TABLE III. Ultrafiltration of Serum Samples Drawn During Mannitol Diuresis and Calcium Infusion.

Sample	Ca (mg/ml)	cpm	S.A. (cpm/mg Ca × 10^{-5})
Tot. serum	.1088	98,124	9.01
Ult_1	.0728	65,972	9.06
Ult_2	.0696	63,452	9.09
Tot. serum	.1120	117,624	10.51
Ult	.0724	76,860	10.59

after injection of the isotope. Table III shows the results of these studies. No real calcium specific activity differences were found between total serum calcium and calcium in the ultrafiltrate. This *in vivo* ultrafiltration, then, served to confirm the view that the exogenous calcium contained in the isotope injection and infusion solution was rapidly equilibrating with the various calcium pools in the total serum.

Moreover, it should be noted from Table III that the calcium concentration in the serum ultrafiltrates was in the range of 3.6 meq/l which, presumably, would be the concentration at which it existed in the plasma ultrafiltrate as the latter entered the proximal tubules *in vivo*. As none of the stop-flow samples had a calcium concentration above 1.12 meq/l, it is readily seen that addition of cellular calcium to the luminal fluid does not, in this case, involve a net secretion of calcium on the part of the tubular epithelium.

Summary. Utilizing ureteral occlusion during low urine flow and the stop-flow technique during mannitol diureses it was noted that the calcium specific activity in the urine fractions obtained upon release of the ureteral clamp was altered in a manner which could be predicted from the state of kidney tissue calcium. It was concluded that the prolonged contact of the luminal fluid with the tubular epithelium when caused by a decrease in glomerular filtration rate resulting either from administration of massive doses of adrenergic drugs or from mechanical obstruction of urine flow, had provided time for a discernible addition of calcium from the epithelial cells to the tubular urine. Calcium concentrations in the stop-flow samples showed that addition of cellular calcium to the luminal fluid did not imply an active or net secretion.

1. Miller, E. J., Neuman, W. F., Bazerque, P. M., *Am. J. Physiol.*, 1964, v206, 755.

2. Bonsnes, R., Taussky, H. H., *J. Biol. Chem.*, 1945, v158, 581.

3. Toribara, T. Y., Terepka, A. R., Dewey, P. A., *J. Clin. Invest.*, 1957, v36, 738.

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Response of Mice to Reovirus Type 3 in Presence and Absence of Ascites Tumor Cells.* (29459)

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In 1960 Tarnowski and the writer described an oncolytic virus of mice and noted that weanlings were largely unresponsive to it in the absence of implanted tumor cells (1). A filtrable and tumor-destroying agent reported by Bennette was also lacking in pathogenicity(2). Our virus was later identified serologically by Hartley *et al*(3) and Eggers *et al*(4) as a murine strain of reovirus type 3. Additional observations on its behavior in mice are herewith presented. The accepted name, abbreviated RV3, is substituted for our earlier descriptive one.

Materials and methods. The original virus strain of 1960 was used in the following experiments. It had been maintained by serial transfer with our ascites tumor in Swiss mice (12 to 15 g) from the random-bred colony of the Rockefeller Institute. Growth of the neoplastic cells was partially suppressed by RV3 in low concentration. Dually injected mice, killed on the 7th day, commonly showed pale

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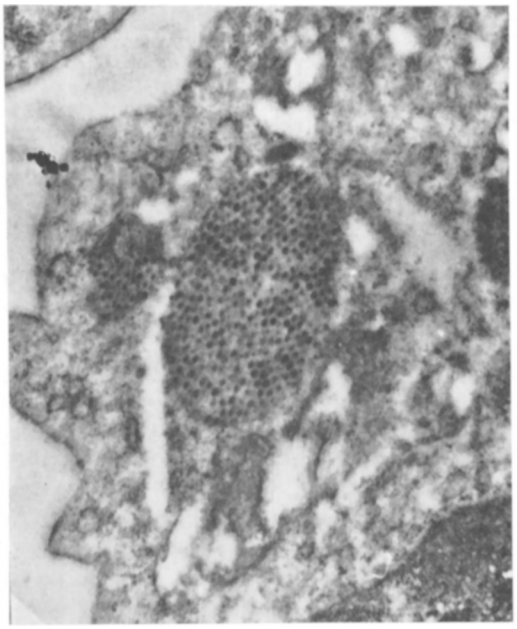

FIG. 1. Nest of virus-like bodies in cytoplasm of an ascites tumor cell. Electron micrograph about × 19,000.

slightly turbid ascitic fluids. They contained reduced numbers of tumor cells together with lymphocytes, macrophages, and much cellular debris. Cytoplasmic nests of well defined virus-like bodies (Fig. 1) with diameters in the area of 70 Mμ were found in some tumor cells by electron microscopy.

