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# THE EFFECT OF GLUCOSE PRETREATMENT ON THE CARCINOSTATIC AND TOXIC ACTIVITIES OF SOME ALKYLATING AGENTS

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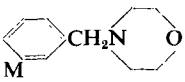
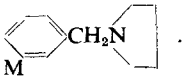
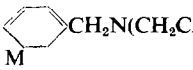
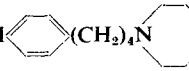
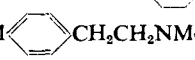
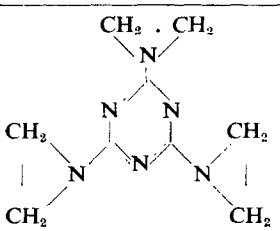
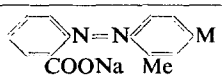
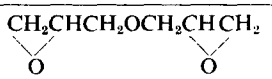
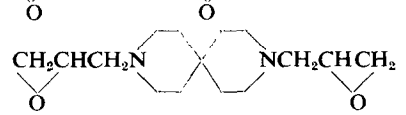
**Abstract**—Glucose pretreatment is known to accentuate the pH difference between most normal tissues and neoplastic tissues. Certain alkylating agents should exert a greater cytotoxic effect in regions of lower pH. This communication reports on the effect of glucose pretreatment on the carcinostatic effect in Walker tumour bearing rats and the toxic effect in normal rats of some aromatic nitrogen mustards, two epoxides, nor-HN2, and triethylenemelamine. Only in the case of triethylenemelamine was a significant increase in the therapeutic index demonstrated.

IT HAS been pointed out[1-4] that a feature of neoplastic tissue is its lower pH relative to normal tissue and that this property can be enhanced by glucose administration. Advantage might be taken of this increased acidity—a tumour tissue pH of 6 could be achieved—to obtain more selective cytotoxic action of alkylating agents towards cancer cells. An earlier communication[3] reported that the growth inhibitory action on the transplanted Walker rat carcinoma of some aromatic nitrogen mustards carrying side-chains containing basic groups with suitable $pK_a$ values is enhanced by glucose pretreatment. Further compounds with appropriate $pK_a$ values have now been examined. In two cases (CB 3039 and CB 1791, see Table 1) a more detailed study of tumour growth inhibitory activity and host toxicity has been made. Only if a differential increase in anti-tumour activity is observed can it be claimed that the combined treatment with a basic drug and glucose leads to an improved therapeutic index.

This approach to the design of drugs specifically toxic towards neoplastic tissue seeks to exploit the selective concentration of basic compounds within such tissue. Another approach is to use drugs which become more effective alkylating agents under conditions of lower pH. 1,2-Epoxides in general and especially some basic derivatives described by Gerzon *et al.*[5] as well as certain ethyleneimido-derivatives, e.g. triethylenemelamine, become more reactive under acid conditions and should be suited to this type of investigation. The effect of glucose pretreatment upon the toxic and carcinostatic action in rats bearing the Walker tumour of two epoxides (CB 1161 and CB 1762) and triethylenemelamine (TEM, CB 1246) is now described.

Various chloroethylamino-derivatives of azobenzene have been shown to inhibit the growth of this tumour and such activity is probably potentiated by reduction *in vivo* of the azo-linkage.[6] Since pH changes will influence the ability of cells to carry out this reduction it was also considered of interest to examine the effect of glucose pretreatment on the activity of one of the most efficient of these azo-compounds, namely 4-di-2″-chloroethylamino-2-methylazobenzene-2′-carboxylic acid (CB 1414).

