TABLE I. Net Weight Gain (%).

Urea conc. (M):	0	.005	.3	1.0	*row means*
pH					
5.0	24.28	26.40	27.96	26.81	26.36
7.4	30.00	27.54	27.63	27.71	28.22
9.5	32.93	28.98	36.23	30.77	32.23
Column means:	29.07	27.64	30.61	28.43	28.94

similar experiment the effect of pH—varying in one-half unit increments from 6.0 to 9.5—was determined. At pH 9.5 the uptake of solution was again accelerated, but it was approximately constant at other pH values tested. The concentration of urea in the medium, either alone or in interaction with pH had no effect on the inflow of solution.

Table II shows the quantities of bound C^{14}-urea as fractions of the total C^{14}-urea concentration in the albumin solutions. In agreement with recent findings of Lassiter *et al*(4), only a small fraction of the total urea was bound to albumin. Analysis of variance of the data showed that at pH values of 7.4 and 9.5 the bound fraction amounted to approximately one per cent and that it was significantly greater ($p<0.01$) than at pH 5.0. At pH 5.0 several negative values were obtained. A t-test indicated, however, that the mean value of all samples at that pH was not significantly different from zero. The concentration of urea in the medium had no significant effect on the binding of urea.

TABLE II. Bound Urea Fraction (%).

Urea conc. (M):	0	.005	.3	1.0	*row means*
pH					
5.0	−1.20	−1.74	−.34	−.13	−.85
7.4	−.65	.51	1.43	2.73	1.01
9.5	2.24	.85	.54	.88	1.13
Column means:	.13	−.13	.54	1.16	.43

These results indicate that interstitial albumin in the renal medulla may affect the withdrawal of fluid from the collecting ducts, provided that pH attains a sufficiently high level in this environment. Further, they do not support the hypothesis that albumin-bound urea might sustain a significant difference in urea concentration between interstitial and collecting duct fluids in the renal medulla.

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Inhibition of Solid Tumor Formation by Prior Immunization with Formalized Neoplastic Spleen Extracts.* (32102)

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A previous report described cytopathic effects and a transformed growth pattern in an established BALB/c mouse cell line (JLS V5) infected with the Rauscher leukemia virus(1). Subsequent studies have shown that the transformed cells are more highly tumorigenic than uninfected, nontransformed cells when injected subcutaneously into newborn BALB/c mice(2). Moreover, the resulting tumors produced by transformed cells are predominantly myxofibrosarcomas, whereas those produced by uninfected, nontransformed cells are spindle-cell sarcomas.

Mirand *et al*(3) have shown that immunization of pregnant mice with Friend virus vaccine conveys passive immunity to the

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newborn mice against subsequent challenge with infectious Friend virus. The present study was undertaken to determine if either the growth or the myxomatous nature of the myxofibrosarcomas produced by transformed cells could be repressed in newborn mice by passive immunization with a Rauscher virus vaccine.

Materials and methods. Two lots of vaccine prepared from formalized, sodium citrate extracts of neoplastic spleen cells from BALB/c mice were used in this study. One lot was obtained through the courtesy of Dr. M. A. Fink.† A second lot was prepared in our laboratory following the procedure described by Fink and Rauscher(4). Control vaccine from normal spleen cells was also prepared in like manner.

Whereas an incidence of myxofibrosarcomas of ≥60% can be obtained in newborn BALB/c mice on subcutaneous injection either of JLS V5 cells transformed *in vitro* or of such cells after growth *in vivo* as myxofibrosarcoma cells, adult BALB/c mice are resistant to challenge with such cells (unpublished data). The resistance of adult mice necessitated the use of newborn BALB/c mice as the test animal.

