Inhibition of Cell Proliferation Caused by Oncogenic DNA-Polyoma Virus

Following inoculation of new-born Wistar rats within the first 30 h p.n. with our polyoma virus strain (derived from Dr. S. E. STEWART[1]) fibrosarcomas of the kidney developed. No other tumors could be found elsewhere[1]. Histological follow-up studies of the kidneys have shown that a period of 18 days elapses between virus inoculation and beginning of tumor growth[1,2].

Aside from the transformation and the already known replicative-cytolytic effect of polyoma virus we found a third one, the inhibition of cell proliferation in non-transformed cells of virus inoculated animals.

Material and methods. Cultivation of polyoma virus was done in mouse embryo cells grown in Eagle's-20% fetal bovine serum with addition of 100 IU penicillin and 100 γ streptomycin. We calculated the infection titer on secondary mouse embryo cell cultures and determined it as $TCID_{50}$/0,3 ml after REED and MUENCH. The titer of the virus solution used amounted to 10^5 $TCID_{50}$.

New-born Wistar rats were inoculated with 0.3 ml, new-born NMRI-mice with 0.1 ml, and young adult Wistar rats with 7 ml of virus solution. Control animals were inoculated with a corresponding amount of virus free tissue solution. Decapitation of neonatal inoculated rats was done 5, 10, 15, 18, 20, 21, 25 and 40 days p.i., of adult rats 5, 10 and 20 days, and of neonatal mice 10, 15 and 20 days p.i., respectively.

We performed determination of 3H-thymidine labelling index (amount of labelled cells/total cell count) by a single i.p. injection of 1 μCi/g ^{3}H-thymidine (specific activity 5 Ci/mmol), 1 h before decapitation. For continuous labelling we made i.p. injections of 0.5 μCi/g over 72 h at the 20th day and over 58 h at the 25th day following inoculation.

Autoradiograms were made by the stripping film method (Kodak AR 10). The total amount of cells counted in the kidney was 4 million epithelial and 520,000 stromal cells, in the liver 9,350,000 parenchymal and 1,900,000 Kupffer cells, and finally 45,000 epithelial cells of the duodenal mucosa. Calculation of silver grain count/nuclear surface was done at the 40th day following inoculation counting 1,900 epithelial cells of the proximal convoluted tubules and 1,000 stromal cells of cortex and medulla. All cell counts starting from the 20th day apply to tissue not affected by neoplastic growth.

Results. A significant reduction of DNA synthesizing cells amounting up to 40% is seen in the kidney of polyoma inoculated rats, beginning from the 10th day following virus inoculation (Table I). This effect is especially conspicuous in the stromal cells of the renal medulla. It even applies to those animals, which do not develop any tumors later on.

The level of mitotic activity is also reduced significantly whereas the total amount of silver grains seen in a labelled cell was the same in virus inoculated as well as control animals. In addition to it, this inhibition of proliferation is quite obvious following continuous labelling: in the stromal cells of the renal medulla, 47% respectively 55% less cells are DNA-synthesized compared to controls. In repeated studies we have seen a significant inhibition of cell proliferation, lasting from the 10th to the 40th day after virus inoculation. Studies of other cell popula-

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Table I. 3H-thymidine labelling index in stromal cells of the kidney, 5–25 days following neonatal inoculation with polyoma virus

Days	5	10	15	18	20	21	25
Cortex							
Virus inoculated	11.60[a]	7.90	5.59[a]	4.99	2.66[a]	2.10[a]	1.28
Controls	6.10	9.24	6.08	5.21	4.26	3.00	1.82
Medulla (outer zone)							
Virus inoculated	9.12[a]	6.70[a]	6.53[a]	4.85[a]	3.48	1.25[a]	1.42[a]
Controls	2.88	9.60	10.15	7.50	5.88	3.10	2.96

[a] Significance below 1%.

Table II. ^{3}H-thymidine-labelling index and mitotic index in epithelial and stromal cells of the kidney and liver and in epithelial cells of the small bowel, 40 days following neonatal virus inoculation

	Kidney				Liver		Small bowel	
	Cortex Virusinf.	Controls	Medulla Virusinf.	Controls	Virusinf.	Controls	Virusinf.	Control
Labelling index								
Epithelial cells	1.19[a]	1.96	0.99[a]	1.92	0.07[a]	0.25	52.7	48.2
Stromal cells	1.95[a]	3.96	1.78[a]	4.15	0.1[a]	0.53		
Mitotic index								
Parenchymal and stromal cells	0.06[a]	0.09	0.06[a]	0.11	0.01	0.03		

[a] Significance below 1%.

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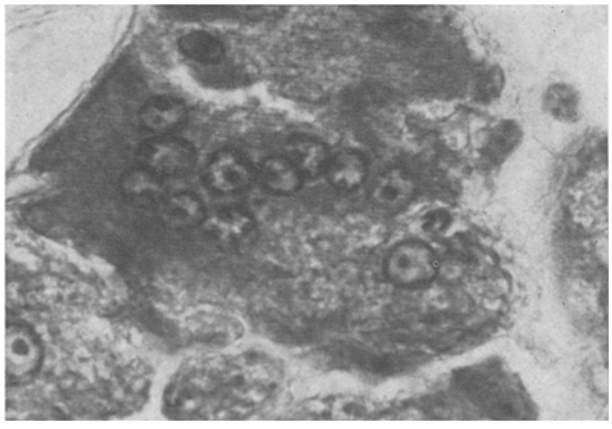
Multinucleated Acinar Cells in the Pancreases of AKR and C58 Mice[1]

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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