TABLE 1. SUMMARY OF PRELIMINARY RESULTS

| Compound CB No. | Structure M = $-N(CH_2CH_2Cl)_2$ | p$K_a$ | $LD_{50}$* mg/kg | MED† mg/kg | Vehicle for i.p. admin. |
|---|---|---|---|---|---|
| 1766¶ |  . picrate | 6·6 | | c.90 | Arachis oil |
| 1770¶ |  . picrate | 9·1 | 180 | 60 | ,, |
| 1774¶ |  . picrolonate | 7·1 | 560 | 90 | ,, |
| 1789 |  . 2HCl | 7·0 | 35 | 19 | Water |
| 1790¶ |  . 2HCl | 7·7 | 140 | c.55 | ,, |
| 1791 |  . 2HCl | 7·4 | 25 | 7·5 | ,, |
| 3039 |  . 2HCl | 9·1 | 24 | 11 | ,, |
| 1246 |  | c.2·0‡ | 1·1 | 0·12 | |
| 1414 |  | | 20 | 9 | |
| 1153 | $HN(CH_2CH_2Cl)_2$ . HCl | 6·9‡ | 130 | c.25 | |
| 1161 |  | | | | |
| 1762 |  | 6·8<br>7·9§ | 35 | ‖ | |

* In tumour-free rats.
† In rats bearing the Walker tumour.
‡ In water.
§ In 66% aqueous dimethylformamide (Gerzon *et al.*[5]): the two nitrogen atoms in this compound are of comparable basicity.
‖ No tolerated dose inhibited the growth of the tumour to 10% of that of the control weight.

## EXPERIMENTAL

### *Materials*

The compounds examined are listed in Table 1. The determination of the p$K_a$ values of the aromatic nitrogen mustards and the preparation of those compounds marked(¶) were carried out by Mr. J. M. Reid: the figure given is for the more basic

nitrogen atom. Because of the low solubility of the free bases in water the $pK_a$ values were determined by potentiometric titration in 50% aqueous acetone. The values in purely aqueous solution will be about one pH unit higher, for example benzylpiperidine has p$K$ 8·2 in aqueous acetone and 9·0 in water. The $LD_{50}$ and MED (minimum effective dose, defined as the dose required to inhibit the growth of the tumour to 10% of the control tumour weight) values given in the Table were obtained in preliminary work on the Walker tumour system.

*Drug administration*

All the drugs used in this study were given as a single intraperitoneal injection, the vehicle used being indicated in Table 1. Where aqueous solutions were used these were given within 5 min of preparation in order to minimize errors due to hydrolysis.

*Glucose administration*

Three i.p. doses of glucose were given at hourly intervals commencing 1 hr before the administration of the drug. The dose used was 5 g/kg as a 50% aqueous solution.

*Toxicity assays*

A group of 40 Chester Beatty male rats was used for assaying the toxicity of each of the compounds studied. Each rat was given either the compound alone or the compound with glucose and the rats were observed for 14 days. Four logarithmically spaced doses of the drug were used in preliminary experiments and two suitably spaced doses in subsequent assays. The number of deaths in each dosage group was recorded and the results analysed by the method of angular transformation in order to determine the relative toxicity of the compound alone and with glucose.

*Carcinostatic assays*

The carcinostatic effects of the drugs studied were evaluated in groups of rats implanted 24 hr earlier with the Walker carcinosarcoma (Table 2).

Preliminary experiments to assess the dose–response curve for each drug alone and with glucose were carried out in groups of 12 implanted rats using three logarithmically spaced doses of the drug. The weights of the dissected tumours were recorded for each dose of the drug. Subsequent assays were conducted as 2 + 2 parallel line dilution assays in randomized groups of 32–40 implanted rats using suitably spaced doses of drug. The results of these assays were analysed by the standard analysis of variance procedures in order to determine the relative carcinostatic activity of the compound alone and with glucose.

In the case of CB 1246 an additional combined toxicity–therapeutic activity comparative assay was carried out in a randomized group of 42 implanted rats. Six animals served as untreated controls. CB 1246 was given to the remaining 36 animals, 3 animals per dose. Six logarithmically spaced doses were chosen for the administration of the drug alone and a further six for use in combination with glucose. The animals were killed on day 12 and the tumours weighed. For each of the two treatments the MED was estimated by linear interpolation of the log dose–response curve between the results at doses straddling the required value. The $LD_{50}$ for each treatment was estimated by the Spearman–Kärber method. In applying this method to the results

presented it was assumed that 100% deaths would have resulted from the administration of the next higher logarithmically spaced dose. This assumption was based on the slopes of the log dose–mortality curves previously obtained for CB 1246 both alone and with glucose in normal and tumour bearing animals. In no case was the $LD_{90}/LD_{10}$ greater than 2. A therapeutic index for each treatment was calculated as the ratio: $LD_{50}$/MED.

