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Growth Inhibitory and Haematological Effects of Alkylating Agents and Attempts at their Modification to give Greater Selectivity of Anti-Tumour Action

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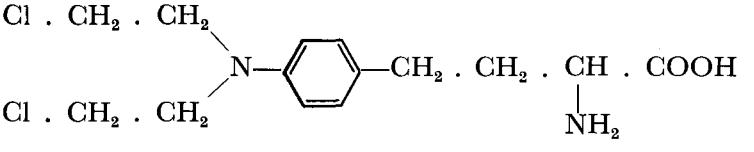
Many of our present cancer chemotherapeutic agents, including radiation, are essentially inhibitors of growth and cell proliferation with little or no specific action on tumour growth as opposed to normal growth and cell division, especially of rapidly proliferating tissues such as the haemopoietic system.

Fig. 1 shows the dose response relations in the rat of the alkylating agent amino-chlorambucil (Calcutt *et al.*, 1963) for inhibition of transplanted Walker carcinosarcoma 256, haemotoxicity (represented by depression of circulating lymphocytes)



growth retardation (L_1) and lethality. All are responses to single doses of the compound dissolved in a propylene glycol-ethanol-HCl buffer and administered by intraperitoneal injection. The growth retardation L_1, obtained from growth curves, represents the weight retardation in steadily growing young rats and is the difference in grams between the weight of the animal when it has regained its steady growth rate (2 to 3 days after the single injection of the drug) and the weight it would have reached if it had maintained its original (untreated) growth rate. An index, $D(L_150)$, the dose causing L_1 equal to 50 gms, can be used for comparison with the median lethal dose LD50 as an extension of toxicity assessment into the sub-lethal dose range (Elson, 1967).

Fig. 1 shows that, under the conditions of the Walker tumour test (injection of the drug 24 hours after tumour implantation) complete inhibition of the tumour can be obtained with doses which have no effect on haemopoiesis, body weight or lethality. With established Walker tumours and with more resistant tumours, however,

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increased doses are required, resulting progressively in effects on bone marrow, body weight, and finally producing death. With aminochlorambucil the slope of the dose response curve appears to be essentially the same for all these parameters, so that if we are to succeed in attaining relatively greater tumour specificity with a drug of

