ACUTE HEPATITIS ASSOCIATED WITH MOUSE LEUKEMIA

I. Pathological Features and Transmission of the Disease

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Plate 8

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During the past year (1951) viral diseases of white mice, characterized by involvement of the liver, were encountered independently in 3 different laboratories. The first report was by Jordan and Mirick (1) at the Johns Hopkins Hospital in Baltimore and was concerned with a chronic infection. An acute type was later described by Gledhill and Andrewes (2) at the National Institute for Medical Research in London, England. The third type, to be considered in the present paper, is also an acute disease, which was found in a particular strain of mice maintained at the Rockefeller Institute, New York.

A variety of agents, namely cysticerci (*Taeniae formis*), coccidia, bacteria, and viruses, which are pathogenic for the mouse, may invade the liver and produce local changes. Liver lesions of unknown etiology characterized by infiltration and necrosis have been reported by Olitsky and Casals (3) in a high percentage of supposedly normal mice of 2 different strains.

A liver disease of particular interest was described in 1917 by Tyzzer (4). It occurred in epidemic form in a colony of Japanese waltzing mice and was highly fatal. Seventy deaths were noted, after an interval of 10 to 40 days, and one recovery. Lesions were limited to the liver and consisted of multiple tubercle-like nodules. A noncultivable spore-forming organism named *Bacillus piliformis* was regularly observed within intact hepatic cells at the margin of the necrotic foci. It was also present in the intestinal mucosa but produced little or no reaction. The disease was transmissible by contact, feeding, and intravenous injection in waltzers but not by intraperitoneal injection. Common albino mice were largely resistant; only 2 cases were noted among several hundred under observation. In 1947, however, Rights, Jackson, and Smadel (5) encountered the same disease in a British strain of Swiss mice. *B. piliformis* was again present in the liver lesions. The disease was apparently not transmissible in Swiss mice by feeding or on intraperitoneal injection. Intracerebral injection resulted in a local development of *B. piliformis* followed by paralysis and death. The organism was propagated in embyonated eggs but rapidly became avirulent. The writers pointed out that the relation of *B. piliformis* to the disease was not clearly established.

The hepatitis of probable virus etiology described by Jordan and Mirick (1) was first observed on the 4th mouse passage following the initial injection of liver from

hepatitis in man, together with urethane. The precise source of the virus was thus uncertain. The disease was of slow onset (3 to 4 weeks), chronic, and rarely fatal (±2 per cent). At autopsy, splenomegaly, focal necrosis of the liver, and serous ascites were generally observed. Mice 1 to 5 months old and of 3 different strains were susceptible although obvious disease occurred in only one-third of them on injection.

The viral hepatitis of Gledhill and Andrewes (2) had its origin in Webster's strain of VS mice following the injection of liver filtrates from mice of the Parks strain. Losses had recently occurred in the breeder stock of the latter mice. In VS weanlings less than 14 gm. in weight, the disease was nearly 100 per cent fatal, with the maximum death rate on the 5th day. The chief manifestation at autopsy was diffuse necrosis of the liver. Older VS and Parks mice were relatively resistant. The latter were regarded as carriers of a latent virus activated by passage in young VS mice. The disease was preventable by the prior injection of aureomycin and terramycin.

Leukemia in Princeton Mice and Its Association with Acute Hepatitis

The circumstances which originally led to the detection of the present disease were intimately connected with the serial passage of leukemia in albino mice of the random bred Princeton strain.

Leukemia of natural origin was not observed in mice of the Princeton strain until 1948. It may well have existed, however, throughout the entire history of this colony, which was started in 1922 while the animal pathology laboratories were still in Princeton, and continued in New York. The disease was of sporadic appearance, with a morbidity rate of somewhat less than 5 per cent, and occurred only in adult mice upwards of 6 months in age. It was of slow progression but ultimately fatal. The pathognomonic cell was an atypical lymphocyte which was present in the circulating blood but was particularly conspicuous in the organs of the lymphatic series. At autopsy a marked enlargement of the thymus, spleen, and all regional lymph nodes was regularly observed. The disease was detectable during life by palpation of the groin nodes.

