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Anti-tumour Activity of Novel Adducts of Ascorbic Acid with Aldehydes*

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Abstract—*A novel adduct of ascorbic acid and methylglyoxal (MGA) inhibited the growth of the Ehrlich ascites carcinoma (EAC) in male CBA/Ca mice, following the i.p. injection of* 5×10^6 *tumour cells on day* 0. *MGA,* 62.5 *and* 125 mg kg^{-1} *twice daily i.p., inhibited tumour growth by* 93% *and* 96%, *respectively, when given on days* 1–5 *following transplantation. Treatment with MGA over days* 5–9 *after tumour cell injection was ineffective. A modified dose schedule, MGA* 250 mg kg^{-1} *i.p., given as a single i.p. dose on days* 1, 3 *and* 5 *following transplantation, significantly increased the survival time of EAC-bearing mice. A number of related ascorbate (AsA) acetals were tested for antitumour activity using an identical experimental protocol. AsA-acetylacrolein was as active a growth inhibitor as MGA, inhibiting the growth of EAC by* 97 *and* 98% (100 *and* 200 mg kg^{-1} *twice daily, respectively). AsA-acrolein and AsA-glyoxal also inhibited the growth of EAC. Under identical conditions ascorbic acid,* 200 mg kg^{-1} *i.p. twice daily, did not inhibit the growth of EAC, while the aldehydes methylglyoxal,* 50 mg kg^{-1}, *and acetylacrolein,* 12.5 mg kg^{-1}, *inhibited tumour growth by* 98 *and* 99%, *respectively. Combination of the aldehydes with ascorbic acid resulted in a considerable reduction in host toxicity; the* LD_{50} *for MG being* 332 mg kg^{-1} *and that for MGA* 959–1462 mg kg^{-1}, *for a single i.p. dose in mice.*

INTRODUCTION

THE GROWTH inhibitory activity of methylglyoxal *in vitro* against viruses, [1] bacteria, [2] and a variety of mammalian cell lines including mouse lymphoma L-5178Y, [3] human fibroblasts [4] and human kidney T-cells [5] is well known. Methylglyoxal has also been shown to inhibit the growth of a number of murine ascites tumours [6–8]. In both bacteria and mammalian cells the primary site of attack appears to be protein synthesis [9–11].

Methylglyoxal, in conjunction with the glyoxalase enzymes, has been considered by Szent-Györgyi [12] to form the basis of a cell growth regulatory system. Methylglyoxal may arise in the cell both enzymically [13–15], and non-enzymically from free trioses [16]; a variety of related keto- and hydroxyaldehydes are also produced during lipid peroxidation that may be enzymic or non-enzymic in character [17, 18]. The glyoxalase enzymes [19] (glyoxalase I, EC 4.4.1.5; glyoxalase II, EC 3.1.2.6) may operate to maintain a homeostatic level [20] of methylglyoxal throughout the cell's life, since the glyoxalase enzymes have been implicated in preventing a 'lethal synthesis' of methylglyoxal in bacteria [21]. In the process of tumourigenesis the glyoxalase enzymes are believed by Szent-Györgyi [22] to have free access to endogenously produced methylglyoxal, so releasing the cell from its quiescent state.

The use of methylglyoxal as a chemotherapeutic agent may be favourably influenced by the reduced activity of the glyoxalase enzymes in tumours [23], although the glyoxalase enzymes also lead to a considerable reduction of the cytotoxic ketoaldehyde following administration. The glyoxalase enzymes and the chemical reactivity of methylglyoxal lead to a short biological half-life, and although some cellular processes may be inhibited by micromolar concentrations of methylglyoxal [24], generally it has been found that millimolar concentrations are re-

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quired for the inhibition of cell growth [18, 25].

Similarly to a number of other cytotoxic aldehydes, methylglyoxal possesses a narrow margin of safety between therapeutic and toxic doses. A number of aldehyde derivatives have been prepared using non-toxic carrier molecules to increase the biological half-life and these show a considerable decrease in toxicity [26–29].

