Biochemical Pharmacology, 1964, Vol. 13, pp. 589–602. Pergamon Press Ltd., Printed in Great Britain.

A TRANSPLANTABLE PLASMA CELL TUMOUR IN THE STUDY OF CARCINOSTATIC AGENTS

V. M. ROSENOER and M. E. WHISSON

Institute of Cancer Research: Royal Cancer Hospital, Fulham Road, London SW 3, Chester Beatty Research Institute

(*Received* 28 *August* 1963; *accepted* 10 *December* 1963)

Abstract—The growth characteristics and serum myeloma globulin production of a transplantable plasma cell tumour, ADJ-PC5, have been studied in the isologous host, BALB/c mice. A screening system for carcinostatic agents has been developed and the therapeutic indices of a selection of alkylating agents estimated. The therapeutic indices of the amino acid mustards melphalan, medphalan, merophan and amino-chlorambucil did not differ significantly, but that of another aromatic drug, aniline mustard, was significantly higher. The potencies of melphalan and medphalan showed differences consistent with their being taken up by active transport mechanisms. The therapeutic index of cyclophosphamide was significantly greater than that of melphalan, although the potency was far lower. These results are discussed in relation to clinical studies of the treatment of multiple myeloma with melphalan and cyclophosphamide.

IN THE past few years several transplantable plasma-cell tumours in mice have become available for experimental study. These neoplasms grow progressively in normal mice and some give rise to the characteristic changes associated with multiple myeloma in man, including a specific hyperglobulinaemia,[1] Bence Jones proteinuria,[2] myeloma kidney[3] and osteolytic bone destruction.[1, 4] They thus provide a unique opportunity to study the chemotherapeutic responses of animal tumours which mimic a human disease. In the present study the plasma cell tumour ADJ-PC5 was used as the test system. The development of an assay system is described and studies with a number of carcinostatic agents are reported.

MATERIALS AND METHODS

1. *Tumour system*

The ADJ-PC5 tumour arose in a BALB/c An mouse following a single i.p. injection of an emulsion of incomplete Freunds adjuvant and heat-killed staphylococci.[5] This tumour was obtained from Dr. M. Potter in its 40th transplant generation (Line A). Subcutaneous implantation by trocar into the right flank of 8-week old female BALB/c mice, using 4 mm^3 fragments of a 14-day donor tumour was found to be the most satisfactory method of transplantation. The mice were maintained on a diet of rat cake supplemented with biscuits, oats, wheat and maize. In the early experiments the mice were weighed daily and the tumours measured in two diameters. A tumour volume index was calculated from the product of the square of the smaller diameter multiplied by the longer diameter. This index was linearly related to the weight of the excised tumours, an index of 2000 corresponding to a tumour weight of 1·1 g.

2. *Protein analysis*

For the study of serum protein changes blood was collected in capillary tubes by puncture of the orbital venous plexus, allowed to clot for 10 min at room temperature

and spun down in a high speed microhaematocrit centrifuge for 5 min. Electrophoresis was carried out on Whatman No. 1 paper in Kohn type Shandon electrophoresis tanks using 0·05 M. Veronal buffer (pH 8·6) at 13 V/cm for 3 hr at room temperature. 5-μl samples were applied to the paper along 2-cm lines with 0·8-cm intervals. The papers were dried at 120°, stained for 16 hr in 15-l. tanks containing 0·03% Ponceau S in 5% trichloracetic acid, washed for 15 min in each of two stirred 15-l. tanks filled with 5% glacial acetic acid. The papers were then dried again at 120° and scanned in a Joyce Loebl reflecting densitometer. The relative concentration of each protein fraction was estimated by weighing the cut out densitometer traces. No correction was made for any variation in dye-protein affinity amongst the different fractions.

Quantitative assays of the amount of myeloma globulin present in the serum were carried out in the following way:

(*a*) *Standard curve.* 5-μl. samples which contained 15, 30 and 60 per cent of a standard ADJ-PC5 serum were electrophoresed and stained as described. The myeloma globulin bands of each sample were then scanned as shown in Fig. 1, and the 'areas' under

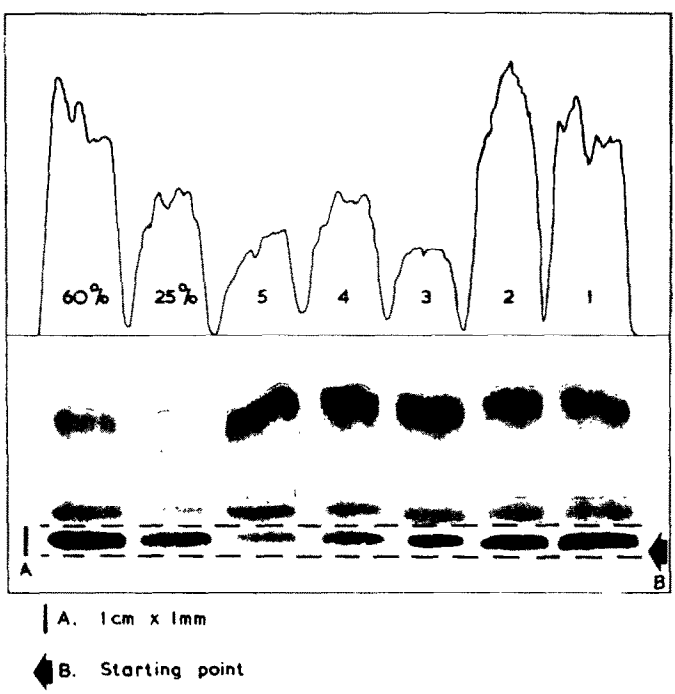


FIG. 1. Method of scanning γ globulin. 5 samples and two standards are applied to each paper at B. After staining, a 2-cm-wide strip is cut out so as to include the γ globulin. This is then scanned in a reflecting densitometer using a 10 mm × 1 mm slit.

the curves measured by weighing the cut out traces. The results of these assays, shown in Fig. 2, indicate a satisfactory linear relationship between the 'areas' so obtained and the log.-concentration of the myeloma globulin over the range studied.

