and Haas strains. At least 12 mice in each strain were examined. All of the mice of the various genetic strains were maintained and fed under the same conditions of husbandry; and the germfree mice were free of fungi, protozoa, bacteria, and mycoplasma. Leukemia virus has been reported in all strains of mice[7], and mice of the Haas strain are persistently infected with lymphocytic choriomeningitis virus[8].

It was of significance that among the above noted mouse strains, only mice of the AKR strain develop a significant incidence of spontaneous lymphatic leukemia. Additional conventional strain AKR mice were obtained from Dr. J. TRENTIN of Baylor Medical College, from whom the original mouse stocks had been obtained 7 years previously; and they too had multinucleated acinar cells in their pancreases. In an additional mouse strain (C58) which also develops leukemia spontaneously[9], similar multinucleated acinar cells have been observed in their pancreases. The average number of nuclei per multinucleated C58 cell was 5, whereas in AKR mice the average number of nuclei was 10 per cell. The C58 and the AKR mouse strains differ in their origins[10], but they are closely related in H-2 histo compatibility patterns[11]. However, other mouse strains which show a relationship to C58 and AKR by the latter criterion have a low incidence of spontaneous leukemia.

The appearance of these unusual multinucleated cells in the pancreases of AKR and of C58 mice may be no more than coincidental and unrelated to their leukemic propensity. They may be the polykaryon effect of an unrecognized virus, or a genetically-related anomaly. The role of the pancreas in leukemogenesis is unknown. The nature of the unque multinucleated acinar cells is unknown, but their pathogenic potentialities should be further investigated.

Résumé. Dans des souches de souris AKR et C58, les cellules exocrines du pancréas ont jusqu'à 12 noyaux par cellule. Tandis que dans des souches d'autres souris, il n'y y en a plus qu'une ou deux par cellule.

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Induction of Active Immune State by Multinucleate Tumour Cells in Mice

Expression of tumour specific transplantation antigens (TSTA's) is generally weak when tumour cells are injected into syngeneic hosts[1]. Immunogenicity of TSTA's could be enhanced by tumour cells whose surfaces had been altered in some way, such as by introducing foreign antigenic materials onto them[1–5]. The studies to be reported here include the induction of active immunity to tumour by Sendai virus-fused multinucleate cells in syngeneic mice. Immunogenicity was indicated by increased resistance of the treated recipients to subsequent challenges with viable tumour cells.

Materials and methods. A/Jax male mice (Jackson Memorial Laboratory, Bar Harbor, Maine) were used for all studies. The principal tumour used was originally induced with methyl-cholanthrene (MC) and had been carried in its indigenous host for 10 years. The MC tumour was histologically characterized as a sarcoma. The MC cells regularly produced 100% tumours with as few as 600 cells and remained specific to A/Jax mice with regard to transplantability throughout the experiment.

Two other tumours, designated as SP_1 and SP_2 were used in some control experiments. These tumours arose spontaneously on the neck area of 2 A/Jax male mice and both were classified as parotid salivary gland myoepithelioma from their early histological appearance. SP_1 and SP_2 were transplantable in A/Jax mice.

The reconstituted Sendai seed virus was passaged at a dilution of 1:10,000 in 10-day-old fertilized hen eggs by the allantoic route. The virus preparation containing 2500 hemagglutinating units (HAU) per ml was inactivated by UV-light before cell fusion.

Fusion was carried out essentially as described by HARRIS et al.[6]. After fusion, the giant multinucleate cells were concentrated by use of discontinuous Ficoll gradients ranged 1.0500–1.1100 g/ml in Ca^{++} free PBS according to SYKES et al.[7]. After centrifugation for 15 min at 8000 *g* in a Spinco Model L2, two major bands were observed. The upper band contained mainly single mononucleate cells

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Table I. Frequency distribution of multinucleate MC cells following treatment with UV-irradiated Sendai virus

	Number of nuclei in fused cells																		
No.	2	3	4	5	6	7	8	9	10	11	12	13	14	15	16	17	18	19	≥20
%	53.8	24.6	10.9	4.1	2.6	1.0	1.3	0.7	0.8	0.2	0.3	0.1	0.1	0.1		0.2	0.1		0.4

while the lower one consisted predominantly of multinucleate cells. The layers were sufficiently far apart, but it was not possible to remove the lower band without some contamination with mononucleate cells.

Results and discussion. Immunization and tests for immunogenicity. After cell fusion by Sendai virus and separation by Ficoll gradients, the mononucleate cells contaminating the 'fused cells' were less than 20%. Scored from the stained preparations, the multinucleate cells were predominantly binucleate (Table I). A million nuclei h/0.1 ml in the fused cell preparation were used for the immunizing dose, and injected i. m. at the hind leg.