Fodor *et al.* [30] were able to report the formation of stable, 2,3-enediol acetals of ascorbic acid with a number of cytotoxic aldehydes including methylglyoxal (NFCR 278021). The ketoaldehyde is believed to be the active constituent of the methylglyoxal complex, and has been shown to inhibit the growth of a mammalian cell line *in vitro* [31], on a molar basis being approximately 1000 times less toxic than the free ketoaldehyde. It has been reported by Ahmed and Slater [32] that the complex is not a substrate for the glyoxalase enzymes of mouse or rat liver supernatants, although the results obtained by Riley [31] suggest that the complex may function as a glyoxalase inhibitor. We report here on the antitumour activity of the ascorbate–methylglyoxal complex and some related compounds against ascites tumours in the mouse.

MATERIALS AND METHODS

Animals and tumour maintenance

Mice. CBA/Ca mice were obtained from an inbred colony maintained by brother–sister monogamous pair mating at Brunel University; CBA/Ca breeding pairs were originally supplied by Carworth Europe in 1974. Mice were fed a standard laboratory diet (CRM(X), Labsure Animal Foods) and supplied with water *ad libitum*. For experimental purposes mice of 20–25 g body weight, within a maximum of 3 g weight range, were used.

Tumours. The Ehrlich carcinoma was obtained from the Chester Beatty Research Institute, and maintained at Brunel by weekly i.p. transplantation in male CBA/Ca mice. Transplantation occurred every seventh day. Sterile phosphate-buffered saline (PBS, Dulbeco "A"), 1 ml, was washed into the peritoneal cavity and the tumour cells were aspirated into a 1 ml syringe. Recipient mice received 0.2 ml of this cell suspension i.p., equivalent to approximately 3×10^6 cells.

Tumour cell suspensions

For experimental purposes, Ehrlich ascites tumour cells (EAC) were harvested from the peritoneal cavities of donor mice 4 days post transplantation. The tumour cells were harvested from the peritoneal cavities by 2 × 5 ml washes of sterile PBS into sterile 'Universal' containers, and sedimented at 1000 rev/min for 4 min in a bench centrifuge. If necessary the cell pellet was resuspended in 5 ml "lysing" buffer (2.06 g Tris–HCl, 7.47 g NH_4Cl per litre, pH 7.2) to remove erythrocytes. The cell pellet was resuspended in 5 ml sterile EMEM (Eagles Minimum Essential Medium). Cell counts were made using a Coulter counter (Model ZF, 100 μ orifice), and cell viability was determined by Trypan blue dye exclusion. The cell suspension was adjusted to 2.5×10^7 cells per ml such that recipient mice received 5×10^6 cells i.p. in 0.2 ml.

Preparation and administration of test materials

Methylglyoxal (MG). Commercial 40% aqueous MG (Sigma) was distilled to yield a pale yellow solution of MG, 0.2–0.3 M. MG was determined by the semicarbazide method of Alexander and Boyer [33]. The distillate was stored at −4°C until required, and discarded after a maximum of 2 months.

Ascorbic acid acetals. The synthesis of the acetals is described elsewhere [30]. All the acetals used in this study were supplied by Prof. G. Fodor (NFCR Workshop, University of West Virginia, U.S.A.) as lyophilised foams. Immediately on receipt all samples were stored at −20°C (anhydrous) and testing was completed within 2 months of receipt.

Administration of test materials. All agents were administered on the basis of 10 ml drug solution equivalent to 1 kg body weight. Appropriate dilutions of the test materials were prepared in PBS and the solutions brought to pH 7.2 by the addition of 20% NaOH before making up to final volume. All solutions were filter sterilised through Millex 0.22 μ filters (Millipore) before use and were stored at −4°C (MG) or −20°C (ascorbate acetals) until required.

Dose schedules and experimental details

A standardised protocol was adopted for all experiments. Treatment courses were begun 24 hr following tumour transplantation and consisted of two daily i.p. injections spaced 6 hr apart for 5 days unless stated otherwise. Experiments were assayed 24 hr following the final injection. Mice were killed by cervical dislocation and the peritoneal cavities washed with at least 4 × 5 ml volumes of PBS, into disposable "Universal" containers. An

erythrocyte-free pellet was obtained as described and resuspended in a known volume of PBS. Cell counts were determined using a Coulter counter and cell viability was determined by Trypan blue dye exclusion.

The mitotic indices of ascites tumour cell populations was determined from acetic acid:methanol (1:1 v/v) fixed cell smears stained with Wright's and Giemsa stains.

