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Hepatitis in Marmosets: Induction of Disease with Coded Specimens from a Human Volunteer Study

Abstract. *Marmosets inoculated with plasma from three early acute hepatitis patients developed hepatitis 30 to 40 days later. Other groups of marmosets receiving preinfection plasmas from the same patients showed no evidence of hepatitis in this experiment. It is, therefore, most probable that hepatitis in marmosets represented transmission of human disease rather than activation of latent "marmoset hepatitis."*

Previous reports from our laboratory (1, 2) have characterized the hepatitis occurring in two species of marmosets after the inoculation of serum or plasma obtained from patients early in the course of acute viral hepatitis. Subsequently, other investigators have suggested that this phenomenon represents the activation of latent "marmoset

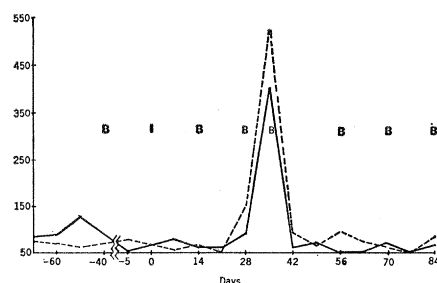


Fig. 1. Changes in serum enzyme activities of a marmoset inoculated with acute-phase plasma from patient G.C. (B) Liver biopsy; (I) inoculation on day 0. Values on the ordinate are levels of serum glutamic oxalacetic transaminase (SGOT) activity (solid line) and of serum isocitric dehydrogenase (SCD) activity divided by 10 (broken line). Methods used for these tests have been previously described (1). Upper limits of normal in our laboratory for marmosets are SGOT of 200 and SCD of 2000.

hepatitis" rather than the transmission of human disease (3). In order to help resolve this issue an experiment was designed in which preinoculation and acute-phase plasmas were obtained from three of ten human volunteers, who had been inoculated orally with infectious plasma containing the Willowbrook MS-1 strain of hepatitis virus (4) and who developed clinical disease 27, 29, and 31 days, respectively, after inoculation. Hepatitis was confirmed by liver biopsy. The acute-phase specimens used in our study were samples of plasma drawn on the 29th day from two of the volunteers and on the 30th day from the third (5). No Australia antigen (6) was detected by complement-fixation tests in the preinoculation, the day-33, and the day-100 (after inoculation) serums of the three human volunteers.

To insure objectivity in the interpretation of results the six specimens were coded (by Col. Marcel E. Conrad, Walter Reed Army Institute for Research) before being sent to us, and the marmoset liver biopsies were studied by one of us (L.W.) under code. Each of the unknown specimens was diluted 1 : 2 in Hanks balanced salt

solution and each marmoset received 0.5 ml of a diluted specimen intravenously. Inoculated animals and their uninoculated controls were bled once weekly for the determination of serum activity of glutamic oxalacetic transaminase and isocitric dehydrogenase, and percutaneous needle liver biopsies were done at least every 2 weeks in all experimental subjects.

The results of the experiment are detailed in Table 1. Only marmosets inoculated with acute-phase human plasma developed hepatitis confirmed both biochemically and by liver biopsy. Incubation periods were fairly consistent at 30 to 40 days from inoculation to detection of first abnormal biochemical tests. The course of the disease was brief, as exemplified in Fig. 1. A single animal in the group receiving preinoculation plasma from patient G.C. had a rise of serum enzyme activities 60 days after inoculation, but these values had returned to normal 4 days later. A liver biopsy done on the 60th day showed no evidence of hepatitis.

From the results of this experiment it would seem likely that human hepatitis can be transmitted with regularity to marmosets. These specimens were obtained quite early in the disease (just before the first abnormal hepatic tests in one of the three volunteers) and for this reason they were ideal for attempted transmission. If we add to these six unknowns two other coded specimens (one proven positive and the other proven negative in human volunteers) the correct identification of which was reported in an earlier publication (1), we have a total of eight unknowns. Considering the marmosets as eight groups, the statistical likelihood of correct identification by chance alone is 0.37 percent. If the marmosets are considered as individuals, this chance becomes far smaller.

The suggestion that the disease observed in the experimental animals is latent "marmoset hepatitis" stems from three points. One is that the agent producing disease in marmosets has different properties (such as heat and ether stability) than those commonly attributed to human hepatitis virus. It should be remembered that the physicochemical studies of human hepatitis virus were done in various experiments with human volunteers, by using different original inocula from experiment to experiment and with a small number of total studies. A careful search of the literature shows, for example, that the dictum of ether stability of infectious

Table 1. Results of inoculation of coded specimens from volunteer patients into marmosets. Incubation period is defined as interval from inoculation to detection of first abnormal hepatic tests.