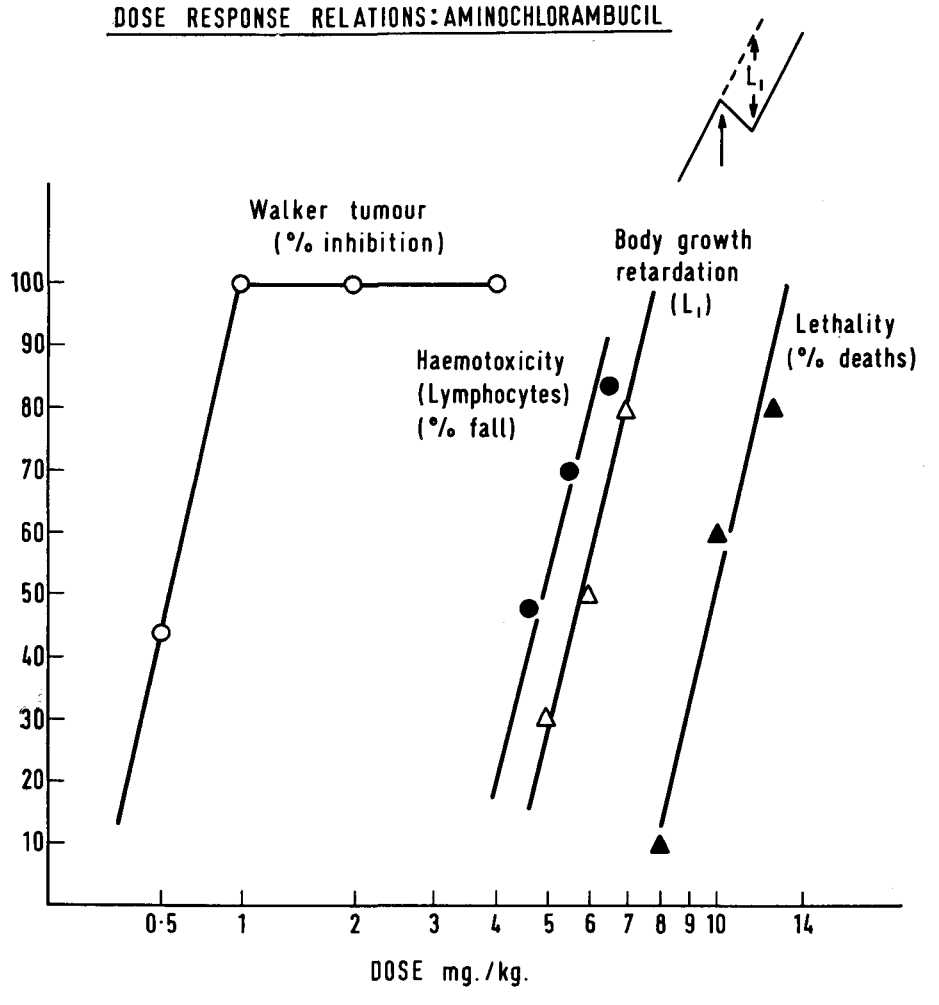

Fig. 1

this type, we must have some means of either, (1) reducing the toxicity towards the host tissues, or (2) increasing the susceptibility of the tumour.

1. Pretreatment of an animal with certain thiol derivatives has some protective action against the toxicity of certain " radiomimetic " alkylating agents (Connors

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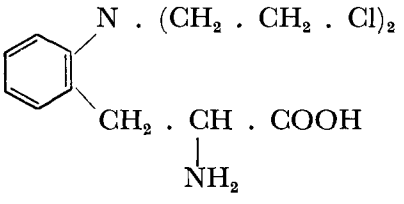
and Elson, 1962). In the rat, treatment of the animal with cysteine about half an hour before administration of the compound Merophan (II)



gives more than four-fold reduction in the lethality. Fig. 2 shows the effect of thiol pretreatment on the growth retardation effect (L_1) of merophan in the rat. Pretreatment with cysteine, but not with cysteamine or sodium thiosulphate, gives a reduction of toxicity (Dose reduction Factor: D.R.F.) of 4.4 which is esentially the same as that obtained against the lethality. The effect of cysteine pretreatment on the depression by merophan of circulating leucocytes in the rat is shown in Fig. 3. Merophan administered in a single dose depresses the number of circulating neutrophils to about twice the extent of the fall of lymphocytes (Elson, 1963). Cysteine pretreatment affords protection against the fall of both types of leucocyte, but gives greater protection against the fall of neutrophils (D.R.F. 5.7) than against the fall of lymphocytes (D.R.F. 4.4).

With aminochlorambucil, which depresses circulating lymphocytes to about twice the extent of the depression of neutrophils cysteine pretreatment affords less protection than in the case with merophan. Tab. 1 compares the dose reduction factors obtained with cysteine administered 30 minutes before single doses of these alkylating agents. Although the protection against the haemotoxicity of aminochlorambucil is much less than is obtained in the case of merophan, again it is seen that better protection is afforded to neutrophils than to lymphocytes. Hence, with thiol pretreatment, considerable protection against the toxicity of certain alkylating agents to the host tissues of a tumour-bearing animal can be obtained, but the advantages of greater selectivity of action will not be achieved if protection is also afforded against their cytotoxic action on the tumour tissue itself. As seen in Tab. 2, however, the dose reduction factor for cysteine pretreatment in the case of merophan is greater for the Walker carcinoma and Yoshida sarcoma than for the host animal (Connors *et al.*, 1965). In these cases, thiol pretreatment would appear to have an adverse effect with regard to selectivity of anti-tumour action. The degree of protection afforded by a thiol against tissue damage by alkylating agents is proportional to the intracellular concentration of thiol obtained in that tissue at the time of administration and duration of action of the the alkylating agent (Calcutt *et al.*, 1963). The increased dose reduction factor shown by these tumours is hence explainable by the higher concentration of cysteine given as a single injection attained in the tumours relative to the concentrations attaining in the normal tissues such as bone marrow and spleen. Although slight increase in therapeutic index was obtained by pretreatment with cys-

