ACUTE HEPATITIS ASSOCIATED WITH MOUSE LEUKEMIA

III. The Natural Resistance of Swiss Mice to Hepatitis

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Earlier work on mouse hepatitis[1] had indicated that different strains of mice varied markedly in their susceptibility to the disease.[2] Based on the mortality rate of infected weanlings Princeton mice were the most susceptible (98 per cent) and Swiss mice the least so (4 per cent) of the strains that were tested in significant numbers. Since the causal virus was occasionally demonstrable in liver suspensions from Swiss survivors it seemed of interest to inquire more minutely into the actual behavior of the agent in this seemingly resistant host. Transmission experiments have now been carried out with different age groups of Swiss mice, under various conditions. The pertinent observations are brought together in the present paper.

The mortality rates just mentioned were derived from 100 Princeton mice (98 deaths) and 50 Swiss mice (2 deaths). Weanlings of either sex, weighing 10 to 12 gm., were used. They were injected intraperitoneally, in groups of 5, with 0.1 ml. of an approximately 10 per cent liver suspension in saline. In these experiments as well as those that follow, the liver suspensions were prepared from a pool of 2 or more diffusely necrotic livers removed from sick Princeton weanlings killed on the 2nd or 3rd day after injection. Since the natural disease was obtainable only by chance during the passage of mouse leukemia, the virus was maintained for experimental purposes by successive passages at intervals of about 10 days. The suspensions prepared from the passaged virus were stored in the refrigerator at 35°F. In the present series of experiments the passages numbered 72 through 95.

Only 1 of the 48 Swiss survivors of the previous work showed evidence of liver damage (surface pitting) at autopsy on the 10th to the 14th day. The presence of active virus in the liver was determined by the injection of Princeton mice, which react to a dilution of 10^{-7}. Virus was demonstrable in but 2 of the 7 groups examined.

Examination of Swiss Weanlings Killed between the 10th and the 14th Day after Injection

The original experiment with Swiss weanlings was repeated in the fall of 1952, beginning with the 72nd passage of the virus. The mice were injected in 3 groups of 10 each and killed after 10, 12, and 14 days. The findings were in

[1] Nelson, J. B., *J. Exp. Med.*, 1952, **96**, 293.
[2] Nelson, J. B., *J. Exp. Med.*, 1952, **96**, 303.

essential agreement with those previously obtained although no deaths were recorded for the series of 30 mice.

The livers from 29 of the mice were normal macroscopically and on examination with a low power dissecting microscope. The liver from 1 animal, killed on the 10th day, showed distinct surface pitting, indicative of earlier injury with repair. For the determination of active virus, pools were made from the livers of 5 mice and the suspensions injected intraperitoneally in 5 Princeton weanlings. The 2 liver pools from the Swiss mice killed on the 10th day contained active virus, though only 3 of the 10 injected Princeton mice died. 1 pool from each of the groups killed on the 12th and 14th day was also active. The deaths, after injection in Princeton mice, were 3 of 5 and 1 of 5.

The injection of Princeton weanlings with the passaged liver suspensions used throughout this work was regularly followed by a high death rate. In the maintenance of the virus by passage 2 or more mice were commonly killed but with 8 groups of 5 mice injected between the 73rd and the 86th passages none of the animals were sacrificed. The mortality rate of the 40 mice in this series was 100 per cent. All deaths occurred between the 2nd and the 5th day.

Examination of Swiss Weanlings Killed at Intervals between the 1st and the 21st Day after Injection

Owing to the possibility that death might be delayed in infected Swiss mice the animals in the preceding experiment were held under observation for at least 10 days. In this experiment the injected mice were examined at earlier intervals and the range extended through the 3rd week. At autopsy the virus was looked for in peritoneal washings as well as in the liver.

20 Swiss weanlings were injected intraperitoneally with the standard inoculum (0.1 ml. of a 10 per cent liver suspension in saline). The animals were killed in groups of 2 at intervals between the 1st and the 21st day.

