TABLE III. Effect of Pertussis Vaccine on Hemorrhagic Necrosis of Tumors Induced by Anaphylactic Shock.

Anaphylaxis induced by horse serum (ml/animal)	Untreated	Treatment before induction of anaphylaxis*			
		Implant. tumor		Implant. tumor + pertussis v.	
	Survivors	Survivors	Tumor necrosis	Survivors	Tumor necrosis
.001	4	10	8	10	7
.005	2	8	7	7	7
.01	0	7	7	6	5
.05	0	5	5	4	4
.1	0	2	2	1	1

* Each group consisted of 10 mice.

served when the hemorrhagic necrosis was induced by anaphylaxis, since under the latter condition pertussis vaccine appeared to have no effect on the outcome of the reaction. It is to be noted that pertussis treatment affects the reactivity of the animals to a variety of substances such as histamine and serotonin as well as to endotoxin(6). Pertussis can also increase susceptibility of the animals to anaphylactic shock and can be used as an adjuvant for production of immune antibodies(2). Thus it is not clear which factors are responsible for the alterations in reactivity to endotoxin following pertussis treatment.

Summary. Pertussis-sensitized mice were tested for their reactivity to endotoxin as expressed by lethality and induction of hemorrhagic necrosis in implanted tumors. It was found that, whereas pertussis treatment increased the susceptibility of animals to the lethal effect of endotoxin, transplanted tumors became more resistant to its necrotizing effects. Induction of tumor necrosis by an anaphylactic reaction was not affected by pertussis treatment. Tumor-bearing mice injected with pertussis vaccine did not become more susceptible to the lethal effects of endotoxin than did animals not bearing tumors.

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Factors Which Affect Plasma Lactic Dehydrogenase in Tumor-Bearing Mice.* (28408)

R. N. ARISON, J. A. CASSARO, AND C. E. SHONK (Introduced by G. E. Boxer)
Merck Sharp & Dohme Research Laboratories, Division of Merck & Co., Inc., Rahway, N. J.

Increased activity of plasma lactic dehydrogenase (LDH) in neoplastic diseases has been observed in human subjects as well as in experimental animals(1,2,3,4,5). Although this phenomenon is encountered in a wide variety of disease states and is therefore by no means diagnostic of cancer, the possible significance of this manifestation in carcinogenesis has been of considerable interest. One or more of the following mechanisms has been suggested to explain the elevation of this enzyme during tumor development: 1. Tissue damage which accompanies tumor growth leads to release of LDH from cells (6). 2. Necrosis of neoplastic tissue results in liberation of LDH into intercellular fluid

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and plasma(6). 3. Greater permeability of tumor cell membrane allows seepage of LDH (6,7). 4. It may be an indication of metabolic changes in the body(3). 5. It is caused by a specific agent(s), possibly viral in nature(8).

There is general agreement that each of the first 4 mechanisms contributes at least in part to the rise in plasma LDH in the presence of neoplasia(3,5,6,7). The last item in the above list has been proposed by Riley (8), who observed that a transmissible agent, capable of raising the plasma LDH level, was associated with many transplantable mouse tumors. Blood and organ extracts of tumor-bearing mice, when inoculated into non-tumor-bearing animals, caused a rise in plasma LDH activity of recipients within 48 hours. The effect was much more marked, however, in tumor-bearing recipients. The "LDH factor" persisted in mice in which tumor had regressed completely. Plasma and tissue fluid passed through bacterial filters retained their ability to elevate plasma LDH. These findings led Riley to suggest that the "LDH factor" may be a virus, and the enhanced effect produced in tumor-bearing animals was called "virus-tumor synergism"(9).

The concept of "LDH factor" has aroused considerable interest, and has become widely accepted. Many investigators believe that the factor is a contaminant commonly associated with transplantable tumors but not comprising an integral part of them(10,11,12, 13). They do not, however, question the likelihood of the factor being viral in nature, and have confirmed the synergistic relationship between the factor and tumors.

Following our observation that infection by *Hemobartonella muris* may lead to tumor destruction in rats, it has been our practice to examine the blood of our stock and tumor-bearing animals for possible parasitization. By light microscopy, the blood of our stock mice was found to be free of obvious infection, but mice implanted with a variety of transplantable tumors were infected, often heavily, with *Eperythrozoon coccoides*. These tumors were: sarcoma 180, Ehrlich carcinoma, Krebs 2, and one line of the Lettré variant of Ehrlich carcinoma. Another line of the Lettré tumor, obtained originally from a different source, was the only one which was free of Eperythrozoon. Transfer of ascites fluid from infected donors resulted in the appearance of Eperythrozoa in the blood of recipients.