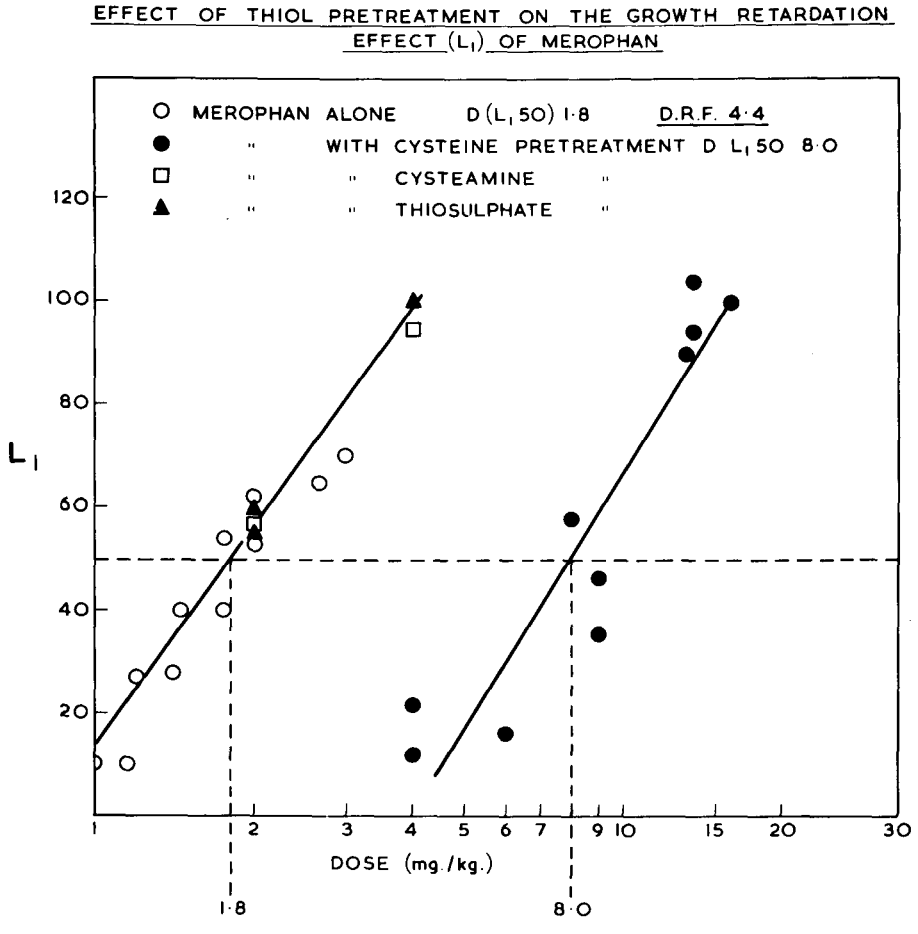


Fig. 2

Tab. 1. Dose reduction factor (D.R.F.) by cysteine pretreatment

	Lethality	Growth inhibition	Haemotoxicity	
			Lymphocytes	Neutrophils
Merophan	4·2	4·4	4·4	5·7
Amino-Chlorambucil	1.6	1.6	1.15	1.4