Eight- to 10-week-old female BALB/c mice were injected intraperitoneally with .5 ml of a mixture that contained equal volumes of Freund's incomplete adjuvant and either Rauscher virus vaccine, control vaccine, or sodium citrate. Five days later the mice were mated. Two weeks after copulation, as judged by the presence of vaginal plugging, the pregnant mice were injected subcutaneously with .1 ml of their respective vaccine without adjuvant. One to three days after birth the newborn mice were injected subcutaneously with 1×10^5 cells of either the transformed JLS V5 culture or of resulting myxofibrosarcomas. The myxofibrosarcoma cells originated from serial mouse passage of transformed JLS V5 cells.

Results. The data from two experiments revealed that offspring of mice immunized with Rauscher virus vaccine before and during pregnancy had a reduced tumor incidence when compared with offspring of mice which received control vaccine (Table I, Fig. 1 and 2). Moreover, the few tumors that did appear in the Rauscher vaccine group occurred in only 2 of 17 litters and were noticeably smaller than those in the control groups. The 40-day observation period does not necessarily differentiate between complete inhibition and temporary tumor repression in the Rauscher virus vaccine group; however, the appearance within 30 days of all 4 of the tumors observed in this group suggests that the incidence had plateaued by the end of the 40-day observation period in question.

Discussion. The data from the two experiments show a lower myxofibrosarcoma incidence in offspring of mice immunized with the Rauscher virus vaccine than in those of the corresponding control mice. One possible explanation of these results is that the offspring became passively immunized against neoplastic spleen extracts and were thereby also protected against challenge with myxofibrosarcoma cells, owing to antigenic similarity between the sarcoma cells and the neoplastic spleen extracts (Rauscher virus vaccine). The presence of specific antigens in Rauscher leukemia cells(5,6) and infected JLS V5 cells(1) has been reported.

It is also conceivable that passive immunization against the Rauscher virus, which was contained in the JLS V5 cells, might have protected the recipients of such cells against viremia and subsequent leukemia, either of which might have affected the resistance to tumor formation. Dent *et al*(7) and Morton and Seigel(8) have recently demonstrated impairment of the immune response in mice infected with the Gross and Rauscher leukemia viruses, respectively. Although the numbers of mice examined histologically were small, all of 19 such mice in the group not immunized against neoplastic spleen developed leukemia, whereas only 6 of 14 such mice in the immunized group developed leukemia. Nevertheless in the immunized group, only one of the 6 leukemic mice developed a tumor, whereas 3 of 8 mice showing no histologic signs of leukemia developed tumors. Hence, although the data suggest that immunization may have affected

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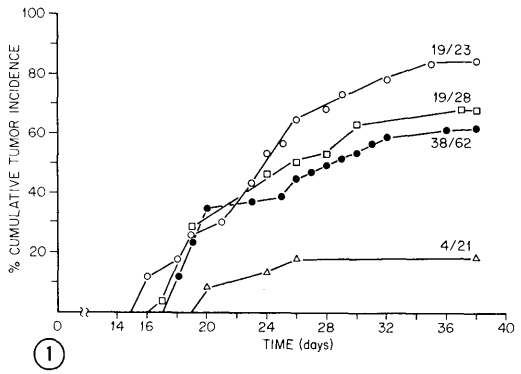
TABLE I. Influence of Maternal Immunization on Incidence of Myxofibrosarcomas in Newborn BALB/c Mice.*

Immunizing material inoculated into pregnant mice	Type of cell used to challenge newborn mice	No. of tumors within 40 days after challenge/ No. of mice challenged
	Exp. I	
Sodium citrate + Freund's incomplete adjuvant	Transformed JLS V5	19/28 (68%)
Formalized, sodium citrate extracts of normal spleen + Freund's incomplete adjuvant	"	19/23 (83%)
Formalized, sodium citrate extracts of neoplastic spleen + Freund's incomplete adjuvant	"	4/21 (19%)
None	"	38/62 (61%)
	Exp. II	
Formalized, sodium citrate extract of normal spleen + Freund's incomplete adjuvant	Passaged myxofibrosarcoma (*in vivo* serially passaged, transformed JLS V5 cells)	45/54 (83%)
Formalized, sodium citrate extract of neoplastic spleen + Freund's incomplete adjuvant	"	0/54 (0%)
None	"	17/18 (94%)

* Also illustrated in Fig. 1 and 2. Descriptions and tumor incidences shown in Table I also apply to the curves seen in Fig. 1 and 2.

the incidence of leukemia, no correlation between the presence of leukemia and tumors is apparent. These observations, and the finding that little or no infectious leukemia virus can be detected in passaged myxofibrosarcoma cells such as were used to challenge the mice in the second experiment, tend to discount the necessity of concomitant leukemia for the development of cell-induced myxofibrosarcomas.