At autopsy the peritoneal cavities were washed with 1.0 ml. of saline and the washings from each lot of 2 pooled. The livers were removed to a Petri dish for examination with the dissecting microscope and a suspension prepared from each pair. 3 Princeton weanlings were injected intraperitoneally with 0.1 ml. of these suspensions. The survivors were killed on the 7th to the 10th day and autopsied.

No deaths occurred in the group of 20 mice. None of the signs indicative of hepatitis in Princeton weanlings were apparent and the weight gains were normal. Liver lesions characterized by small, rather indistinct foci, minute areas of hemorrhage, and surface pits or depressions were first observed on the 3rd day after injection and continued through the 7th day. The livers from all the mice were deep red in color. The mice killed between the 10th and 21st day showed normal livers with no apparent structural alteration.

Active virus in sufficient concentration to kill a majority of the injected Princeton mice was demonstrable in the liver from the 3rd through the 5th day. By the 7th day the virus titer was evidently decreased as the suspension resulted in the death of only 1 test mouse. In this series the virus was not detectable in the liver after the 7th day, though in the earlier experiments it had been observed in one group through the 14th day. Active virus was present in the peritoneal cavity throughout the entire interval. There was no significant change in the action of the washings until the 21st day. The suspension injected at this time killed but 1 of the 3 test mice. The 2 survivors showed normal livers at autopsy.

The results of this experiment, which are summarized in Table I, indicated survival of the virus in the liver through the 7th day. Its presence was attended

by discrete areas of necrosis which failed to progress as they did in Princeton weanlings. The persistence of the virus in the peritoneal cavity, after its disappearance from the liver, was an unexpected finding.

TABLE I

The Duration of Liver Injury and the Survival of Hepatitis Virus in Swiss Weanlings

No. of Swiss mice injected	No. of days to autopsy	No. of mice with liver lesions	No. of deaths in each group of 3 Princeton mice	
			Injected with liver suspension	Injected with peritoneal washings
2	1	0	3	3
2	2	0	3	3
2	3	2	3	3
2	4	2	2	2
2	5	2	3	3
2	7	2	1	2
2	10	0	0	3
2	11	0	0	3
2	14	0	0	3
2	21	0	0	1

Supplementary Examinations of Swiss Weanlings after Subcutaneous and Intraperitoneal Injection

Two additional but unrelated experiments were carried out with Swiss weanlings to determine the presence of virus in the liver and peritoneal cavity on subcutaneous injection and in the blood on intraperitoneal injection. Earlier work with Princeton weanlings had indicated that subcutaneous injection was attended by liver lesions with death and that intraperitoneal injection was followed by the appearance of virus in the circulating blood.[2]

6 Swiss mice were injected subcutaneously with the standard inoculum and killed in groups of 3 on the 3rd and the 5th day. All the mice were normal in appearance. Liver lesions (surface pits) were observed at autopsy in only 1 of them,—from the group killed on the 5th day. Pooled liver suspensions and peritoneal washings from each of the groups were injected intraperitoneally in 3 Princeton weanlings. The results of this test were somewhat inconsistent but indicated the presence of virus in both loci on both days. The liver suspension prepared on the 3rd day killed 3 of the 3 Princeton mice and the peritoneal washings 1 of the 3. The 2 survivors showed no lesions at autopsy. The 3 mice injected with the liver suspension of the 5th day survived, but on subsequent autopsy 2 of them showed surface pits and petechial hemorrhages. 1 of the mice injected with peritoneal washings died. The liver from 1 of the survivors showed scattered foci of necrosis and the other, cirrhosis.

For the detection of virus in the blood, 5 injected Swiss weanlings were killed on the 3rd day. The livers from 3 of the mice showed minute surface hemorrhages at autopsy. Immediately after death by ether anesthesia, 0.1 to 0.2 ml. of blood was aspirated from the heart with a capillary pipette and added to 0.5 ml. of saline containing 0.1 ml. of heparin (27 mg. per cent). Virus was demonstrable in all 5 blood samples on intraperitoneal injection 0.1 ml.)

in Princeton weanlings. 2 mice were used with each sample. 9 of the 10 Princeton mice died on the 3rd or 4th day with typical manifestations of hepatitis. The liver from the single survivor was normal.