The immunity induced by 3 separate injections of fused MC cells, over 20 days, was tested by challenge with untreated MC tumour cells. 12 groups of such experiments were performed. Each group consisted of 5, 6 or 10 mice. 15 days after the last immunizing dose was given, each animal was challenged by 1×10^5 untreated MC cells. 2 control normal mice were innoculated at the same time with the same dose of the tumour cells. The results are summarized in Table II. Treatment with virus-fused MC cells increased the immunogenicity to MC cells as indicated by the outcome of the first challenge: 86% (92/107) of the mice failed to develop tumours within a month, while control mice had 100% tumour takes and died in approximately 1 month. The resistant animals were subsequently given a second and a third challenge of 1×10^5 live MC cells at monthly intervals and the percentages of resistant animals were 77.6 (83/107) and 71.9 (77/107), respectively. Control mice at each test level died with tumours. It is concluded that Sendai virus-fused multinucleate MC cells lost their transplantability and stimulated an immunoprotective reaction in the recipient mice.

Table II. Transplantation resistance to MC cells induced in A/Jax mice by virus fused tumour cells

Experiment No.	No. of animals tested	No. of animals not developing tumour		
		1st Challenge	2nd Challenge	3rd Challenge
1	5	5	4	4
2	10	9	9	9
3	10	7	6	6
4	6	5	4	3
5	10	8	8	8
6	10	8	7	7
7	10	9	9	9
8	10	10	10	6
9	10	9	7	7
10	6	6	5	5
11	10	8	6	6
12	10	8	8	7
	107	92 (86.0%)	83 (77.6%)	77 (71.9%)

Table III. Effects of single unfused MC cells on the growth of transplanted tumour in recipient mice

Pretreatment of cells	Immunization			Tumour challenge	
	1st	2nd	3rd	1st	2nd
10,000 cells					
single unfused cells	5/5[a] (43.6)[b]	–	–	–	–
untreated cells	5/5 (30.0)	–	–	–	–
1,000 cells					
single unfused cells	0/5	2/5 (51.6)	3.3	–	–
untreated cells	5/5	2/2[c] (32.5)	–	–	–
100 cells					
single unfused cells	0/5	0/5	0/5	2/5	3/3
untreated cells	0/5	0/5	0/5	5/5	–

'Untreated cells' indicate dispersed MC Tumour cells not treated with Sendai virus. [a] No. of mice developing tumour/No. of mice tested. [b] Figure in the parenthesis represents the mean of survival time (days). [c] Two untreated control mice were used only for the second challenge experiment as controls in the indicated group.

A group of mice which were rendered immune to MC cells were tested against other tumour cells 15 days following the last challenge. The immunized and untreated control mice were injected either with SP_1, SP_2 cells or untreated MC cells. All control animals died with tumour after injection of 10^5 untreated tumour cells. Immunized mice were found resistant to MC cells, but died at the same rate as controls when challenged with SP_1 or SP_2 cells. Thus, the immunity to MC cells was specific.

Sendai virus has been shown to exhibit neuraminidase activity[8]. The immunogenicity of Sendai virus-fused cells may have been due to a viral neuraminidase effect on the tumour cells[9]. This seemed unlikely for the following reasons: The pH of Sendai virus suspension harvested from allantoic fluid of hen eggs ranged from 7.6 to 7.8. After storage in the cold (4 °C) for 2 to 3 weeks, the pH of the suspension rose to 8.0 to 8.2. The optimal pH for neuraminidase of egg-grown Sendai virus was shown to be 5.0 to 5.5 and very low activity could be found when the pH was at or above 7.0[10]. In our cell fusion process pH of virus suspension was always higher than 7.4.

Controlled experiments. It is possible that single cells exposed to Sendai virus (but not fused), or interferon induced by Sendai virus may have been responsible for inducing immunity. To test these possibilities, the following experiments were designed: 1. Single unfesed cells were separated from fused cells after treatment with Sendai virus in usual fusion and separation procedures. Dosages of 100, 1000 and 10,000 cells were separately injected into groups of animals. Each group consisted of 5 mice. 2. Tumour cells and Sendai virus, which had been mixed but without allowing time for fusion to take place, were injected into 10 mice in a dose of 1×10^6 cells. 3. Tumour cells (1×10^6) were injected into one leg and Sendai virus (552 HAU) in the other leg at the same time. 10 mice were used in this experiment.

The results of the first experiment are summarized in Table III. In no instance was immunity induced. At the higher dose levels, tumours actually developed from the 'immunizing' injections. This occurred at the first injection of 10,000 cells and later treatment with 1,000 cells. No tumour resulted from 3 injections of 100 cells, whether virus treated or not. However, none of these treatments induced immunity to more than one challenge with 1×10^5 cells.

