Prolongation of Homograft Survival in Mice with Single Doses of *Cyclo*phosphamide

It is customary, in prolonging the survival of homografts with a chemical agent, to give frequent doses of the agent over a prolonged period. Effective agents are generally toxic, and their use is attended by considerable mortality, in both laboratory animals and man. The immune response is not uniformly susceptible to inhibition by these agents throughout its course[1], nor is it uniformly active. It is likely, therefore, that dose-schedules not closely adapted to this changing sensitivity and activity of the immune response may, on one hand, subject the recipient to unnecessary toxicity and, on the other, fail to attack the immune response sufficiently during its most vulnerable period. It is therefore of some importance to establish the principles on which optimum dose-schedules may be based.

The complexity of this problem necessitates a stepwise approach. Initially we have investigated whether a single dose of a drug can significantly prolong the survival of a homograft and whether there is an optimum time for administering this dose.

Tail or abdominal skin from $(C57Bl \times A2G)$ F_1 hybrid male mice was grafted on to suprapannicular beds on the chest wall of male *A2G* recipients. The animals weighed 14–22 g at the start of the experiment. The drug chosen was *cyclo*phosphamide, since we found it to have the highest therapeutic index of several effective agents when used to suppress the antibody response to a bacterial vaccine[2].

At various times before or after grafting, recipients were given *cyclo*phosphamide, 200 mg/kg subcutaneously, dissolved in physiological saline, 1 ml./20 g body-weight. Grafts were first inspected 9 or 10 days after grafting and then every 1–2 days thereafter. The end-point adopted was the time of complete breakdown of the graft.

The results are shown in Fig. 1, from which it appears that a single injection of *cyclo*phosphamide is maximally effective in prolonging the survival of homografts in the mouse if given at any time from shortly after grafting to about the 4th day afterwards. Administration before grafting, or on the sixth day, has relatively little effect. The results are analysed statistically in Table 1.

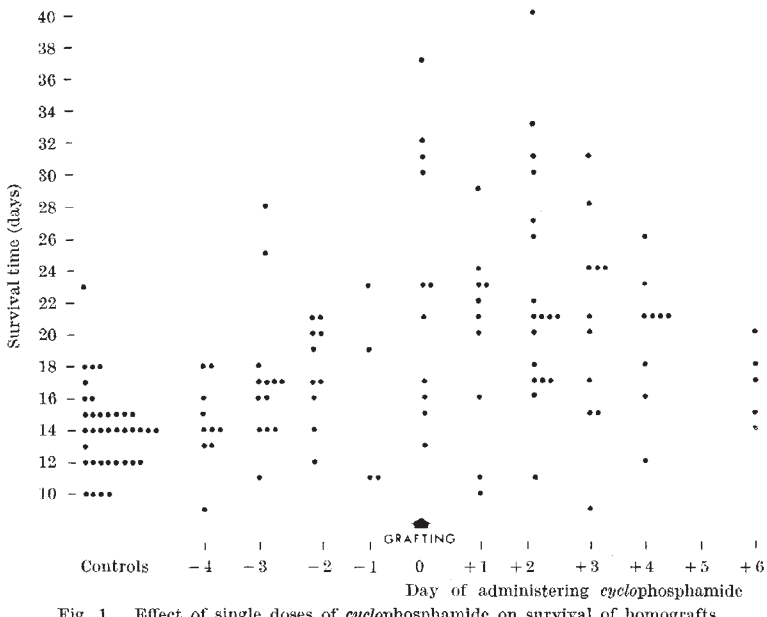

Fig. 1. Effect of single doses of *cyclo*phosphamide on survival of homografts. Points indicate animals in which grafts were completely rejected at the time shown in the ordinate. Recipients were grafted on day 0. The *cyclo*phosphamide injection on day 0 was given 1–2 h after grafting

We have also found that a single injection of 200 mg/kg *cyclo*phosphamide given 2 days after grafting to *A2G* recipients bearing grafts of *C57Bl* skin prolonged the mean survival time from the normal of $12{\cdot}0 \pm 2{\cdot}2$ days to $17{\cdot}0 \pm 2{\cdot}3$ days. This difference is highly significant ($P = < 0{\cdot}001$). A single injection, therefore, given at the appropriate time, significantly prolongs homograft survival even in the presence of strong *H2* incompatibility. In further experiments we hope to find the optimum times of injection of two or more doses of drug.

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PATHOLOGY

Action of Tumour Growth and Tissue Products on the Zonal Distribution of Liver Glycogen in Rats and Mice

In 1947, Young *et al.*[1] reported a decreased deposition of glycogen in the livers of tumour-bearing mice given a glucose load after a fasting period. Goranson *et al.*[2] were unable to demonstrate any real difference in the levels of liver glycogen in fasted controls and tumour-bearing rats and showed that the phosphorylase activity in the liver was about the same in both groups. On the other hand, the blood sugar-levels of tumour-bearing animals according to Goranson *et al.*[3] is reduced in alloxan-induced diabetes. As pointed out by Boyd *et al.*[4] the carbohydrate metabolism in human beings may be abnormal in malignant disease. These authors demonstrated a decrease in glucose tolerance in cancer patients which was reversed to normal by treatment with œstrogens.

It has been suggested that the disturbance of the carbohydrate metabolism in tumour-bearing individuals, including the low liver glycogen deposition, is due to the shunt of available glucose to the tumour on account of its high glycolytic rate[5,6].

We studied the zonal distribution pattern of the glycogen in the livers of rats and mice bearing tumours or injected with tumour and normal tissue supernatant and now report our results.

Male Wistar rats, weighing 150–200 g, were implanted subcutaneously with the

Table 1. Effect of Single Doses of *Cyclo*phosphamide on Survival of Homografts

Group	Controls	−4	−3	−2	−1	0	+1	+2	+3	+4	+6
Mean survival time (days)	14·1	14·4	17·2	17·7	16	23·5	19·9	22·7	20·7	19·9	16·8
No.	37	10	13	10	4	11	10	18	11	9	5
Variance	6·66	6·7	20·9	9·34	36	64·1	35·2	51·7	47·8	16·6	5·7
P*	—	> 0·5	0·02–0·05	0·002–0·01	> 0·5	0·001–0·002	0·01–0·02	< 0·001	0·01–0·02	0·002–0·01	0·05–0·1

* The significance of each difference from controls was determined by a modified t-test, taking into account the differing variances.