Response of Infant Swiss Mice to Hepatitis

Since Princeton weanlings were highly susceptible to mouse hepatitis the testing of infant mice was largely neglected and only a few tests were made. The results, not previously reported, were indicative of equal susceptibility. The resistance of Swiss weanlings focused attention on the use of nurslings and in this experiment their response to the virus was determined by intraperitoneal injection.

TABLE II

The Mortality of Infant Swiss Mice Infected with Hepatitis

Age of infants	No. of mice injected	No. of deaths	Average period until death
days			*days*
1	8	8	3.7
3	8	8	8.6
5	8	6	7.1
7	6	5	6.1
10	6	6	7.0
14	7	0	—

Six litters of nursing Swiss mice, varying in age from 1 to 14 days, were removed with their mothers from the breeding colony to individual cages. The young were injected in the usual way, except that the inoculum was reduced to 0.05 ml. and a special 30 gauge needle used with the very young litters. The injected nurslings were returned to their respective mothers and the cages held under observation for 10 days.

The results of this experiment, summarized in Table II, indicated that the natural resistance characteristic of Swiss weanlings was not acquired until nearly the 2nd week after birth.

The findings with the infant mice injected between the 1st and the 10th day after birth were reasonably uniform. There were 36 mice in this category and 33 of them died. The interval between injection and death compared favorably with that of Princeton weanlings only in the group of day old infants. In the groups born on the 3rd through the 10th day the interval was noticeably prolonged. 2 of the 3 survivors showed liver lesions when killed and virus was demonstrable on passage in Princeton weanlings. The mice injected on the 14th day survived and developed normally. At autopsy their livers showed no structural alteration but passage in Princeton mice indicated that active virus was present.

A number of the mice that died were partially or entirely eaten by their mothers and others showed advanced postmortem changes. 19 which were autopsied showed liver lesions varying from focal to diffuse necrosis.

The 6 uninjected mothers remained normal throughout the period of observation and showed no liver lesions at autopsy. Liver suspensions from the mothers of the first 5 litters

were innocuous on passage in Princeton weanlings. The liver suspension from the 6th adult produced a reaction in Princeton mice indicative of a very low concentration of virus.

Passage of Hepatitis Virus in Swiss Weanlings

A passage series was begun in Swiss weanlings to determine the effect of continued residence on the pathogenicity of the virus. The results were indicative of a modification in the direction of increased virulence and were confirmed by the findings of a 2nd passage series. The combined data from these two experiments are summarized in Table III.

TABLE III

The Effect of Passage in Swiss Weanlings on the Pathogenicity of Mouse Hepatitis Virus

Passage No.	Experiment 1		Experiment 2	
	No. of deaths in each group of 5 mice	Average period until death	No. of deaths in each group of 5 mice	Average period until death
		days		*days*
1	0	—	0	—
2	0	—	0	—
3	0	—	2	4
4	2	3	0	—
5	4	3.5	0	—
6	3	3	0	—
7	5	3	1	5
8	4	4	3	3.7
9	3	4	5	3.4
10	5	2.6	5	3.6

The two passage series were conducted in much the same way. 5 Swiss weanlings were injected intraperitoneally with the standard inoculum and killed on the 4th day. A suspension was prepared from the pooled livers for the subsequent passage. This procedure was continued at intervals of 3 or 4 days with all groups in which there were no deaths. In the event of death 2 mice were killed on the same day or, if this was not possible, dead mice which showed the least postmortem deterioration were selected. To provide an added control on the activity of the suspension each one was injected into 2 or 3 Princeton weanlings. 10 successive passages were carried out in both experiments.