Mice with reduced amounts of fluid often showed a white cellular membrane which partially covered the liver. Extension of the membrane into the mesenteric folds was accompanied by intestinal dilation with rectal soilage or plugging. Chalky white areas of necrosis were also present in renal and genital fat deposits.

Copious amounts of fluid which was blood-red, due to early hemorrhage, and rich in tumor cells was regularly found in mice killed on the 7th day after injection of the tumor alone.

In practice, weanlings in groups of 5 were injected intraperitoneally with .1 ml of freshly drawn ascites tumor fluid diluted 10^1 with saline and after a brief interval with .1 ml of virus fluid diluted 10^1 and 10^5. Ascitic fluids were aspirated at autopsy on the 7th day, pooled, and examined by phase contrast. Supernatants, removed after low speed centrifugation, contained the virus in high concentration and were stored in the cold for use. This procedure has also proved to be a reliable diagnostic test for RV3 and as used here is termed the ascites tumor-virus assay.

Suspensions employed in the passage series were prepared from a mince of 5 livers homogenized in a glass tissue grinder with sufficient saline for a 10% concentration. Swiss weanlings were injected intraperitoneally in groups of 5 with .1 ml of fresh suspensions and killed with ether 7 days later, unless otherwise specified. The peritoneal cavities were aspirated and the livers removed to petri dishes for examination with a dissecting microscope.

Earlier observations had indicated that virus alone produced well defined pathologic changes in infant mice(1). The reaction pattern corresponded to that originally described by Stanley *et al*(5) and recently Walters *et al*(6) for the type 3 strain formerly designated hepato-encephalomyelitis virus. The highly sensitive ascites tumor-virus assay was supplemented at times by the use of 2- or 5-day-old Swiss nurslings. They were injected intraperitoneally with .03 to .05 ml of inocula and caged with their untreated mothers.

Results. Tumor-free fluid from the 108th combined passage was used to establish the virus in Swiss weanlings. It was then maintained for 52 weeks in a total of 260 mice by serial transfer of liver suspensions. All of the injected mice were alive when brought to autopsy. They had gained weight normally and showed no outward signs of illness. Their livers were free from necrotic foci or any gross pathologic change. The other abdominal organs and the regional fat deposits were unaffected. The only indication of peritoneal irritation was the presence of slightly turbid fluid in small amounts. A pool from 5 mice never exceeded one ml and was usually less. Examination by phase contrast showed variable numbers of lymphocytes and macrophages but no tumor cells. The fluid was detectable visually and was observed in one or more mice from 42 of the 52 passages. In most instances it was not present in injected mice held for longer intervals.

RV3 was detected in the liver suspension from the 3rd passage by ascites tumor-virus assay. The limiting dilution with oncolytic activity was 10^5 as compared with 10^8 for the supernatant of ascites fluid from a combined transfer. Liver suspensions diluted 10^1 and 10^5 were then tested from 10 of the subsequent passages including the 52nd. All of the dually injected mice showed atypical tumor reactions pathognomonic of RV3. Replication of the virus in the liver with no observable gross pathologic change was clearly indicated. The prior injection of *Eperythrozoon coccoides,* known to increase the pathogenicity of weakly virulent murine hepatitis virus (Niven *et al*(7)), had no effect on RV3.

The virus was also detected by nursling injection. Liver suspensions or peritoneal fluids were used without addition of tumor cells. The findings with fluid from the 31st liver passage are presented as characterizing the behavior of passaged RV3 in infant mice.

Seven 5-day-old nurslings injected with undiluted fluid were held with 2 uninfected litter mates. Four died on the 5th day. Examination of dead mice was usually prevented by cannibalism. One mouse killed at this time was underweight but otherwise normal. Two killed on the 10th day had small pale yellow foci of necrosis in the liver and white foci in heart muscle. Neither mouse showed oiliness of the fur but one was stunted, prone, and tended to roll. The 2 controls which had gained weight normally were also killed. They were lesion-free at autopsy but RV3 was demonstrated in a pool of washings from their intestinal tracts.

RV3 is readily maintained *in vitro* by serial transfer in myco-plasma-free cultures of HeLa cells. A high titered inoculum results in almost complete loss of the monolayer by the 7th-10th day. Numerous cells in various states of degeneration are present in the supporting fluid. Supplementary verification of the virus in liver suspensions was obtained by inoculation of freshly prepared cultures with supernatants. The initial transfer was attended by the characteristic cytopathic reaction but it was delayed until the 14th day or later.

TABLE I. Distribution of Reovirus 3 in Injected Weanlings at Weekly Intervals.