(*b*) *Routine procedure.* In the routine assays two of three dilutions of the standard serum were run on each paper together with 5 test sera. 5-μl samples were used in each case, but when the protein concentration was very high it was diluted 1:1 with

Veronal buffer. Each standard and test serum was run in triplicate and the mean 'areas' under the densitometer traces estimated. A standard curve was plotted and the myeloma globulin concentration in each test serum read as a percentage of that in the standard.

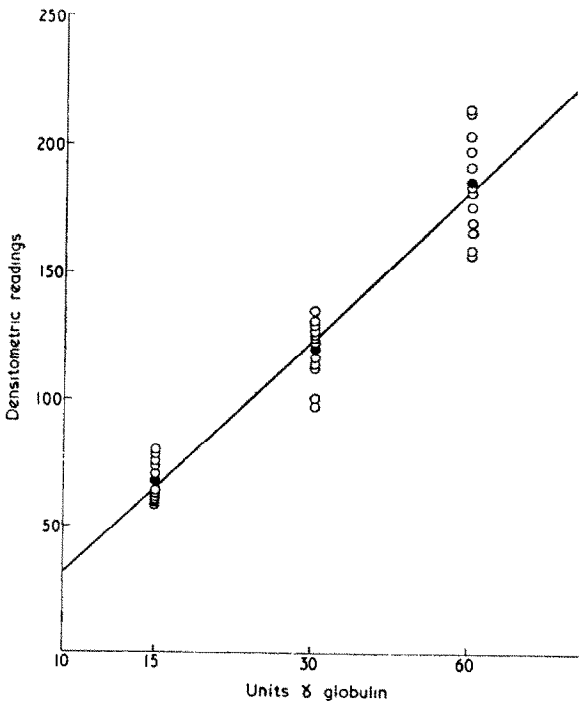


FIG. 2. Standard curve obtained by using the method described in Fig. 1. Densitometric readings are obtained from the weight of the cut out densitometer traces. Units of γ globulin represent percentage concentrations of the standard serum.

Vertical starch-gel electrophoresis was carried out by a modification of the method of Smithies[6] using Tris-citrate buffer (pH 8·65) for preparation of the gel, and sodium borate (pH 8·45) in the electrode chambers. Ultracentrifugal analysis of the serum proteins was carried out in a Spinco model E ultracentrifuge by Mr. P. A. Edwards, and Agar-gel immuno-electrophoresis was carried out by the method of Scheidegger[7] using an antiserum developed in rabbits against the 5563 plasma cell tumour γ globulin by Dr. B. A. Askonas. Analysis of urinary proteins was made by the paper electrophoretic method using 10-μl samples of fresh urine.

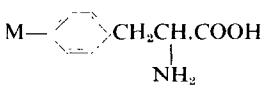
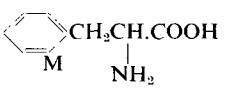
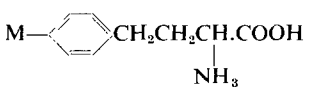
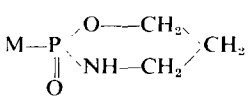
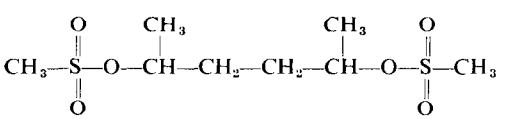
3.*Biological assays*

The carcinostatic and toxic effects of single intraperitoneal doses of melphalan and medphalan were compared in a series of parallel line dilution assays. Animals bearing

10-day established tumours in the range 4 × 4 mm to 8 × 8 mm were selected as it was found that more consistent results were obtained than when all the implanted animals were used. The mean weight of such selected tumours was in the region of 100 mg. Mortality was used as the index of toxicity; tumour weight and serum myeloma globulin concentration 10 days after treatment were used as measures of carcinostatic activity.

In preliminary experiments, using 3 animals per dose over a threefold dose range, approximately linear relationships were found between log. dose and log. tumour weight, and between log. dose and serum myeloma globulin concentration. From the results of these experiments doses were selected for 4 point assays of toxic and carcinostatic activities. Twenty tumour-bearing mice, randomized for dose, were used for each assay. Standard analysis of variance procedures were used for the carcinostatic assays, and an angular transformation procedure for the toxicity data. The fiducial limits were calculated according to Finney.[8]

TABLE 1.

Compound	Formula $M = Cl.CH_2CH_2\diagdown\diagup N\diagup\diagdown Cl.CH_2CH_2$	Solvent*
Melphalan (L) CB3025		Buffer
Medphalan (D) CB3026		Buffer
Merophan (DL) CB1729		5% methanol
Aminochlorambucil (DL) CB1385		Buffer
Aniline mustard CB1074		Arachis oil
Cyclophosphamide (DL) CB4564		Buffer
Dimethyl "Myleran" CB2348		10% propylene glycol

* See text.

