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Induction of Hepatitis in Adult Syrian Hamsters by H-1 Virus (38736)

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(Introduced by A. S. Lubiniecki)

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H-1 is a small (23 nm) single stranded DNA virus that is a member of the parvovirus group. The natural host of H-1 and many of the parvoviruses is the wild or laboratory rat (1). The diseases caused by these agents in laboratory animals are dependent upon the age of the host and also on the growth rate of cells in specific organs of the host. Parvoviruses infect growing cells while nongrowing cells appear resistant to virus destruction (2). The pathology induced by these viruses is explained by this selective affinity for proliferating tissue. Therefore, parvoviruses are highly pathogenic for fetal, neonatal and nursing laboratory animals particularly the hamster (3) and the rate (4). The rat virus (RV), minute virus of mice (MVM) and H-1 produce skeletal defects (6), cerebellar hypoplasia (1), and hepatitis in these animals (7, 8).

As the animal ages and the growth rate of brain and liver cells decreases the animal becomes more resistant to infection. It has been reported that the liver can become susceptible again to viral replication upon regeneration after surgical or chemotoxic destruction (9). Parvoviruses have also been isolated in other types of rapidly growing cells such as tumor (5) and placenta tissue.

This report focuses on the hepatitis induced by H-1 and demonstrates that this virus can also induce hepatitis in adult hamsters. The pathology observed in the adult liver is not as striking as reported for the newborn; however, liver necrosis, elevated enzymes (SGOT, SGPT, LDH) and isoenzyme studies are a definite indication of hepatitis in the adult animals.

Materials and Methods. Virus: The H-1 virus (Toolan) used in this study was obtained from the American Type Culture collection (VR No. 356) and passed once in HeLa cells. The hemagglutination (800 hemagglutinating units, HU, with guinea pig cells) and TCD_{50} (hamster embryo culture tubes, 106.5 TCD_{50}/ml) titers were determined and the virus stored in liquid nitrogen.

Inoculation of animals. Syrian golden hamsters (4–6 mo old) were obtained from Lakeview hamster colony. One week prior to virus inoculation the animals were bled by cardiac puncture (1 ml vol) to obtain a base line for subsequent enzymatic assays. H-1 virus (80 HU) was inoculated (0.1 ml) intraperitoneally into each animal and also one drop of the virus suspension (8 HU) was deposited in the mouth of the hamster for oral inoculation. Other animals were left uninoculated. The animals were bled under anaesthetic approximately every 1–3 wk by cardiac puncture. One ml of blood was taken from each animal and centrifuged for subsequent removal of the serum. If an animal died in the course of the experiment, it was necropsied and the liver, large and small intestines were fixed in 10% buffered formalin for histological observation and in glutaraldehyde for electron microscopy. The methods for electron microscopy have been published previously (10). Certain animals that had very high enzyme levels (SGOT, SGPT) were sacrificed and their organs processed for EM and histologic observation.

Enzyme activity analysis. All assays were conducted at 30° utilizing a Gilford Model 300-N micro-sample spectrophotometer equipped with a thermo–cuvette temperature regulating accessory.

Serum glutamic oxaloacetic transaminase (SGOT) was assayed based on the methods of Karmen (11) and Henry (12); glutamic pyruvic transaminase (SGPT), based on the methods of Wroblewski and LaDue (13) and Henry (14); gamma glutamyl transpeptidase (γ-GTP), based on the method of Szasz (15); lactate dehydrogenase (LDH), based

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TABLE I. SGOT AND SGPT LEVELS IN HAMSTERS INOCULATED WITH H-1.

Animal	Prior to inoculation		Weeks post inoculation[a]											
			1		3		4		6		9		11	
	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT	SGOT	SGPT
1-control	37	47					51	41			42	48		
2-control	31	20					25	31			30	22		
3-control			105	81	71	61								
4-control			14	41	57	61			45	57				
5-control			44	68	64	68	55	61	52	63				
6-control			30	41	57	61								
1-expt.	34	27			88	44	173	217	193	128				
2-expt.	51	47					302	564	54	61	112	61	7	34
3-expt.	57	27					428	302	27	27	71	54	24	37
4-expt.	44	41					1022	1303	71	27	47	41	7	34
5-expt.	44	34			88	18	30	61	78	88	355	406		
6-expt.	44	40			54	47	85	122	71	74	132	122	71	95
7-expt.	40	51					200	199			784	1240		
A-expt.[b]	35	28			422	473	25	32						
B-expt.	26	31			247	412	34	38						
C-expt.	21	32			534	473	50	78						

[a] 0.1 ml. H-1 inoculated IP (80 HU) and 8 HU given orally, control animals were not inoculated.
[b] The blood from four animals was pooled and analyzed.

on the method of Wacher *et al.* (16) and alkaline phosphatase based on the method of Bessey, Lowry and Brock (17). Total bilirubin was assayed based on the method of Malloy and Evelyn (18).

