Modified Acute Canine Viral Hepatitis—a Model for Physiologic Study[1] (36070)

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Viral hepatitis is a common disorder among animal species (1). Definition of the hepatic excretory and splanchnic circulatory changes in these hepatitides has been hindered by a number of factors including the small size of some of the animals, the need for anesthesia during such investigation, and inability to control the severity and duration of the disease. Our previous attempts to study the physiologic changes in hepatic function during acute infectious canine hepatitis (ICH) were particularly hampered by these latter factors (2).

The present report describes a reliable method for production of a modified form of acute ICH characterized by anorexia, fever, lethargy, serum enzyme elevations, sulfobromophthalein (BSP) retention, clotting disturbances, and a lengthy course leading to recovery. By means of this system, which depends on the administration of antiserum to the canine hepatitis virus at a time of only slight impairment of hepatic function as measured by fractional clearance of BSP ($k/_{BSP}$), sequential measurement of multiple indices of hepatic function through the entire course of a severe experimental hepatitis seems feasible.

Methods and Materials. Animals. All dogs were purebred beagles (3–8 kg) which had been raised in isolation and immunized against distemper and leptospirosis as previously described (2). Upon arrival in the laboratory, each dog was housed in a special isolation cage to prevent spontaneous infection with ICH virus. The diet consisted of horse meat and dry meal. Water was not restricted. Prior to blood sampling, each animal was subjected to an overnight fast.

Virology and immunology. Canine hepatitis virus was prepared from infected liver homogenates as described in an earlier report (2). Infection was produced by inoculation of 300 $TCID_{50}$ of ICH virus into the anterior chamber of the topically anesthetized eye. Serum titers of anti-ICH neutralizing antibody were determined in a standard tissue culture neutralization assay with 100 $TCID_{50}$ of the virus as the test dose. Hyperimmune ICH antisera were obtained from an adult dog which had been repeatedly inoculated with ICH virus. The final pool of antiserum had a neutralization titer of 8×10^4 against a test dose of 100 $TCID_{50}$ of ICH virus. Intravenous injection of 2.5 ml of this material produced an antibody titer of approximately 1:500 in a dog with a plasma volume of 400 ml.

Procedure. Prior to infection, control measurements including rectal temperature, body weight, white blood cell count, hematocrit, venous clotting time, serum glutamic oxaloacetic transaminase (SGOT), serum glutamic pyruvic transaminase (SGPT), and serum anti-ICH antibody titer were made in each dog. Only dogs with no detectable antibody were subsequently infected. The peripheral disappearance rate of BSP was estimated following injection of 8 mg/kg of BSP, a dose within the recommended range (3). Plasma samples were drawn through a polyethylene catheter (No. 19 Deseret) in a peripheral vein at 2, 4, 6, 8, 10, and 15 min after injection. Precutaneous liver biopsy for routine histologic examination was performed with a Menghini needle via the subxiphoid

[1] Supported by U.S. Public Health Service, Research Grant AI 08890.

