tions, besides the potential target cells have also shown this effect of inhibition of the epithelial cells of renal tubules and parenchymal and Kupffer cells of liver. In contrast to these slowly regenerating cells, the rapidly regenerating mucosal cells of the small bowel (duodenum) did not show this effect (Table II). Mice and adult rats were also studied after virus inoculation, which do not develop sarcomas. Our effect of inhibition of cell proliferation could be seen only in the renal medulla of adult rats; whereas no evidence of this effect could be observed in the liver of adult rats, and definitively not in mice.

Discussion. The results of our autoradiographic studies with decrease of labelling and mitotic index, without change in the total amount of silver grains found in a single cell, reflect an inhibition of cell proliferation. This effect is not a transient one, compared to findings after application of chemical carcinogens, where an inhibition is seen to last only a few days, followed by an increased proliferation[3–5].

In addition to that the effect is not comparable to virus infection in vitro, in which cell specific DNA-synthesis is inhibited on behalf of virus replication[6–9]. Correspondent findings are missing concerning the transformative effect of virus, until now. Tumor-bearing animals display this effect much stronger than virus-inoculated, tumor-free rats. In addition to this the effect becomes even more obvious following neonatal thymectomy[10]. All these facts lead to the final conclusion, that the inhibiting influence on cell proliferation might possibly be caused by newly formed antigens in a changing immunological milieu of the animal.

Zusammenfassung. Eine eindrucksvolle, über 30 Tage beobachtete Hemmung der Zellproliferation in Niere und Leber wird verursacht durch Infektion neugeborener Ratten mit unserem PV-Stamm. Dieses Phänomen ist bei adult infizierten Ratten nur im Nierenmark nachweisbar und fehlt bei Mäusen ganz.

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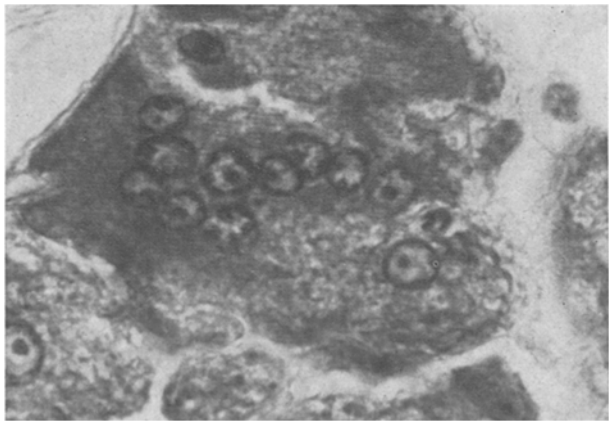
Multinucleated Acinar Cells in the Pancreases of AKR and C58 Mice[1]

In the course of examining healthy germfree AKR mice[2,3], an unusual histological structure was observed in the acinar cells of the pancreas. All of the mice were killed by ether inhalation. The entire pancreas of each mouse was fixed in Bouin's solution, embedded in paraffin, and 6 μm sections thereof were stained with hematoxylin and eosin. As described in the literature[4], the pancreatic acini usually contain cells in which the levels of activity are interpreted by their size and content: in some the cytoplasms are distended with acidophilic zymogen granules, and in others the cells are smaller, have less prominent but clear cytoplasms. In mice, the acinar cells are usually mononucleated and occasionally binucleated. This is the cytological pattern that we associate with and expect in the mouse pancreas. In contrast to this, all of the pancreases of AKR mice were normal on gross inspection; but, by microscopic examination significant numbers of the cells contained up to 12 nuclei, most of which were smaller than the nuclei of the mono- and binucleated cells (Figure). There was no evidence of inflammatory nor other reactive cellular infiltration in the tissues. The cytoplasm of the multinucleated cells appeared homogeneous and of ground glass appearance. Multinucleated acinar cells were observated in all of the pancreases of 60 germfree AKR mice, representing both sexes and age range from 1 to 10 months. A similar pattern of multinucleated acinar cells was observed in the pancreases of disease-free conventional counterpart AKR mice, as well as of leukemic germfree and conventional AKR mice. These multinucleated cells were also observed in the pancreatic cells of germfree AKR mice with so-called 'puny' or secondary-type disease[5], and in AKR mice which had been subjected to extensive therapy with cyclophosphamide[6].

By contrast, the multinucleated cells were not observed in the pancreases of disease-free, nor in radiation-induced leukemic germfree and conventional mice of C3H, Balb/c, CFW, ICR, C57 Bl, DBA, Swiss-Webster,



Multinucleated acinar cell in pancreas of C58 mouse. Hematoxylin and eosin stain. ×160.

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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