The naturally acquired leukemia of mature animals was transmissible experimentally to Princeton weanlings of either sex by the injection of suspensions from any of the affected organs. In practice, young females weighing 12 to 15 gm. were commonly used. They were injected intraperitoneally, in groups of 5, with 0.1 to 0.2 ml. of saline suspensions prepared from minced and ground splenic tissues in a concentration of approximately 10 per cent. In comparison with the naturally acquired disease there was a marked increase in the activity of the tumor cells after several intraperitoneal passages. The course of leukemia from the time of injection to death was 10 days or less and the morbidity rate was 75 to 90 per cent. The manifestations at autopsy were similar to those of the long continued natural disease but appreciably less conspicuous. In the injected mice the liver was commonly involved and had a swollen, pallid appearance. Females often showed bilateral hypertrophy of the ovary and its supporting tissues. Swiss mice of comparable age were largely resistant to the passaged leukemia. A single positive result was obtained in 50 mice injected intraperitoneally with suspensions which were active in Princeton mice.

Contrary to experience elsewhere with other strains of mice, difficulties were encountered in maintaining the leukemia by intraperitoneal injection in Princeton weanlings. In 2 successive series the customary leukemic reaction ceased

after 30-odd passages. In a 3rd series the leukemia was again lost but in an earlier passage—the 12th—which was made during February, 1951. At this time an explanation was sought.

Two of the mice in this passage died on the 9th day after injection. At autopsy there was no indication of leukemia but the livers were noted as having a granular surface and speckled with gray. Three of the mice survived and when killed on the 14th day were normal in appearance. Their livers were also granular, but there were no signs of leukemia.

Serial passage was then begun in Princeton weanlings, with a suspension from the livers of the 2 mice that died. It was soon apparent that the original leukemic syndrome had been superseded by one typical of acute hepatitis. Unless otherwise noted, all the following observations were made with this strain of the disease.

TABLE I

Number of Deaths by Days after Intraperitoneal Injection of Liver Suspensions into Weanlings

No. of mice injected	No. of deaths	Interval between injection and death in days								
		1	2	3	4	5	6	7	8	9
		No. of deaths per day								
100	100	0	8	34	34	14	6	2	2	0

Characteristics of the Experimental Hepatitis

General Manifestations.—Acute hepatitis was regularly reproducible in Princeton weanlings by the intraperitoneal injection of liver suspensions from dead or sick mice. Beginning with the first liver injection from the 3rd leukemia series the disease was maintained consecutively for 60 passages over a period of 13 months.

In practice, several acutely ill mice were killed on the 2nd or 3rd day after injection. Their livers were removed aseptically to a Petri dish, minced, and ground with saline in a glass tissue grinder. An approximate 10 per cent suspention was made in the same diluent. Princeton mice of either sex, less than 15 gm. in weight (commonly 10 to 12 gm. and 3 to 4 weeks old) were injected intraperitoneally, in groups of 5, with 0.1 ml. of the whole, unsedimented liver suspension.

The injected mice regularly showed objective signs of illness and well marked pathological changes at autopsy. Evidence of leukemia was not observed macroscopically during the entire series of injections. The incubation period was brief, usually 24 to 48 hours. The course of the disease was also short and after the first few passages commonly terminated fatally during the 1st week after injection. As indicated in Table I, 90 per cent of the deaths in a group of

100 mice representing 20 different passages, occurred between the 2nd and 5th day.

During the brief interval prior to death, injected Princeton mice showed outward signs of illness which were definite but not specific. In most instances they became progressively inactive from the 2nd day on and were sometimes found in a prone position shortly before death. Trembling of the extremities was not uncommon. There was a significant loss in weight, up to several grams, and considerable roughening of the coat. There was no evidence of diarrhea and no external indication of jaundice.

Macroscopic Pathology.—At autopsy the only constant pathological changes were in the liver. Other manifestations, namely pallor of the kidneys, blood in the upper intestinal tract, and distention of the bladder by a deep yellow urine, were observed in varying combinations. Ascites was very rarely encountered.

The color change in the liver was of particular value in diagnosis. The liver of a normal mouse *in situ* is uniformly dark red. In moribund (killed) or dead animals the intact liver was diffusely pale, the color varying from a light pink to ivory or yellowish white. The uncut surface was often studded with tiny pits and with pin-point hemorrhages.