RESULTS

The growth of the Ehrlich ascites carcinoma (EAC) following the i.p. injection of 5×10^6 tumour cells is shown in Fig. 1(a) and (b). Log phase growth commences with little evidence of a lag phase following transplantation. A log phase doubling time of 10.6 hr was obtained from the line of best fit through the plotted points. From day 5 onwards the tumour enters a plateau phase, there being only 1.5 doublings of cell number between days 5 and 10. Following transplantation a mitotic index of 2.5 was observed which steadily declined to 0.9 by day 10, corresponding with entry of the cell population into plateau phase growth. Over the 10-day observation period the tumour cell viability remained between 96 and 98%.

The response of EAC to treatment with the ascorbic acid:methylglyoxal acetal (MGA) is shown in Table 1. MGA, 62.5 and 125 mg kg^{-1} i.p. twice daily on days 1–5 following transplantation, inhibited the growth of EAC by 92.8 and 95.6%, respectively ($P<0.001$). The viability of the surviving cell population was also reduced, the fraction of cells killed being dose dependent. Under identical experimental conditions MGA was ineffective against a plateau phase population (treatment on days 5–9). A small but significant ($P<0.05$) reduction in cell number, 17%, was found in mice receiving 125 mg kg^{-1} twice daily. The viability of the surviving cell population, however, was unaffected.

The antitumour activity of a number of ascorbate (AsA) aldehyde acetals is shown in Tables 2 and 3. Where sufficient material was available two dose regimens were investigated, 100 and 200 mg kg^{-1} i.p. for 5 days (days 1–5). All of the acetals tested displayed some degree of anti-tumour activity, the most active compound being AsA-acetylacrolein. AsA-acrolein and AsA-glyoxal also exhibited anti-tumour activity at the higher dose levels.

Indirect evidence as to the mode of action of the acetals may be inferred from the results of Table 4. Assuming a 1:1 stoichiometry of ascorbic acid and component aldehyde in the acetals, it was found that the dose of ascorbic acid, 200 mg kg^{-1} i.p. twice daily, far in excess of that expected to be released from the breakdown of the acetals into constituent molecules, did not inhibit the growth of EAC.

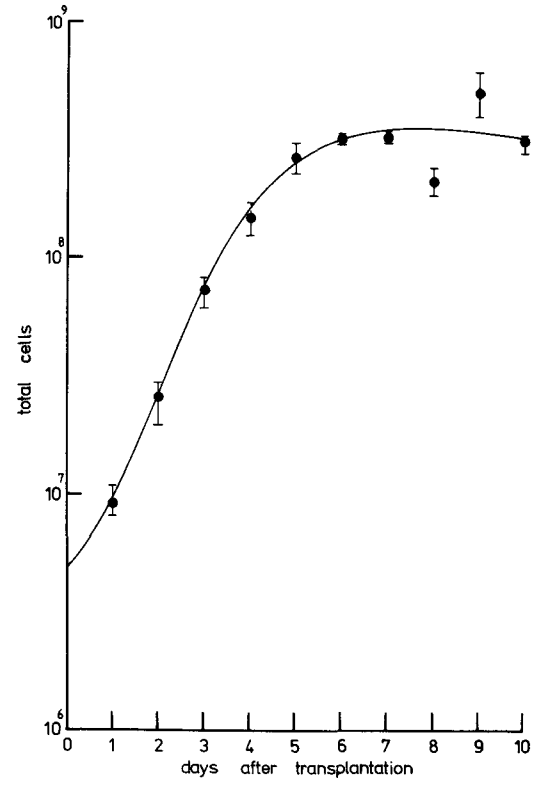

Fig. 1(a). The growth of the Ehrlich ascites carcinoma in male CBA/Ca mice following the i.p. transplantation of 5×10^6 *tumour cells on day 0. Each point represents the mean total recoverable cell count from three animals. Vertical bars indicate maximum and minimum values.*

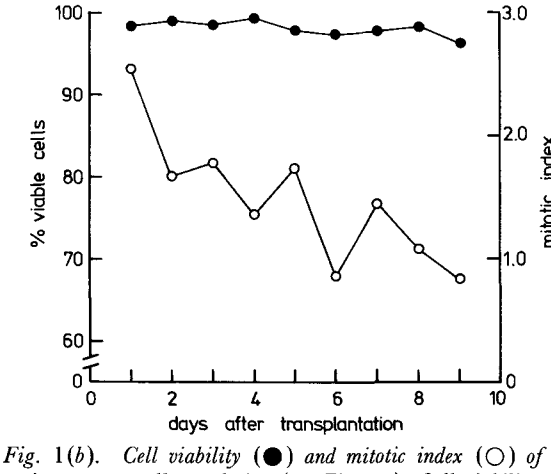

Fig. 1(b). Cell viability (●) and mitotic index (○) of the ascites tumour cell population (see Fig. 1a). Cell viability was assessed by trypan blue dye exclusion counting cells over at least four fields of view. The mitotic index was based on the number of metaphase figures observed in a minimum of 1000 *cells*

$$\left(MI = \frac{\text{No. of metaphases}}{\text{Total No. cells examined}} \times 100\right)$$