Locus	1 wk	2 wk	3 wk	4 wk
Liver	+	+	—	—
Brain	+	+	—	—
Heart's blood	+	—	—	—
Peritoneal cavity	+	+	+	+
Intestine	+	—	—	—

In addition to its presence in the liver and peritoneal fluid RV3 was also demonstrated in heart's blood, brain suspensions, and intestinal washings of injected weanlings killed on the 7th day. At least 2 assays representing different passages were made with the suspensions from each locus. The number of mice was increased in some of the passages to determine how long the virus survived in the above areas. All of these mice were normal in appearance when killed and showed no hepatic involvement at autopsy. Saline washings of the abdominal cavity were substituted for peritoneal fluid after the first week. The findings, based on the assay of 10 or more mice with each suspension, are summarized in Table I.

RV3 was recovered from each of the 5 loci of mice killed after one week but was found only in peritoneal washings after 3 weeks. It was also detected in the washings from one or more mice of 2 groups held for 13 and 18 weeks, respectively. Two pools of blood serum from these animals failed to neutralize the oncolytic activity of the virus on injection together with tumor cells.

Work was in progress at this time with a murine hepatitis virus (MHV) which was fully active only in nurslings. The pathologic reaction was confined to the liver and characterized by necrotic foci which were larger, more sharply defined, and more numerous than those produced by RV3. They were regularly white with no yellowing but often showed red central areas. An experiment carried out to determine the effect of injecting a mixture of the 2 viruses pointed to the suppression of MHV by RV3. Interference of some sort was indicated by the results of 6 additional tests in 2-day-old infants. The reactions of 23 nurslings killed 5 to 7 days after injection with a suspension containing an equal mixture of the respective viruses

were pathognomonic only of RV3. The distinctive lesions of MHV were regularly lacking. Other types of MHV have not been studied in this connection.

Summary. A passaged strain of reovirus type 3 (oncolytic virus of Nelson and Tarnowski) was established in weaned Swiss mice and maintained for 52 weeks by liver transfer in the absence of ascites tumor cells. The only pathologic response was the presence of peritoneal fluid in trace amounts during the first week. Pathogenicity was not altered by prior injection of *Eperythrozoon coccoides*.

Active virus was recovered from the liver and brain through the 2nd week after injection. It was present in heart's blood and intestinal washings only during the first week but was detectable in peritoneal washings for at least 18 weeks. The virus was identified by oncolysis in the presence of tumor cells, the response in infant mice, and the cytopathic reaction in HeLa cell cultures. Interference with the development of a particular murine hepatitis virus by reovirus 3 was observed in nurslings injected with a mixture of the 2 viruses.

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1. Nelson, J. B., Tarnowski, G. S., *Nature,* 1960, v188, 866.

2. Bennette, J. G., *ibid.,* 1960, v137, 72.

3. Hartley, J. W., Rowe, W. P., Huebner, R. J., PROC. SOC. EXP. BIOL. AND MED., 1961, v108, 390.

4. Eggers, H. J., Gomatos, P. J., Tamm, I., *ibid.,* 1962, v110, 879.

5. Stanley, N. F., Dorman, D. C., Ponsford, J., *Aust. J. Exp. Biol. and Med.,* 1953, v31, 147.

6. Walters, M. N-I., Joske, R. A., Leaks, P. J., Stanley, N. F., *Brit. J. Exp. Path.,* 1963, v44, 427.

7. Niven, J. S. F., Gledhill, A. W., Dick, G. W. A., Andrewes, C. H., *Lancet,* 1952, v2, 1061.

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Enhanced Methylglyoxal Toxicity in Oxythiamine Induced Thiamine Deficiency in Mice.* (29460)

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In contrast to earlier reports, recent studies have indicated that thiamine deficient animals do not excrete methylglyoxal(1,2). However, it has been shown that methylglyoxal can accumulate *in vitro* when the tissues of thiamine deficient animals are incubated with fructose-diphosphate(3). Hence it is possible that a transient, localized accumulation of methylglyoxal in certain tissues may be a contributing factor in the thiamine deficiency syndrome in animals.

Although the administration of methylglyoxal has not been shown to produce the characteristic symptoms of thiamine deficiency in normal animals, it does exhibit considerable toxicity. One might expect greater toxicity from methylglyoxal administered to a thiamine deficient animal if such material accumulates during deficiency or if oxidative degradation is inhibited. In the present study, the toxicity of methylglyoxal was determined in mice made deficient by dietary lack of thiamine as well as by the use of thiamine antagonists. The urine, brain and muscles of normal and deficient mice were also analyzed for the presence of methylglyoxal.

Materials and methods. Male Swiss-Webster mice weighing 18-22 g were either pretreated with pyrithiamine or oxythiamine, or maintained on a commercial thiamine deficient diet† for 10 days. Methylglyoxal solu-

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