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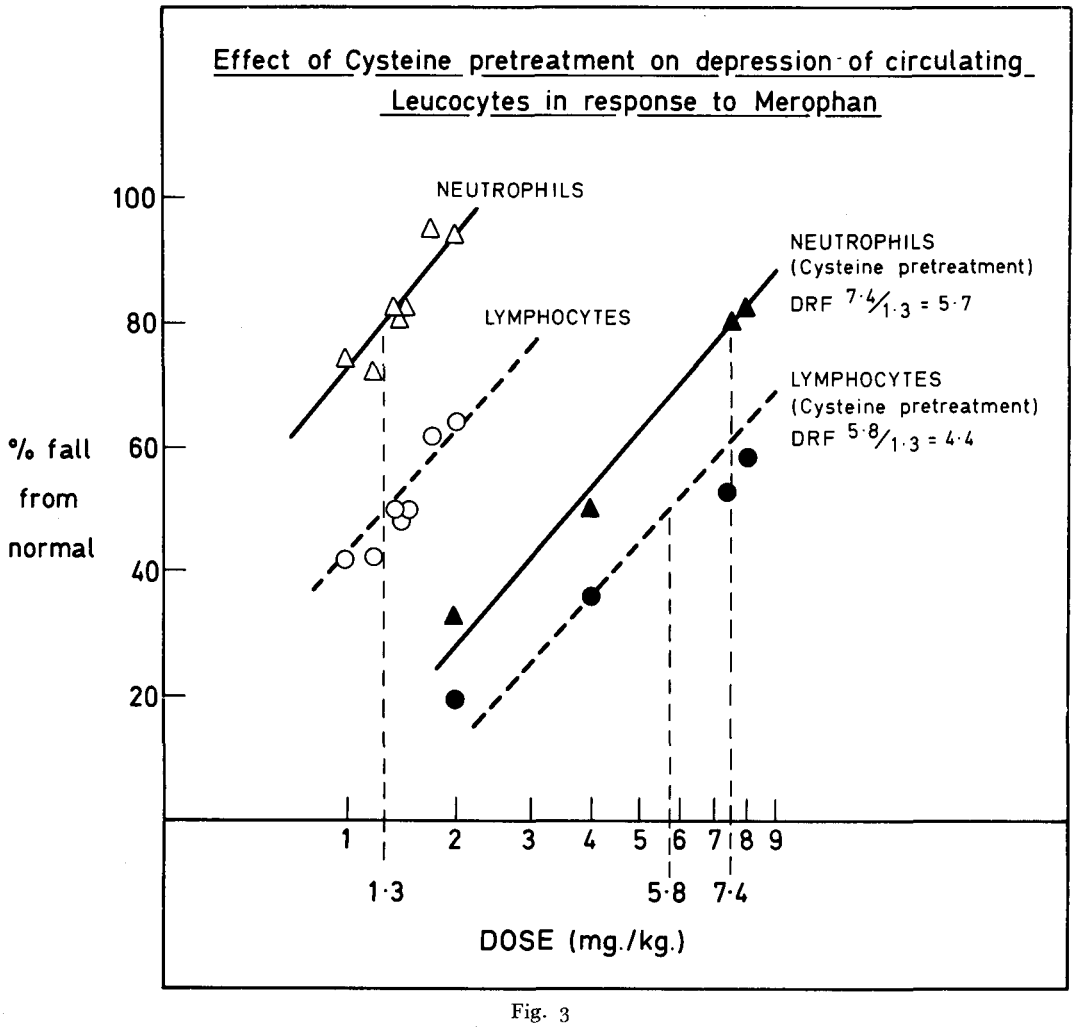

Fig. 3

Tab. 2. Modification of pharmacological action of Merophan in the rat by cysteine pretreatment

	Haematological effects			Body growth inhibition	Lethality	Tumour inhibition (ID^{22})	
	Bone marrow DNA	Blood					
		Lymphocyte depression	Neutrophil depression	L^2	LD^{22}	Walker carcinoma	Yoshida sarcoma
Dose reduction factor (D. R. F.)	4·4	4·4	5·7	4·4	4·2	6	11

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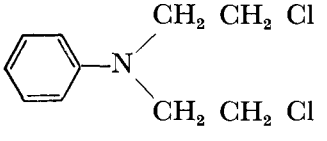
teine or A.E.T. in merophan-treated mice bearing the ADJ/PC5 plasma cell tumour (Connors *et al.*, 1865), thiol pretreatment does not at present appear very promising as an adjunct in general chemotherapy of malignant disease, it may possibly have some place in regional perfusion methods in affording some degree of protection to the host against " overspill" of the cytotoxic agent.

