Infection of Tumor-Bearing Mice with the Lactic Dehydrogenase Agent. (27400)

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A close correlation between tumor size and plasma lactic dehydrogenase (LDH) was reported by Riley and Wroblewski(1). Further studies by these investigators uncovered a transmissible agent (factor) which was associated with 26 types of mouse tumors. This agent was capable of producing a 5- to 10-fold increase in plasma LDH activity within 48 hours after inoculation into normal mice (2). Recent work in this laboratory(3) demonstrated that the transmissible lactic dehydrogenase elevating agent was a contaminant which was being carried by many serially transplanted mouse tumors. Examination of the plasma from a number of tumor-bearing mice revealed several tumors that were not contaminated with the LDH agent (3). One of these, plasma-cell tumor SS (70429), was selected for further study. With this tumor it was possible to follow the changes in plasma activity of lactic dehydrogenase in a tumor-bearing animal that was not contaminated with the LDH agent. Such a tumor also made it possible to study changes in plasma enzyme activity following experimental infection with the LDH agent. The present report describes these enzyme changes.

Materials and methods. Animals. C3H HeN male mice, 6 weeks of age, were used for tumor implantation. CAF-1 males of the same age were used for transfer and titration of the LDH agent. *Tumor.* Plasma-cell tumor SS (70429) of C3H origin and specific to the C3H host(4) was kindly supplied by Dr. Morris Barrett of Nat. Cancer Institute. *Plasma.* Plasma for enzyme determinations was obtained by orbital bleeding as described by Riley(5). *LDH agent.* The 7th serial weekly intraperitoneal passage in normal CAF-1 male mice of plasma originally obtained from an animal which had been infected with the mouse Moloney leukemia virus and shown to carry the LDH agent as a contaminant(3), served as the source of the LDH agent. *Enzymes. Lactic dehydrogenase* was assayed as described by Wroblewski and LaDue(6). The method was slightly modified and adapted to the Bausch and Lomb Spectronic 505. The change from DPNH to DPN was measured at 340 mμ in the pyruvate to lactate reaction. One unit of activity is defined as the amount of enzyme that produces a decrease in O.D. of 0.001 per one minute. *Glutamic-oxalacetic transaminase* (GOT) was assayed by the method of Steinberg, Baldwin and Ostrow(7). The change from DPNH to DPN was measured at 340 mμ on the Bausch and Lomb Spectronic 505. One unit of activity is defined as the amount of enzyme that produces a decrease in O.D. of 0.001 per one minute at 25°C. All data are expressed as units of activity per ml of plasma. Enzyme determinations were performed with 20 μl or less of plasma.

Procedure. C3H/HeN male mice 6 weeks old were numbered and divided into 4 groups of 8 animals each. Groups III and IV received 0.025 ml of a 50% plasma cell tumor suspension in saline. The material was inoculated subcutaneously in the right flank. Groups I and II did not receive tumor. Three days later Groups II and IV were inoculated intraperitoneally with 0.1 ml of a 1 in 10 saline dilution of the LDH agent. Groups I and III received 0.1 ml of saline (0.85% NaCl) intraperitoneally. At intervals following injection of the LDH agent, animals were bled and both individual and pooled specimens of plasma were assayed for LDH activity. Only pooled specimens were assayed for GOT activity. Hemolyzed specimens were discarded. Weight of the animals was recorded throughout experiment and gross tumor size was noted. Actual tumor weight was not determined because of the undelineated growth of the plasma-cell tumor.

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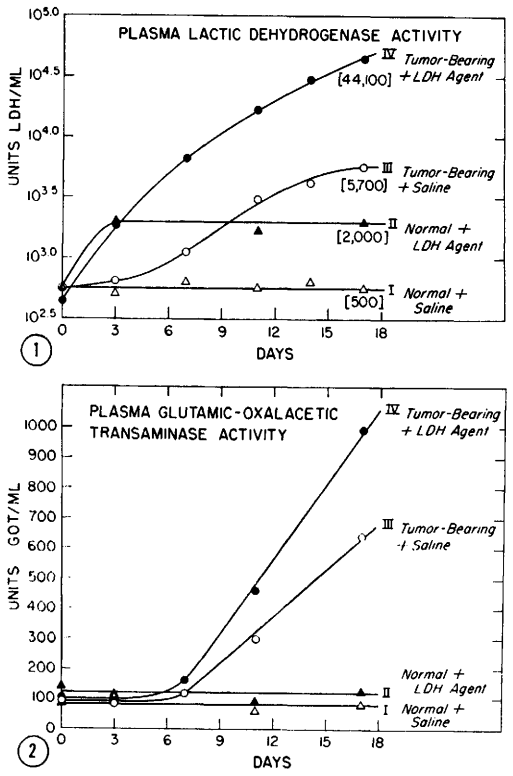

FIG. 1. LDH activity per ml of plasma (expressed logarithmically) in normal and tumor-bearing mice with and without the LDH agent. The LDH agent was inj. on day zero. Tumors were implanted 3 days earlier. Numbers in brackets represent the arithmetic units of LDH on day 17. The mean day of death of uninfected tumor-bearing mice was 18.3 ± stand. dev. 2.14. Mean day of death of infected tumor-bearing mice was 18.5 ± stand. dev. 3.4.

