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Enhanced growth of transplanted tumours after treatment with cytotoxic agents

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DURING the course of some experiments involving the treatment of mice carrying large transplanted tumours with a wide range of doses of various cytotoxic drugs it was found that the dose response curves showed two curious features. One was that even very low dosages caused some inhibition of tumour growth, and the other that higher, but still usually small, doses could cause an enhancement of tumour growth. This is illustrated in Fig. 1 for sarcoma 180 (Crocker) when treated with either urethane or Thiotepa. In both experiments the tumours measured approximately 8 × 5 × 5 mm at the time of treatment.

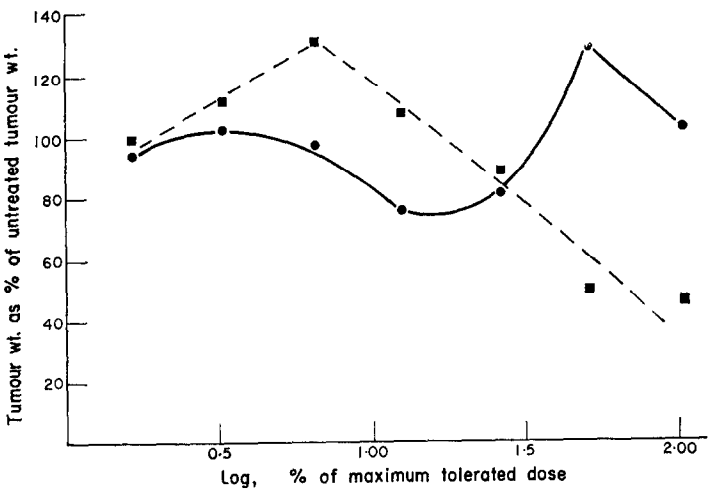

FIG. 1. Dose response curves for Sarcoma 180.
●——● treated with Urethane; ■——■ treated with Thiotepa.

TABLE 1. ENHANCEMENT OF TUMOUR GROWTH AFTER DRUG TREATMENT GROWTH AS A PERCENTAGE OF THAT OF UNTREATED CONTROLS

Tumour	T.E.M.	Mannitol Myleran	Eponate	A.C.P.C.	Thiotepa	Dimethyl Myleran	Degranol	Merophan	Methotrexate	Chlorambucil
NK (lymphoma)	106% (1/8)			122% (1/32)						129% (1/32)
NK/R (lymphoma)	108% (1/8)	118% (1/8)	134% (1/16)	140% (1/16)	118% (1/32)				122% (1/4)	141% (1/32)
S.180 (sarcoma)				116% (1/4)	132% (1/16)	114% (1/32)	111% (1/4)			
Plasma cell tumour							121% (1/8)	116% (1/4)		

In brackets is the fraction of the maximum tolerated dose of the drugs which gave the enhanced growth.

Extension of these experiments showed that similar findings occurred with other tumours and other cytotoxic agents. The results have been summarised in Table 1. In all cases the experiments were carried out when the solid tumours were at least 5 mm^3 in size or, in the case of ascites tumours, a minimum of five days after transplant of 1×10^6 cells. At this stage all animals bearing ascites tumours were showing obvious abdominal distension.

A number of experiments run in parallel using the N.K. ascites lymphoma and the resistant form of this tumour (NK/LY/R) which was originally induced by repeated treatment of the normal tumour with Degranol showed two interesting features. The first was cross resistance to other types of cytotoxic agent; these including 1 aminocyclopentanecarboxylic acid (A.C.P.C.); Eponate; Mannitol Myleran and Tri-ethylene melamine (T.E.M.). Secondly it was found that any growth enhancement was more pronounced in the resistant tumour than in the normal one. This is illustrated in Fig. 2.