TABLE 2. PROTOCOL FOR CARCINOSTATIC ASSAY

| Routine passage | | | Therapeutic evaluation | | | |
|---|---|---|---|---|---|---|
| Host | Transplantation technique | Transfer time | Host | Donor for test | Dosage schedule | Criterion of response |
| Chester Beatty male rats | 4–6 $mm^3$ fragments No. 8 E.G. trocar implant subcut. | 6–8 days | Chester Beatty male rats | 6–8 days after implant | single i.p. day 1 | Tumour weight on days 10–14 |

TABLE 3. EFFECT OF GLUCOSE ADMINISTRATION ON CARCINOSTATIC ACTIVITY AND TOXICITY

| Compound | Glucose enhancement of, | |
|---|---|---|
| | Carcinostatic activity | Toxicity* |
| CB 1791 | 1·1 (0·8–1·2) | 1·2 (1·0–1·4) |
| CB 3039 | 1·7 (1·2–2·4)† | 1·3 (1·2–1·4)† |
| CB 1246 | 4·7 (3·1–8·3) | 2·4 (2·1–2·8) |
| CB 1414 | 1·1 (0·6–2·1) | 1·1 (0·8–1·6) |

* In tumour-free rats.
† Fiducial limits, P = 0·95.

## RESULTS

Preliminary experiments on Walker tumour bearing animals with CB 1766, 1770, 1774, 1789, and 1790 and also with the epoxides CB 1161 and 1762, and with nor-HN2 (CB 1153), carried out as previously described[3] showed that the glucose pretreatment produced a less than two-fold potentiation of carcinostatic activity.

The results of more precise parallel line dilution assays on Walker tumour bearing rats and toxicity assays on normal rats are shown in Table 3. No significant enhancement of anti-tumour activity has been produced by the administration of glucose with CB 1791 or 1414. With CB 3039 a significant potentiation of anti-tumour activity has been demonstrated (Fig. 1) together with a parallel increase in host toxicity. No improvement in the therapeutic index has been achieved. However with CB 1246 there was a significantly greater potentiation of the carcinostatic activity than of the toxicity giving an approximately two-fold increase in the therapeutic index. This result was confirmed by the combined toxicity–therapeutic activity comparative assay with CB 1246. The results of this assay are shown in Fig. 2. The therapeutic index for CB 1246 alone was 9 and for the drug with glucose was 28.

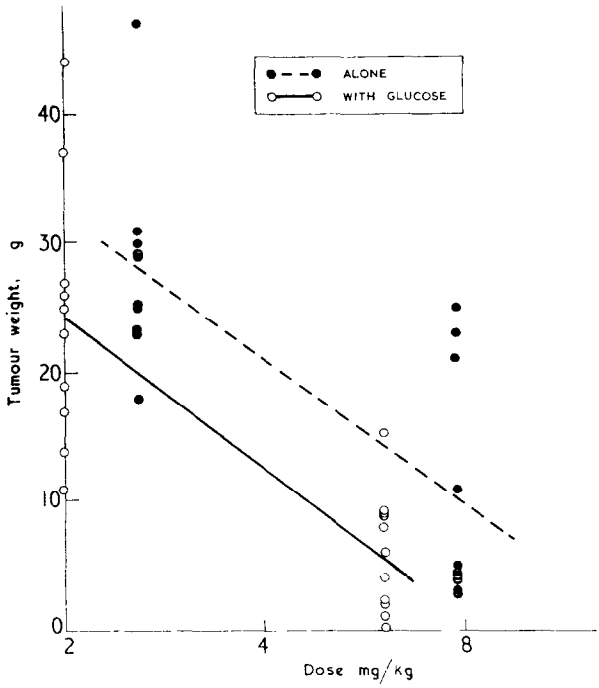



FIG. 1. Comparison of CB 3039 i.p. single dose alone and with glucose i.p.