In order to estimate therapeutic indices, 4 or 5 logarithmically spaced doses, chosen so as to span the therapeutic and lethal ranges, were given to 15–30 randomized mice bearing established tumours. 3–6 implanted but untreated animals were kept as controls. An estimate of the LD 50 was made by the Spearman–Kärber method. The

minimum carcinostatic dose (M.C.D.) was defined as the dose which produced tumour growth inhibition such that the mean tumour weight, measured 10 days after treatment, was 100 mg, i.e. the same as the mean tumour weight on the day of treatment. This definition permitted the optimal use to be made of tumour bearing animals. Untreated control animals were not essential to the experimental design, though it was considered desirable to include some untreated animals to check that tumour growth was within normal limits. The minimum carcinostatic dose was estimated by linear interpolation on the log. dose–response curve between the results at doses straddling the required value. The therapeutic index, defined as the ratio LD 50/M.C.D., was then calculated.

Drugs

The drugs used are listed in Table 1. In order to minimize errors due to hydrolysis drugs were injected not longer than 5 min after being dissolved in the appropriate solvent. Merophan and dimethyl Myleran were first dissolved in methanol or propylene glycol respectively, then made up to the appropriate volume with water. Melphalan, medphalan and aminochlorambucil were first dissolved in one volume of 2% ethanolic HCl then made up with 9 vol. of 40% propylene glycol in phosphate buffer (pH 6·8). The purity of the samples of melphalan and medphalan used was confirmed by optical rotation studies, i.r. spectrography and paper chromatography.

RESULTS

1. *The tumour system*

Macroscopically the tumours were well defined vascular structures supplied by two large vessels, one from the axilla and one from the inguinal region. The tumours were easily dissected free from the surrounding tissues, but when very large (over 4 g) tended to become adherent to the overlying skin, which eventually ulcerated. No metastases were found in any of the animals studied in these experiments. Microscopically the neoplastic plasma cells showed a variety of cytological appearances: in most cells the cytoplasm was strongly pyroninophilic with a clear juxtanuclear zone, the nucleus being lobulated or duplicated with densely clumped chromatin. Mitotic figures were frequent. Electron microscopy revealed large cysternae of endoplasmic reticulum surrounded by numerous ribosomes, and virus-like particles free in the cytoplasm. These appearances were identical with those described by Dalton *et al.*[9]

In Fig. 3 is shown the electrophoretic pattern of normal mouse serum compared with that of tumour-bearing mouse serum. The narrow slow moving band of myeloma globulin is clearly seen. Starch-gel electrophoresis demonstrated that this myeloma globulin could be fractioned into at least 3 components; immunoelectrophoresis and ultracentrifugal analysis confirmed that the abnormal protein was a γ globulin. The sedimentation coefficient was found to be 6·4 S ($S_{20}W$).

In Fig. 4 paper electrophoretic patterns of serum and urine from a mouse bearing an advanced tumour are shown. The marked proteinuria is evident, the urine containing all the serum protein components although there is a relative predominance of albumin. On heating the urine, precipitation of protein begins at about 80° and increases on further heating.

