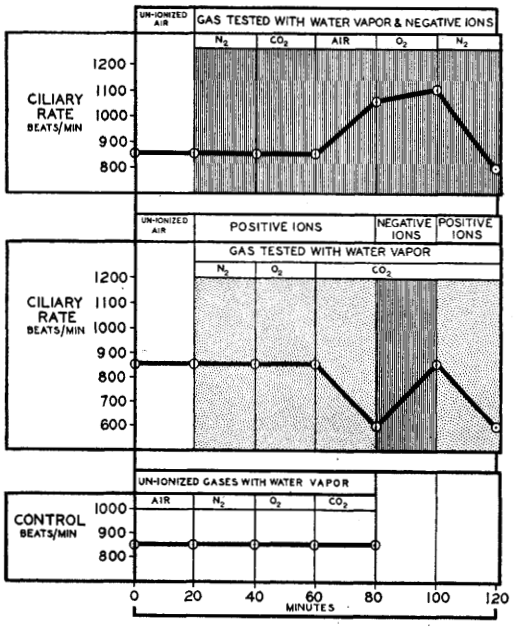

FIG. 2. Effects of ionized and un-ionized gases on ciliary activity in trachea of living rabbit.

Un-ionized N_2, O_2 and CO_2 had no effect on ciliary activity. Under conditions permitting formation of (+)ions, typical inhibition of the ciliary beat occurred in an atmosphere of CO_2 but not in N_2 or O_2. When the rectifying circuit was reversed so that only (−) ions could be generated, atmospheres of N_2 and CO_2 were without effect, while O_2 and ordinary air accelerated the rate of beat (Fig. 2).

N_2, O_2 and air in the un-ionized state or under conditions favoring (−)ion formation failed to induce EVT. This also was true for CO_2 as a rule, but occasionally a barely detectable EVT was produced by untreated CO_2 bled directly from a tank. Probably this resulted from an ionized fraction of the CO_2 produced by natural forces; extremely small numbers of positively charged CO_2 ions will induce a state of EVT(5). With the rectifying circuit reversed so that only (+)ions would be generated N_2 and O_2 were without effect, but CO_2 or air containing CO_2 permitted the prompt formation of a marked EVT.

These findings corroborate and extend our earlier experiments conducted with excised tracheal strips and living tracheotomized rabbits.

It is clear that positively charged CO_2 is the agent responsible not only for reduction of ciliary activity but also for the following effects: (1) Contracture of the trachael smooth muscle; (2) Pronounced mucosal ischemia; (3) Exaggerated lability of the mucosal vessels (enhanced vulnerability to trauma).

Summary. The trachea of the living rabbit was observed during exposure to un-ionized and ionized N_2, O_2 and CO_2 using a separate airway for respiration. Positively charged CO_2 was found to be responsible for: reduction of ciliary activity, contracture of smooth muscle, ischemia and enhanced vulnerability to trauma.

1. Krueger, A. P., Smith, R. F., PROC. SOC. EXP. BIOL. AND MED., 1957, v96, 807.
2. ———, *J. Gen. Physiol.*, 1958, v42, 69.
3. ———, PROC. SOC. EXP. BIOL. AND MED., 1958, v98, 412.
4. Krueger, A. P., Hicks, W. W., Beckett, J. C., *J. Frank. Inst.*, 1958, v266, 9.
5. Krueger, A. P., Smith, R. F., *J. Gen. Physiol.*, 1959, v42, 959.

Received July 28, 1959. P.S.E.B.M., 1959, v102.

Emergence of Hepatitis Virus in Mice Injected with Ascites Tumor. (25247)

JOHN B. NELSON
The Rockefeller Inst., N. Y. City

An ascites tumor that originally arose in Princeton mice during transfer of *Eperythrozoon coccoides* was reported from this laboratory in 1956(1). After a long series of passages with ascitic fluid, carrying the tumor cells, lesions that were similar to those produced by the virus of murine hepatitis, MHV (Pr) (2), were observed in the livers of the

injected mice. The hepatic reaction was reproducible in the absence of the neoplastic cells and was indeed referable to a virus of the mouse hepatitis group.

Material and methods. The ascites tumor, which closely resembled the Ehrlich tumor, had been maintained for several years by weekly transfer in Princeton mice. Weanlings, 4 to 5 weeks old, were injected intraperitoneally in groups of 5 with .1 ml of undiluted fluid or of a 10% saline dilution. They were killed and autopsied on the 7th day. At this time, they usually showed marked abdominal distension and yielded a considerable amount of creamy peritoneal fluid, either white or slightly tinged with pink. Tumor cells were demonstrable by phase microscopy. Extension of the holding period was followed by massive peritoneal hemorrhage which resulted in a blood-red fluid and death by the 10th to the 14th day. The visceral organs were not involved and the livers were normal in appearance. Swiss mice were equally susceptible but commonly showed terminal hemorrhages with a deep red fluid by the 7th day after injection.