FIG. 2. GOT activity per ml of plasma (expressed arithmetically) in normal and tumor-bearing mice with and without LDH agent. Refer to text and legend of Fig. 1.

The second part of the experiment was designed to detect and titrate the LDH agent. Seventeen days after injection of the LDH agent, plasma from each of the 4 groups was collected and pooled. Serial dilutions in saline were made from each pooled specimen. 0.1 ml of each dilution was injected intraperitoneally into CAF-1 male mice. Three mice were used for each dilution. Four days after inoculation the animals were bled and their plasma assayed for LDH activity. A 4- to 5-fold increase in plasma LDH activity over normal was considered a positive transfer.

Results. Plasma LDH activity was followed over a period of 17 days (Fig. 1). Inoculation of the LDH agent into normal mice produced a 4- to 5-fold increase in LDH activity within 72 hours (Group II). This elevation persisted throughout the experiment. The uninfected tumor-bearing animals (Group III) showed a gradual increase in plasma LDH activity, which paralleled the gross increase in tumor size. On day 17, plasma LDH reached 5,700 units. The tumor-bearing animals which had been infected with the LDH agent (Group IV) showed the most dramatic rise in plasma LDH activity, reaching 44,100 units, 88 times greater than plasma LDH activity of the normal animals (Group I), 22 times greater than that of the infected non-tumor-bearing animals (Group II), and 8 times that of the uninfected tumor-bearing animals (Group III). Weight of the uninfected and infected tumor-bearing animals increased by 91.8% and 96.1% respectively, as compared to 39.5% and 26.0% for the uninfected and infected non-tumor-bearing mice.

Plasma GOT determinations were done on the same pools used to assay LDH (Fig. 2). Plasma GOT activity of animals which had been infected with the LDH agent (Group II) was slightly higher (and more clearly so in later experiments) than that of the uninfected animals (Group I). The uninfected tumor-bearing animals (Group III) showed a gradual but marked increase in plasma GOT activity. Twenty days following tumor implantation plasma GOT was 8 times that of the normal animals (Group I). Plasma GOT activity from tumor-bearing animals which had been infected with the LDH agent (Group IV) was 8 times that of the infected non-tumor-bearing animals (Group II) and slightly higher (55%) than that of the uninfected tumor-bearing animals (Group III).

The second part of the experiment demonstrated that the transmissible LDH agent as of day 17 had not contaminated the normal (Group I) or uninfected tumor-bearing mice (Group III), but was still present in Groups II and IV (Table I). However, the plasma LDH activity of the infected tumor-bearing animals (Group IV) had reached 44,100 units, while that of the infected non-tumor-bearing animals (Group II) was only 2,000

TABLE I. Transfer and Titration of LDH Agent.

Group		Units LDH/ml donor plasma on day of transfer	Transfer of LDH* agent (donor plasma) to normal recipients	ID_{50} of donor*† plasma
I.	Normal + saline	500	0	
II.	Normal + LDH agent	2,000	+	$10^{-4.8}$
III.	Tumor-bearing + saline	5,700	0	
IV.	Tumor-bearing + LDH agent	44,100	+	$10^{-4.8}$

* Pooled donor plasma from each group was serially diluted starting at 10^{-1} and 0.1 ml of each dilution was inj. intraper. into 3 recipient mice. Four days later plasma was obtained from the recipients and assayed for LDH activity. A 4- to 5-fold increase in LDH activity over normal was considered a positive transfer (+). A transfer was considered negative if the plasma LDH activity of the recipient remained within the normal range (0).

† The ID_{50} (dose which infects 50% of the animals) was determined by the method of Reed and Muench(9).

units. Therefore, it was important to see if this difference in enzyme activity was due to a difference in titer of the LDH agent. Dilutions of donor plasma from Groups II and IV, injected into normal recipient mice, failed to reveal a difference in plasma titer of the LDH agent. Another experiment showed that a 10^{-6} dilution of the LDH agent produced the same increase in plasma LDH activity as that which occurred after a 10^{-1} dilution. The assay was performed 96 hours after injection of the LDH agent.