In both curves each point represents the mean of the determinations from three animals.

Table 1. *Effect of MGA on the growth of Ehrlich ascites carcinoma* in vivo

Treatment	Total cells ± S.E.M.	Inhibition (%)	% Viable Cells ± S.E.M.
Days 1–5			
Controls (10)	$2.51 \pm 0.26 \times 10^8$	—	97.03 ± 0.74
MGA 62.5 mg kg^{-1} 2 × daily (10)	$1.81 \pm 0.23 \times 10^7$*	92.8	92.51 ± 1.67
MGA 125 mg kg^{-1} 2 × daily (10)	$1.07 \pm 0.10 \times 10^7$*	95.6	85.97 ± 1.40
Days 5–9			
Controls (10)	$2.81 \pm 0.13 \times 10^8$	—	95.89 ± 0.44
MGA 62.5 mg kg^{-1} 2 × daily (10)	$2.82 \pm 0.25 \times 10^8$‡	—	89.05 ± 3.15
MGA 125 mg kg^{-1} 2 × daily (10)	$2.33 \pm 0.14 \times 10^8$†	17.0	95.31 ± 0.97

5×10^6 tumour cells injected i.p. on day 0 in male CBA/Ca mice. Treatment was scheduled over days 1–5 or days 5–9 following transplantation. Control animals received PBS i.p., treated animals received MGA 62.5 or 125 mg kg^{-1} i.p. Solutions were administered twice daily on the basis 0.1 ml/10 g body weight. The table shows total recoverable cells and cell viability 24 hr after the finish of the treatment.
*$P < 0.001$ with student's *t*-test.
†$P < 0.05$.
‡Not significant.
Figures in parentheses are numbers of animals/group.

Table 2. *Antitumour activity of a number of ascorbic acid/aldehyde acetals, against the Ehrlich ascites carcinoma in male CBA/Ca mice*

Treatment	Total cells ± S.E.M.	Inhibition (%)	% Viable Cells ± S.E.M.
Controls (12)	$2.46 \pm 0.14 \times 10^8$	—	97.64 ± 0.30
AsA-Acrolein			
100 mg kg^{-1} 2 × daily (5)	$1.57 \pm 0.13 \times 10^8$	36.2	92.16 ± 1.47
200 mg kg^{-1} 2 × daily (5)	$0.99 \pm 0.17 \times 10^8$†	59.8	89.40 ± 0.91
AsA-Acetylacrolein			
100 mg kg^{-1} 2 × daily (5)	$8.07 \pm 0.59 \times 10^6$†	96.7	96.58 ± 0.86
200 mg kg^{-1} 2 × daily (5)	$5.40 \pm 1.22 \times 10^6$	97.8	88.48 ± 1.78
AsA-Glyoxal			
100 mg kg^{-1} 2 × daily (5)	$1.12 \pm 0.21 \times 10^8$†	54.5	95.74 ± 1.20
200 mg kg^{-1} 2 × daily (5)	$3.14 \pm 0.36 \times 10^7$†	87.2	90.44 + 3.04
AsA-Crotonaldehyde			
100 mg kg^{-1} 2 × daily (5)	$1.86 \pm 0.11 \times 10^8$‡	24.4	95.92 ± 0.54
AsA-Malealdehyde			
100 mg kg^{-1} 2 × daily (5)	$1.68 \pm 0.23 \times 10^8$§	31.7	96.20 ± 1.10

5×10^6 cells injected i.p. on day 0, treatment schedules on days 1–5 following transplantation. Experimental protocol as for Table 1.
*$P < 0.005$ with Student's *t*-test.
†$P < 0.001$.
†$P < 0.02$.
§$P < 0.01$.
Figures in parentheses are numbers of animals/group.

Similarly the viability of the tumour cells remained unchanged.