In the first experiment no deaths occurred until the 4th passage but at autopsy 12 of the 15 mice showed liver lesions. From the 4th through the 10th passage 26 of the 35 mice died between the 2nd and the 5th day after injection. Lesions were observed in the livers from the 9 survivors. In the second experiment 2 deaths were recorded during the 3rd passage but consistent deaths were delayed until the 7th passage. 22 of the 28 survivors in the first 6 passages showed liver lesions when killed. 14 of the 20 mice in the last 4 passages died. There was no significant variation in the intervals between injection and death. At autopsy 5 of the 6 survivors showed lesions in the liver. All the injected Princeton mice died.

The livers of the surviving mice in these experiments were generally dark red and the structural alterations rather inconspicuous macroscopically, although well defined by low magnification. The lesions varied considerably in number and kind. Surface depressions or pits, pits

with a red peripheral ring or a red point at the base, small red foci, red foci with a white peripheral zone, and small white foci were observed at one time or another. 2 of the survivors showed a diffuse reaction in the liver as did the dead mice that were autopsied. The diffuse reaction in the livers of Swiss weanlings was noticeably different from the almost structureless coalescing necrosis commonly observed in Princeton mice. In the Swiss mice the livers had a speckled appearance with bright red areas interspersed in a white network of necrotic parenchymal tissue.

A difference was also noted in the appearance of the Swiss weanlings prior to death. Princeton mice usually showed signs of illness before they died. They were inactive, thin, tended to huddle, and their fur was roughened. Swiss mice appeared normal up to the time of death, save for terminal tremors and convulsive jumpy movements. As in Princeton mice there was no consistent involvement of any organ other than the liver.

TABLE IV

The Effect of Passage in Swiss Weanlings on the Titer of Hepatitis Virus in the Liver

Dilution of liver suspension	No. of deaths in each group of 5 Princeton mice		
	Suspension from Princeton mice	Suspension from 1st Swiss passage	Suspension from 9th Swiss passage
10^{-3}	5	4	5
10^{-4}	5	5	4
10^{-5}	5	3	5
10^{-6}	5	1	5
10^{-7}	5	1	5
10^{-8}	4	0	1
10^{-9}	0	0	0

Titration of Virus in Liver Suspensions from the Swiss Passage Series

The virus titer in liver suspensions from the 1st and 9th groups of the 2nd passage series was determined from the mortality of Princeton mice injected with 10-fold dilutions and compared with that of a suspension from Princeton weanlings.

In the preparation of the suspension from Princeton weanlings the livers from 2 mice of the 83rd passage were used. These mice died on the 2nd day after injection and were autopsied while still warm. A 10 per cent suspension of the weighed livers was made in saline and subsequently diluted by 10-fold intervals through 10^{-9}. Princeton weanlings, in groups of 5, were injected intraperitoneally with 0.1 ml. of dilutions 10^{-3} through 10^{-9}. The volume of inoculum was not considered in expressing the dilution value.

The 10^{-1} dilution of this suspension was used in injecting the 1st group of mice in the 2nd Swiss passage experiment. In preparing the suspension for titration from them the livers of all 5 mice, killed on the 4th day, were used. 3 of the livers showed lesions. The suspension from the mice of the 9th passage was made from the pooled livers of 2 mice that died on the 4th day. They were autopsied almost immediately after death.

The results of the 3 titrations are given in Table IV. All the 25 mice injected with dilutions 10^{-3} through 10^{-7} of the suspension from Princeton weanlings

died. With dilution 10^{-8}, 4 of the 5 mice died and with dilution 10^{-9} there were no deaths.

The titration of the suspension from the 1st Swiss passage gave rather inconsistent results. The 3 dilutions through 10^{-5} resulted in the death of 12 out of the 15 injected mice but with dilutions 10^{-6} and 10^{-7} only 2 of the mice died. There were no deaths with dilutions 10^{-8} and 10^{-9}. The results of the 9th passage titration were less irregular. 24 of the 25 mice injected with dilutions from 10^{-3} through 10^{-7} died, as did 1 of the 5 which received the 10^{-8} dilution. No deaths were recorded with the 10^{-9} dilution. The majority of all deaths occurred on the 4th or the 5th day after injection.