Since Eperythrozoa parasitize erythrocytes, it seemed likely that destruction or alteration of cell surface of red cells may contribute significantly to the rise in LDH in infected mice. The possibility that the organisms themselves may add to the level of plasma LDH was also considered. The experiments to be described were undertaken to study the effect of Eperythrozoon infection on the activity of plasma LDH in mice, with or without tumors.

Materials and methods. Female Swiss mice weighing 20 $\pm$ 2 g, divided into groups consisting of not less than 10 animals each, were subjected variously to the following treatments:

1. Implantation of infected tumors: Mice bearing sarcoma 180, Ehrlich carcinoma, and the Lettré variant of Ehrlich tumor, all in the ascites form, were used as donors. After examination of blood smears to ascertain that the donors were infected with Eperythrozoon, ascites fluid was drawn and diluted with 3 volumes of Gey's solution. 0.5 ml of the diluted fluid containing 5 to 10 $\times$ 10^6 cells was inoculated subcutaneously or intraperitoneally into each recipient.

2. Implantation of "clean" tumors: Tumor-bearing donors were rendered non-infectious by injecting them subcutaneously with 0.5 mg 2-amino-4-arsenosophenol hydrochloride immediately following inoculation with ascites fluid. Blood smears taken at intervals following arsenotherapy were negative for Eperythrozoon. The treatment had no apparent effect on the development of either the ascites or the solid form of tumor. By transferring ascites fluid from treated donors to fresh recipients, a "clean" line of donors was maintained.

3. Transfer of infected blood: Blood, obtained by cardiac puncture of infected donors, was heparinized and injected either intravenously or intraperitoneally into recipients.

4. Splenectomy: Removal of spleen, which

TABLE I. Effect of Tumor Implanation, Splenectomy, and Eperythrozoon Infection on Plasma LDH in Mice.

	Days after treatment								
	3 – 5			6 – 7			8 – 13		
	No. of groups*	Eperythrozoon in blood†	LDH, U/ml	No. of groups	Eperythrozoon in blood	LDH, U/ml	No. of groups	Eperythrozoon in blood	LDH, U/ml
Intact									
Controls	7	0	.8	6	0	1.0	4	0	.7
Infected tumor	7	+++	5.3	10	++++	7.2	2	++++	10.7
Treated tumor	9	0	3.7	4	0	3.0	2	0	7.7
Infected blood	7	++++	5.4	3	±	3.4	2	±	3.3
Treated blood	2	0	3.0	3	0	1.3	2	0	.7
Splenectomized									
Controls	3	0	1.1	3	0	1.1	2	0	.8
Infected blood	2	++++	8.8	3	++++	6.0	2	++++	6.6
Treated blood	1	0	3.3	3	0	2.0	2	0	.7

* Each group = minimum of 8 mice.
† ++++ = 10 or more per RBC; +++ = 1 to 5 per RBC; ± = 1 or less per oil immersion field.

enhances proliferation of Eperythrozoon, was accomplished under ether anesthesia. Tumor implantation or injection of blood was performed either immediately following operation or one day later.

Blood smears were prepared from each animal at intervals, and the severity of Eperythrozoon infection was expressed in terms of average number of organisms per erythrocyte.

Collection of blood for determination of LDH activity was made by cardiac puncture without anesthesia, using heparinized syringes. LDH activity was determined by a procedure previously described(14). The results are expressed in international units; *i.e.*, micromoles of substrate utilized per minute.

In a separate series, the effects of infection and splenectomy were studied in non-tumor-bearing mice. One hundred eight mice were segregated into 3 groups of 36 mice each. One group was given intraperitoneal injection of whole blood containing Eperythrozoa. Another group was inoculated with blood from animals treated with 2-amino-4-arsenosophenol hydrochloride. The third group was untreated. At 2 and 5 days following treatment, 6 mice from each group were bled at each interval. Plasma from 3 mice was pooled to obtain 2 duplicate samples from each group. On the fifth day, one-half of the remaining animals in each group was splenectomized. Blood collection was performed on the seventh and twelfth days after infection.

Results. The LDH values and the approximate number of organisms in blood are presented in Table I. The results in tumor-bearing mice were obtained in 194 animals with Ehrlich carcinoma in the ascites form, 100 mice with sarcoma 180 in the solid form, and 36 mice with Lettré tumor in the ascites form. Since the LDH values were comparable in all groups, composite figures are presented in the table. The data for non-tumor-bearing mice were obtained in 316 mice.