In the other 2 experiments, the first immunizing injections invariably produced tumours although the latent period for appearance of palpable tumours was significantly increased when compared with controls ($p < 0.001$). The survival times of the mice for both experimental groups were, however, not statistically

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different from those for the control groups. It appears, therefore, that virus exposed mononucleate cells or the interferon induced by Sendai virus cannot be the explanation for the demonstrated immunogenicity of fused cells.

The possibility of direct viral oncolytic effect[12–14] on the tumour cells could be excluded in the immune reaction elicited by the fused cells, as the virus used had previously been UV-inactivated.

It is difficult to fully explain the mechanism of action of the fused cells. However, it appears clear that during tumour cell fusion by Sendai virus the coat components of the virus would become incorporated into the cell membrane and alter the structure of the cell periphery[11]. Although the modification of cell surface in the terms of molecular configuration has still not been understood, the viral lipoproteins might well be bound to the TSTA's in some way within the cell membrane. The modified fused cells are merely attenuated, in the sense that their growth rate is slowed down and they lose their transplantability, and/or render themselves more immunogenic in isogenic hosts. This principle was recently discussed by MITCHISON[1,15].

Résumé. Une immunité active au sarcome, produit par le cholanthrène de méthyle, a été obtenue en utilisant des cellules tumorales multinucléées, associées au virus de Sendai; les réactions ont été spécifiques à la tumeur. A la suite de ces injections, plus de 70% des souris furent résistantes aux trois inoculations, avec 10^5 cellules vivantes tumorales.

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Metabolism of Dimethylnitrosamine by Amphibians and Fish in vitro

Nitrosamines have a toxic and carcinogenic effect in a wide range of animal species[1,2], including primates[3,4] and fish[5–7]. There is much evidence to show that nitrosamines are not themselves active but that a chemical reaction occurring during their decomposition causes cellular injury and tumours[2,8].

Although dimethylnitrosamine (DMN) induces tumours of the liver in rainbow trout[5], KRÜGER et al.[9] found no evidence of methylation of nucleic acids or proteins of the liver in vivo. These findings suggest that trout liver lacks the capacity to metabolise DMN and indicate that in trout the carcinogenic effect is not related to the alkylation of cellular constituents.

It was decided to investigate further the capacity of various amphibians and fish to metabolize DMN. In a series of experiments, liver slices of rainbow trout, gold fish and 3 species of amphibians (*Triturus helveticus, Triturus cristatus* and *Ambystoma mexicanum*) were incubated with (^{14}C)-DMN and the production of labelled CO_2 was measured.

The Table shows the rates of production of labelled carbon dioxide. The highest activity was observed in

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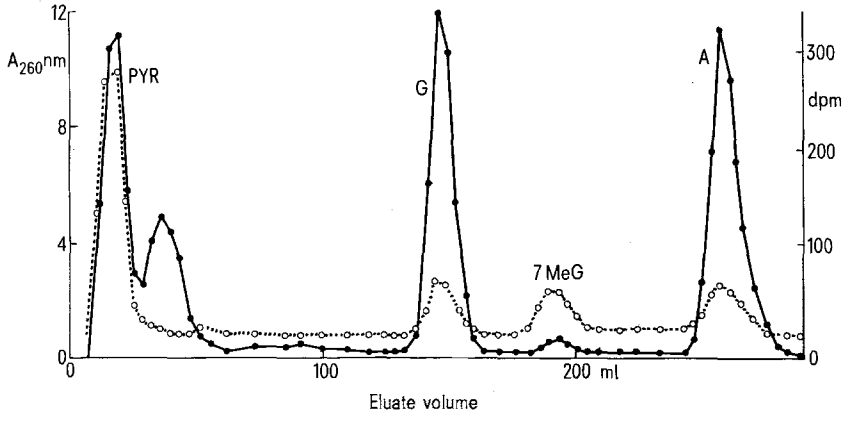

Fig. 1. Ion-exchange chromatography of nucleic acids of trout liver incubated with (^{14}C)-DMN in vitro, conditions of incubations as in the Table. Nucleic acids (DNA and RNA) were extracted from pooled slices by the SCHNEIDER procedure[17], followed by hydrolysis in 1*N* HCl at 100 °C for 1 h and ion-exchange chromatography on Dowex 50 (×12, H form) with exponential 1–4*M* HCl gradient solution, 3.6 ml fraction volume. ●, A_{260}; ○, radioactivity; PYR, pyrimidine nucleotides; G, guanine; 7-MeG, 7-methylguanine; A, adenine. Carrier 7-methyl-guanine was added to the hydrolysate.