Detection of Virus in the Blood of Swiss Weanlings on Subcutaneous Injection

Mouse hepatitis virus is detectable in the blood of Princeton weanlings as early as 24 hours after subcutaneous injection. Tests were made for its presence in the blood of Swiss weanlings following the subcutaneous injection of unmodified virus and of virus modified by passage.

3 groups of 3 Swiss weanlings were injected intraperitoneally with the standard inoculum and killed at intervals of 24, 48, and 72 hours. Blood was drawn from the heart by the method earlier described. The pooled blood from each group of 3 mice was injected intraperitoneally in 3 Princeton weanlings. The experiment was repeated in the same way with Princeton weanlings as a control on the activity of the liver suspension. The same test was again carried out with Swiss weanlings injected subcutaneously with a liver suspension from the 10th group of the 2nd Swiss passage experiment.

The presence of virus in the blood of the Princeton weanlings, following subcutaneous injection, and of the Swiss weanlings which received the virus modified by passage was indicated by the death of all 18 test mice. There was no indication, however, that virus was present in the blood of the Swiss weanlings injected subcutaneously with unmodified virus. The 9 test mice survived and at autopsy showed no involvement of the liver.

Although the unmodified virus was not demonstrable in the blood by actual test, its presence there is indicated by the earlier noted recovery from the liver and peritoneal washings on subcutaneous injection. It may well be that the agent is transported by the blood in increments too small to detect.

DISCUSSION

The hepatitis virus used in the preceding experiments was originally obtained from Princeton weanlings during the passage of a lymphocytic leukemia which is of natural occurrence in old breeders of this strain. The hepatitis produced by the virus has been previously encountered only under these circumstances. Leukemia of natural origin has never been observed in adult Swiss mice, and Swiss weanlings have been highly resistant to the passaged tumor cells introduced by intraperitoneal injection. Although no direct relation is inferred, it is of interest that Swiss weanlings were also resistant to mouse hepatitis.

From the experimental findings it was evident that the resistance of weaned Swiss mice was more effective in restricting the activity of the virus than in eliminating it. The terminal manifestations which characterize hepatitis in Princeton mice were commonly lacking in Swiss weanlings. The virus was demonstrable, however, for a considerable period of time in the peritoneal cavity and the liver. Its recovery from the peritoneal washings 21 days after injection was indeed suggestive of actual multiplication rather than mere survival. Carriage of the virus to the liver was followed by the appearance of lesions which generally failed to progress as they did in Princeton mice. Although repair promptly ensued, the virus was still detectable in some instances.

The susceptibility of infant Swiss mice, until about the 10th day of life, indicated that the factors responsible for the resistance of weanlings were not acquired prenatally by placental transfer nor postnatally through the medium of their mothers' milk.

The ability of Swiss weanlings to restrict and ultimately eliminate the virus of hepatitis was far from being a state of absolute resistance. Modification of the virus, resulting from continued passage in these mice, enabled it to overcome the hosts' defense and produce the terminal manifestations, characteristic of the disease in a fully susceptible animal. In connection with the increased titer of the modified virus it may be noted that some increase was also observed during the long continued passage in Princeton mice.

The factors which underlie the natural resistance of Swiss weanlings remain unexplained. Neither antibodies nor phagocytic cells appear to play a significant role in its expression. Whatever the factors may be they determine a property which is commonly shared by these mice and is seemingly under genetic control.

SUMMARY

The mortality rate for 80 Swiss weanlings infected with mouse hepatitis was 2.5 per cent in comparison with 98 per cent for 140 Princeton weanlings.

In Swiss weanlings discrete lesions, which generally failed to progress, were observed in the liver from the 3rd through the 10th day after intraperitoneal injection. The causal virus was demonstrable in peritoneal washings through the 21st day and less regularly in the liver through the 14th day. It was also detectable in both loci after subcutaneous injection.

Infant Swiss mice were susceptible through the 10th day of life, intraperitoneal injection being commonly followed by death.

The pathogenicity and titer of the virus were significantly increased by successive passage in Swiss weanlings.

The virus was detected in the blood of Swiss weanlings on subcutaneous injection only after it had been modified by passage.