STUDIES ON THE TRANSMISSION AND THE EXCRETION OF THE LACTIC DEHYDROGENASE AGENT

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In 1960, Riley *et al.* (1) found a transmissible agent in the plasma of many tumor-bearing mice which upon injection into normal mice produced a 5- to 10-fold increase in the activity of the plasma enzyme lactic dehydrogenase. The enzyme elevation occurred within 72 hours after inoculation of this lactic dehydrogenase–elevating agent (LDH agent) and remained elevated for months despite the lack of gross pathology. Since that time Notkins *et al.* (2–5) showed that isocitric dehydrogenase, malic dehydrogenase, phosphohexose isomerase, and glutamic-oxalacetic transaminase were also elevated and that much of the increase in the activity of plasma enzymes which previously had been attributed to tumor growth was in fact due to contamination with the LDH agent. Similar findings have been reported by Plagemann *et al.* (6).

Recently, Notkins and Shochat (7) reported studies on the growth and properties of the LDH agent. They found that the titer of the LDH agent in mouse plasma reached $10^{10.8}ID_{50}$/ml within 24 hours after inoculation, dropped to $10^{7.8}ID_{50}$/ml within 96 hours, and remained at about $10^{4.5}ID_{50}$/ml for over 1 year. The present report is concerned with the excretion of the LDH agent in the urine, feces, and saliva and with its transmission from infected mothers to their offspring.

Materials and Methods

Animals.—Strain CAF-1 male mice 4 to 6 weeks old were routinely used. In the vertical transmission studies strain BALB/c females were mated with strain ALN males. Mice were obtained from the Animal Production Section of the National Institutes of Health.

LDH Agent.—The source and passage of the LDH agent was described earlier (2, 3). The virus pools employed throughout these studies were obtained from the plasma of mice 24 hours after intraperitoneal injection of a stock preparation of the LDH agent. The infected plasma was distributed in individual vials, sealed under vacuum, and stored at −55°C. The titer was approximately $10^{10.5}ID_{50}$/ml.

Lactic Dehydrogenase Enzyme Assay.—The lactic dehydrogenase activity of the plasma was assayed by the method of Wroblewski and LaDue (8). Plasma was obtained from mice by

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orbital bleeding with heparinized micropipettes (9) and stored at 4°C until assayed. Hemolyzed specimens were not used.

Titration of the LDH Agent.—The procedure used to determine the number of infective units of the LDH agent in a given preparation was described elsewhere (7). In brief, mice infected with the LDH agent showed a 5- to 10-fold increase in the activity of the plasma enzyme lactic dehydrogenase within 72 hours after infection. The titer in a given preparation was determined by making serial tenfold dilutions of the original material in Eagle's minimum essential medium (10) with 20 per cent veal infusion broth (MEM-VIB) and injecting 0.1 ml of these dilutions intraperitoneally into normal mice. Five mice were used for each dilution. These mice were bled 72 to 96 hours later and the plasma was tested for the characteristic 5- to 10-fold increase in the activity of the enzyme lactic dehydrogenase. The number of animals infected at each dilution was determined and the dose which infected 50 per cent of the animals (ID_{50}) was calculated by the method of Reed and Muench (11) and expressed on the basis of 1.0 ml of material.

Collection and Preparation of Specimens.—Urine was collected in sterile test tubes by applying gentle pressure over the mouse's bladder. Specimens from 10 mice were pooled, diluted 1:30 in Eagle's MEM-VIB, and passed through a 450 mμ Millipore filter. The filtrate was diluted in tenfold steps in Eagle's MEM-VIB and the infective titer was determined. After the urine was collected, fecal pellets were obtained from the same 10 mice. The pellets were weighed, diluted 1:30 in Eagle's MEM, homogenized for 90 seconds with a Potter-Elvehjem tissue grinder, and centrifuged at 1500 *g* for 10 minutes. The supernatant fluid was centrifuged a second time at 1500 *g* for 10 minutes and the upper 75 per cent was removed and passed through a 450 mμ Millipore filter. The filtrate was diluted in tenfold steps in Eagle's MEM-VIB and the infective titer was determined. Saliva was obtained from the same mice by gently swabbing the buccal surfaces of the mouth with sterile cotton. Each swab was allowed to soak for 10 minutes in 1.0 ml of Eagle's MEM-VIB. The swab was discarded and the specimens were pooled. The pooled material was passed through a 450 mμ Millipore filter and the filtrate was designated as a 10^1 dilution. Serial tenfold dilutions were performed in the usual manner in Eagle's MEM-VIB and the infective titer was determined.