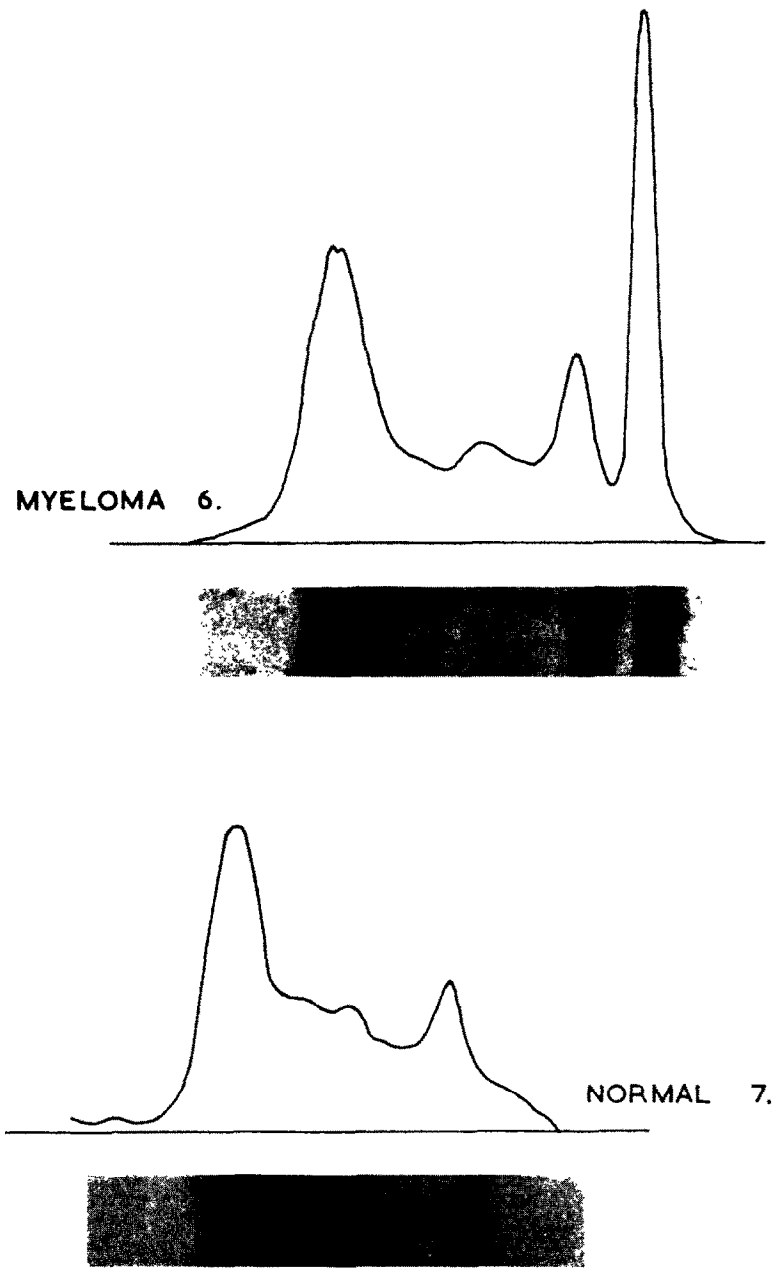


FIG. 3. Paper electrophoretograms of serum from a normal BALB/c mouse and from one carrying the ADJ-PC5 tumour. The sharp band of myeloma globulin scarcely moves from the baseline.

Growth characteristics. The characteristic growth curve of the subcutaneously transplanted ADJ-PC5 tumour in BALB/c mice is shown in Fig. 5, together with the changes in the relative amounts of the various serum protein fractions. The tumours grew in all the animals implanted and the serum myeloma globulin increased in parallel with the growth of the implanted tumours. Tumour excision caused a rapid

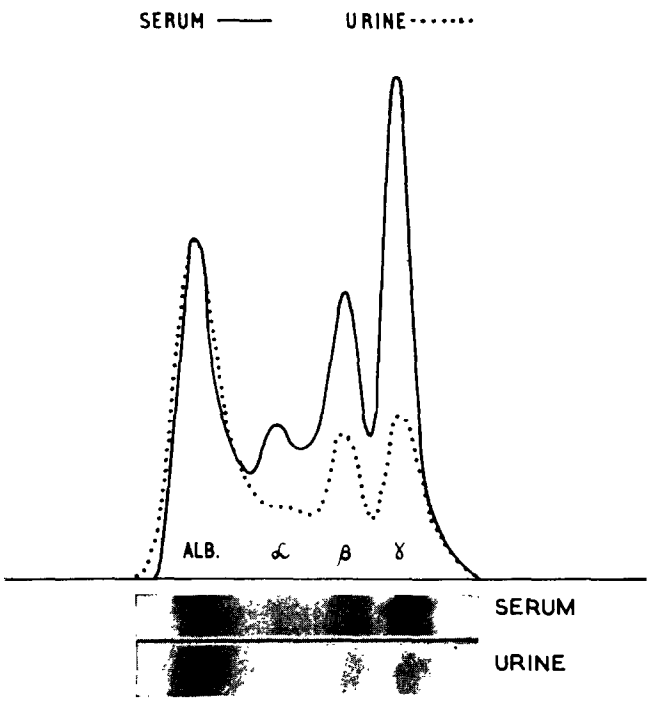


FIG. 4. Paper electrophoretograms of serum (5 μl) and urine (10 μl) from a mouse with a 4 wk ADJ-PC5 implant. The albumin concentration in the urine is half that in the serum.

fall in serum myeloma globulin concentration followed by a slow rise as the tumour regrew (Fig. 6).

Food restriction. It is well known that loss in body weight of the host may be associated with marked growth inhibition in implanted tumours. To assess the extent of this effect on the ADJ-PC5 tumour a controlled feeding experiment was carried out. Three groups of separately housed mice bearing 10-day tumours were fed on rat cake (a) ad libitum, (b) 2·5 g/mouse per day and (c) 1·25 g/mouse per day for 10 days. The results are plotted in Fig. 7. Dietary restriction produced profound loss in body weight of the host. The tumours continued to grow, but the size achieved at the 20th day after implantation was inversely proportional to the weight loss suffered by the host animal. Thus animals which had lost 8 g body weight on the 1·25 g/mouse diet carried tumours weighing only 22% of those carried by animals fed ad libitum.

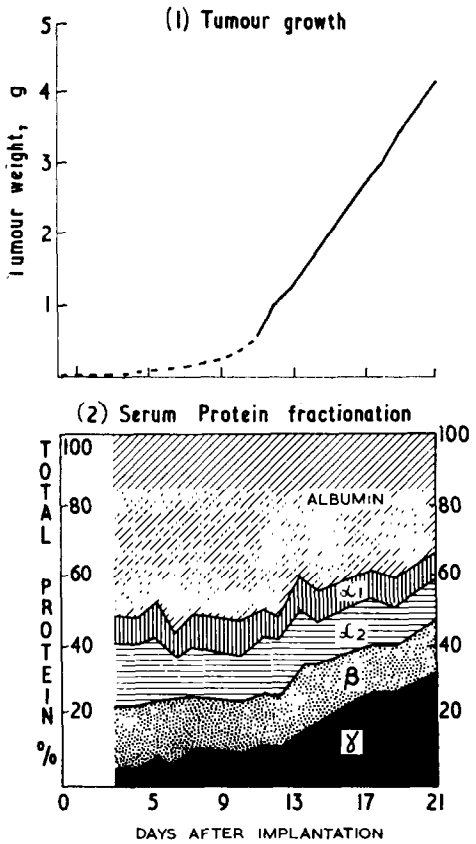


FIG. 5. Tumour growth and serum protein changes in mice carrying the ADJ-PC5 tumour. Each point represents the mean values obtained from 5 mice. Protein fractions are expressed as a percentage of the total serum protein.