It may be seen that, within 3 to 5 days after tumor implantation, plasma LDH was significantly elevated. During the same period, the animals implanted with fluid from donors treated with 2-amino-4-arsenosophenol hydrochloride exhibited lower LDH activity. The same magnitude of increase was found in mice, both intact and splenectomized, which had been inoculated with blood from treated donors. Injection of infected

TABLE II. Effect of Eperythrozoon Infection and Splenectomy on Plasma LDH of Non-Tumor-Bearing Mice.

Treatment	Plasma LDH (U/ml) Day 2	Day 5	Day 7	Day 12
Infected blood	1.7	3.5	3.3	3.3
Treated "	.5	.6	1.4	.7
No treatment	.7	.7	1.0	1.8
Infected blood, splenectomized on day 5	—	—	6.6	6.6
Treated blood, splenectomized on day 5	—	—	1.3	2.5
No treatment, splenectomized on day 5	—	—	1.3	.8

blood caused a rise in plasma LDH almost identical with that in infected tumor-bearing animals. Splenectomized mice infected with blood containing Eperythrozoa showed a marked rise in plasma LDH. Splenectomy alone had no effect on plasma enzyme level.

During the next few days, the plasma LDH of infected tumor-bearing mice continued to rise. In contrast, the enzyme level in mice bearing treated tumors remained unchanged. LDH activity in animals injected with blood showed a drop during this period. In splenectomized, infected mice, LDH level remained high.

After one week, the plasma LDH activity of all tumor-bearing mice was increased, whether Eperythrozoon infection was present or not, although the average level was still higher in infected mice. The enzyme activity in intact mice injected with infected blood remained at the slightly elevated level; while in animals given treated blood, LDH activity had subsided to a normal level. Splenectomized mice given infected blood continued to show a high LDH activity.

Table II shows data obtained in non-tumor-bearing mice in which splenectomy was performed subsequent to infection. It may be seen that intact mice given infected blood show a moderate increase in plasma LDH, which is maintained at this level. Upon splenectomy, plasma LDH activity is abruptly elevated, and remains high. Splenectomy in untreated animals and in mice injected with treated blood had little effect on plasma LDH.

Autopsy of animals inoculated with ascites tumor revealed that, in the presence of Eperythrozoon infection, the liver contained grossly visible areas of necrosis, and frequently the viscera were covered with fibrinosis exudate.

Discussion. Our finding that administration of an arsenical results in lowering of plasma LDH in tumor-bearing animals suggests that an arsenic-sensitive agent, which is only incidentally associated with transplantable tumors, contributes to the early rise in plasma LDH. It appears that, in the animals used in our study, this agent is probably *Eperythrozoon coccoides*. The effect produced by splenectomy supports this deduction. The frequently observed hepatic necrosis in infected tumor-bearing mice would almost certainly add to the rise in enzyme level. The LDH agent may not be necessarily a virus, since Eperythrozoa pass through millipore filters of 0.3 μ diameter (15).

The terminal rise in plasma LDH in tumor-bearing mice, with or without the infection, is attributable to tissue destruction accompanying the extension of tumor.

The phenomenon of "virus-tumor synergism" is also consistent with the effects produced by Eperythrozoon. When an intact mouse is infected with Eperythrozoon, the plasma LDH rises moderately, then remains stationary because of the clearing action of the spleen. This is observed in tumor-bearing animals also, during the early stage of tumor growth. With the continued growth of tumor, the ability to clear the blood of Eperythrozoa apparently becomes impaired, for the organisms persist in the blood (Table I). If, at this stage in tumor growth, the "LDH factor" should be introduced in animals heretofore uninfected, the result may be comparable to injecting Eperythrozoa into splenectomized animals, and the plasma LDH would rise markedly and abruptly.

The above report does not suggest that Eperythrozoon is the LDH factor, or that Eperythrozoon alone is responsible for the drastic rise in plasma LDH. There are doubtless other infections, some as yet unknown, which would affect the level of plas-

ma enzymes, particularly if hematologic disorders are involved.

Summary. 1. Administration of 2-amino-4-arsenosophenol hydrochloride to Eperythrozoon-infected mice bearing transplantable tumors resulted in a lowering of plasma LDH early in the course of tumor development. 2. Splenectomy of Eperythrozoon-infected mice resulted in a marked rise in plasma LDH. 3. One week or more after tumor implantation, plasma LDH was elevated in all mice, with or without the presence of demonstrable Eperythrozoon in the blood. 4. It is suggested that Eperythrozoon infection contributes to the early rise in plasma LDH in mice inoculated with transplantable tumors.

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