RESULTS

Titer of the LDH Agent in Urine, Feces, and Saliva.—Mice were injected intraperitoneally with a 10^2 dilution of a stock preparation of the LDH agent. At various intervals thereafter, urine, feces, and saliva were collected and the titer of the LDH agent in these materials was determined. As seen in Table I, the highest titer was found at 24 hours but decreased over the following days. Sixteen days after inoculation of the LDH agent less than $10^{2.5}ID_{50}$/ml of virus was found in the urine and at 28 days less than $10^{2.0}ID_{50}$/ml was present in the saliva. At the end of 135 days the titer of virus in the feces fell to $10^{2.5}ID_{50}$/ml.

Infection by the Oral Route.—Ten mice were placed in each cage and deprived of water for 24 hours. The mice were then given 20 to 50 ml of distilled water containing approximately $10^{6.4}$ to $10^{8.8}ID_{50}$/ml of the LDH agent. Most of the water was consumed within 6 to 12 hours. Earlier studies (7) showed that exposure to distilled water for a short time did not inactivate the LDH agent. As seen in Table II, 18 to 50 per cent of the mice became infected when exposed to over $10^{8.1}ID_{50}$/ml. However, none of the mice became infected when exposed to a lower concentration of virus ($10^{6.4}ID_{50}$/ml). Further studies (Table II) showed that 60 to 100 per cent of the mice became infected when 0.1 to 0.2 ml of material was delivered directly into the oral pharynx of each mouse with a soft plastic tube.

TABLE I

Titer of the LDH Agent in the Urine, Feces, and Saliva

Time after injection of LDH agent	ID_{50}/ml (log 10)		
	Urine	Feces	Saliva
days			
1	4.3	5.9	4.2
2	3.3	4.3	3.5
3	3.3	4.7	2.8
9	3.1	4.0	2.4
16	<2.5*	3.8	2.8
28	<2.5*	4.3	<2.0*
42	<2.5*	5.0	<2.0*
60	—	3.9	—
135	—	2.5	—

* Virus was not detected at these concentrations.

TABLE II

Infection of Mice by the Oral Route

Procedure and experiment No.	Approximate titer of LDH agent in water ID_{50}/ml (log 10)	Volume of infected material	No. mice infected / Total No. mice exposed	Infected
		ml Per 10 mice		*per cent*
Infected material placed in the water bottle of each cage*				
1	6.4	30	0/20	0
2	8.1	50	2/11	18
3	8.1	50	2/10	20
4	8.4	30	10/20	50
5	8.8	20	3/10	30
6	8.8	20	5/10	50
		Per mouse		
Infected material delivered directly to the oral pharynx of each mouse with a plastic tube				
1	9.0	0.1	12/20	60
2	8.8	0.2	10/10	100

* 10 mice per cage.

Transmission of the LDH Agent to Offspring.—As previously reported (7) mice infected with the LDH agent prior to mating rarely transmitted the LDH agent to their offspring. Additional experiments are reported herein. One week prior to mating both parents were injected subcutaneously in the back of the neck with $10^{7.5}ID_{50}$ of

TABLE III

*Transmission of the LDH Agent to Offspring**

Time of infection of mothers‡	No. litters with infected progeny§ / Total No. litters	No. progeny infected / Total No. progeny	Progeny infected
			per cent
Prior to mating‖	1/14	1/108	0.9
During gestation¶	17/17	62/68	91.2
Postpartum**	14/14	51/99	51.5

* CAF-1 offspring from BALB/c females mated with ALN males.

‡ Mothers received $10^{7.5}ID_{50}$ of the LDH agent subcutaneously in the back of the neck at the times indicated. Mothers and progeny were assayed for infectivity 2 to 3 weeks after birth.

§ All the mothers were shown to be infected with the LDH agent at the time that their progeny were assayed for infectivity.

‖ In this group both parents were infected with the LDH agent 1 week prior to mating and remained with their progeny throughout the experiment.

¶ Mothers infected between the 7th and 18th days of gestation.

