obtained in the present series of experiments. It is apparent that the calculations from Deyrup's experiments are in close agreement with the values obtained in the present experiments, and that all values listed in the Table fall below the range of normal values. The difference between average post-histamine F_{cells} value and average normal F_{cells} value, determined by Rawson, *et al.*(9) also utilizing Cr^{51} and T-1824, is highly significant. This is also shown by direct comparison of control and post-histamine F_{cells} values in individual dogs of Group A (Table I).

The term "extra plasma" refers to the percent of plasma left over when one calculates the plasma required to contain all the cells in the circulation at the concentration obtaining in the large vessels from which samples are drawn. In the normal unanesthetized splenectomized dog, extra plasma amounts to about 13% of the total blood volume(1,2). After injection of histamine, the proportion of "extra plasma" increases to 22% as given by the F_{cells} value. Since histamine causes widespread dilatation of the minute vessels(10), it may be inferred that the increase in percent "extra plasma" is the result of a larger proportion of the blood being present in the small vessels. However, it could also be a consequence of a decrease in cell percentage of the blood of the small vessels. Both factors may be operative, but the former seems the more likely explanation.

Summary. From ½ to 2 hours after subcutaneous injection of histamine (3.0-4.7 mg/kg) the average F_{cells}Factor of splenectomized dogs decreased to 0.78 from the control value of 0.87.

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A New Member of Hepatoencephalitis Group of Murine Viruses. (24810)

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Frequent occurrence of latent agents of infectious diseases in apparently healthy laboratory animals may be responsible for error in interpretation of isolation procedures. A number of transmissible agents have been procured within recent years by serial passage of supposedly "normal" mouse tissues. Some of these, namely the ascites-hepatitis agent isolated in mice in Mirick's laboratory(1) and the mouse ascitic agent recovered in mice at Walter Reed Army Inst. of Research(2), are associated principally with ascites and hepatosplenomegaly. In addition to these agents, mice may harbor latent viruses which produce massive macroscopic necrosis of liver and which on intracerebral inoculation into suckling or weanling mice may or may not induce fatal encephalitis. The latter group of agents is designated murine hepatoencephalitis group of viruses(3). The disturbing frequency with which members of this group of viruses were encountered in commercially procured mice in our laboratory in Japan in 1956 through 1958, in human hepatitis isolation studies prompts this report. It is the purpose of this paper to delineate murine hepatoencephalitis group of viruses and to add to it a new member.

Materials and methods. Viruses. Three

TABLE I. Members of Hepatoencephalitis Group of Viruses.

Virus	Origin	Area where originated	Year	Author & ref.
MHV	Albino mouse, Parkes strain	London	1950	Gledhill & Andrewes(4)
EHF120	*Idem*, unknown "	Tokyo	1952	Buescher(5)
H747	" " "	"	1953	Morris(3)
JHM	" , Schwentker strain	Boston	1947	Cheever, *et al.*(6)

viruses were employed. The source of each and area of origination are given in Table I. MHV virus(4) in form of lyophilized suspension of infected mouse brain was kindly sent to us from London in 1951 by Dr. C. H. Andrewes. The EHF120 virus(5) in lyophilized mouse brain infected with 9th mouse passage of the agent was received from Major E. L. Buescher in 1952. The H747 agent (unpublished) was recovered in 1953 in Japan in suckling mice from tissues of naturally infected albino mice. JHM virus(6) was not available to us at the time these experiments were performed. MHV, EHF120 and H747 viruses were maintained in our laboratory by intracerebral or intraperitoneal inoculation of suckling mice. *Immune sera* against H747 virus was produced in adult mice by multiple intraperitoneal and intracerebral inoculation of infectious suckling mouse brain and against EHF120 and MHV in adult mice by single intraperitoneal injection of suckling mouse brain infected with appropriate virus mixed with Freund's adjuvant(7). The JHM immune serum was prepared and supplied by Dr. F. S. Cheever. *Neutralization tests* were performed in usual manner employed in U.S. Army laboratories(8) employing dilutions of virus in the form of infected suckling mouse brain and inactivated (56°C for 30 minutes) undiluted serum inoculated intracerebrally into 1-3-day-old mice. *Complement fixation tests* were performed in standard way(8) using antigens prepared from brains of suckling mice infected with H747 agent or with EH120 virus. The antigens represented 13,000 rpm supernatants of 20% brain suspensions.

