parallel phenomenon, the renal synthesis of urea, has been described(8). Confirmation that the kidney can also serve as a source of creatinine might reorient interpretation of available experimental observations.

Summary. In slices of rabbit kidney cortex, active transport of 4-aminohippurate was uninfluenced by creatinine even when the concentration of creatinine was 60 times greater than that of hippurate. Previously published claims that creatinine and 4-aminohippurate compete for a single transport mechanism are thereby refuted. A small but significant rate of synthesis of creatinine chromogen by renal tissue conceivably can serve as a renal source of creatinine during clearance measurements.

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Plasma Enzymes Erythropoiesis and R.E.S. Function in Mice Following Infection with an LDH Agent.* (28993)

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Riley(1) reported that a number of murine tumors were contaminated with a virus-like agent which could be propagated independently of tumor by serial passage of plasma from infected mice. The agent was detected only by a sharp rise of 5- to 10-fold in plasma lactic dehydrogenase level about 2 to 3 days following infection(2). General properties of the agent, as described by Riley, have been confirmed by a number of workers (3,4,5). An agent having certain similarities to LDH agent was reported by Old *et al*(6) in mice bearing sarcoma 180. This agent caused hyperactivity of the reticulo-endothelial system. No observations of plasma LDH were reported.

In a previous study(3) no difference in LDH enzyme content of a number of tissues of both normal and agent-infected mice was found. There was, however, a slightly but significantly lower hematocrit in infected mice which suggested red blood cells as a possible source of elevated plasma LDH.

As a possible means of identifying the target tissue for the LDH agent, therefore, it was of interest to determine levels of a number of different enzymes in plasma of mice both before and after infection with agent. Similar studies have recently been reported by Notkins and by Plagemann(9,10). Erythropoiesis in normal and infected mice was studied during an intensive bleeding schedule. R.E.S. function was examined by measuring rate of carbon clearance from plasma. Gross autopsy studies were made on fixed and stained sections of liver, kidney, brain, lung, spleen and thymus of infected mice.

Materials and methods. Source of LDH agent used in this work was CD-5 lymphoma carried in BALB/c mice.† Pattern of replication of agent, demonstration of virus-like nature and similarity to agent isolated by Riley have been reported(3).

Mice of BALB/c strain were bled from

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† Original tumor kindly supplied by M. K. Barrett, Nat. Cancer Inst., N.I.H.

orbital plexus following light anesthesia with diethyl ether using technique of Riley(8). Care was taken to avoid mechanical hemolysis. Hematocrit values were measured following centrifugation of blood under standard conditions (25 minutes at 1750 × *g*) in small heparinized sample tubes.

Plasma enzyme determinations. LDH was determined by modification of method of Wacker *et al*(7), described previously(3). Acid phosphatase, alkaline phosphatase, DFPA, ZF, MDH, GOT, GPT and SODH were determined using commercially available enzyme assay kits.‡ Methods used to determine activities of SODH, GOT, GPT and MDH were micro modifications of spectrophotometric procedures given in literature accompanying test-combinations. These were adapted for use with Beckman DB recording spectrophotometer. Activities of DPN- or TPN-linked enzymes studied (*i.e.*, LDH, GOT, GPT, MDH, SODH, ZF, FDPA) are expressed in units/ml; unit is defined as that amount of enzyme causing a change in optical density of .001/minute at 340 mu at 30-31°C in 1 ml reaction mixture. When readings were made at 366 mu, resulting activity was multiplied by a factor of 1.89 to convert to units defined above.

Red blood cell enzyme determinations. Whole blood samples were centrifuged at 1750 × *g* in the heparinized blood collecting tubes described above. Tubes were calibrated for volume per unit length. Suitable lengths containing cellular portion of blood were cut off and hemolyzed in 10 volumes of distilled water. Following treatment for 3 minutes in a sonic vibrator (MSE 20 KC at 60 watts with ice cooling), hemolysates were centrifuged and suitable dilutions of supernatant made for enzyme determinations. Enzyme assays were calibrated with standardized enzyme source (small samples of pooled horse serum stored individually frozen until use). This corrected for day-to-day variations in quality of reagents and temperature. Standard values for horse serum used were: LDH 170, MDH 700, GOT 160, GPT 7 and SODH 2.2 units per ml, respectively.

‡ C. F. Boehringer & Sons, Mannheim.