Under low magnification (× 3) the architecture of the liver was profoundly altered. The normal liver on removal to a dissecting microscope showed a characteristic pattern of pale lobules separated by red interlobular spaces. In the diseased livers the sharp demarcation between these areas was lost. Those from advanced cases showed almost complete lobular coalescence and the appearance of small opaque spots. Minute depressions were often seen on the uncut surface, giving a dimpled appearance when numerous. Some of these pits contained blood, accounting for the hemorrhages seen in the gross.

This picture (Fig. 3) was characteristic of at least 90 per cent of the Princeton weanlings injected with active liver suspensions. Three additional types of reaction were occasionally seen: a dark red liver with flat, opaque, white surface foci up to 2 mm. in diameter (Fig. 1); a dark red liver with few to many surface pits without hemorrhages; and a moderately dark liver with coarse cirrhotic lobulations (Fig. 5). The so called granular livers observed in the mice of the 12th passage in the 3rd leukemic series were probably of this type. These reactions generally occurred in mice injected with suspensions of known or suspected low titer, and seemingly preceded recovery.

Microscopic Pathology.—The predominant microscopic feature in liver sections was necrosis of the parenchymal cells. In most instances the reaction apparently began in many small areas throughout the organ and progressed rapidly with eventual coalescence. The few kidney and spleen sections that were examined showed no definite pathological changes.

Most of the tissue sections were stained with Giemsa. Hematoxylin-eosin was occasionally used. In livers which showed surface foci in the gross the necrosis was limited to small circumscribed areas which bore no consistent relation to the blood vessels. In some instances, as in Fig. 2, they were located between 2 central vessels. These areas were surrounded by liver cells of normal appearance. Within the focus the tissue was thinned out and replaced by pink-staining masses together with nuclear debris. At the periphery there was some infiltration of leucocytes and lymphocytes.

In livers which showed a diffuse macroscopic reaction the degree of necrosis varied from many small irregular areas interspersed with intact parenchymal cells to nearly complete cellular destruction. The necrotic areas were composed of amorphous pink material with many nuclear fragments. Some of the latter were intensely basophilic. Commonly, inflammatory cells were scattered throughout the section but were not conspicuous. There was no evidence

of cuffing around blood vessels. Some of the larger vessels were distended with blood. Fig. 4 is illustrative of partial necrosis. The outer margin of the livers often showed small depressions, some containing red blood cells. They conformed to the pits and petechial hemorrhages seen in the gross. The pits were probably the result of circumscribed surface necrosis with resulting loss of tissue.

In livers from recovered mice the surface depressions were visible without necrosis but with a backing of connective tissue. More extensive damage with recovery was attended by cirrhosis of the liver The pits were more numerous and much deeper, giving a coarsely lobular appearance. There was a significant increase in connective tissue elements. These sections also showed regeneration of liver cells without organization and many open spaces. A low power view of such a liver is shown in Fig. 6.

Transmission by Extraperitoneal Routes

Princeton weanlings in groups of 10 or more were injected subcutaneously (0.1 ml.), intranasally, intracerebrally, and in the foot pad (0.03–0.05 ml.) with 10 per cent liver suspensions of known activity. All the mice in the 4 series developed signs of illness and died. In the nasal and foot pad experiments, the interval between injection and death was somewhat longer than average, up to 8 days. At autopsy there was no significant departure from the customary hepatic reaction. There were no local changes in the skin, foot pads or joints, lungs, or brains of the mice injected by the corresponding routes. Brain suspensions from the intracerebral group, made on the 3rd day, produced hepatitis on intraperitoneal passage. Sections from the brain showed no apparent pathology.

Oral transmission experiments were also conducted with Princeton weanlings.

Four separate groups of 5 mice were fed liver suspensions. The inoculum, about 0.05 ml. in volume, was introduced into the mouth with a capillary pipette. 5 of the 20 mice died on the 7th to the 10th day and showed typical liver lesions. 9 of the 15 survivors, killed on the 10th to the 14th day, also showed unmistakable evidence of hepatitis at autopsy. The livers from 5 were pitted and cirrhotic but inactive on passage, while 4, with focal areas of necrosis, produced acute hepatitis on intraperitoneal injection. 6 of the survivors showed neither macroscopic nor microscopic evidence of hepatitis and their liver suspensions were inactive on passage.