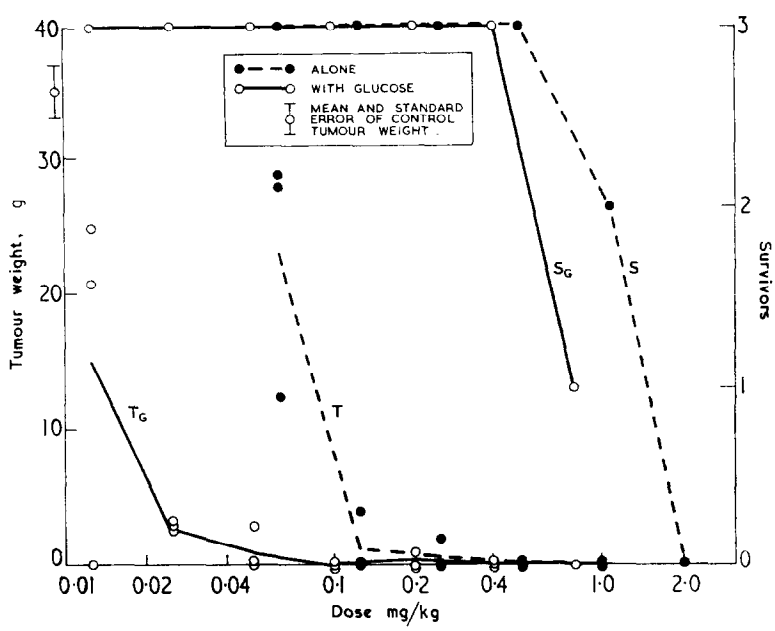



FIG. 2. Comparison of CB 1246 i.p. single dose alone and with glucose i.p.

## DISCUSSION

The present results confirm the earlier finding of enhanced anti-tumour activity when compound CB 3039 is administered in conjunction with glucose. Consideration of the results already published[3] indicate that the effect of a dose of CB 3039 given alone can be obtained with 63% of that dose if glucose is also given: that is, an enhancement factor of 1·6. This compares well with the value of 1·7 obtained in the present investigation. However it has now been shown that there is also an enhancement of host toxicity and therefore no increase in the therapeutic index.

CB 3039 is a relatively strong base ($pK_a$ 9·1) and its cationic form will predominate at physiological pH. This form will not readily pass through cellular membranes and this restriction to diffusion will hinder the establishment of the equilibrium conditions under which selective concentration within the more acidic cancer cell would be expected. It is also essential that the cationic form of the alkylating agent should be as reactive chemically as the uncharged form or the advantage of selective concentration will be lost. By lengthening the side chain carrying the more basic centre or by inserting the side chain in the *m*-position the effect of the acquired positive charge on the reactivity of the chloroethylamino group should be limited. These considerations led to the synthesis and testing of CB 1791, which is less basic and has a longer side chain than CB 3039, and of the *m*-substituted derivatives CB 1766, 1770, and 1774. However no appreciable enhancement of carcinostatic activity was observed with any of these compounds.

The failure to produce a modified activity in the case of CB 1414 suggests that the pH change does not influence the rate of reduction of the azo-linkage in this compound *in vivo*.

There was no indication of enhanced activity following glucose pretreatment in the case of a neutral (CB 1161) and a basic (CB 1762) diepoxide or in the case of nor-HN2 (CB 1153) which probably forms 2-chloroethylethyleneimine is aqueous solution. The increase in the rate of reaction of triethylenemelamine (CB 1246), which has a much higher acid catalysis coefficient[1, 4] than the epoxides studied, should be at least two-fold for the pH change from 7·0 to 6·0. It is encouraging that there is a significant potentiation of the anti-tumour activity of this compound when it is given with glucose. The toxicity to the host is not increased to the same extent and an improved therapeutic index results. This finding supports the view that a differential increase in toxicity towards cancer cells can be achieved by exploiting pH differences. The examination of more ethyleneimido-derivatives with high acid catalysed reaction coefficients is clearly indicated.

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