Determination of carbon clearance rates. India ink (Higgins #4415) was centrifuged for 30 minutes at 2400 × *g* to remove larger carbon particles. 0.05 ml of the supernatant was injected into tail veins of normal and infected BALB/c mice using #27 needle. Successful administration was followed by darkening of skin and eyes. Samples of blood (approximately 0.05 ml) were withdrawn at intervals using calibrated heparinized sampling tube (see above) and the intraorbital technique(8). Blood samples were hemolyzed by 1:50 dilution in 0.1% sodium bicarbonate solution, and were treated for 1 minute in a sonic vibrator (MSE 20 KC, 200 watts) to break up aggregates and formed blood elements. Optical density was read at 620 mu in 1 cm cell against blank (0.05 ml normal blood prepared by a similar hemolysis procedure).

Autopsy studies. Organs and tissues were removed from normal and infected mice. Tissue slices of lung, spleen, thymus, brain, heart, kidney and liver were fixed and stained with hematoxylin eosin using standard procedures. Sections were examined by bright field microscopy. No differences between normal or infected mice were seen in any of the tissues mentioned above.

Results and discussion. A preliminary survey of levels of 8 enzymes in plasma of a number of normal and LDH-agent-infected mice was made. Despite a considerable range in age and duration of infection, there were no significant differences in plasma levels of acid or alkaline phosphatase, GOT, GPT or FDPA.

Levels of MDH and SODH were somewhat higher in infected mice, but because of fairly wide range for mice of different ages and experimental backgrounds, the values could not be considered significant. To eliminate the variable of using different mice to compare enzyme levels, these 3 enzymes and also GOT (because of high RBC content of the latter) were assayed simultaneously in numbers of single mice of the same age at frequent intervals following infection with LDH-agent. Levels of all enzymes in plasma (except GPT) fell rapidly in the first 24

TABLE I. Time Course of Plasma Enzyme Levels in Mice Following Infection with LDH Agent.

Time*	No.†	LDH‡	No.†	MDH‡	No.†	GOT‡	No.†	GPT‡	No.†	SODH‡
0	4	226 ± 60	4	3031 ± 427	4	486 ± 37	3	77 ± 4.3	2	31 ± 2.6
3:45 hr	4	164 ± 60	4	1190 ± 147	4	230 ± 45	4	84 ± 13	2	16 ± .70
8:15 "	4	55 ± 27	4	1134 ± 147	4	354 ± 46	4	146 ± 10	2	7.0 ± 0
22:30 "	3	73 ± 60	3	1351 ± 154	3	214 ± 24	3	82 ± 3.2	2	8.8 ± .81
29:30 "	4	151 ± 42	4	1582 ± 84	4	227 ± 26	4	50 ± 4.3	2	7.3 ± 1.1
3 days	3	423 ± 41	3	2226 ± 133	3	254 ± 40	3	45 ± 7.1	2	21.1 ± 3.7
4 "	4	915 ± 70	4	2604 ± 84	4	213 ± 22	4	74 ± 20	2	17.9 ± 1.8
5 "	4	1209 ± 116	4	3185 ± 574	4	246 ± 35	4	80 ± 24	2	18.8 ± .70
6 "	4	1057 ± 141	4	3276 ± 553	4	209 ± 70	4	61 ± 6.4	2	10.0 ± .92
7 "	4	1148 ± 51	4	3584 ± 406	4	218 ± 19	4	94 ± 9.5	1	10.1
8 "	4	1119 ± 37	4	3612 ± 511	4	221 ± 29	4	74 ± 7.1	1	10.3
9 "	4	1125 ± 110	4	2884 ± 98	4	253 ± 45	4	91 ± 6.0	1	13.5

* Indicates time from infection.
† No. of mice for each value given.
‡ ± standard error of mean. All values in units of enzyme/ml (see *Methods* section).
A group of BALB/c mice was infected with LDH agent by intraperitoneal injection of 0.01 ml infectious plasma. At frequent intervals following injection, mice were bled and plasma enzymes were determined as described in *Methods* section. The rapid drop in plasma enzymes noted over the first 24 hours was not evident in a group of 6 control mice and corresponds to the rapid replicative phase of the agent.

hours following infection (Table I). This rapid drop in plasma enzymes did not occur in control mice and was too large to be accounted for by hemodilution effects. It corresponds to the rapid replication phase of the LDH agent which has been shown by Notkins(9) to reach maximum titer in the first 24 hours following infection.