In light of the recent report by Gross(9) to the effect that the Rauscher virus is a mixture of the Friend and Gross viruses, studies are presently underway to test Friend and Gross virus vaccines regarding their effectiveness in immunizing against the growth of our myxofibrosarcomas. The involvement of avian leukosis viruses in the maturation of the Rous sarcoma virus(10), the rescue of the defective genome of the Moloney sarcoma virus by various murine leukemia viruses(11), the derivation of many of the murine leukemia viruses from solid tumors (12), and the detection of the gross leukemia antigen in sarcomas induced with methylcholanthrene(13) raise the question as to



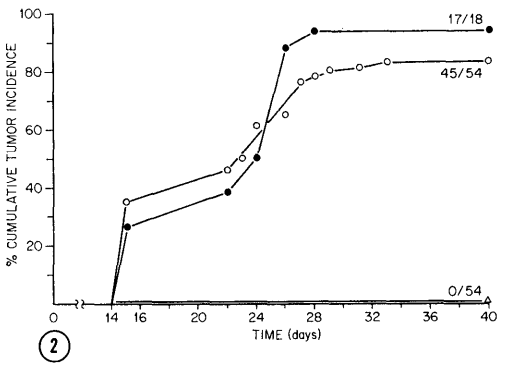


FIG. 1. Experiment I.
FIG. 2. Experiment II.

whether the growth of solid tumors other than that described herein might be inhibited by immunization with leukemia virus vaccines. This question, as well as the relation between the leukemogenic and sarcomagenic effects exerted by the same virus preparation, is under study.

Summary. Offspring of mice inoculated during pregnancy with a Rauscher virus vaccine prepared from neoplastic spleen cell extracts were resistant to challenge with cells rendered myxofibrosarcomatous by transformation *in vitro* with the Rauscher virus. Offspring of mice similarly inoculated with normal spleen cell extracts showed no such resistance. The results suggest that cells transformed to a myxofibrosarcomatous pattern of growth by infection with the Rauscher leukemia virus *in vitro* share a specific antigen with spleen cell extracts rendered neoplastic by the same virus *in vivo*. The possible significance of the relationship between leukemia induction by the Rauscher virus *in vivo* and myxofibrosarcomatous transformation of cells by the same virus *in vitro* is discussed.

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E. coli Agglutinating Activity of Ulcerative Colitis Sera. (32103)

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It has been suggested(1) that enteric bacteria play a role in the pathogenesis of chronic ulcerative colitis (UC). It is known that strains of *E. coli* which contain capsular ("K") antigens, particularly of the "B" type, are more pathogenic than non-capsular organisms(2). Serum gamma globulins represent one aspect of the defense against Gram-negative bacteria(3); we therefore measured the antibody titer against "B" antigens in the sera of patients with UC compared with the titer in patients free of gastrointestinal disease and patients with liver disease. The slide agglutination technique was used(4).

Methods. Serum was taken from 16 ambulatory patients with well documented, chronic UC. The patients were in various stages of clinical activity, but none were in full proctoscopic remission. Eight patients were receiving salicylazosulfapyridine, and none were receiving other medication. These 16 sera were compared with sera from 17 individuals free of gastrointestinal disease, 23 patients with advanced Laennec's cirrhosis confirmed by recent liver biopsy, and commercial pooled gamma globulin adjusted to physiologic concentration. The contribution of immunoglobulin-M to the agglutinin titer was determined