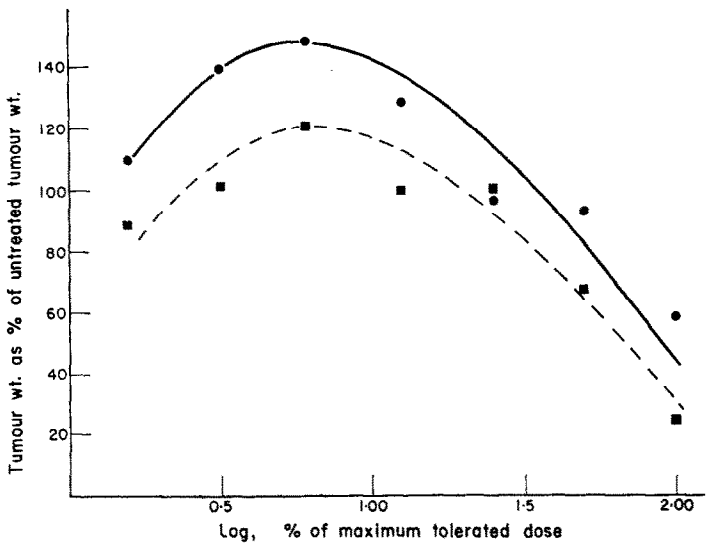

FIG. 2. Dose response curves for Lymphoma N.K. and Lymphoma N.K. (Resistant) treated with A.C.P.C. ■——■ Normal tumour; ●——● Resistant tumour.

All the experiments referred to above have been carried out using animals in groups of five for each dose applied. In the case of the two ascites tumours checks have shown that the growth of the normal N.K. lymphoma shows a standard deviation of 6 per cent when ten untreated groups are considered together. The growth of the resistant form—NK/LY/R—is only slightly more irregular with a standard deviation of 8 per cent. Thus in the case of the two lymphomas the enhancement of growth found is statistically significant since in many cases it exceeds three times the S.D. In the case of the solid tumours it has been practice to transplant a large number of tumours and then select those nearest the required size. After randomisation into groups of five the S.D. in the growth of various groups has been about 18 per cent. So the significance of the results in the case of the solid tumours is rather more doubtful. However the factor which determines the validity of the findings is that they are repeatable.

The experimental results would not appear to be the direct result of a stimulatory effect by a small dose and inhibitory effect by a large dose since doses even smaller than those causing enhanced growth are regularly inhibitory. A more likely explanation would be that inhibition of tumour growth occurs at all dose levels of the cytotoxic agent, but at a certain minimal level suppression of immunity responses occurs. Once the dose level reaches this required value the removal of the immune response then allows an increase of tumour growth which is greater than the direct inhibitory effect

of the drug. At even higher dose levels the direct effect of the drug on the tumour becomes sufficiently large to overcome the enhanced growth occurring as the result of the removal of the immune response. Since the tumours used in these experiments cannot be regarded as syngeneic, removal of the immune response should allow considerable growth stimulus, as has been found.

The treatment of animals with cytotoxic drugs prior to tumour transplantation has been shown to cause enhanced tumour growth.[1-4] Schmael and Sattler[4] suggested that this effect was dose dependent, a high dose of cytotoxic agent being required. Schmid, Schmid and Sugiura[3] also used high doses and concluded that the effect depended upon lymphocidal activity. The present findings differ considerably in that they have usually been obtained with a single small dose of cytotoxic agent. In fact, in some cases, the doses are too small to have produced any detectable lymphopenia.

In contrast to these findings is the suggestion[5] that the successful treatment of the Burkitt lymphoma by, often quite small, doses of various cytotoxic drugs is the result of immunity responses together with the drug action. Whilst this may mean that there are differences in the responses of mice and men to cytotoxic agents it does lead to the overall conclusion that further investigations are required in this field.

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Tetrahydrofolate-dependent enzymes in Sarcoma 180 cells sensitive and resistant to amethopterin

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AMETHOPTERIN-resistant sublines of mouse Sarcoma 180 cells (S-180) have been developed *in vitro* in the presence of hypoxanthine (AH/67) or thymidine (AT/174).[1] Both of these resistant sublines contain greatly increased amounts of folate reductase (tetrahydrofolate dehydrogenase), which by many different criteria is identical with the enzyme in the parent cells.[1, 2] The purpose of the present study was to compare the activity of several tetrahydrofolate-dependent enzyme systems in these cells. This was done to determine whether amethopterin resistance might be associated with changes other than the increase in folate reductase. Thymidylate synthetase activity in these cells had been studied earlier and was found to vary widely and independently from folate reductase.[3]

The origin of the parent S-180 cells, the development of the amethopterin-resistant sublines (AH/67 and AT/174), their maintenance media and harvesting have been previously described.[1] The media now used contain 5 per cent instead of 10 per cent of horse serum.

The cell pellets were stored at —75° and homogenized in a Potter–Elvehjem homogenizer in ice-cold 0·05 M K-maleate buffer, pH 7·0, containing 0·1 M 2-mercaptoethanol. The homogenate