2. It has been pointed out by Ross (1961; 1962) that neoplastic tissues generally, consequent on accumulation of lactic acid due to their high glycolysis, have a lower pH relative to normal tissue, and this property can be enhanced by glucose administration. It should be possible to take advantage of this increased acidity — a tumour tissue pH of 6 could be achieved — to increase the susceptibility of the tumour to cytotoxic agents without at the same time increasing toxicity towards normal tissues. By glucose administration, some enhancement of growth-inhibitory action on the Walker rat carcinoma of a number of aromatic nitrogen mustards carrying side chains containing basic groups with suitable p.Kα values have been reported (Ross, 1962). Perhaps the best result, however, was reported by Connors *et al.* (1964), who found that the carcinostatic activity of triethylene melamine (T.E.M.) against the Walker tumour was enhanced with glucose treatment of the animal by a factor of 4.7, whilst the toxicity was only enhanced by a factor of 2.4. There is, thus, an improved therapeutic index. To obtain this effect, three i. p. doses of glucose were given at hourly intervals commencing 1 hour before administration of the chemotherapeutic drug. The dose of glucose is high (5 g/kg as a 50% aqueous solution) and whether this method at present represents a practical clinical approach is uncertain. The findings, however, do not support the view that a differential increase in cytotoxicity towards cancer cells can be achieved by exploiting pH differences and clearly warrant further investigation. Combinations of methods (1) and (2) might in certain cases be profitably explored.

Perhaps the most encouraging approach towards greater selectivity of anti-tumour action however is to explore and take advantage of biochemical variations from the normal tissue pattern which may be found in certain tumours. An example of this is the very successful treatment of the mouse plasma cell tumour with N : N--di-2-chloroethyl aniline " Aniline mustard ".

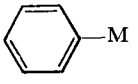
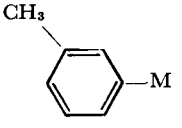
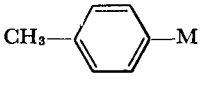
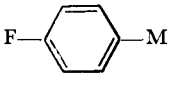
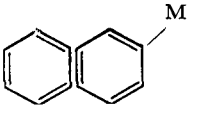
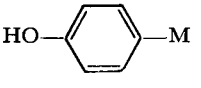
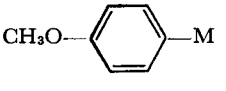


(Rosenoer and Whisson, 1964; Whisson and Connors, 1965).

Tab. 3 adapted from Whisson and Connors (1965) shows the effect of a number of closely related aromatic nitrogen mustard derivatives on the ADJ/PC5 mouse myeloma. The aniline and toluidine derivatives A and B show high activity and a favourable therapeutic index (about 4) whilst compounds C and D in which the position para to the di (chloroethylamino) group (M) is blocked by a substituant group, are inactive.

Evidence is accumulating that aniline mustard (A) undergoes metabolism to the highly cytotoxic but relatively unstable para hydroxy derivative (F) which is conjugated in the liver to the glucuronide in which form the drug reaches the tumour. The glucuronide itself would be relatively inactive but the ADJ/PC5 tumour has

Tab. 3. Effect of aromatic nitrogen mustards on the mouse myeloma ADJ/PC5

Compound		10 Day tumour		
		LD_{50} (mg/kg)	ID_{90} (mg/kg)	Therapeutic index
A		117	30	3.9
B		191	47	4.1
C		430	>430	<1
D		415	>415	<1
E		1,300	>1,300	<1
F		29	19	1.5
G		170	61	2.8

been shown to have a very high glucuronidase activity and it is presumed that this enzyme, acting on the glucuronide, releases the cyto-active p-hydroxy aniline mustard *in situ* within the tumour. (Connors and Whisson, 1966). This mechanism of action would be similar to that postulated for the carcinogenic action of aromatic amines (Elson, 1958) based on the finding (Elson *et al.*, 1958) that metabolism and excretion as glucuronide rather than as ethereal sulphate appears to be a major factor in

determining carcinogenicity. This demonstration of selectivity of anti-tumour action against a mouse myeloma gives some hope of a more rational treatment of certain forms of human cancer by taking advantage of any divergences in enzyme pattern or other biochemical differences. Estimations of glucuronidase activity in human tumours are being carried out. A relatively high glucuronidase activity shown by any particular type of tumour or any individual tumours would certainly be an indication for treatment with aniline mustard or chemically similar chemotherapeutic agents.

Summary

Attempts to induce greater relative tumour specificity in cancer chemotherapeutic drugs may be aimed at (1) reducing toxicity towards host tissues or (2) increasing the susceptibility of the tumor.

