 54 |

Each pair of Swiss Webster host mice was bled at zero time, then received 0.1 ml hemolyzed RBC's in 1 ml saline intraperitoneally, from the stated donor. Blood samples were taken 2 hr or 8 hr* later as indicated. LDH and "hemoglobin" content were measured as described in text.

units/ml/hr in normal controls (Table I).

*Clearance of MDH, GOT and GPT.* Initial plasma levels of MDH in infected mice showed a small (50%) but significant elevation above normal. Initial levels of GOT and GPT were not significantly different from those of uninfected controls. Enzyme levels increased in plasma following either i.v. or i.p. injection (Fig. 1). The rise in GOT following i.v. injection was slower than for either LDH or MDH. Clearance of both MDH and GOT from plasma of infected mice (58 and 5.1 units/ml/hr) was significantly slower than in normal mice (98 and 10.6 units/ml/hr) (Table I).

*Plasma LDH levels following i.p. injection of RBC's from different species.* Pairs of Swiss Webster mice were injected i.p. with 0.1 ml of hemolyzed RBC's from either Swiss Webster or DBA/2 strain mice, rabbits or human donors (Table II). Plasma LDH and hemoglobin were elevated in all mice within 2-8 hours following injection.

*Discussion.* Administration of enzyme intravenously through the tail vein gave a more rapid increase in plasma than did intraperitoneal administration. The intravenous method, however, was subject to considerable variation due to formation of blood clots in the tail which were dissipated only slowly. Clearance of intravenously administered enzyme from plasma probably depends upon a rapid tissue equilibration followed by a slower decrease which represents true clearance. Because of the variabilities in administration *via* the tail vein, more consistent measurements of clearance can be made following intraperitoneal injection. Also because of the requirement for passage from the peritoneal cavity into plasma, much of the tissue equilibration phase is included in the time taken to reach maximum plasma levels. The subsequent decrease of enzyme in plasma following i.p. injection therefore gives a closer approximation to the true clearance rate than that immediately following i.v. injection.

Interpretation of the clearance of GOT was complicated by unexplained increases in the enzyme observed in control mice injected i.v. with saline. This resulted possibly from tissue damage associated with the injection procedure or with the intra-orbital bleeding technique, and has been noted previously (11). In some instances also, particularly following i.p. injection (Fig. 1), the total MDH found in plasma was greater than the amount injected.

Passage of intraperitoneally administered red blood cell enzymes into plasma appeared to be a general phenomenon. It was noted with all combinations of relationships (autologous, isologous, homologous and heterologous) of donor and subject tested (Table II), and was not related therefore to the "self recognition" process. It represents a rapid and fairly quantitative passage of intact, functionally active proteins across membrane barriers in the host animal from the peritoneal cavity into the systemic circulation.

Administered LDH from homologous RBC's was cleared at about 64 units/ml/hr from plasma of normal mice, and the plasma level returned close to normal within 24 hours. Clearance from infected mice was much slower (32 units/ml/hr) and plasma levels did not return to the original (elevated) level for 48 hours (Fig. 2). A similar impairment of clearance of MDH and GOT in infected mice was also found (Table I).

*Proof that tissue destruction is not the source of additional enzyme in infected mice.* In addition to suggesting an alternative explanation for the increase in plasma enzymes following infection, the magnitude of the observed enzyme clearance rates also eliminates the possibility that tissue necrosis could provide the increased plasma enzyme, as shown by the following arguments: From the measured clearance in normal mice the rate of excess LDH production by infected mice required to maintain an elevated plasma level would be approximately 1500 units per 24 hours. From previously published figures on the total body pool of LDH present in various organs or tissues (Bailey *et al*(7)), the degree of tissue necrosis (or tissue leakage of LDH) required to provide this in 24 hours is as follows: brain (11%), heart (20%), kidney (20%), lung (10%), spleen (19%), WBC's (45%), liver (0.85%), and RBC's (3.3%). The first 6 tissues (brain, heart, kidney, lung, spleen and WBC's) can be eliminated as a source of the LDH since no degree of necrosis of this magnitude is noted (9,11). Furthermore, kidney, heart and lung contain considerable amounts (2500-3500 units/g(6)) of GPT. No increase in this enzyme was noted and the clearance rate observed (1.4 units/hr) (Table I) is too low to mask releases of this magnitude. Liver can also be eliminated as a source because of its high content of GPT (106,000 units/g) (6).

The red blood cell content of GOT is 16,750 $\pm$ 1260 units per ml(11). Red blood cell hemolysis required to liberate 1500 units of LDH per 24 hours (3.3%) would also liberate therefore 534 units of GOT.‡ The clearance of GOT in normal mice (10.6 units/hr), however, would not mask this amount of released enzyme. In addition, Plagemann *et al*(6) and Notkins *et al*(8) have shown that plasma isocitric dehydrogenase (ICDH) also is 7-8 fold elevated in infected mice (18 *vs* 140 units/ml). Red blood cell content of ICDH (57 units/ml(6)) is insufficient to provide this elevation. We have also shown (unpublished data) that the fragility of RBC's from infected mice as measured by resistance to osmotic shock, does not differ significantly from that of normal RBC's. These arguments taken in conjunction with the apparently normal histological picture in infected mice(11) would appear therefore to invalidate the hypothesis that virus-induced necrosis of any tissue or blood cell type is the source of increased enzyme in plasma.

*Impairment of clearance as explanation of elevated plasma enzymes.* Assuming that the lower plasma enzyme clearance rate in in-

‡ All calculations of this type refer to a 25 g mouse having a blood volume of approximately 8% of body weight.

fected mice is real,§ the following considerations could serve to explain the observations following infection with an LDH agent: It can be seen from the results (Table I) that LDH enzyme in plasma of normal mice has a much smaller half-life than either MDH or GOT. Based on the observed clearance rate of injected enzymes, the total LDH in normal plasma is cleared every 3 hours as compared to 18 hours and 22 hours for MDH and GOT, respectively. A nonspecific impairment of the normal clearance mechanism for plasma enzymes would therefore affect plasma LDH to a greater extent than the other enzymes. It is possibly significant in this respect that 2 of the enzymes showing major elevations during infection with LDH agent (LDH and ICDH)(6,8,9) are also those which have the highest tissue/plasma ratios in normal mice.

*Summary.* 1. Plasma enzyme levels in mice rose rapidly following either intravenous or intraperitoneal injection of enzymes from hemolyzed red blood cells. The increase following i.p. injection represented a rapid and fairly quantitative passage of functionally active proteins across membrane barriers in the host animal into the systemic circulation. It was independent of the RBC donor species. 2. Plasma clearance rates of injected LDH, MDH and GOT were measured in normal mice and in mice infected with an LDH agent from the CD/5 lymphoma. Clearance of exogenously administered enzymes was about double in normal as compared to infected mice. 3. Clearance data used in conjunction with comparative enzyme levels in infected mice eliminate the possibility that a nonspecific virus-induced tissue necrosis is the source of increased plasma enzyme. 4. It is concluded that the increase in certain plasma enzymes following infection with an LDH agent most probably results from an impairment of plasma enzyme clearance mechanisms.

§ The results might also be interpreted as an apparent impairment of clearance due to continuous overproduction of LDH in infected mice overloading the normal clearance mechanism. Notkins, however (personal communication), has also observed impaired clearance in infected mice when injected with much larger quantities of LDH than normally present in infected mouse plasma. It is concluded therefore that the observed impairment of clearance is real.

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