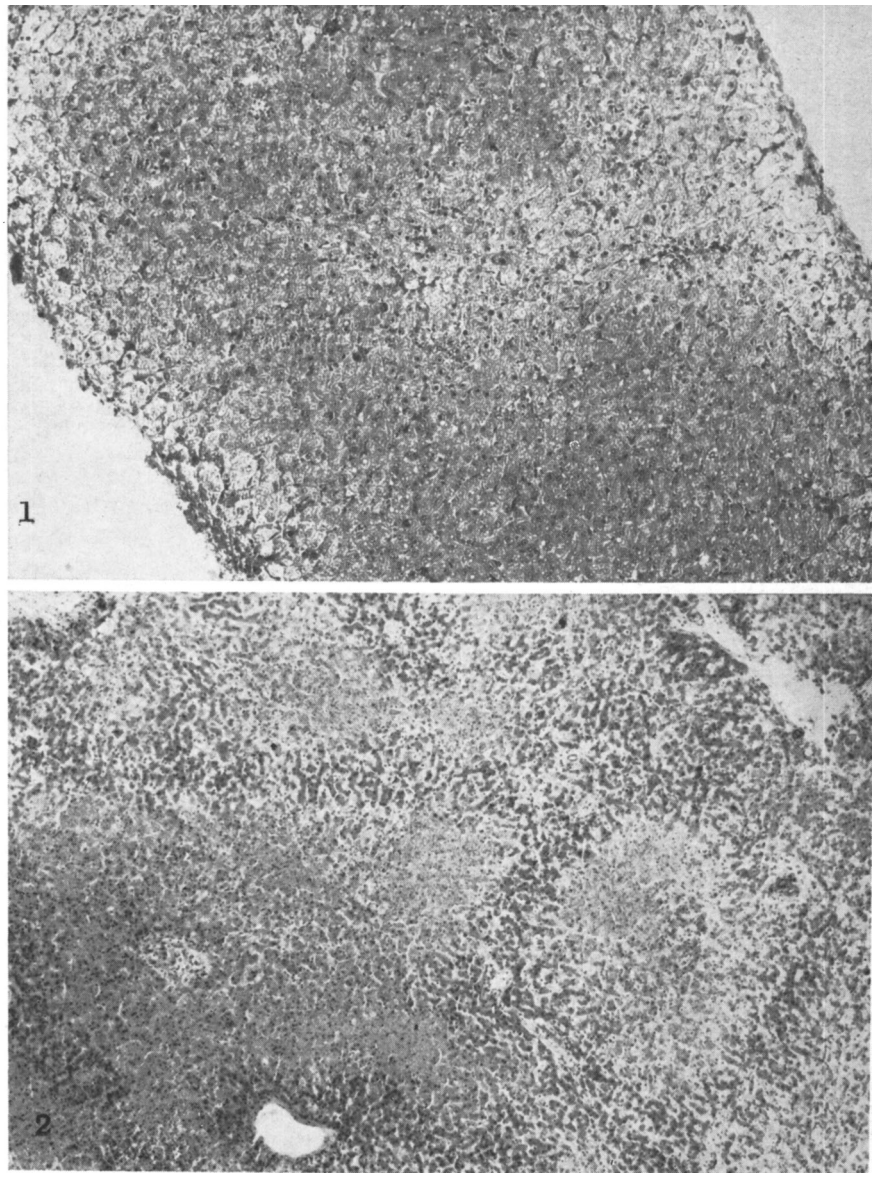

FIG. 1. Liver biopsy on day 5 after infection at the time of administration of anti-ICH antiserum. A small focus of necrosis and inflammation is seen with some cells containing the intranuclear inclusions typical of ICH virus; H & E; 32×.

FIG. 2. Liver biopsy showing severe, confluent parenchymal necrosis and inflammation; H & E; 32×.

route.

After inoculation, daily measurements of BSP clearance were made. At the first sign of impaired disappearance of BSP from the plasma, 5 ml of anti-ICH antisera were administered intravenously. Thereafter, measurements of BSP clearance were continued through the period of recovery. The additional clinical and laboratory indices noted above were also followed serially.

Analytical methods. BSP plasma concentrations were measured as previously described (4), and plotted on semilogarithmic paper. The slope was drawn by eye, and the

disappearance half time ($t_{½}$) estimated to permit calculation of the disappearance constant, k_{BSP}, from the formula, $k_{BSP} = 0.694\ t_{½}$. Serum enzyme levels were measured with conventional methods.

Results. Hepatitis with a severe and protracted course leading to survival was successfully produced in six of seven animals. The dog which failed to recover died on day 7 with severe hepatitis and upper gastrointestinal bleeding. The subsequent data apply only to the six survivors.

Clinical signs. The appearance of fever (range, 103 to 106°) on day 2 or 3 after infection, in conjunction with the development of a permanent corneal opacity in the inoculated eye, was the most reliable early sign of an active hepatitis. In general, pyrexia continued only until the end of the first week of disease, when all dogs manifested severe anorexia and marked lethargy. These latter signs persisted into the second week of disease, the period of greatest hepatic dysfunction. Return of appetite and normal activity always heralded recovery which usually began within the second week of disease and was complete by the end of the third week.

Immunology. Prior to infection and until administration of anti-ICH antisera, sera from all dogs were free of neutralizing antibody to the ICH virus. Thereafter, antibody titers of greater than 1:500 were found in all serum samples.

Histology. All liver biopsies obtained prior to infection were completely normal. Tissue obtained on days 3 to 5 after infection contained collections of inflammatory cells including lymphocytes, plasma cells, and polymorphonuclear leukocytes in the sinusoids and scattered small foci of hepatic necrosis involving only a few cells, some with characteristic intranuclear inclusions indicative of the presence of ICH virus particles (Fig. 1). Following administration of immune serum, intranuclear inclusions were either very scarce or absent. Inflammation and necrosis of hepatocytes, however, increased markedly by the seventh to eighth day and some dogs (Q, T, F) showed confluent areas of hemorrhagic necrosis during the middle of the second week (Fig. 2). Thereafter, repair of the tissue injury as evidenced by mitotic figures and phagocytosis proceeded slowly toward

TABLE I. Sequential Changes in Fractional Clearance of BSP (k_{BSP}) and Serum Enzymes During the Course of Hepatitis in Dog T.

Day	k_{BSP}	SGOT/SGPT[a]	AP[b]
0	0.347	26/24	15
4	0.277	42/31	15
5	0.187	210/115	17
6	0.102	330/290	29
8	0.094	460/480	78
11	0.158	200/475	119
13	0.193	55/255	120
19	0.347	37/38	52
25	0.408	37/22	24

[a] SGOT/SGPT, serum transaminase; normal: 10–40 Sigma units (3).

[b] AP, alkaline phosphatase; normal: 4–12 King Armstrong units (2).

TABLE II. Fractional Clearances of BSP (k_{BSP}) During Control Studies and Administration of Passive Immunity Plus the Maximal Change in k_{BSP}, Serum Enzymes, and Venous Clotting Times for Each Dog.

