PATHOLOGY

Evidence for the Cell-free Transmission of chemically induced Mammary Carcinoma in the Rat

THE possibility that chemically induced cancer might be the result of the activation of a latent virus has been considered in the past. Some evidence has been reported[1] which indicates transmission of chemically induced tumours by subcellular materials prepared from the latter. Data implicating a virus aetiology of this class of tumours have been inconclusive[2].

In the investigations described here, we have attempted to produce mammary carcinomata in the rat with cell-free preparations derived from the same type of tumour induced in the rat by administration of 7,12-dimethylbenzanthracene (DMBA), as originally described by Huggins *et al.* and by Geyer *et al.*[3].

Female rats of the random-bred Sprague–Dawley strain, approximately 50 days old and weighing 130–150 g, were given a single oral dose of 20 mg DMBA. About 65 per cent developed single or multiple mammary carcinomata within a 6-month period. These tumours were used in the experiments described here.

Tumour tissue preparations from one rat were never pooled with those derived from another rat; however, in some instances, multiple tumours from the same rat were combined. Aseptic procedures were followed at all times. The tumour tissue was rinsed once in Hanks's balanced salt solution, minced with scissors, and the mince was divided into two portions. One portion was ground in a mortar with 'Alundum' in 0·15 M potassium citrate solution containing 1 mg per cent hyaluronidase. This extract was freed from tissue debris and cells by three cycles of centrifugation at 1,900*g*, 2,400*g*, and 12,000*g*, for 20, 20, and 10 min each, respectively. The supernatant from the third cycle of centrifugation was centrifuged at 73,000*g* for 1 h. The resulting pellet was suspended in 0·05 M potassium citrate to give a concentration of 0·5–1·0 g equivalents of the original tumour tissue per ml.

The second portion of the same tumour mince was rinsed twice with Hanks's solution and gently agitated as a suspension in a 0·25 per cent solution of trypsin for 1 h at 37° C. The trypsinized cells were washed twice in Hanks's solution and suspended in complete medium to a concentration of 5–10 × 10^5 cells per ml., and grown on glass in Brockway Flint prescription bottles at 37° C for 2–4 weeks. (The complete medium consisted of 55 per cent minimum essential medium, 30 per cent beef amniotic fluid, 15 per cent calf serum (heated at 56° C for 30 min), 100 U. penicillin per ml. and 100 μg streptomycin per ml. The *p*H was adjusted with a 7·5 per cent sodium bicarbonate solution. All ingredients were obtained from Microbiological Associates, Inc., Bethesda, Maryland.) When the cell sheets from a number of bottles became confluent, the cells and medium were collected and pooled. This suspension was frozen and thawed three times. The suspension thus obtained was centrifuged in the same manner as the first portion of the mince. The final pellet was resuspended in a volume of 0·05 M potassium citrate equivalent to 1/10–1/20 of the original volume of the culture fluid.

Inocula prepared from each tumour by both processes were injected into new-born female Sprague–Dawley rats. Groups of 10–15 new-born rats were each injected with 0·1 ml. of the inocula subcutaneously, intraperitoneally, or, in a few instances, with 0·025 ml. intracerebrally. The injected animals were weaned when they were 3 weeks old and palpated for the presence of tumours at bi-weekly intervals. New-born rats of the same sex and strain, serving as controls, were injected either with similarly concentrated extracts of liver–spleen–lung tissue from tumour-bearing rats, of rat embryo cell cultures, 0·05 M potassium citrate or with 1–10 × 10^5 intact trypsinized cells from DMBA-induced tumours. None of the 800 rats given the foregoing control materials developed mammary carcinomata during 9 months of observation. On the other hand, when inocula derived from 54 DMBA-induced tumours were injected into a total of 1,180 new-born rats, fourteen mammary carcinomata (identified by histological examination) developed from thirteen of the DMBA tumours within 9 months. The results of these experiments, presented in Table 1, show that: (*a*) the incidence of tumour response of the injected animals was very low even in the positive groups; (*b*) the average latent period was about 6 months; (*c*) tumour production was equally successful with tissue culture preparations and tumour tissue extracts; (*d*) tumours also resulted from the injection of extracts that were filtered.

Table 1. PRODUCTION OF MAMMARY CARCINOMATA BY INJECTING NEW-BORN RATS WITH PREPARATIONS DERIVED FROM CHEMICALLY (DMBA) INDUCED MAMMARY CARCINOMATA OF THE RAT

DMBA-induced tumours		Tumour response	
Source of inoculum	Route of injection	Latent period (days)	Tumour*/ total
Cell culture	intraperitoneal; subcutaneous†	100; 224	2/9
Cell culture	intraperitoneal; intracerebrally†	187	1/6
Cell culture	intraperitoneal; intracerebrally†	205	1/12
Cell culture	intraperitoneal	250	1/3
Cell culture‡	intraperitoneal	264	1/10
Cell culture‡	intraperitoneal	283	1/13
Tumour tissue	intraperitoneal	95	1/13
Tumour tissue‡	intraperitoneal	141	1/12
Tumour tissue	intraperitoneal; subcutaneous†	171	1/12
Tumour tissue	intraperitoneal	178	1/11
Tumour tissue	intraperitoneal	193	1/12
Tumour tissue	intraperitoneal	201	1/15
Tumour tissue	intraperitoneal	210	1/22

* Histologically, all tumours were papillary carcinomata or adenocarcinomata.

† Same animal injected by two routes.

‡ Filtered through 'Millipore *HA*' filter impermeable to bacteria (0·45μ).

The small number of tumours obtained in the experimental groups would seem to be significant, however, since none developed in control animals during the same period of time. Further investigations are being undertaken in an attempt to increase the tumour incidence.

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Deoxyribonucleic Acid Synthesis of Hydatidiform Moles in Organ Culture: an Autoradiographic Investigation

MIDGLEY *et al.*[1], using tritiated thymidine, have demonstrated that placental syncytiotrophoblast in the monkey originates from cytotrophoblast. Electron microscopic and immunochemical investigations of benign and malignant trophoblast have supported this concept and have also shown that the differentiated syncytiotrophoblast produces chorionic gonadotrophin[2–4].

A hydatidiform mole is a chorionic growth which morphologically and clinically represents an immature and pathologic trophoblast[5,6]. In the investigation reported here, an attempt was made to label selectively and to follow the maturation pattern seen in trophoblast