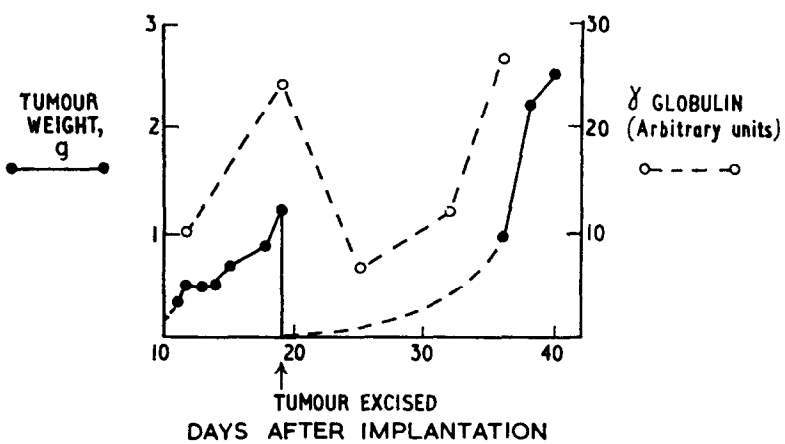


FIG. 6. Effect of tumour excision on serum myeloma globulin level. The tumour was excised on day 19 and was palpable again after 7 days.

The loss of 5 g body weight however was associated with less than 50% inhibition of tumour growth.

2. *Time relations of drug effects*

In the preliminary studies with merophan, aminochlorambucil and dimethyl Myleran the acute toxicity of a single i.p. dose was determined for each drug in at least 30 normal BALB/c mice. The mice were weighed daily for 20 days and an estimate

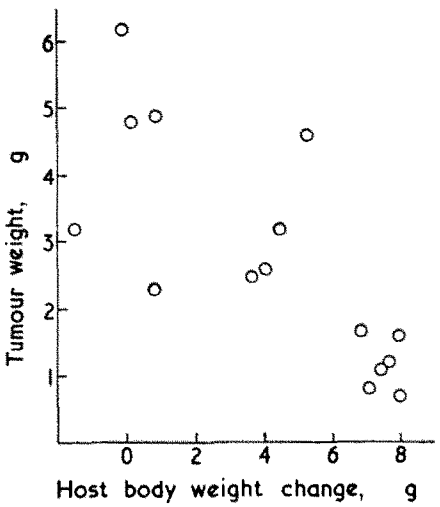


FIG. 7. Effect of host body weight change on tumour growth. Mice were placed on restricted diets. Figures for host body weight change represent weight loss.

obtained of the maximum weight loss in each animal and of the LD 50. The maximum tolerated dose for each drug was defined, for the purpose of subsequent tumour-inhibitory studies as the highest non-lethal dose which would result in less than 5 g host body weight loss.

The mean effects in groups of 6 tumour bearing animals given these maximum tolerated doses of merophan (1·5 mg/kg i.p.), aminochlorambucil (12·5 mg/kg i.p.), and dimethyl Myleran (10 mg/kg i.p.) on tumour growth and serum myeloma globulin concentration are shown in Fig. 8. Both tumour growth and globulin production were held up for a time which, in other experiments was found to be partly dependent on the dose. The optimal time to demonstrate the drug effects appeared to be 5–10 days after drug administration.

3. *Comparative assays*

A series of estimates of therapeutic indices was carried out with a selection of drugs in order to provide a comparative assessment of their therapeutic effectiveness against this tumour. The results of these assays are shown in Table 2. The potencies of the three phenylalanine mustards melphalan, medphalan and merophan, measured in terms of the LD 50 differed significantly ($P > 0{\cdot}95$) but their specific anti-tumour