By comparison, the free aldehydes, methylglyoxal (Table 4) and acetylacrolein (Table 3), inhibited the growth of EAC by 98 and 99%, respectively. Acetylacrolein was considerably more toxic than the corresponding acetal; the AsA-acetylacrolein acetal given at doses of 200 mg kg^{-1} caused little obvious side effects in the treated mice, while free acetylacrolein at 100 mg kg^{-1} resulted in the death of all treated animals after 1 day of treatment. Methylglyoxal was found to have an LD_{50} of 332 mg kg^{-1} for a single i.p. dose; the single i.p. dose LD_{50} for MGA was 959–1462 mg kg^{-1} for many different preparations tested over a 1-year period.

Modification of the dose schedule for MGA

Table 3. Antitumour activity of AsA-hydroxyethylglyoxal, and acetylacrolein against the Ehrlich ascites carcinoma in male CBA/Ca mice. Experimental protocol as for Table 2

Treatment	Total cells ± S.E.M.	Inhibition(%)	% Viable cells ± S.E.M.
Controls (10)	$2.87 \pm 0.20 \times 10^8$	—	97.6 ± 0.46
AsA-Hydroxyethyl-glyoxal			
10 mg kg^{-1} 2 × daily (5)	$3.56 \pm 0.36 \times 10^8$*	—	98.1 ± 0.22
20 mg kg^{-1} 2 × daily (5)	$2.36 \pm 0.16 \times 10^8$*	17.8	98.2 ± 0.56
Acetylacrolein			
6.25 mg kg^{-1} 2 × daily (5)	$3.34 \pm 0.59 \times 10^5$†	99.9	98.1‡
12.5 mg kg^{-1} 2 × daily (5)	$1.63 \pm 0.25 \times 10^6$†	99.4	83.2 ± 7.89

*Not significant.
†$P < 0.001$ with Student's *t*-test.
‡Too few to count, figure is mean of two determinations.
Figure in parentheses are numbers of animals/group.

Table 4. Effect of ascorbic acid and methylglyoxal, on the growth of the Ehrlich ascites carcinoma in vivo

Treatment	Total cells ± S.E.M.	Inhibition(%)	% Viable cells ± S.E.M.
Controls (9)	$2.09 \pm 0.15 \times 10^8$	—	98.12 ± 0.42
Ascorbic acid			
200 mg kg^{-1} 2 × daily (5)	$2.12 \pm 0.15 \times 10^8$*	—	97.5 ± 0.29
Methylglyoxal			
50 mg kg^{-1} 2 × daily (5)	$4.22 \pm 0.72 \times 10^6$†	98.0	96.9 ± 0.61

5×10^6 tumour cells injected i.p. on day 0 in male CBA/Ca mice. Treatment was scheduled over days 1–5 following transplantation. Experimental protocol as for Table 1.
*Not significant.
†$P < 0.001$ with Student's *t*-test.
Figures in parentheses are numbers of animals/group.

to a single i.p, dose of 250 mg kg^{-1} on days 1,3 and 5 following tumour transplantation in two experiments resulted in an increase in life span (ILS) of 62.9% (exp. 1) and 87.6% (exp. 2) for treated mice compared to saline-treated controls (Table 5). The cause of death in MGA-treated mice was due to the presence of a large solid tumour in the peritoneal cavity, there being almost no ascitic fluid present as compared to control animals where death occurred with gross ascites. Since the solid tumours were centered around the injection site in all cases, it may be that the solid tumours developed from tumour cells that had been drawn into the body wall by the repeated withdrawal of needles, leaving a small population of cells relatively inaccessible to the drug given i.p.

DISCUSSION

The results obtained with MGA, and separately with the parent molecules ascorbic acid and methylglyoxal, suggest that this new series of ascorbate derivatives exert their antitumour properties either as the intact complex or by a gradual release of growth inhibitory aldehyde from the complex. The former conclusion was reached by Riley [31], who found that treatment of the MGA complex with ascorbate oxidase did not result in the liberation of methylglyoxal. Evidence from the toxicity and antitumour activity of MGA and the AsA-acetylacrolein complex tend to support the view of an active complex.