** Mothers infected within 48 hours after giving birth.

the LDH agent. The parents and progeny were assayed for infectivity 2 to 3 weeks after birth. As seen in Table III, only 0.9 per cent of the progeny were infected. However, if the mothers were infected between the 7th and 18th days of gestation with the LDH agent, 91.2 per cent of the offspring were infected. On the other hand, if the mothers were infected with the LDH agent within 48 hours after giving birth, only 51.5 per cent of their offspring were infected.

DISCUSSION

The LDH agent was found in the urine, feces, and saliva of infected animals. The titer was highest at 24 hours but decreased to less than $10^{2.5}ID_{50}$/ml in the urine and the saliva within 1 month. At the end of 4½ months, $10^{2.5}ID_{50}$/ml of virus was still present in the feces. It was previously shown that the titer of the LDH agent in the plasma of infected animals was $10^{10.8}ID_{50}$/ml at 24 hours, decreased rapidly over the next 72 hours, but remained at about $10^{4.5}ID_{50}$/ml for many months thereafter (7). The concentration of virus in the urine, feces, and saliva therefore appears to be directly related to the decreasing titer of the virus in the plasma.

Infection by the oral route occurred when a high concentration of virus was used. A titer of $10^{6.4}ID_{50}$/ml failed to infect mice, while $10^{8.1}$ to $10^{8.8}ID_{50}$/ml infected 18 to 50 per cent of the animals. Although direct inoculation of the LDH agent into the oral pharynx of each mouse with a plastic tube infected 60 to 100 per cent of the animals, aspiration may have been partially responsible for this higher figure. Even though the LDH agent was recovered from the urine, feces, and saliva of infected mice, the above findings suggest that it was

present in concentrations lower than that required to infect cagemates by the oral route. This could account for the previously reported low rate of cross infection from infected to uninfected mice housed in the same cage (7).

If mothers were infected with the LDH agent within 48 hours after giving birth, 51.5 per cent of their offspring became infected. Since it was shown that mice could be infected by the oral route if given a high concentration of virus, the above findings suggest that the transmission of the LDH agent probably occurred through the mothers' milk. Transmission through the urine and feces of the infected mothers seems unlikely because of the low concentration of virus in these materials. Transmission by parenteral inoculation of infected saliva through biting cannot be excluded.

If the mothers were infected during pregnancy 91.2 per cent of the offspring became infected. This higher incidence of infected progeny from animals infected during pregnancy as compared to the progeny from animals infected prior to mating (0.9 per cent) or infected postpartum (51.5 per cent) suggests that the LDH agent crossed the placenta. Examination of the growth curve of the LDH agent (7) offers a possible explanation for the high incidence of infected offspring from animals infected during pregnancy and the low incidence of infected progeny from animals infected prior to mating. Since the plasma titer of the LDH agent reached $10^{10.8}ID_{50}$/ml within 24 hours after inoculation but dropped to $10^{7.2}ID_{50}$/ml by the end of 1 week, mice infected with the LDH agent 1 week prior to mating would have a relatively low titer during pregnancy while those infected during pregnancy would have a very high titer immediately thereafter. The titer of $10^{10.8}ID_{50}$/ml may have allowed a sufficient number of virus particles to pass through the placenta to infect the fetus. Thus, the transmission of a virus from an infected mother to her offspring appears to depend not only on the time of infection but also on the actual titer of the virus in the maternal circulation. Even in a chronically infected animal with a persistent viremia, vertical transmission may not occur if the concentration of the virus in the blood is low. On the other hand transmission through the placenta or by milk may occur in an acutely infected animal with a high blood titer. Failure by a number of investigators to demonstrate the vertical transmission of tumor and non-tumor viruses may have been due to the relatively low blood titer of these viruses during the gestation period.

SUMMARY

The lactic dehydrogenase agent (LDH agent) was found in the urine, feces, and saliva of mice within 24 hours after inoculation. The titer of virus in these materials appears to be directly related to the titer in the plasma. Infection by the oral route occurred only when a high concentration of virus was used.

Animals infected prior to mating rarely transmitted the LDH agent to their progeny. However, 91.2 per cent of the progeny of mothers infected during

gestation and 51.5 per cent of the progeny of mothers infected within 48 hours after giving birth became infected with the LDH agent. Evidence is discussed which suggests that the transmission of the LDH agent from the infected mother to her offspring is related to the titer of the LDH agent in the maternal circulation.

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