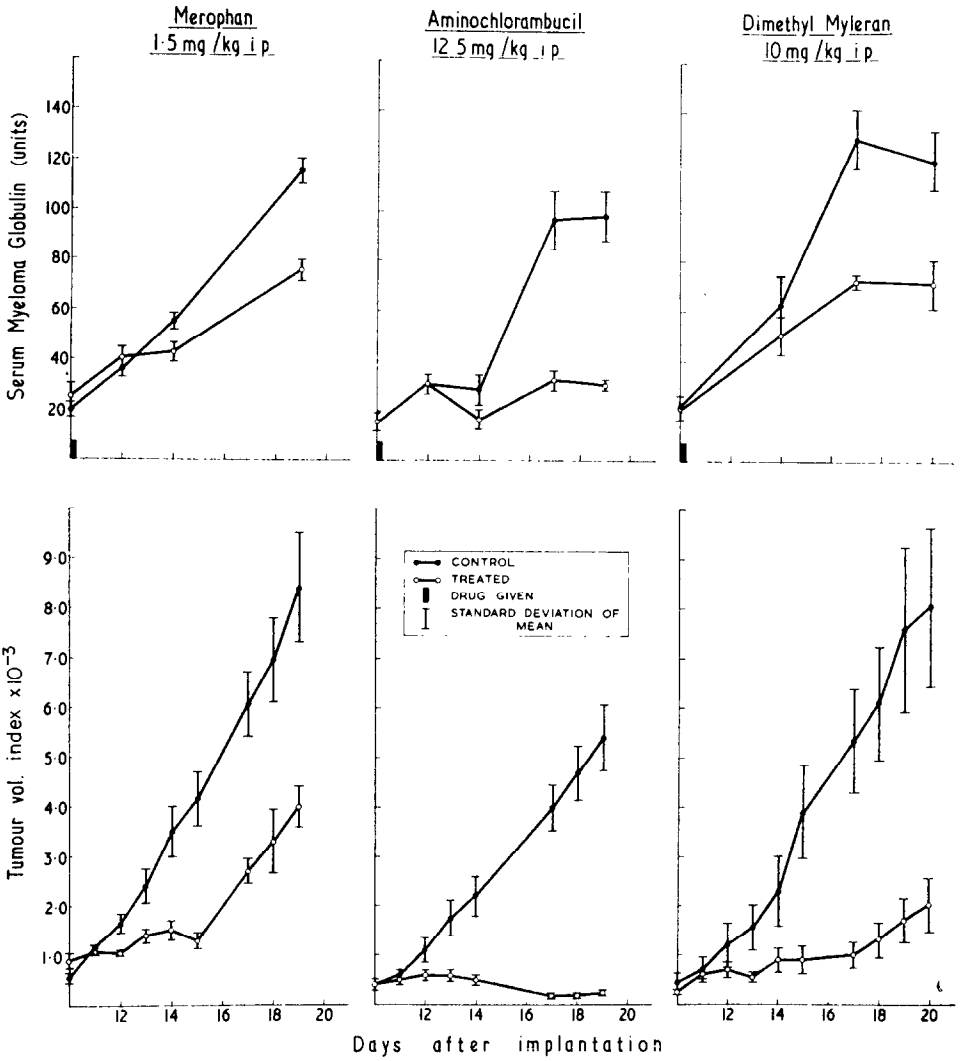


FIG. 8. Effect of merophan, aminochlorambucil and dimethyl Myleran on tumour growth and serum myeloma globulin concentration. Each point represents the mean value from 6 mice. Injections were given on the 10th day after implantation.

TABLE 2.

Compound	Mean LD 50 (μM/kg)	Mean therapeutic index (LD 50/MCD)	Range
Melphalan (L)	60	1·2	0·8 – 1·7*
Medphalan (D)	110	1·7	1·5 – 1·9†
Merophan (DL)	9	1·3	–
Aniline mustard	580	3·7	3·0 – 4·4†
Amino-chlorambucil (DL)	45	1·5	–
Cyclophosphamide	1360	2·6	2·1 – 3·2*

* 4 independent estimates.
† 2 independent estimates.

activities, measured in terms of the therapeutic index, were not significantly different. Aniline mustard and cyclophosphamide were less potent than melphalan ($P > 0{\cdot}99$) but their therapeutic indices were significantly higher ($P > 0{\cdot}99$).

In view of the known differences in the uptake of L and D amino acids it was of interest to make a critical comparison of the activities of melphalan and medphalan. This was done by comparing their toxicities and effects on tumour growth and serum myeloma globulin production in a series of parallel line dilution assays using groups of tumour-bearing animals radomized for drug and dose. The results are shown in Fig. 9 and analysed in Table 3. In order to arrest tumour growth 12 mg/kg of mel-

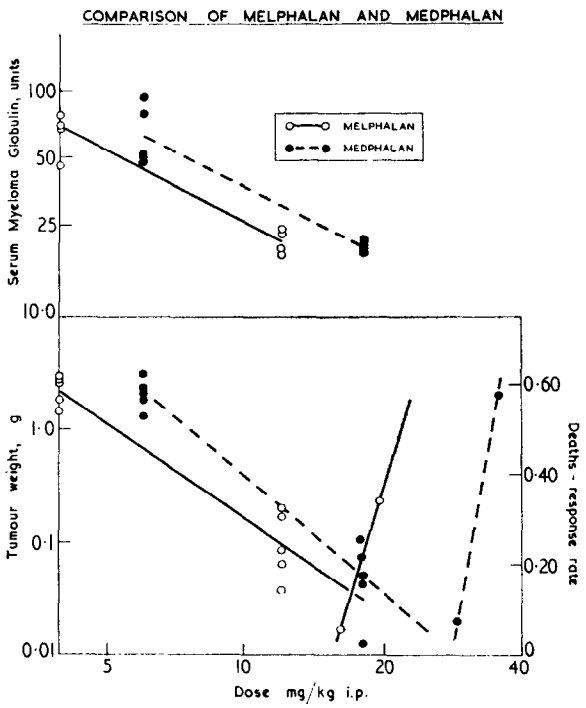


FIG. 9. Result of an experiment comparing the potencies of melphalan and medphalan. Melphalan was found to be 1·8 times as toxic as medphalan and 1·4 times as effective in inhibiting tumour growth and protein production.

phalan or 18 mg/kg of medphalan were required, whilst to produce 50% mortality 21 mg/kg of melphalan and 34 mg/kg medphalan were required. No consistent differences were detected for any of these compounds between the doses inhibiting tumour growth and those inhibiting myeloma globulin production.