However, ascorbic acid is known to affect many aspects of tumour growth [34], acting either directly on the tumour or modifying host–tumour relationship. The possibility of some synergistic activity between the components of MGA on tumour growth cannot be overlooked; ascorbate is a strong inhibitor of glyoxalase I [32, 35, 36]; potentiation of the growth inhibitory effects of methylglyoxal by an inhibition of its metabolism by ascorbate may be of special relevance with regard to the distribution of the glyoxalase enzymes in tumours [13].

The main advantage of the acetals as chemotherapeutic agents is the considerable re-

Table 5. Effect of the ascorbate:methylglyoxal acetal on the survival time of EAC-bearing mice

Treatment	Day of death	Mean day of death	% ILS
Experiment 1			
Controls (13)	16, 16, 16, 16, 16, 16, 16, 16, 17, 18, 18, 18, 18	16.7	
Treated (5)	22, 26, 28, 29, 31	27.2	62.9*
Experiment 2			
Controls (13)	14, 14, 15, 15, 15, 15, 16, 16, 16, 16, 16, 16, 16	15.4	
Treated (10)	14, 15, 21, 27, 31, 34, 34, 35, 39†	28.9	87.6*

*$P<0.001$ with Student's t-test.

†One indefinite survivor past day 40. Included in calculation as a death of day 39. Figures in parentheses are numbers of animals/group.

Male CBA/Ca mice 20–22 g body weight were injected i.p. on day 0 with 4×10^6 (exp. 2) or 5×10^6 (exp. 1) tumour cells. On days 1, 3, 5 following transplantation control mice received PBS i.p., treated mice received MGA 250 mg kg^{-1} i.p. as a single dose.

duction in host toxicity that has been achieved with a retention of strong anti-tumour activity when given i.p.

In preliminary studies, we have given considerable doses (250 mg/kg/day) of MGA i.v. by the continuous infusion technique of Conroy and Elvin [37] for several days without obvious signs of toxicity. In mice bearing a lymphosarcoma this treatment appeared to decrease liver metastasis but not to influence the primary solid tumour. At present, little is known of the metabolism and tissue distributions of MGA and related acetals, but their relatively low toxicity together with marked effects on ascitic cells suggests that they may be useful in attenuating metastasis, for example post-surgically.

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REFERENCES

1. Underwood GE, Weed SD. Glyoxal and related compounds as potential blood sterilising agents. *Proc Soc Exp Biol Med* 1956; **93:** 421–424.
2. Együd LG. Studies in cell division: the effect of aldehydes, ketones and ketoaldehydes on the proliferation of *Escherichia coli*. *Curr Mod Biol* 1967; **1:** 14–20.
3. Gregg T Ch. Inhibition of mammalian cell division by glyoxals. *Exp Cell Res* 1968; **50:** 65–72.
4. Klamerth OL. Influence of glyoxal on cell function. *Biochem Biophys Acta* 1968; **155:** 271–279.
5. Scaife TF. Mitotic inhibition induced in human kidney cells by methylglyoxal and kethoxal. *Experientia* 1969; **25:** 178–179.
6. Apple MA, Greenberg DM, Arrest of cancer in mice by therapy with normal metabolites (1) 2-oxopropanal (NSC-79019). *Cancer Chemother Rep* 1967; **51:** 455–464.
7. Jerzykowski T, Matuszewski W, Otrzonzek N, Winter R. Antineoplastic action of methylglyoxal. *Neoplasma* 1970; **17:** 25–35.
8. Együd LG, Szent-Györgyi A. Cancerostatic action of methylglyoxal. *Science* 1968; **160:** 1140.
9. Együd LG, Szent-Györgyi A. On the regulation of cell division. *Proc. Natl Acad Sci USA* 1966; **56:** 203–207.
10. Fraval HN, McBrien DCH. The effect of methylglyoxal on cell division and the synthesis of protein and DNA in synchronous and asynchronous cultures of *E. coli* B/r. *J Gen Microbiol* 1980; **117:** 127–134.
11. Otsukah Együd LG. Nucleic acid and protein synthesis of malignant ascites cells in the presence of liver extract and methylglyoxal. *Cancer Res* 1967; **27:** 1498–1499.

