of small amounts of Ritalin. Maximum effect occurs twenty-four hours after the initial dosage. Adjustment to normal levels occurs in the ensuing 48 and 72 hour periods. It is hypothesized that the stimulating effect of analeptics is related to brain sterol metabolism.

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Pathogenicity of Murine Hepatitis Virus Recovered from Infant Swiss Mice.* (30438)

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In the spring of 1963 a natural outbreak of disease with high mortality occurred in infant Swiss mice maintained at the Virus Laboratory of the Rockefeller Foundation. A filterable agent identified as a virus of the hepato-encephalitis (H-E) group of Morris *et al*(1) was isolated in our laboratory from survivors of one litter. Observations on its behavior in nurslings and weanlings were indicative of differences from other members of the group and are reported in the present paper.

Materials and methods. Three 7-day-old survivors from a litter of 9 were received and killed for autopsy. Two showed focal necrosis of the liver. One was lesion-free but proved to be an intestinal carrier of reovirus type 3. All mice used in the subsequent experiments were drawn from specific pathogen-free colonies (SPF) of the Rockefeller Institute and unless otherwise noted were random-bred Swiss. Intracerebral injections were made with a .02 to .03 ml of liver or brain supernatants. Intestinal washings were tested by intraperitoneal injection of supernatants containing 2000 units of penicillin per 2 ml of saline suspension. All other methods were described earlier(2).

Results. Peritoneal transmission. The hepatic reaction was reproduced in one-day-old nurslings by intraperitoneal injection of a liver suspension from the 2 naturally infected infants and was subsequently maintained for 40 passages. The causal agent was resistant to penicillin and was not cultivable in bacteriologic media. Its activity was retained on filtration through Millipore filters of 300 and 220 mμ but not 100 mμ. The highest dilution which consistently resulted in hepatic involvement was 5×10^{-5}. The necrotic foci in the liver resembled those produced by other members of the H-E group. The agent was regarded, hence, as a murine hepatitis virus and designated MHV (SR4).

A study of host susceptibility was carried out with 3 different age groups of nurslings and with weanlings of nearly uniform age.

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TABLE I. Reaction of Infant and Weaned Mice to Intraperitoneal Injection of MHV (SR4).

Age of mice	No. injected	No. of deaths	% Mortality	No. killed	No. with liver lesions
1-day	70	32	45	38	38
2-day	76	30	39	46	46
5-day	34	3	8	31	14
3-4 wk	70	1*	1	69	1

* Accidental non-specific death.

The results are summarized in Table I. One- and 2-day-old nurslings were most responsive and 10 litters of each were used to determine the pathogenic potential of the virus. The respective mortality rates were subject to some error from accidental deaths and early killing. Most of the natural deaths occurred 2-5 days after injection. The high rate of maternal cannibalism generally precluded postmortem examination and it was necessary to kill some nurslings of each litter to determine their internal response. The external appearance of survivors on the 5th-7th day varied considerably. Some were erect and normal while others were stunted and sickly. A few were prone and showed signs of incoordination.

Hepatic involvement was the only gross pathologic finding and occurred in all nurslings which were killed. The reaction was either focal or less often semi-diffuse. Small white foci varying from less than 10 to innumerable were distributed on the surface of the liver or arranged in clusters. Some foci had either red rims or centers but all contrasted sharply with the darker red of the uninvolved parenchymal tissue. Minute hemorrhages were also present in some livers. Histologic examination showed discrete necrotic foci in the midst of normal parenchyma and in more advanced cases coalesced foci (Fig. 1) interspersed with islands of intact liver cells. Blue remnants of nuclei, lymphocytes, erythrocytes, and occasionally large multi-nucleate cells were present in the necrotic areas but masses of pink cytoplasmic fragments were not conspicuous. There was usually some lymphocytic infiltration of the parenchyma.

MHV (SR4) was also established in 2-day-old Princeton nurslings with essentially similar results but only a few litters were available for testing.