Subsequent rapid rise in plasma LDH began 29 hours following infection and reached maximum of 5 times normal in 5 days. During this latter period there was no significant change in plasma levels of MDH, GOT, GPT or SODH except for slow return toward normal levels obtaining originally (Table I). Elevated level of plasma LDH persisted for at least 15 days; levels of other enzymes did not change significantly from initial normal values.

Hematocrits and erythropoiesis in normal and infected mice. Hematocrits dropped rapidly both in normal and infected mice during initial period of frequent bleeding, reaching minimum of approximately 30% in 72 hours (Fig. 1). Fall in hematocrit agreed well with values calculated for hemodilution effects. At 48 hours erythropoietic process was initiated. After 72 hours hematocrit values rose rapidly, returning to normal in 6-7 days. No difference was observed in rate of erythropoiesis in infected as compared to normal mice. Previous analysis of hematocrits of much larger numbers of animals(3) has shown a small but highly significant difference (47.14% ± 0.47 *vs* 50.17 ± 0.52) in steady state hematocrit levels.

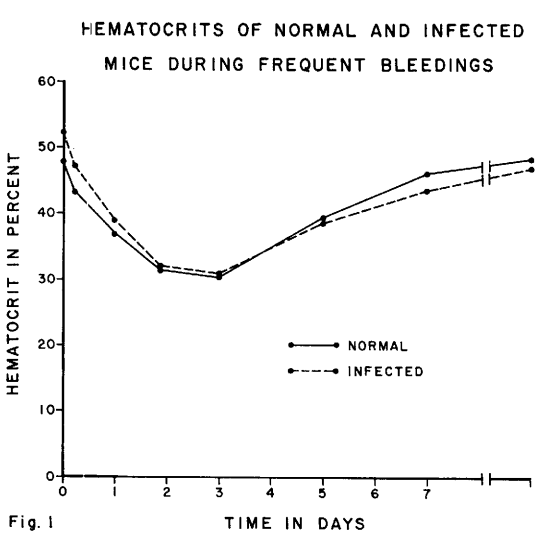
Enzyme levels in red blood cells. It was shown previously(3) that additional LDH enzyme in plasma of infected mice (5 times normal) corresponded to about 1000 LDH units per mouse.

Erythrocyte contents and plasma levels of various enzymes in normal mice are shown in Table II. It can be calculated that hemolysis required for the release of 1000 additional units of LDH from red cells would result in 1.2-fold, 2.5-fold and 2.3-fold in-

TABLE II. Effect of Hemolysis on Plasma Enzyme Levels.

Enzyme	RBC content*	Plasma content*	Calculated plasma content with 4.1%† hemolysis
LDH	24,140 ± 675	232 ± 14	1200
GOT	16,750 ± 1260	530 ± 54	1200
MDH	46,000 ± 3100	1287 ± 87	3200
GPT	231 ± 45	60 ± 43	70

* Mean ± S.E.M. measured in enzyme units per ml of plasma or packed red blood cells.
† The figure 4.1% represents the fraction of RBC's which on hemolysis would release 1000 units of LDH. This was used to calculate increase in plasma levels of the other enzymes listed.



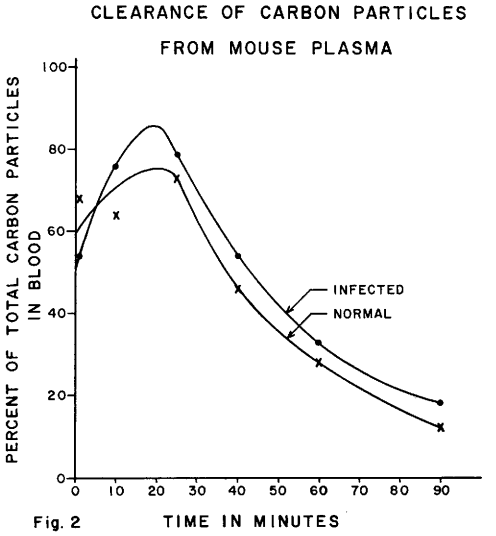


FIG. 1. Hematocrits in normal and LDH agent infected mice during frequent bleeding schedule. Figures given are average for 5 mice (normal) and 6 mice (infected). Infected mice were injected with 0.05 ml infectious plasma at 0 time. Measured enzyme levels in plasma are given in Table II.