12. Szent-Györgyi A. Cell division and cancer. *Science* 1965; **149:** 34–37.
13. Cooper RA, Anderson A. The formation and catabolism of methylglyoxal during glycolysis in *E. coli*. *FEBS Lett* 1970; **11:** 273–276.
14. Elliot WH. Methylglyoxal formation from aminoacetone by ox plasma. *Nature* (*Lond*) 1960; **185:** 467–468.
15. Sato J, Wang Yeu-ming, van Tys J. Methylglyoxal formation in rat liver cells. *J Biol Chem* 1980; **225:** 1046–2050.
16. Riddle V, Lorenz FW. Non-enzymic, polyvalent anion-catalysed formation of methylglyoxal as an explanation of its presence in physiological systems. *J Biol Chem* 1968; **243:** 2718–2724.
17. Conroy PJ. Ph.D. Thesis, Brunel University, Uxbridge, 1967.
18. Schauenstein E, Esterbauer H, Zollner H. *Aldehydes in Biological Systems*. London: Pion, 1977.
19. Racker E. The mechanism of action of glyoxalase. *J Biol Chem* 1951; **190:** 685–696.
20. Szent-Györgyi A. Bioelectronics. *Science* 1968; **161:** 988–990.
21. Ackerman RS, Cozzarelli WR, Epstein W. Accumulation of toxic concentrations of methylglyoxal by wild type *E. coli* K12. *J Bacteriol* 1974; **119:** 357–362.
22. Szent-Györgyi A. The living state and cancer. *Proc. Natl Acad Sci USA* 1977; **74:** 2844–2847.
23. Jerzykowski T, Winter R, Matuszewski W, Piskorska D. Re-evaluation of studies on the distribution of glyoxalase in animal and tumour tissues. *Int J Biochem* 1978; **9:** 853–860.
24. Dianzani MU, Biological activity of methylglyoxal and related akdehydes. In: *Submolecular Biology and Cancer*. CIBA Symposium No. 67. Wolstenholme G, Fitzsimons DW, Whelan J, Eds. Amsterdam: Elsevier, 1979, 245–270.
25. Együd LG, Szent-Györgyi A. Cell division, SH, ketoaldehydes and cancer. *Proc Natl Acad Sci USA* 1966; **55:** 388–393.
26. French FA, Freedlander BL. Carcinostatic action of polycarbonyl compounds and their derivatives 1) 3-ethoxy-2-ketobutyraldehyde and related compounds. *Cancer Res* 1958; **18:** 172–275.
27. Conroy PJ, Nodes JT, Slater TF, White GW. The inhibitory effects of a 4-hydroxypentenal-cysteine adduct against sarcoma 180 cells in mice. *Eur J Cancer* 1977; **13:** 55–63.
28. Tillian HM, Schauenstein E., Ertl A, Esterbauer H. Therapeutic effects of cysteine adducts of α, β-unsaturated aldehydes on Ehrlich ascites tumour of mice–I. *Eur J Cancer* 1976; **12:** 989–993.
29. Tillian HM, Schauenstein E, Esterbauer H. Therapeutic effects of cysteine adducts of α, β-unsaturated aldehydes on Ehrlich ascites tumour of mice—II. *Eur J Cancer* 1978; **14:** 533–536.
30. Fodor G. The search for new cancerostatic agents. In: *Submolecular Biology and Cancer*. CIBA Symposium No. 67. Wolstenholme G, Fitzsimons DW, Whelan J, Eds. Amsterdam: Elsevier, 1979, 165–174.
31. Riley PA. Inhibition of protein and DNA synthesis in tissue culture cells by a derivative of methylglyoxal and ascorbate. *Chem Biol Interact* 1980; **31:** 73–80.
32. Ahmed SM, Slater TF. Effects of ascorbic acid and various ketoacids on the glyoxalase enzyme system in the mouse and rat liver. *IRCS Med Sci* 1979; **7:** 505.
33. Alexander NM, Boyer YL. A rapid assay for the glyoxalase enzyme system. *Anal Biochem* 1971; **41:** 29–38.
34. Cameron E. Pauling L, Leibowitz B. Ascorbic acid and cancer: a review. *Cancer Res* 1979; **39:** 663–681.
35. Ito M, Okabe K, Omura H. Effect of reductones on glyoxalase *J. Nutr Sci Vitaminol* 1976; **22:** 53–61.
36. Fox SW, Syren RM, Windsor CR. Thermal copoly (amino acids) as inhabitors of glyoxalase I. In: *Submolecular Biology and Cancer*. CIBA Symposium No. 67. Wolstenholme G, Fitzsimons DW, Whelan J, Eds. Amsterdam: Elsevier, 1979, 175–193.
37. Conroy PJ, Elvin P. (to be published).