Dog	k_{BSP}		Maximal abnormalities			
	Control	Immunity	k_{BSP}	SGOT/SGPT[a]	AP[a]	VCT[b]
M	0.385	0.289	0.151	85/70	15	15
Q	0.365	0.301	0.105	1220/470	105	11
T	0.347	0.277	0.094	460/480	120	10
U	0.385	0.347	0.204	100/94	86	11
B	0.301	0.216	0.158	270/175	154	13
F	0.349	0.238	0.069	558/—	28	>60

[a] SGOT/SGPT, AP, see Table I.

[b] VCT, venous clotting time; normal range: 5–7 min [J. Exper. Med. 90, 97 (1949)].

normal. At the end of the third week after infection biopsy specimens still showed foci of histiocytes and chronic inflammatory cells.

Hepatic function. BSP clearance was the most sensitive index of impaired hepatic function, a finding in keeping with earlier experience (2). Typical serial changes in k_{BSP} are shown in Table I. The functional course of the disease could be followed precisely with this technique and abnormalities detected when serum enzyme measurements were not markedly elevated (Dog T, Table I). Recent studies have indicated that fever alone is not sufficient cause for impaired plasma clearance of BSP (5, 6), a finding in keeping with the stepwise changes during development of and recovery from this modified form of acute hepatitis.

Fractional clearances of BSP (k_{BSP}) during control studies and at the times of antibody administration and maximal BSP retention are shown in Table II. Although the control values were closely bunched (0.355 ± 0.03), proper timing of antibody administration required individual study since one control (T, B) might represent the point at which another (U, Q) received antibody. Administration of immune sera at a later stage was totally ineffective and did not prevent the previously described fulminant course (2).

Maximal increments in serum transaminase, alkaline phosphatase, and venous clotting times (Table II) were also indicative of a severe hepatitis. In general, transaminase elevations were most marked when BSP retention was greatest. Alkaline phosphatase levels rose more slowly and tended to lag behind SGOT and BSP in returning to normal (Table I).

Discussion. Impetus for development of this modified form of acute infectious canine hepatitis arose from a spontaneous recovery observed during a previous study of the therapeutic efficacy of specific neutralizing anti-ICH antibody in exchange transfusion treatment of fulminating canine hepatitis (7). The single survivor of treatment with antibody negative blood developed a severe and protracted hepatitis in association with an unusual antibody response appearing during the first week after infection, a time when all other completely susceptible dogs had died (2). The goal of the present study was to produce a similar disease with passive immunity. In six of seven attempts, a protracted and severe hepatitis leading to recovery within about 3 weeks was produced. This success probably resulted from a delicate balance between the numbers of hepatocytes invaded by infectious viral particles and those remaining unaffected and depended on the ability to prevent continuous viral replication and cellular invasion beyond a "point of no return." Administration of anti-ICH antibody effectively removed viral particles from the circulation and interrupted this cycle. Nevertheless, virus already established intracellularly continued to exert deleterious effects on the liver as indicated by continued progression for the next few days. Anti-ICH antibody was effective in producing modified acute hepatitis only when given at the first sign of significant impairment of BSP clearance, by far the most sensitive index of hepatic function. Delay in this treatment did not prevent the usual fatal outcome in the completely susceptible dog. It is possible that similar success could have been achieved by only reducing the infectious dose of ICH virus or simply changing the route of administration. To date, however, hepatitis of similar duration and severity has not been produced with either of these techniques.

Clinically, all dogs developed signs of typical hepatitis. Fever and the appearance of a permanent corneal opacity in the inoculated eye were present early in the disease and preceded the development of marked anorexia and lethargy. Peak abnormalities in serum enzyme and blood clotting were found in conjunction with marked impairment of BSP clearance and severe hepatic necrosis during the early part of the second week. Recovery then ensued through the next 10 days. It is noteworthy that in keeping with the low renal threshold for bilirubin in the dog (3), jaundice was not observed in these animals.

As a model for the study of hepatic function during a severe viral induced injury to the liver, modified acute infectious canine hepatitis offers several desirable characteristics. Notably dogs have a proved ability to

tolerate without anesthesia not only splanchnic circulatory measurements (8) but also duodenal cannulas to permit studies of bile formation (9). In addition, their size permits repeated blood sampling without serious reduction of plasma or red cell volumes. The ease of percutaneous liver biopsy and the well-defined character of the ICH virus present an opportunity to correlate physiologic measurements with virologic and histologic findings. Lastly, the predictable recovery permits sequential studies from the normal healthy state through a severe hepatitis to recovery. Definition of this sequence may yield additional information and understanding of the disturbed physiology in hepatitis.

Summary. A method for production of a severe and prolonged form of canine hepatitis is presented. The clinical course, which is characterized by fever, lethargy, anorexia, marked elevations of serum enzymes, and impaired BSP removal, is most severe 7 to 10 days after infection. Recovery ensues by the end of 3 weeks. Study of this disease will hopefully yield additional insights concerning the physiologic abnormalities in hepatitis.

The authors gratefully acknowledge the skillful assistance of Misses Eleanor Bachthaler, Linda Green, and Emily Bidwell.

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Received Mar. 19, 1971. P.S.E.B.M., 1972, Vol. 139.