Electrophoresis. Serum protein electrophoresis was performed on cellogel utilizing the microzone system (Beckman, Fullerton, CA 92634).

LDH isoenzyme electrophoresis was performed on polyacrylamide gels utilizing an analytical vertical-gel apparatus (Canalco, Rockville, MD 20852).

Results. In two experiments combined in Table I an increase in the level of SGOT and SGPT was noted in those animals that received H-1 inoculation. The highest enzyme levels were obtained approximately 2–4 wk after inoculation. The mean enzyme levels for infected animals was 244 and 49 for control animals. All the animals inoculated with H-1 displayed an increase in their enzyme level, although several animals showed only moderate increases. A couple of animals showed maximum enzyme levels at 9 wk postinfection. The serum electrophoresis pattern of H-1 infected and control animals was similar, also the alkaline phosphatase, gamma glutamyl transpeptidase (γ-GTP) and bilirubin were similar for both sets of animals. The lactic dehydrogenase (LDH) isoenzyme pattern was then analyzed on the sera and a striking difference was noted between the infected and control animals (Fig. 1). The total LDH was elevated on one animal to 1150 units while the value on a matched control was 118 units. The electrophoresis pattern of the LDH isoenzymes showed a marked elevation in the liver fraction five (Fr. 5) indicating liver damage in those animals that received H-1 virus.

Attempts were made to isolate H-1 from the animals that had increased enzyme levels. Liver and intestinal tissue as well as feces were planted on hamster embryonic tissue for virus isolation. No cytopathic effect or hemagglutination activity was observed from these cultures over a 2-wk culture period. Feces and organs were collected for virus isolation studies 2–3 days after increased enzyme levels were observed.

Viral manifestation observed in the livers of newborn hamsters (8) infected with H-1 such as leukocyte infiltration, intranuclear inclusions, giant cell formation and nuclear enlargement were rarely observed in histological sections of infected adult hamsters. However, certain pathological differences were noted in comparison to control animals (Fig. 2a). Focal areas of hepatic cell degeneration were observed (Fig. 2b) in infected animals. This is in comparison to the general, infiltration and necrosis of the liver reported for H-1 induced hepatitis in newborn hamsters (8). By electron microscopy the hepatic cells in these focal areas appeared disrupted and displayed cytoplasmic vacuolization, glycogen infiltration and lipid body formation (Fig. 3). The EM

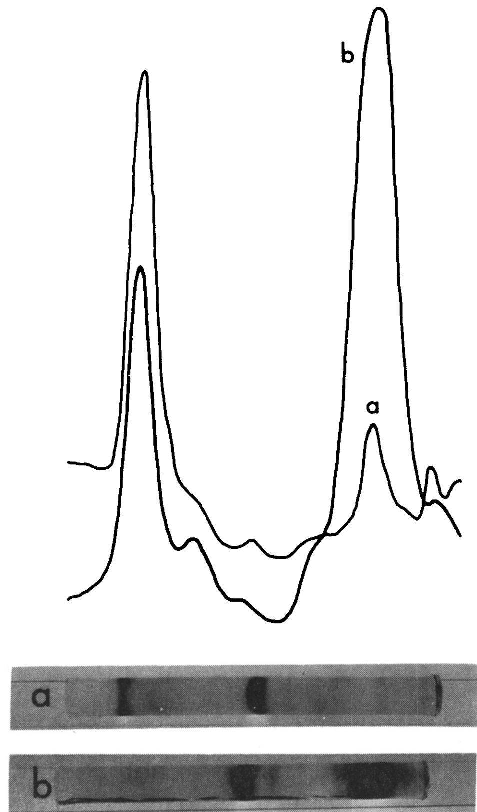

FIG. 1. LDH isoenzyme pattern of H-1 infected and control animals. Fraction 5 in the infected animals is highly elevated in comparison to the controls. (a) represents the control animals and (b) represents the infected animals. The infected animals are listed in Table I as Expt. 5.