As an extension of the oral series, transmission by direct contact was attempted.

In each of 2 experiments 5 normal mice were placed in the same cage with 5 infected by intraperitoneal injection. The latter mice were removed as they died, and in no case was cannibalism observed. The exposed mice were in contact with at least one sick individual over a period of 5 to 7 days. All of them survived and when killed on the 14th day, showed no hepatic lesions. Suspensions of their livers proved inactive on passage. In 2 additional experiments, otherwise carried out in the same way, one or more of the dead injected mice were left in the cage until partially eaten. In this series, 5 of the exposed mice died between the 3rd and 8th day after cannibalism was first noted. They showed liver lesions at autopsy. 2 of the 5 survivors which were killed on the 10th to the 14th day also gave evidence of hepatic

involvement. The liver suspensions from these mice were active on passage. Three of the survivors were normal at autopsy and their liver suspensions inactive.

Additional Observations on the Association of Leukemia and Acute Hepatitis

Reexamination of the autopsy records from the first 2 leukemia series revealed unmistakable evidence of hepatitis in the injected mice of the passages during which it superseded the leukemia. The findings from one series are presented as illustrative of the replacement of leukemia by hepatitis.

This series was begun in February, 1949, with a spleen suspension from 2 leukemic breeders. It was successfully continued for 35 successive passages, at intervals of 7 to 14 days, in groups of 5 young Princeton mice. Of the 175 mice used in this series, 156 (89 per cent) showed typical manifestations of leukemia at autopsy. In the 35th passage, 3 of the mice were positive and showed no unusual pathology. In the 36th passage, begun in April, 1950, 1 mouse died on the 7th day and 4 that were sickly were killed. At autopsy there was no indication of leukemia but it was noted that the livers were unusually pale. In the 37th passage 2 mice died on the 6th day and 3 were killed. The livers were again pale and had a pitted granular appearance. There were no manifestations of leukemia. The series was discontinued at this time.

A typical involvement of the liver was again encountered during a 4th series of leukemia passages, in this case under somewhat different circumstances.

A number of passages were carried out with a mixture of leukemic cells and pleuropneumonia-like organisms (PPLO) of the catarrhal type. On intraperitoneal injection these bacteria ordinarily multiply together with the tumor cells but produce no pathological changes in the liver. The liver of a mouse in the 5th combined passage (23rd of the leukemia), that died on the 7th day, showed focal involvement at autopsy. The livers of 4 mice killed at this time were normal. There were no indications of leukemia. 4 subsequent passages begun with the pathologic liver, aureomycin being added to eliminate PPLO, resulted in clear cut evidence of acute hepatitis. The general manifestations were identical with those of the previously described disease. Several passages from the normal livers were also suggestive. The livers of these mice showed pitting although active disease did not develop.

Attempts to Initiate Hepatitis in the Absence of Leukemia

(*a*) *By Serial Passage of Liver Suspensions in Normal Mice.*—The breeding colony of Princeton mice is maintained in semiquarantine at all times. Save for leukemia in adults, there is little evidence of naturally acquired disease. Numerous young mice removed from the colony for experimental purposes and later killed have been examined. In no instance were lesions suggestive of hepatitis observed. From time to time, however, a few unweaned or recently weaned mice with a sickly appearance were seen. Most of these mice, which were commonly from large litters, recovered. 10 individuals of this description were autopsied. 2 of them showed rather pale livers, but microscopic examination failed to reveal any of the customary hepatic changes. Liver suspensions injected into weanlings and passaged once were innocuous.

As a possible means of detecting carriers or of activating latent liver infec-

tions, a passage series with liver suspensions from normal weanlings was started and continued throughout a period of 22 weeks.

5 normal mice weighing 10 to 12 gm. were removed at random from the breeding colony and killed. A 10 per cent suspension was made from their pooled livers and 0.1 ml. injected intraperitoneally into each of 5 mice. The series was maintained for 15 passages in the same way. The interval between injection and autopsy was 10 days, which corresponded to the average period in the passage of leukemia. The sex varied from group to group but was the same in each individual one. At autopsy all livers were examined *in situ* and, on removal, with a dissecting microscope.