Results. Findings in neutralization tests performed with H747 virus and antisera against H747, EHF120, JHM and MHV are summarized in Table II. The results indicate that antisera we prepared against 2 other mouse viruses, *i.e.* EHF120 and MHV and JHM mouse immune serum supplied by Dr. Cheever, neutralized the H747 agent to approximately the same degree as did homologous antiserum. Selected normal mouse serum and antiserum prepared in rabbits against Theiler's GDVII virus as well as antisera prepared in guinea pigs against lymphocytic choriomeningitis virus did not neutralize H747 agent. In addition, serum of guinea pigs, rabbits and monkeys which had been repeatedly inoculated with H747 virus in every instance failed to neutralize the homologous agent. Reciprocal neutralization tests were not done since neither EHF120 nor MHV, the other 2 viruses available to us at time of this study, consistently induced fatal disease in suckling mice inoculated with low dilutions of infectious material.

Results of complement fixation tests using antigen prepared from brains of suckling mice infected with H747 agent or with EHF120 virus are summarized in Table III. It is seen

TABLE II. Neutralizing Capacity of Serum of Adult Mice Hyperimmunized against H747, EHF120, JHM or MHV Viruses.

(Suckling mice, 1-3 days old, inoculated intracerebrally.)

Serum	Dilutions of H747 virus 10^{-1}	10^{-2}	10^{-3}	10^{-4}	10^{-5}	LD_{50}	Immunity index (log)
Normal mouse		7/7*	8/8	2/7	1/6	$10^{-3.7}$	
H747	5/5	0/5	0/4			$10^{-1.5}$	2.2
EHF120	2/8	0/7				$10^{-1.0}$	2.7
JHM	3/6	1/7	0/8			$10^{-1.1}$	2.6
MHV	0/10	1/7				$10^{-1.0}$	2.7

* No. mice dead/No. mice inoculated.

TABLE III. Results of Complement Fixation Tests with Materials from Hepatoencephalitis Group of Viruses.

Serum	Suckling mouse brain antigens H747	EHF120	Normal	A. C. control (saline)
Normal mouse	0*	0	0	0
H747	1:8	1:8	0	0
EHF120	1:8	1:8	0	0
JHM	1:8	1:8	0	0
MHV	1:4	1:4	0	0

* 0 = no fixation at 1:4 dilution of serum.

that complement was fixed in presence of H747 and EHF120 infected suckling mouse brain and their respective antisera as well as antisera against JHM and MHV. In control tests none of the antisera fixed complement in presence of normal mouse brain antigen.

Discussion. H747, EHF120, and JHM and the mouse hepatitis virus of Gledhill and Andrewes are apparently closely related antigenically. Gledhill and his colleagues(4) suspected an immunologic relationship between MHV and JHM, but these workers could not establish a clear cut relationship between agents by neutralization technic since the neutralization values of immune sera prepared against the viruses were too low or the virus titrations were uneven. Buescher, working with EHF120 virus, was unable to demonstrate a satisfactory neutralization test since all mice inoculated with low dilutions of this virus did not succumb to their infection(5). The H747 virus, however, consistently induces fatal disease in suckling mice following intracerebral inoculation of appreciable dilutions of infectious material and this property provided a satisfactory technic to study immunologic relationships of this group of agents. It seems probable that the murine hepatoencephalitis group of viruses which are essentially suckling mouse viruses are distinct from viruses of the Coxsackie group. The latter agents, when injected into rabbits, guinea pigs and monkeys, are capable of evoking specific neutralizing antibody in large amounts, whereas injection of JHM, H747, MHV and EHF120 into the same hosts is not followed by development of demonstrable neutralizing antibody. Furthermore, the infectious titer in suckling mice of Coxsackie viruses is generally of the order of 10^{-8} to 10^{-9}; whereas the infectious titer of viruses described here seldom exceeds 10^{-4} or 10^{-5}. Recovery of members of this antigenically related group of viruses in United States, England, and Japan, indicates wide dissemination of this family of murine viruses. Accordingly, it is emphasized that viruses of the murine hepatoencephalitis group may be encountered with varying frequency in laboratory mice and that presence of these viruses may confuse workers who attempt to propagate the agent of human hepatitis in this host.

Summary. A virus (H747) which consistently produces fatal encephalitis in suckling mice following intracerebral inoculation was recovered in Japan from tissues of naturally infected mice. The new virus was shown by means of neutralization and complement fixation procedures to be antigenically related to JHM, EHF120 and MHV viruses. The agents comprising this antigenically related family of viruses is designated the hepatoencephalitis group of murine viruses.

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