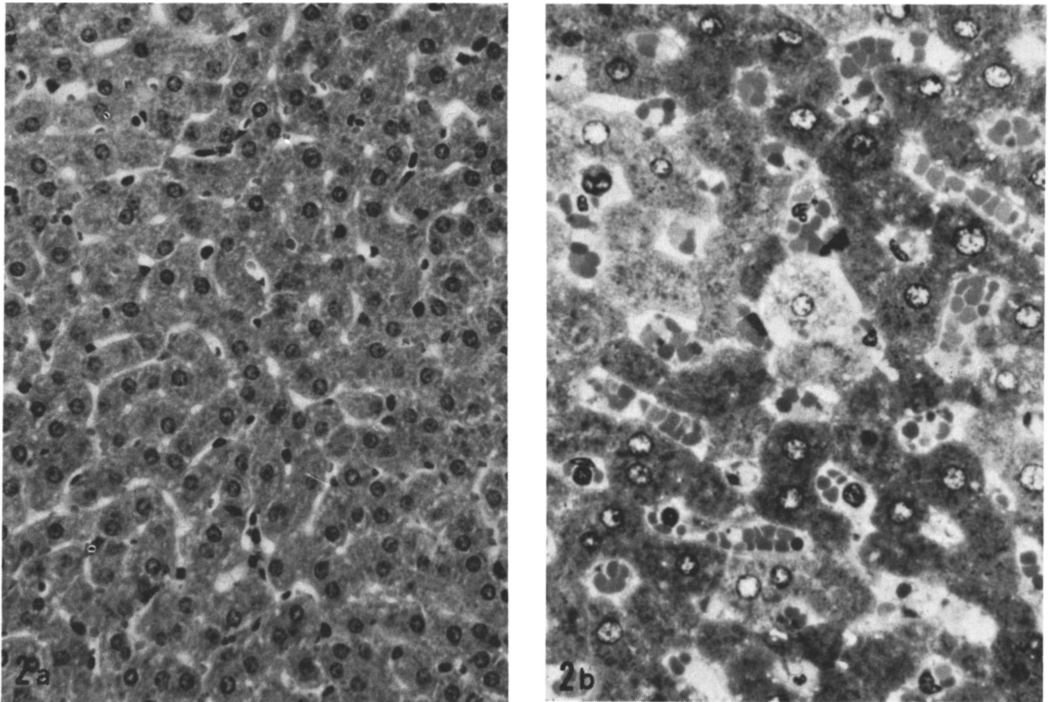

FIG. 2. (a) A micrograph of the liver from a control animal ×320. (b) A micrograph of the liver from an H-1 infected animal. Focal necrosis has occurred in this area while other areas of the liver appear unaffected. ×500.

picture of a control animal is presented in Fig. 4.

Preliminary serological analysis (complement fixation tests) of sera and liver tissue from infected and control animals indicates that H-1 antibody is produced in diseased animals (average CF titer 1/32) in low titers. The presence of H-1 antigen in diseased liver homogenates was also detected in low titers (CF 1/8–1/16).

A generalized increase in the number of red blood cells was noted in the livers of infected animals suggesting congestion or capillary leakage. There was little, if any, white cell infiltration into the liver, which may have been due to the short duration of the hepatitis (1–2 wk). No virus particles were observed by electron microscopy (EM) in the liver of infected animals although our period of EM observation was in the latter stages of the disease.

Discussion. The parvoviruses such as H-1, RV, MVM and panleucopenia virus (PLV) have a general propensity for proliferating tissue (2) and infect the external germinal layers of the developing cerebellum of newborn rats, hamsters and kittens (9, 20) causing cerebellar hyperplasia and atoxia. These agents as well as a relatively new parvovirus (porcine virus, 21) have the ability to produce transplacental infections causing death or inducing specific deformities in the fetus. However, until this report parvoviruses were generally regarded to have little effect on adult animals. H-1 has been reported (19) to produce pathology in newborn hamsters as well as destroy the fetuses in pregnant hamsters, although the virus was regarded to have no effect on the mother. In our experiments we used 4–6 mo old animals and a relatively small dose of virus for inoculation. Two of the female hamsters (1 and 3 expt.) in the experiment were pregnant; however, they reacted similarly to H-1 inoculation as the other females in displaying increased enzyme levels. The newborn animals from these females appeared to have no deformities or abnormalities. This was apparently due to the low dose of virus inoculated. No clinical symp-