1 mouse in the 3rd passage showed early signs of illness and was killed. At autopsy there was an obvious bacterial involvement of the liver with a marked leucocytic reaction. The liver suspension from this mouse was passed separately, with the addition of penicillin. There was no repetition of the reaction. All the other mice appeared normal during the period of observation, gained weight rapidly, and showed no pathological changes in the liver or elsewhere when killed.

(*b*) *By Non-Specific Bacterial Injection.*—In connection with another experiment, many young Princeton mice, numbering at least 200, were injected intraperitoneally with exudates containing PPLO of the catarrhal type and with pure cultures of these latter. In view of the positive result obtained in the combined passages of the 4th leukemia series, particular attention was paid to the livers of these mice. Both single and successive passages of liver suspensions were made, the animals being killed at varying intervals. None of the injected mice showed signs of illness and there were no fatalities. At autopsy there was no specific involvement of the liver throughout the entire series.

DISCUSSION

The hepatitis now considered was quite unlike any of the well recognized infections of the albino mouse and was obviously different from the chronic disease reported by Jordan and Mirick (1). In its general nature, however, there was a close resemblance to the hepatitis described by Gledhill and Andrewes (2). Both of these diseases were characterized, under experimental conditions, by rapid onset, high mortality rate, and necrotic involvement of the liver.

The origins of the two diseases were quite dissimilar. The hepatitis of Gledhill and Andrewes (2) was carried by a strain of mice which were largely resistant to this malady. The full expression of the disease was brought out by the injection of liver suspensions into a second, non-carrier strain. No mention is made of leukemia. Immature Princeton mice in which the present hepatitis had its inception were later shown to be almost uniformly susceptible to it on intraperitoneal transmission. The animals in which it arose had been injected with a suspension of leukemic cells.

As yet the underlying source of the hepatic infection has not been determined. Endemic carriage of the causal agent within the breeding colony pro-

vides the most logical explanation. If present in low titer or in a latent state the agent might well declare itself during the long continued passage of leukemia. The favorable milieu provided by some tumors for certain extraneous pathogenic agents would lend support to this view. As already remarked, the disease was communicable by cannibalism. A means of maintaining the natural infection, at a low level, might thus be provided.

Consideration of the carrier theory must be continued but to date no factual evidence in support of it has been forthcoming. Many attempts have been made to detect a latent infection in mice that appeared normal, but all were unsuccessful. At present it can only be stated that the acute hepatitis had not been observed prior to the leukemia. What stimulus, if any, the presence of the tumor cells provided, must await further enquiry. The two disease syndromes did not continue to coexist, at least under natural conditions. In the 4th passage series which terminated in hepatitis, leukemia was not present and there was no indication of its reappearance during the subsequent passages.

SUMMARY

On four occasions a naturally occurring mouse leukemia, which was maintained by serial passage in weanlings of the Princeton strain, was superseded by a syndrome typical of acute hepatitis. Once initiated, the disease was regularly transmissible by the injection of liver suspensions of sick mice. It was also passed, though irregularly, by feeding such suspensions, and it also followed cannibalism.

The course of the disease after intraperitoneal injection of liver suspensions into normal weanlings was commonly less than 7 days and the mortality rate nearly 100 per cent.

Focal or diffuse necrosis of the liver was the only constant lesion at autopsy. On recovery, which occurred only in exceptional cases, cirrhosis was often found.

The primary source of the disease was undetermined. Latent carriage by healthy mice was not detectable on direct examination nor by the serial passage of suspensions of normal livers.