Results. Ascitic fluid was passaged 131 times in 655 Princeton weanlings with no significant departure from the described course of events. In the 132nd passage, however, 2 of 5 mice showed diffuse necrosis of the liver and 3 discrete surface foci. Free fluid was present in the peritoneal cavity but was altered in amount and color. Similar results were obtained with 2 additional passages and it was obvious that a contaminating agent had appeared. The nature and distribution of the liver lesions were pathognomonic of mouse hepatitis. Cell-free liver suspensions were active on injection and were usually free from bacteria. The associated agent was filterable through Coors filters (No. 3) and was identified as a virus of the murine hepatitis group.

Attempts to eliminate the virus by successive washings of the tumor cells with saline solution were unsuccessful. Swiss weanlings known to be less responsive than those of the Princeton strain were used in a second attempt to eliminate the contaminant. This method was also unsuccesful but injections with undiluted ascitic fluid were continued for 10 passages in both mouse strains. Continued development of the virus was indicated by alteration of the peritoneal fluid and involvement of the liver. Both reactions were much more conspicuous in Princeton than in Swiss mice.

Additional experiments were carried out to determine the behavior of the virus in the absence of the tumor cells. An intraperitoneal passage series was begun in Princeton weanlings with a washed liver suspension from 3 of the mice in which hepatic lesions were first observed and continued for 20 transfers. Liver suspensions from the 6th through the 14th passage were also injected intraperitoneally in Swiss weanlings. In addition, 3 successive liver transfers were made in Swiss mice beginning with the 17th passage. The 3rd suspension from the latter series was tested for activity by injection in Princeton weanlings.

The results of the passage series in Princeton mice are summarized in Table I. By reason of an increase in virulence of the virus on continued injection, the series is divided into 2 groups of 50 mice each. The survivors were killed and autopsied on the 7th day. With the exception of one mouse which showed paralysis of the hind legs, the pathologic reaction was limited to the liver and characteristic of hepatitis virus.

All of the 60 Swiss mice survived and were normal in appearance when brought to au-

TABLE I. Intraperitoneal Injection of the Virus Alone in Princeton Mice.

No. of passages	No. of mice	No. of deaths	No. of survivors	Liver reaction in survivors: No. with discrete lesions	No. with confluent lesions	No. with no lesions
1-10	50	2	48	35	6	7
11-20	50	15	35	15	20	0
Totals	100	17	83	50	26	7

topsy on the 7th day. Three showed a few scattered hepatic foci. The livers from the other mice were normal in the gross and on examination with the dissecting microscope. In the absence of hepatic lesions, active virus was demonstrable in the livers of the mice that received the 3rd passage suspension. Subperitoneal injection in susceptible Princeton weanlings was attended by a characteristic liver reaction.

The ascites tumor had been carried meanwhile, in an uncontaminated state, by successive intramuscular injections in Princeton mice. A second intraperitoneal series was begun with a suspension of the resulting solid growths from several of these mice and has now been maintained for 95 passages in the absence of the virus. Ascitic fluid from this series was used to determine the behavior of an artificial mixture of the uncontaminated tumor cells and the virus. A stored liver suspension from the 12th transfer of the contaminated fluid in Princeton mice was the source of the virus. Fourteen subsequent passages were made with undiluted ascitic fluid at weekly intervals.

The results of this series were similar to those in Princeton mice injected with the naturally contaminated fluid. Changes in the physical state of the ascitic fluid were regularly accompanied by liver lesions.

Pathologic findings. The color change in the contaminated fluids varied from a barely detectable yellowish tinge to a distinct lemon yellow and was presumably due to the influx of bile salts. There were no outward indications of jaundice. Tumor cells were regularly demonstrable in the fluids regardless of their physical state. In the more viscous ones the cells were closely packed and sometimes aggregated. In the limpid fluids they were markedly reduced in number and commonly discrete. At a magnification of 950 diameters with the phase microscope many of the cells showed degenerative changes but normal ones were always present. The fluids also contained cellular debris and spherical masses, with no internal structure, which appeared to be pinched off from the cytoplasmic rim of the cells.

The liver reactions produced by the contaminant alone and by MHV(Pr) were essentially similar but differed somewhat in detail. In both cases, the prodominant histologic feature was necrosis, either diffuse or focal, with little or no cellular infiltration. On examination with the dissecting microscope the livers from mice infected with the present virus showed many, pale, opaque areas separated by narrow red strands or smaller discrete ones with red centers. This admixture of color was not characteristic of the hepatic reaction produced by MHV(Pr). In the livers of some mice, particularly Swiss, frank petechial hemorrhages were also present.