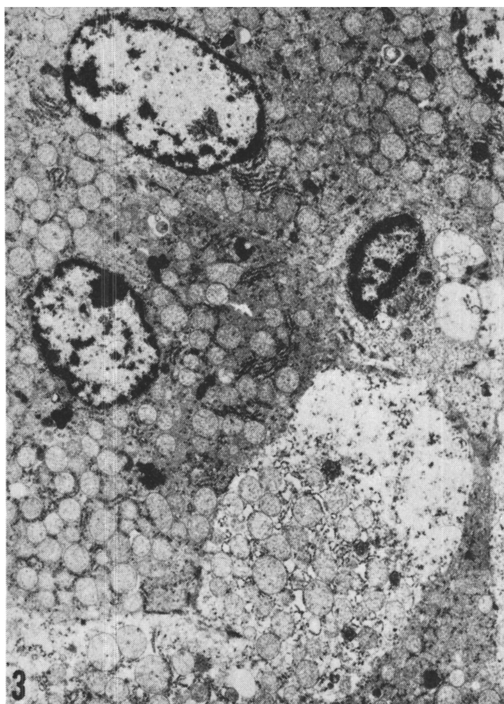

FIG. 3. An electron micrograph of the liver from a 4-mo old hamster infected with H-1 for 4 wk. The cytoplasm of these cells is degenerated and has lost the ribosomes required for protein synthesis. ×3500.

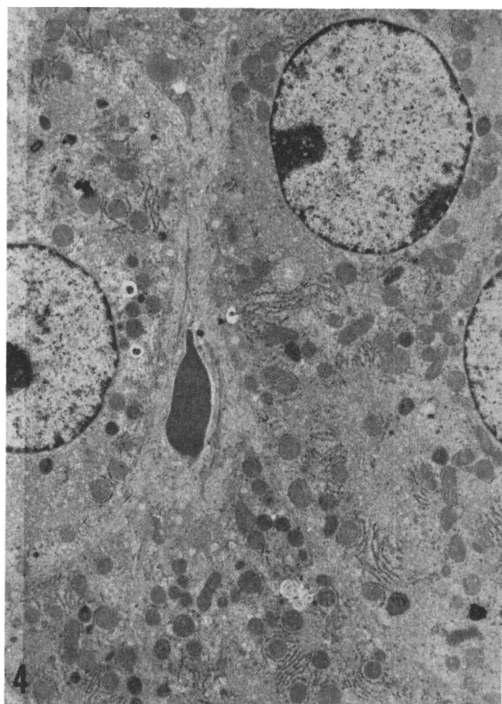

FIG. 4. An electron micrograph of the liver from a control animal. ×3500.

toms were noticed to be associated with the hepatitis as the animals did not appear to deviate from their normal physical routine. Also, no loss of weight or jaundice was noticed. The biochemical analysis of the H-1 induced hepatitis appeared similar in some respects to that observed in man. An increase in SGOT, SGPT, and LDH levels is consistent with infectious hepatitis in man. The LDH isoenzyme pattern (an increase in the liver Fr. 5) observed in infected hamsters is also observed in viral hepatitis in man. Another consistent finding indicative of viral hepatitis in experimental hamsters is the normal values of gamma glutamyl transpeptidase. Hepatitis in man is usually associated with an increased alkaline phosphatase and bilirubin although these values were normal in H-1 induced hepatitis. The pathology observed in diseased hamster livers was different from that usually associated with man. No infiltration of white cells in the liver was observed although the latent period (2–9 wk) of the disease is indicative of a viral multiplication process and not an acute toxic hepatitis. The latter type of hepatitis was observed with another virus (frog virus 3, FV3) in mice (22). Acute toxic hepatitis initiated in adult mice by FV3 inoculation was observed 18–24 hr postinfection. The authors felt that some component of the virus particle was toxic to hepatic cells initiating the hepatitis. H-1 induced hepatitis does not appear to be of this type because high concentrations of FV3 virus (10^{7-8} TCD_{50}) had to be given to induce hepatitis in mice while we gave relatively low concentrations of virus. However, the possibility that a combination of virus replication followed by toxic hepatitis cannot be excluded. H-1 replication in the villi of the gut with subsequent initiation of an acute toxic hepatitis in hamsters is a possibility and could explain the liver necrosis without infiltration of leukocytes. No virus particles were observed by electron microscopy in hepatic cells; however, these animals were generally sacrificed in the healing stages of the disease. Further studies are underway to characterize the pathological

sequence of H-1 induced liver disease and also to study the immunological parameters or viral hepatitis in this animal model system.

Summary. The induction of hepatitis in adult hamsters by H-1 virus was documented by demonstrating an increase in serum SGOT and SGPT at 3–9 wk postinoculation. The electrophoresis pattern of LDH isoenzymes showed a marked increase in the liver fraction (fraction 5) indicating liver damage in infected hamsters. The pathology displayed in diseased livers revealed a focal degeneration of hepatic cells although infiltration of white cells was not observed. H-1 virus is apparently capable of producing hepatitis (without symptoms) in adult hamsters as well as cause hepatitis and severe cerebral disease in newborn hamsters.

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