DISCUSSION

Investigation of the actions of chemotherapeutic agents on experimental tumours is of interest in two related fields. Firstly, the effects of such agents may assist in elucidating differences between normal and tumour metabolism. Secondly, the inhibition of tumour growth by these agents may assist in the selection of drugs for clinical trial.

The ADJ-PC5 tumour is of considerable interest in both these fields. Since it retains an easily measurable special function, the synthesis of γ globulin, it may be possible to find metabolic inhibitors which will affect differentially tumour growth and γ globulin synthesis. Selective inhibition of growth might be correlated with high anti-tumour specificity, whilst selective inhibition of γ globulin production might well indicate a drug capable of supressing specifically immune responses to homografts. In the present study we could find no evidence of such a differential effect with the agents examined. The protein production followed tumour size very closely.

TABLE 3.

Index of activity	Potency ratio Melphalan/Medphalan	Fiducial limits (P = 0·95)
Inhibition of tumour growth	1·4	1·1–1·6
Inhibition of myeloma globulin production	1·4	1·2–1·7
Toxicity	1·8	1·5–2·1

As a screening system for anti-neoplastic agents, the ADJ-PC5 tumour has the same defects as other tumour screens. The tumour is transplanted and not spontaneous; it grows much more rapidly than do human neoplasms; it is subcutaneous and does not invade readily or metastasize. In view of the histological and biochemical similarities to myelomatosis in man, however, it seems reasonable to suppose that the responses of of the ADJ-PC5 tumour to chemotherapy will be more closely related to that of multiple myeloma than the responses of other commonly used experimental tumour systems. In addition the consistent production of the same γ globulin over some 80 transplant generations gives some assurance that the specific characteristics of the tumour are stable.

The therapeutic indices of melphalan and medphalan in this system were not significantly different, although an approximately twofold difference in potency was found. The comparative data presented by Bergel *et al.*,[10] using the Walker 256 tumour are consistent with these findings both in respect of the similar therapeutic indices and the lower potency of the D-isomer. In other tumour systems little difference between the therapeutic indices of melphalan and *p*-DL-phenylalanine mustard (merphalan) have been found.[11]

Koller and Veronesi[12] presented evidence of a delayed action by the D-isomer and suggested that racemization was necessary before the drug became active. This would explain the lower potency since some loss would occur by hydrolysis and excretion during racemization. Nyhan[13] showed that, in contrast to HN_2, melphalan exhibited a high degree of specificity in inhibiting the incorporation of amino acids into the proteins of the Walker tumour. He concluded that the amino acid moiety produced selective uptake of the drug in this tumour system. Although L-phenylalanine is concentrated somewhat faster than D-phenylalanine,[14] it is likely that the final distribution ratios over two or three hours would be closely similar.[15] This would provide an alternative explanation for the delayed effect observed by Koller and Veronesi and is not inconsistent with the greater potency of melphalan. Greater specificity of the L-isomer would be expected only if the preferential concentration of L-amino acids

were more marked in tumour tissues than in normal tissues. In the present experiments the therapeutic indices of the related amino acid mustards, melphalan, medphalan, merophan and amino-chlorambucil did not differ significantly: their potencies did, however, show significant differences. It is likely that hydrolysis rate is an important factor in determining potency. The most potent member of the group, merophan has the highest hydrolysis rate, whilst aniline mustard, the least potent has the lowest.[16] The high therapeutic index of aniline mustard may be associated with its lipid solubility. It is the only member of the group which is soluble in arachis oil.

The therapeutic index of cyclophosphamide was significantly higher than that of melphalan. It is of interest that cyclophosphamide was also effective in inhibiting the hamster plasmacytoma P-1[11] and the mouse plasma cell tumours MPC-1, MPC-2, MPC-3 and ADJ-PC5.[17] Carbone[18] has reported similar results with the LPC-1 plasma cell tumour. He found cyclophosphamide to be superior to melphalan in prolonging the survival time of mice bearing the established tumour in the ascites form.

The therapeutic index of 2·6 found in the present study with cyclophosphamide in the ADJ-PC5 system is interesting. However, in a subsequent study of animals treated with cyclophosphamide 10 days after tumour implantation, ratio LD50/Sur50 (dose producing tumour-free survival for 6 weeks of 50% of the animals) was only 1·3 (Rosenoer[19]).

A number of clinical reports on the value of phenylalanine mustards and cyclophosphamide in the treatment of multiple myeloma have been published recently. *p*-DL-phenylalanine mustard was reported to be effective by Blokhin *et al.*,[20] Larionov,[21] Castellano and Cattaneo.[22] In comparative clinical trials, Bergsagel *et al.*,[23–25] Brown et al.[26] and Austin *et al.*[27] found that, of the 5 agents tested, only melphalan was therapeutically effective by their criteria:[28] meta-L-phenylalanine mustard, however was ineffective. Bernard *et al.*,[29] Speed *et al.*[30] and Waldenström[31] have also reported favourable clinical responses to melphalan.

Matthias *et al.*[32] reported that of 14 patients with myelomatosis treated with cyclophosphamide, 7 responded favourably. Rivers *et al.*[33] studied 54 patients with multiple myeloma who were given either cyclophosphamide or a placebo in a double-blind study. Objective improvement was noted in 6 of the 29 patients receiving cyclophosphamide and marked subjective improvement in 3 others. None of the placebo treated patients showed any improvement. The survival times of the cyclophosphamide treated patients were significantly longer than those of the placebo treated.