Neoplastic cells were rarely observed in liver sections from mice injected intraperitoneally with the tumor cells alone. In the presence of the virus, however, nests of tumor cells, varying in size and number, were often present and replaced the normal liver parenchyma. Not infrequently, the larger of these cell groupings showed a central area of necrosis.

Discussion. Despite the difference in virulence, it seemed appropriate, at least tentatively, to regard the present virus as a variant of MHV(Pr). On its emergence and subsequent passage the characteristics of the neoplastic cells and the ascitic fluid were noticeably altered. Retrogressive changes were more pronounced in Princeton mice than in Swiss. The virus was readily maintained in both mouse strains by intraperitoneal injection of the contaminated ascitic fluid. The infected tumor cells showed varying degrees of degeneration which resulted in a suppression of growth but not in complete inhibition.

The behavior of the 2 agents in the mouse was suggestive of a synergistic reaction. The virus, in some unknown way, provided favorable conditions for migration of the neoplastic cells to the liver. The tumor cells, in turn, constituted a suitable milieu for multiplication of the virus and also acted as a vehicle for its carriage. In the highly susceptible Princeton mouse there was little indication that the pathogenicity of the virus was appreciably affected by the tumor. Liver lesions occurred as readily in its absence as in its presence. In the more resistant Swiss mouse, however, activity of the virus was increased

and the liver reaction significantly enhanced. There was reason to believe that the attendant necrosis began in the nests of tumor cells and spread outwardly to the adjoining parenchyma.

It is probable that the virus was actually carried by one or more mice of the ascites passage series before it was detected by the appearance of liver lesions. The ascites tumor may now be added to the list of agents which have been instrumental in activation of viruses of the murine hepatitis group. In addition to the tumor, the list now includes *Eperythrozoon coccoides,* Niven *et al.*(3), leukemic lymphocytes, Nelson(2), and the reagents urethane and methylformamide, Braunsteiner and Friend(4). The initial emergence of hepatitis virus in Princeton mice has never been observed in the absence of one or another of these agents.

Summary. 1) A contaminant, subsequently identified as a virus of the mouse hepatitis group, appeared in Princeton weanlings during the 132nd intraperitoneal passage of an ascites tumor. The virus was regularly demonstrable in the ascitic fluid and was readily maintained in both Princeton and Swiss mice by serial transfer of fluid. In its presence, livers showed necrotic lesions and the ascitic fluid was altered in amount, consistency, and color. An artificial mixture of the 2 agents behaved in much the same way as the natural mixture. 2) The 2 mouse strains were equally susceptible to the tumor but not to the virus. Liver lesions produced by the virus alone were much more pronounced in Princeton mice. Presence of both agents facilitated migration of tumor cells to the liver and resulted in a marked enhancement of the hepatic reaction in Swiss mice.

1. Nelson, J. B., *J. Exp. Med.,* 1956, v103, 743.
2. ——— *Bull. N. Y. Acad. Med.,* 1957, v33 2nd ser., 811.
3. Niven, J. S. F., Gledhill, A. W., Dick, G. W. A., Andrewes, C. H., *Lancet,* 1952, v2, 1061.
4. Braunsteiner, H., Friend, C., *J. Exp. Med.,* 1954, v100, 665.

Received July 28, 1959. P.S.E.B.M., 1959, v102

Delayed Development of Righting Reflexes in Offspring of Manganese-Deficient Rats.* (25248)

LUCILLE S. HURLEY AND GLADYS J. EVERSON (Introduced by C. W. Asling)
Dept. of Home Economics, University of California, Davis

Our previous reports(1,2) have shown that a maternal dietary deficiency of manganese in rats and guinea pigs results in the birth of offspring which exhibit ataxia with incoordination and loss of equilibrium. These symptoms appear to result from a congenital defect or defects occurring during latter part of gestation in the rat(1). Observation of defective young during early part of life span raised questions concerning development of the righting reflexes. The present communication reports results of tests designed to examine the effect of a maternal manganese deficiency in rats on development of righting reflexes in their young. Two tests were used: (1) time the animal required to right itself when placed on its back, and (2) ability of animal to right itself in air when dropped upside down.

Methods. Procedures used to obtain manganese-deficient offspring were the same as previously reported(1). Briefly, female rats of the Sprague-Dawley strain were maintained from time of weaning on fortified milk diet deficient in manganese. When they reached maturity, they were mated with normal males receiving a stock diet, and the resulting offspring were tested. First, second and third litters were used. Control animals were treated in the same way, except that the diet was supplemented with manganese. Turnover time was tested beginning at 1 day of

* This investigation supported in part by research grant from Nat. Inst. of Arthritis and Metab. Dis., P.H.S.