Discussion. Following implantation of the plasma cell tumor that was not contaminated with the LDH agent, plasma LDH and GOT activity increased 11- and 8-fold respectively by the end of experiment (compare Groups I and III, Fig. 1 and 2). Experimental infection of the tumor-bearing animal with the LDH agent produced a further 8-fold increase in plasma LDH activity, but a relatively small increase (55%) in GOT activity (compare Groups III and IV, Fig. 1 and 2). Infection of the normal animal with the LDH agent similarly produced a small increase in plasma GOT activity (about 50%) but a 4- to 5-fold increase in LDH activity. Thus, both the LDH agent and the tumor without the agent are capable of increasing plasma activity of LDH and GOT, but differ in the magnitude of the increase. A number of other enzymes are now under study. Preliminary experiments have revealed an increase in plasma activity of malic dehydrogenase, phosphohexose isomerase, and isocitric dehydrogenase following injection of the LDH agent into normal animals.

The 88-fold increase in plasma LDH activity of the infected tumor-bearing animals opens interesting areas for speculation and investigation. Riley(8) recently reported a marked elevation in plasma LDH following inoculation of his LDH agent into an uninfected pituitary tumor-bearing animal. This elevation was considerably higher than that which could be accounted for additively by the plasma LDH activity of the infected non-tumor-bearing animal and that of the uninfected pituitary tumor-bearing animal. Riley concluded that a virus-tumor synergism therefore existed. Plasma LDH activity from our infected plasma-cell tumor-bearing animals on day 17 (44,100 units) was also higher than that which could occur by simply adding the enzyme activity of the infected non-tumor-bearing animals (2,000 units) to that of the uninfected tumor-bearing animals (5,700 units). To explain the higher plasma LDH activity of the infected tumor-bearing animals (IV) as compared to the infected non-tumor-bearing animals (II), the relationship between plasma LDH activity and titer of the LDH agent was investigated. Dilution experiments failed to reveal a difference in plasma titer of the LDH agent in the 2 groups (II and IV), even though there was a 22-fold difference in enzyme activity. Thus, there is no evidence that the higher plasma LDH activity of the infected tumor-bearing animal is due to a higher titer of the LDH agent. Before examining other possible explanations one should consider the possible mode of action of the LDH agent in the normal animal. The first and most likely explanation for the

increase in plasma LDH activity in the normal animal is that the LDH agent destroys, damages, or alters the permeability of cells. A second possibility is that the LDH agent increases enzyme production by altering the metabolism of cells. Thirdly, the LDH agent may increase the activity of already existing enzyme or activate a proenzyme. Lastly, the LDH agent may impair the rate of clearance of an enzyme from the plasma. Since the LDH agent acts in the non-tumor-bearing animal, there is no reason to assume that it acts through a different mechanism in the tumor-bearing animal. It is more likely that in the tumor-bearing animal infection with the LDH agent results in higher plasma enzyme activity due to a greater abundance, susceptibility, or enzyme content of the target cells. Experiments are being designed to test some of these possibilities.

Summary. Normal and tumor-bearing mice were experimentally infected with the LDH agent and followed for changes in plasma LDH and GOT activity. 1) Injection of the LDH agent into normal animals produced a 4- to 5-fold increase in plasma LDH activity within 72 hours and a small increase in plasma GOT. 2) Plasma from animals bearing the uninfected plasma-cell tumor SS (70429) showed a gradual increase in both LDH and GOT activity which paralleled the gross increase in tumor size. Transfer experiments failed to reveal a transmissible LDH agent. 3) The tumor-bearing animals which had been infected with the LDH agent showed up to 8 times more plasma LDH activity than the uninfected tumor-bearing animals and up to 88 times more activity than the normal animals. 4) Up to a 22-fold difference in plasma LDH activity was noted between the infected non-tumor-bearing animals and the infected tumor-bearing animals. Serial dilution of plasma from these 2 groups failed to reveal a difference in titer of the LDH agent.

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Experimental Cholelithiasis in the Guinea Pig.* (27401)

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In addition to many other biologic effects, the occurrence of flocculant precipitate and small firm masses in gall bladder bile followed parenteral injection of Klebsiella polysaccharide in the guinea pig(1). The initial gall bladder distension and apparent stasis, the edema of the gall bladder and adjacent structures resembled acute cholecystitis in man. Following intravenous injection of C^{14}-labeled polysaccharide into guinea pigs, considerable label was found within protein-rich ascitic and retroperitoneal fluid, and label appeared in the bile(2). It was not known whether the label in bile represented excreted metabolites of the injected polysaccharide or the exudation of polysaccharide-containing

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