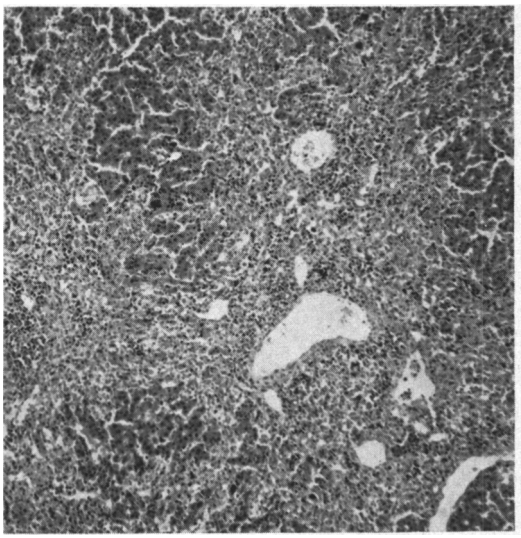

FIG. 1. Semi-diffuse necrotic lesion in liver of a 2-day-old nursling. Section stained with hematoxylin-eosin × 70.

Forty of the Swiss nurslings, representing 18 of the 20 litters, were uninjected and held in continuous contact with their infected litter mates. All survived and developed normally. They were killed on the 10th-21st day of life together with their respective mothers. Neither the young nor the adults showed any evidence of liver involvement at autopsy.

Initial testing of 5-day-old nurslings had indicated that they were significantly more resistant to MHV (SR4) than 1- or 2-day-old young and fewer litters were subsequently used. The mortality rate was much reduced and less than half of those killed showed hepatic lesions. Twelve uninjected but exposed nurslings were normal throughout life and at terminal autopsy.

Seventy weanlings were injected in groups of 5 with active liver suspensions from 14 of the 1- and 2-day-old litters. One early and accidental death was recorded. All of the

other mice gained weight normally and with a single exception had lesion-free livers when killed after 7-8 days. The liver of one mouse showed 2 foci but was not infective on subsequent passage in 1-day-old nurslings. Pooled livers from 2 of the weanling groups were also passaged in nurslings to determine whether or not the virus survived in absence of lesions. None of the injected young showed any pathologic response when finally killed.

Activity tests of MHV (SR4) in weanlings were also made in presence of our ascites tumor. A combined series with the tumor was started with a liver suspension from the 2nd nursling passage and continued for 6 transfers with ascitic fluid. Swiss weanlings injected intraperitoneally with the tumor alone generally survive through the 7th day. If killed at this time they commonly show copious ascitic fluid rich in tumor cells and blood-red as the result of early hemorrhage. Thirteen of 35 used in the combined series were dead by the 7th day. Eighteen were killed for examination and had pale or yellowish ascitic fluid with reduced numbers of tumor cells. They also showed focal or semidiffuse liver involvement. The tumor response in 4 mice was normal and was not accompanied by hepatic lesions. The response to the combined agents was markedly affected, however, by the use of virus which had been repeatedly passaged in nurslings. Fifteen weanlings injected with tumor cells and liver suspensions from the 38th, 39th, and 40th nursling passages survived through the 7th day and only 3 showed an occasional liver lesion when killed.

Oral transmission. Introduction of the virus *per os* was limited to 1- and 2-day-old mice. Thirty-five of 47 nurslings from each age group were fed active liver suspensions. Thirteen of the 1-day-old nurslings (37%) died on the 5th-7th day. Fourteen of 22 survivors, killed on the 7th-10th day, showed liver lesions and 8 were normal. Similar results were obtained with the 2-day-old young. Fourteen deaths (40%) occurred during the first week. Seventeen of 21 survivors, killed at intervals, had focal involvement of the liver and 4 were normal. Signs of nervous disorder were observed in only 2 mice.

