PATHOLOGY

Effect of 'Captan' on the Mouse Ascites Tumour of Ehrlich

THE inhibitory effect of various seed-extracts on the development of the mouse ascites carcinoma was traced to the presence of 'Captan', a fungicide, with which the seeds had been treated.

We therefore injected 0.15 g/kg of 'Captan' (*N*-trichloromethylthio-4-cyclohexene-1-2, dicarboximide) i.p. daily for 14 days into white Swiss mice (25–28 g) which had been inoculated one day before injection with 2×10^6 ascites tumour cells. Higher doses of 'Captan' were found to be too toxic.

'Captan' was extracted by means of 95 per cent ethanol from its inert carrier in the commercial preparation (Stauffer Chemical Co.). The alcohol extract was diluted with sterile water (one part to 27 parts) and 2 c.c. of this opalescent solution containing the foregoing stated amount of 'Captan' was injected. The amount of ethanol present was found to exert no influence on the growth of the mouse ascites tumour cells.

Fig. 1 shows a representative experiment; six other experiments gave similar results. With the injection of 'Captan' the mean survival of mice inoculated with ascites tumour cells was increased from 26 to 80 days.

Fig. 1. Survival of mice inoculated with 2×10^6 Ehrlich ascites tumour cells: (1) no treatment, (2) treated with 'Captan'.

It was of interest that all the mice treated with 'Captan' were found to have solid tumour on autopsy apparently originating from the injection site in the abdominal wall.

There are several entries concerning 'Captan' in the *Negative Results of the Cancer Chemotherapy Screening Data of the U.S. Public Health Service* (Entry numbers: 492, 3160, 3321, 6458, 17374, 24836, 28998, 32419). These experiments differ from ours not only as to the tumours which were used as test material, but also in the vehicle which was employed and the amount of 'Captan' injected.

In view of our findings the tumour spectrum of *N*-trichloromethylthio-4-cyclohexene-1-2, dicarboximide will be investigated.

FRITZ STECKERL

MARY LOUISE TURNER

Boston University School of Medicine,
Department of Medicine,
Boston, Massachusetts.

Inhibition by Nucleoside Analogues of Tumour Formation by Polyoma Virus

THE inhibition of an oncogenic virus by a halogenated deoxyribonucleoside was first demonstrated by co-operating groups at the U.S. National Institutes of Health and Yale University¹. They showed that the development of adenovirus 12-induced subcutaneous tumours in neonatal hamsters could be suppressed by either a single injection of 5-iodo-2'-deoxyuridine (IUdR) given at the same subcutaneous site as the virus, or even at a site distant from the virus injection using 10 subcutaneous doses of IUdR given on alternate days. This dosage of drug was apparently quite non-toxic.

The work recorded here was initiated to determine whether tumours produced at a site distant from the virus injection and by a different DNA virus could be inhibited. The 'Toronto' strain of polyoma virus² produces diffuse kidney tumours and nodular heart tumours which lead to death about 16 days after injection of undiluted virus into new-born hamsters^{3,4}. When diluted virus is used, all tumours tend to be nodular, death is delayed, and subcutaneous tumours are common.

The polyoma virus was grown in mouse embryo tissue and concentrated to a titre of approximately 10^8 tissue culture infective doses 50 per cent effective ($TCID_{50}$). 0.1 ml. of the virus was injected subcutaneously over the right shoulder and 0.1 ml. of a sterile solution of either the drug or of saline was injected subcutaneously over the left rump shortly thereafter. Survivors were weaned on the twenty-first day. All dead animals which could be recovered were subjected to autopsy and histological sections were prepared when indicated.

When undiluted virus was used, the control animals had an average survival time of only 16.1 days (Table 1). When IUdR, 5-iodo-2'-deoxycytidine (ICdR) or 6-azauridine was given for a single day, there was no indication of a change in time of survival. On the other hand, when repeated doses of 0.5 mg of either IUdR or ICdR were given for 5 consecutive days, the average survival was increased to 34.6 and 26.8 days, respectively; nevertheless, all treated animals died with tumours within 71 days.

Table 1. NEONATAL HAMSTERS INJECTED WITH UNDILUTED POLYOMA VIRUS

Drug	Dose	Day	No. of animals	Average survival (days)
None (saline)	0.1 ml.	1 or 5	16	16.1
5-Iododeoxyuridine (IUdR)	0.5 mg	1	3	17.3
	0.5 mg	5	5	34.6
5-Iododeoxycytidine (ICdR)	0.5 mg	1	5	17.6
	1.0 mg	1	7	20.8
	0.5 mg	5	4	26.8
6-Azauridine	0.5 mg	1	6	18.5

In a second experiment, several dilutions of virus were used and the treatments with 0.5 mg of IUdR were prolonged to 10 subcutaneous injections, given on alternate days. The results at 120 days are summarized in Table 2. The striking decrease in incidence of tumours at dilutions of 10^{-1} and 10^{-2} confirms the efficacy of IUdR in the inhibition of viral oncogenesis, not only on tumours formed at the site of virus injection, but also on those formed at sites distant from the site of virus injection.

Table 2. NEONATAL HAMSTERS INJECTED WITH DILUTIONS OF POLYOMA VIRUS

	Virus dilution	10^0	10^{-1}	10^{-2}	10^{-3}
No treatment (saline)	Tumours/total	17/17	20/20	11/13	9/17
	% Tumours	100	100	85	53
5-Iododeoxyuridine	Tumours/total	5/5	3/13	2/12	1/18
0.5 mg on alternate days $\times$ 10	% Tumours	100	23	17	6

Although viral oncogenesis in new-born animals is an artificial system, this investigation does provide some evidence that drugs may be used to modify the consequences of virus infection. These experiments do not provide a definitive answer to the question of whether IUdR acts to prevent replication of the virus or to inhibit the neoplastic cell in the intact animal. Pertinent to this point, however, Herrmann⁵ and Cramer *et al.*⁶ have shown that IUdR may act selectively against a DNA virus without stopping cell growth in tissue culture, and Munyon *et al.*⁷ have shown that IUdR inhibits the production of polyoma virus in tissue culture. Hence, it would seem that viral inhibition is the more probable explanation of the present results. Perhaps inhibition of viral growth reduces the rate and frequency of cell transformation and allows the suckling hamster sufficient time to mature and to mobilize its immunological defences both against virus and against the new antigen⁸ of transformed cells. Such a mechanism would be compatible with the virus dosage effects which we have observed. Further pharmacological