FIG. 2. Reticuloendothelial function followed in normal and LDH agent infected mice by i.v. injection of carbon particles. Figures given are average for 5 animals (infected) and 2 animals (normal). Mice were injected through tail vein at 0 time. Blood samples, 0.05 ml, were taken at intervals and clearance of carbon particles followed by measuring optical density at 620 mu. (See *Methods* section).

creases in plasma levels of GPT, MDH and GOT. No increase of this magnitude was noted for these enzymes (except for an early brief rise in GPT level of unknown cause) following infection. If RBC's are not the source of the increased plasma LDH, the slightly lower hematocrit values in infected mice must result from some secondary effect of LDH agent. That this does not influence erythropoiesis is however also apparent from Fig. 1 where no significant retardation of return of hematocrit to steady-state values is observed in infected mice.

Carbon clearance rates. Maximum concentration of circulating carbon particles was reached within about 10 minutes in both normal and infected mice (Fig. 2). Half-life T 100/50 of carbon in the blood stream was about 28 minutes for normal and 29 minutes for infected mice. Half-life T 25/50 was approximately the same (28 minutes for normal and 30 minutes for infected mice). Except for some variability over the first 10 minutes following injection, clearance curve for infected mice followed very closely that observed with normal mice. Old *et al*(6) reported that sarcoma 180 agent produced up to 5-fold increase in rate of carbon clearance in infected mice. It would appear from our results therefore that sarcoma 180 agent and LDH agent from CD/5 lymphoma bear no similarities in their action on the reticuloendothelial system.

Summary. 1. Evidence is presented that red blood cells are not the source of additional LDH in plasma following infection of mice with LDH agent from CD/5 lymphoma. Of 9 enzymes tested, only 1 (LDH) responded to LDH agent with a significant rise in plasma level. 2. Plasma levels of LDH, MDH, SODH and GOT fell rapidly in first 24 hours following infection. At 29 hours LDH enzyme increased to a final value 5-10 times normal. Other enzymes returned slowly to normal pre-infection levels. 3. Hematocrit levels were slightly lower in infected mice, but erythropoiesis itself was not directly affected by LDH agent. 4. Half-life of circulating plasma carbon particles was about 30 minutes in both normal and infected mice.

LDH agent from CD/5 lymphoma thus differs from R.E.S. stimulating agent of sarcoma 180.

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Temperature Induced Cellular Resistance to the "Lipovirus."* (28994)

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"Lipovirus" is a new transmissible cytopathic agent isolated from (and ostensibly present in) the acute phase blood of a patient with infectious hepatitis. It is capable of inducing marked DNA and thymine degradation in, and the release of a lipogenic toxin from, infected human cells(1,2,3). Electronmicrographic study reveals the presence of electron-dense ring structures of about 15 mu in diameter and their apparent aggregation to form spongiform bodies of about 100 mu as they migrate from the nucleus to the cytoplasm(4). Recent study shows that this agent is a new immunological entity, not related to most of the DNA-containing viruses or the myxoviruses capable of infecting man(5). Antibody to this agent has also been found to be prevalent in man, particularly in those patients suffering from a disease clinically diagnosed as infectious hepatitis(6). This paper describes the creation of a reverisble state of cellular resistance to the "lipovirus" by elevating the incubation temperature to 38.5°C.

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Materials and methods. Cell culture. Cell suspensions were prepared from cultures of permanent cell lines or from minced tissues by the standard trypsinization procedure. The cells were then grown directly on glass as a monolayer in nutrients consisting of 5% heated horse serum (56°C 30 minutes) in Eagle's basal medium(7), as modified in this laboratory(8). Unless otherwise specified, a permanent cell line derived from a human liver biopsy(9), referred to hereafter as the Lich-1 cell, was used in all experiments.

Temperature. Cultures to be maintained at 38-40°C or at 32-34°C were kept in standard laboratory incubators; those to be maintained at 36.5, 37.5, or 38.5°C were immersed in standard water baths, and those to be kept at 25°C were simply left at room temperature which fluctuated between 20 and 28°C. Temperatures in the incubators and water baths were measured with thermometers calibrated to 0.5°C. The thermometers were immersed in stoppered tubes filled with water, and readings were taken each morning when the incubators or water baths were opened for the first time.

"Lipovirus." A strain, which has undergone numerous passages through Lich-1 cells since its original isolation in 1954, was used. Inoculum for the present series of experi-