Until further comparative trials, both experimental and clinical, are made it will not be possible to assess the predictive value of the ADJ-PC5 tumour system. A clinical trial of aniline mustard in myelomatosis would be of considerable interest.

Acknowledgements—This investigation has been supported by grants to the Chester Beatty Research Institute (Institute of Cancer Research: Royal Cancer Hospital) from the Medical Research Council, the British Empire Cancer Campaign, the Tobacco Research Council, the Anna Fuller Fund, and the National Cancer Institute of the National Institutes of Health, U.S. Public Health Service.

One of us, M. E. W., gratefully acknowledges the support of the Anti-Cancer Council of Victoria (Australia).

We wish to thank Miss Monica Hindle, Miss Norma E. Topping and Mr. C. Smith for excellent technical assistance.

REFERENCES

1. M. Potter, J. L. Fahey and H. I. Pilgrim, *Proc. Soc. exp Biol., N.Y.* **94**, 327 (1957).
2. J. L. Fahey and M. Potter, *Nature*, **184**, 654–655 (1959).

3. R. S. COLEMAN, C. H. LUPTON and J. S. A. MCMANUS, *Arch. Pathol.* **71**, 18–27 (1962).
4. H. KOBAYASHI, M. POTTER and T. B. DUNN, *J. nat. Cancer Inst.* **28**, 649–677 (1962).
5. M. POTTER and C. L. ROBERTSON, *J. nat. Cancer Inst.* **25**, 847–861 (1960).
6. O. SMITHIES, *Biochem. J.* **71**, 585–587 (1959).
7. J. J. SCHEIDEGGER, *Int. Arch. Allergy*, **7**, 103–110 (1955).
8. D. J. FINNEY, *Statistical method in Biological Assay*, Charles Griffin, London (1952).
9. A. J. DALTON, M. POTTER and R. M. MERWIN, *J. nat. Cancer Inst.* **26**, 1221–1267 (1961).
10. F. BERGEL, J. A. STOCK and R. WADE, "Peptides and macromolecules as carriers of cytotoxic groups" in *Biological Approaches to Cancer Chemotherapy* ed. R. J. C. HARRIS, pp. 125–138, Academic, New York (1961).
11. H. E. SKIPPER and L. SCHMIDT, *Cancer Chemotherapy Rep.* **17**, 1–144 (1962).
12. P. C. KOLLER and H. VERONESI, *Brit. J. Cancer*, **10**, 703–714 (1956).
13. W. L. NYHAN, *J. Pharmacol.* **130**, 268–274 (1960).
14. H. N. CHRISTENSEN, T. R. RIGGS, H. FISCHER and I. M. PALANTINE, *J. Biol. Chem.* **198**, 1–15 (1952).
15. H. N. CHRISTENSEN, H. AKEDO, D. L. OXENDER and C. G. WINTER, in *Amino Acid Pools*, ed. J. T. HOLDEN, p. 531. Elsevier, New York (1962).
16. T. A. CONNORS, personal communication (1963).
17. D. M. HAYES and C. L. SPURR, *Clin. Res.* **11**, 46 (1963).
18. P. P. CARBONE, *Ann. intern. Med.* **58**, 1027–1029 (1963).
19. V. M. ROSENOER. Unpublished. (1963).
20. N. BLOKHIN, L. F. LARIONOV, N. I. PEREVODCHIKOVA, L. CHEBOTAREVA and N. MERKULOVA, *Ann. N.Y. Acad. Sci.* **68**, 1128–1132 (1958).
21. L. F. LARIONOV, *Acta Un. Int. Cancer* **15**, 171–176 (1959).
22. M. CASTELLANO and C. CATTANEO, *Minerva Med.* **50**, 2365–2369 (1959).
23. D. E. BERGSAGEL, S. W. ROSS and P. DAVIS, *Cancer Chemotherapy Rep.* **21**, 75–80 (1962).
24. D. E. BERGSAGEL, S. W. ROSS and D. T. BAKER, *Cancer Chemotherapy Rep.* **21**, 101–106 (1962).
25. D. E. BERGSAGEL, C. C. SPRAGUE, C. AUSTIN and K. M. GRIFFITH, *Cancer Chemotherapy Rep.* **21**, 87–100 (1962).
26. C. L. BROWN, D. E. BERGSAGEL and W. C. LEVIN, *Cancer Chemotherapy Rep.* **21**, 81–86 (1962).
27. C. AUSTIN, D. E. BERGSAGEL and C. C. SPRAGUE, *Cancer Chemotherapy Rep.* **21**, 107–112 (1962).
28. D. E. BERGSAGEL, C. C. SPRAGUE and S. W. ROSS, *Cancer Chemotherapy Rep.* **21**, 69–74 (1962).
29. J. BERNARD, M. SELIGMANN and F. DANON, *Nouv. Rev. France Hemat.* **2**, 611–616 (1962).
30. D. E. SPEED, D. A. G. GALTON and A. SWAN, *Melphalan in the treatment of myelomatosis*. Lancet, (in press).
31. J. WALDENSTRÖM. Lecture given at the Chester Beatty Research Institute—to be published (1963).
32. J. G. MATTHIAS, J. J. MISIEWICZ and R. B. SCOTT, *Brit. Med. J.* **2**, 1837–1840 (1960).
33. S. L. RIVERS, R. M. WHITTINGTON and M. E. PATNO, *Cancer Chemotherapy Rep.* **29**, 115–119 (1963).