1. Pretreatment with certain thiol derivatives has some protective action against the host toxicity of X-irradiation and of certain alkylating agents. However, in many cases even better protection against the tumour-inhibitory action occurs so that the therapeutic index is unfavourably affected.

2. Pretreatment with glucose which accentuates the pH difference between normal and tumour tissues can, in some cases, produce an increase in therapeutic index.

3. The most encouraging aspect is to explore and take advantage of biochemical variations from the normal tissue pattern which are found in certain tumours, e.g. the successful treatment of the ADJ/PC5 mouse myeloma with « aniline mustard » is believed to be related to the very high glucuronidase activity of this tumour.

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RIASSUNTO

I tentativi d'indurre una maggiore specificità tumorale relativa nei farmaci chemioterapici del cancro possono tendere, o a ridurre la tossicità verso i tessuti dell'ospite, oppure ad accrescere la suscettibilità del tumore.

1. Il pre-trattamento con alcuni derivati di tiolo ha una certa azione protettiva contro la tossicità di raggi X e di certi agenti alchilanti. Tuttavia, in molti casi, si verifica una protezione anche migliore contro l'azione inibente del tumore, di modo che l'indice terapeutico viene ad essere sfavorevolmente alterato.

2. Il pre-trattamento con glucosio, che accentua la differenza di pH fra tessuti normali e tumorali, può in alcuni casi determinare un aumento dell'indice terapeutico.

3. L'aspetto più incoraggiante consiste nell'esplorare e sfruttare le variazioni biochimiche dalla normalità tissutale che si riscontrano in alcuni tumori; ad esempio, il trattamento positivo del mieloma del topo ADJ/PC5 con « mostarda d'anilina » viene ritenuto collegato alla elevatissima attività glucoronidasica di tale tumore.

RÉSUMÉ

Les tentatives d'augmenter la spécificité tumorale relative des médicaments chimiothérapeutiques du cancer peuvent avoir pour but de réduire la toxicité envers les tissus de l'hôte, ou bien d'augmenter la susceptibilité de la tumeur.

1. Le pré-traitement par certains dérivés du thiol a une action protectrice envers la toxicité de rayons X et de quelques agents alchylants. Par ailleurs, dans certains cas, une protection encore plus élevée se vérifie envers l'action inhibitrice de la tumeur, de façon que l'index thérapeutique en résulte défavorablement influencé.

2. Le pré-traitement par glucose, qui augmente la différence en pH entre tissus normaux et tumoraux, peut en certains cas déterminer une augmentation de l'index thérapeutique.

3. L'aspect le plus encourageant consiste dans l'exploration et l'utilisation des variations biochimiques de la normalité tissutale qui se manifestent dans certains tumeurs; par example, le succès du traitement du myélome de la souris ADJ/PC5 par « moutarde d'aniline » est rapporté à la très élevée activité de glucuronidase de cette tumeur.

ZUSAMMENFASSUNG

Die Versuche, die chemischen Krebsbekämpfungsmittel je nach dem Tumor spezifischer zu gestalten, dienen dazu, entweder die Toxizität gegenüber den Geweben des Gastorganismus zu verringern, oder aber die Empfänglichkeit des Tumors für das Mittel zu steigern.

1. Eine Vorbehandlung mit einigen Thiolderivaten bewirkt einen gewissen Schutz gegen die Schädlichkeit der Röntgenstrahlen und gewisser alkylierender Wirkstoffe. In vielen Fällen bewirken sie jedoch zugleich eine noch bessere Protektion gegen die Inhibitionswirkung des Tumors, so dass der therapeutische Index dadurch ungünstig verändert wird.

2. Eine Vorbehandlung mit Glukose, wodurch der pH-Unterschied zwischen normalem und Krebsgewebe erhöht wird, kann in einigen Fällen eine Erhöhung der therapeutischen Wirkung hervorrufen.

3. Am Ermutigendsten erscheint es, die in einigen Tumoren festgestellten biochemischen Abweichungen von der Gewebsnormalität zu erforschen und auszunutzen. Der positive Erfolg der Behandlung des Myeloms bei Mäusen ADJ/PC5 mit « Anlin-Senf » soll angeblich mit der ungeheuren Glukoronidase-Aktivität dieses Tumors zusammenhängen.