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PLATE

EXPLANATION OF PLATE 8

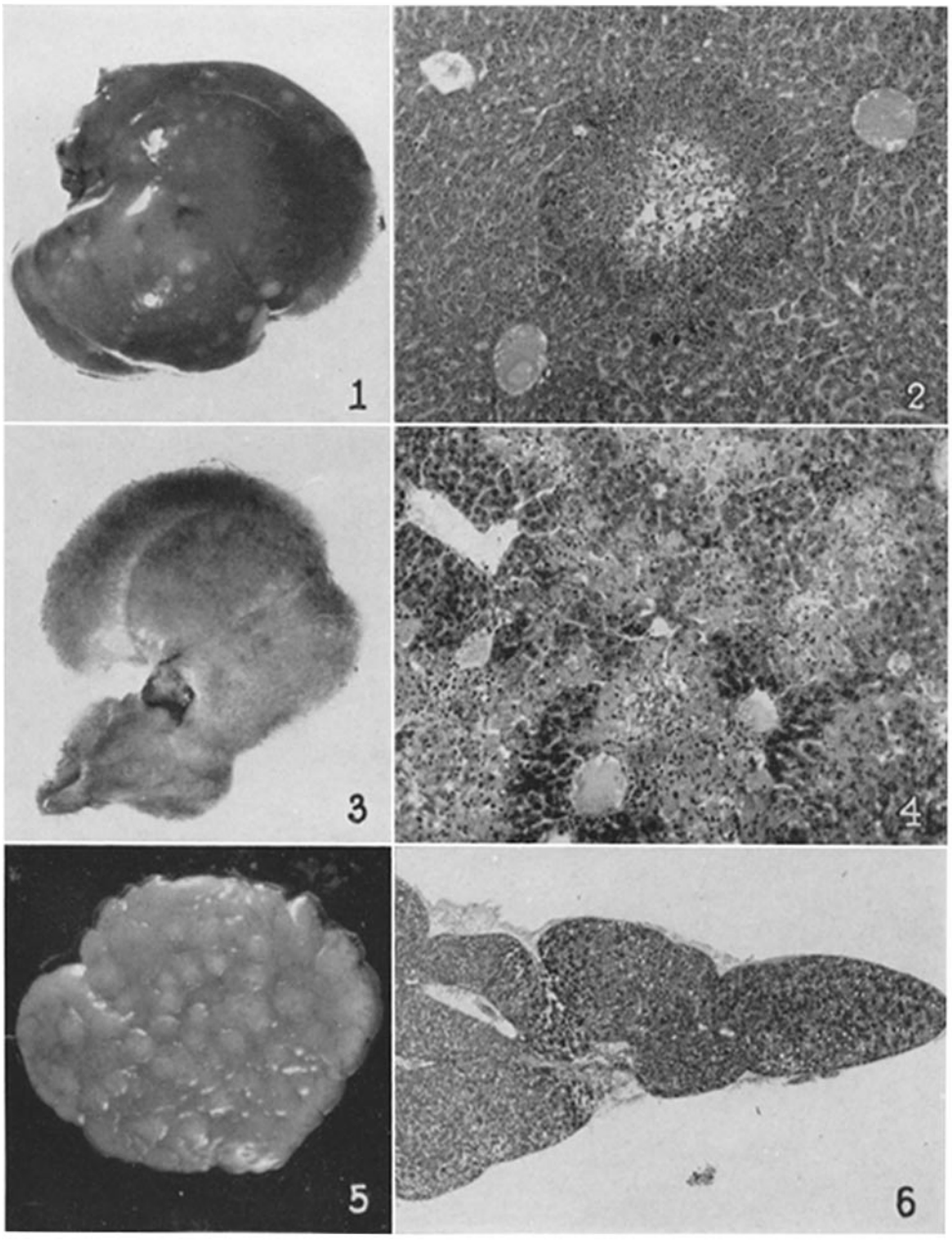
The three principal types of liver involvement in the acute hepatitis are illustrated in the following figures.

FIGS. 1 and 2. Focal necrosis. Specimens from a sick, weanling Princeton mouse killed on the 8th day after intraperitoneal injection of 0.1 ml. of a 10^{-5} dilution prepared from a pathogenic liver suspension. Entire liver × 3 and Giemsa-stained section × 113.

FIGS. 3 and 4. Nearly diffuse necrosis. Specimens from a moribund, weanling Princeton mouse killed on the 4th day after intraperitoneal injection of 0.1 ml. of a 10 per cent pathogenic liver suspension. Entire liver × 3 and Giemsa-stained section × 113.

FIGS. 5 and 6. Cirrhosis. Specimens from a normal appearing, weanling Princeton mouse killed on the 15th day after oral administration of a 10 per cent pathogenic liver suspension. Entire liver × 3 and Giemsa-stained section × 44.

The photographs were made by J. A. Carlile.



(Nelson: Acute hepatitis associated with mouse leukemia)