Twelve untreated nurslings in each of the 2 age groups were held in contact with their injected litter mates. All of the 24 exposed young survived and were normal at autopsy when finally killed. Pooled intestinal washings from fed mice of one litter, in each age group, were tested for viral activity by intraperitoneal transfer. One of 14 injected nurslings died, 11 survivors showed liver lesions, and 2 were normal. Intestinal washings from the untreated but exposed nurslings of 6 litters, representing both age groups, were similarly tested. Four accidental deaths occurred shortly after injection. The 47 survivors developed normally and showed lesion-free livers at terminal autopsy.

Cranial transmission. The incoordination of an occasional injected or fed nursling was suggestive of central nervous system involvement. Intracerebral injection was carried out, accordingly, in the 3 age groups of nurslings and also in weanlings. The infant mortality rates were high and nearly uniform: 74% (20 of 27) for 1-day-old young, 74% (19 of 26) for 2-day, and 75% (21 of 28) for 5-day. The results with the latter group were surprising in view of the low rate after peritoneal transmission. Seven nurslings of each group were killed on the 3rd-5th day when most deaths occurred. Nineteen of the 21 had liver foci and 2 from the 2-day-old group were normal. Nearly all of the injected nurslings were prone by the 3rd or 4th day and showed convulsions, rolling, or rigidity of the hind legs. Twenty-four weanlings injected in groups of 6 displayed no external signs of illness and were normal at autopsy when killed 2-3 weeks later.

Histologic examination of brains from the injected nurslings showed signs of encephalitis which were less marked than these previously reported(3) in Swiss and Balb C weanlings with another type of MHV. The reaction was characterized by restricted nerve cell necrosis, some cuffing around blood vessels, infiltration of phagocytic cells, and presence of newly formed capillaries. There was little or no meningeal inflammation and no fluid.

Discussion. The SPF colony from which the mice used in the preceding tests were obtained has a very low rate of infant mortality and has shown no evidence of natural hepatic infection for an extended period. The results clearly indicated that nurslings from the colony were susceptible to MHV (SR4) under experimental conditions.

The virus was not transmissible, however, by direct contact from infected nurslings to litter mates exposed to them or by indirect contact to nurslings in adjoining cages. How the virus was acquired during the natural outbreak is left in doubt. Inspection of the infected colony was not possible. Infestation with ecto- and endo-parasites was known to occur. Presence of other viruses was intimated by infant diarrhea and our single recovery of reovirus type 3. The impact of these factors on the spread of MHV (SR4) is undetermined but their relation is suggestive.

Our findings in regard to enteric transmission were different from those reported by Rowe *et al*(4) in infant mice of another source with a similar virus described by them and designated MHV strain S. Their virus was reported as highly communicable and transmissible by both direct and indirect contact. It is not clear, however, what role another agent, namely, (LIVIM) Kraft's virus of lethal intestinal infection of mice(5), may have played in the spread of their infection.

It was noted earlier(6) that MHV (Pr) could be acquired by Princeton weanlings through cannibalism. There was no evidence, however, that any of the different types of MHV that we had studied in mice from both Swiss and Princeton colonies were communicable in the ordinary sense.

Summary. A murine hepatitis virus designated MHV (SR4) was recovered from infant Swiss mice of an outside source during a natural outbreak of disease. Intraperitoneal injection of 1- and 2-day-old nurslings from the Swiss colony of the Rockefeller Institute resulted in focal or semi-diffuse necrosis of the liver and mortality rates of 45 and 39%. The virus was less active in 5-day-old nurslings and essentially inactive alone in weanlings. It was established in the latter, however, on combination with ascites tumor cells.

MHV (SR4) was also transmitted to 1- and 2-day-old nurslings by feeding and was demonstrable in their intestinal washings. It was not communicable from either injected or fed nurslings to untreated litter mates exposed to them. One-, 2-, and 5-day-old nurslings injected intracerebrally with the virus showed incoordination and mortality rates of 73-75% but weanlings were unaffected. Necrotic lesions were demonstrable in the brains and livers of the nurslings.

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