Patient	Plasma specimen	Marmosets inoculated	Marmosets showing hepatitis	Incubation days
R.F.	Preinoculation	6	0	
R.F.	Acute phase (day 29)	6	5	34, 41, 41, 41, 48
F.K.	Preinoculation	6	0	
F.K.	Acute phase (day 30)	6	5	33, 33, 40, 40, 40
G.C.	Preinoculation	5	0*	
G.C.	Acute phase (day 29)	6	5	36, 36, 36, 36, 51
	None (controls)	8	0	

* A single animal in this group had elevated serum enzyme activities on day 60 but a normal liver biopsy.

hepatitis virus appears to be based on a single experiment in which the original investigator questioned the validity of the results (7). The properties of the agent which we have studied in previous experiments are that it is at least partially heat labile (2, 3), ether sensitive [according to data from Dr. Melnick's group (3)], and that it bands on density gradient ultracentrifugation in cesium chloride at a density of 1.210 ± 0.05 (8).

Failure of pooled human gamma globulin and serum from convalescent marmosets (2) to protect marmosets against experimental hepatitis has been cited as the second reason why the marmoset disease could not have been produced by human hepatitis virus (3). The experimental design used in our studies involves parenteral transmission, and detection of disease not by clinical but by laboratory criteria. In an analogous human situation, namely post-transfusion hepatitis and detection of both icteric and anicteric cases by serial serum enzyme follow-up, there is little evidence, if any, for protection by pooled gamma globulin. Previously infected marmosets are, however, almost solidly resistant to reinfection (2, 3). Susceptibility rates of marmosets in given experiments are high (1), and if this disease were due to a virus indigenous to marmosets one would expect more resistance to infection in randomly selected animals caught in the wild.

The third objection is based on the development of apparent hepatitis in control animals. This has not been observed in our laboratory [and at least one other (9)], in either inoculated or uninoculated control animals, nor have we seen the development of hepatitis following the repeated hepatic trauma of needle biopsy. The transitory elevations of serum enzyme in one of our inoculated controls, with a concurrent liver biopsy *not* showing hepatitis, stresses the importance of correlated biochemical and morphological observations in these studies.

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Excitatory and Inhibitory Motoneurons in the Central Nervous System of the Leech

Abstract. *The locomotion and reflex responses of the leech are brought about by muscles that are arranged in a regular, simple pattern in the body wall and that flatten, shorten, lengthen, or bend the animal. In the segmental ganglia, it is possible to recognize by morphological and physiological criteria the individual motoneurons that cause contractions and relaxations of these muscles.*

Invertebrate preparations have been used extensively for the study of problems that at present seem too complex for analysis in the vertebrate brain [see, for example (1, 2)]. The medicinal leech offers particular advantages for an analysis of the integrative mechanisms that underlie purposeful movements. These accrue principally from the rigorously segmented form of the animal's body and nervous system. Each of the segments over most of the length of the worm is equipped with a similar set of muscles and a ganglion containing only about 350 nerve cells. In a relatively small population of neurons such as this, one can hope to recognize individual nerve cells, determine their function, and eventually, perhaps, establish the way in which sensory cells, interneurons, and motor cells are connected to produce coor-

dinated movements of the animal. One might, for example, learn what cells are involved when the animal shortens in response to a noxious stimulus applied to the skin, what cells enable him to swim rhythmically, and whether inhibition of muscles occurs at the periphery.

It is already known that 14 sensory cells in each ganglion provide the animal with information about cutaneous mechanical stimuli. Their receptive fields have been mapped and their synaptic connections with each other in the central nervous system (CNS) have been traced (2). In this report it is shown that individual motor cells can also be identified. Each motor cell, like a sensory neuron, occupies a characteristic position in the ganglion and possesses a specific set of properties by which it can be recognized in animal

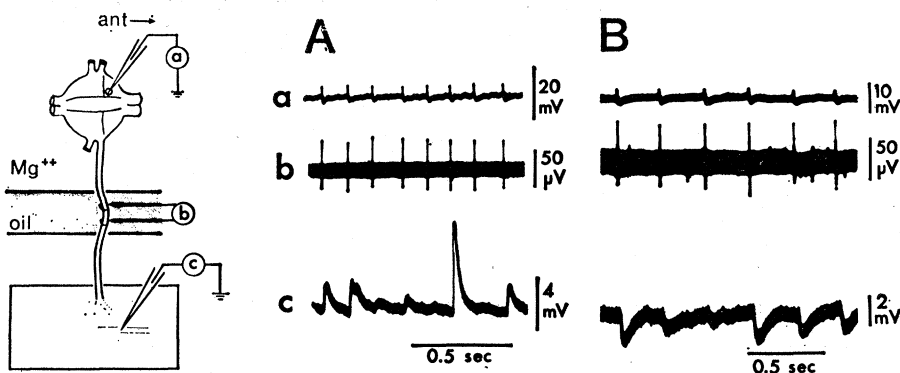


Fig. 1. Evidence that the axon of a motor cell body leaves the ganglion through a root and innervates muscle fibers. (A) Excitatory cell; (B) inhibitory cell. At left is a diagram of the experimental arrangement. The lines drawn within the ganglia in this diagram and in Fig. 2 represent the "packet" margins (see 2) which serve as convenient landmarks for locating cells. The motor neuron is stimulated by passing a depolarizing current through the recording electrode (a). Each impulse in the motor cell's axon is monitored in the root (b). At the neuromuscular junction, each action potential in the motoneuron sets up a junction potential in the muscle fiber (c); ant, anterior.