L-α-methylcholine, and Fig. 2 shows that in D-α-methylcholine the substituted methyl group is further from the OH group than in the L-isomer. With L-α-methylcholine the substituted methyl group may affect, by steric hindrance, the interaction between the acetyl group provided by acetyl-CoA and the hydroxyl group on the substrate molecule. The steric hindrance effect of the methyl group in the β-substituted methylcholines is probably greater than in the α-substituted compounds as reflected in the lower acetylation rates, and in the case of L-β-methylcholine no acetylation is observed.

It is of interest that in the case of hydrolysis of the acetyl-β-methylcholines by bovine erythrocyte acetylcholinesterase the D-isomer is not hydrolyzed.[11] The rate of the hydrolysis of L-acetyl-β-methylcholine is much lower than that of the α-isomers, suggesting a similar effect of steric hindrance by the substituted methyl group.

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REFERENCES

1. S. R. KOREY, B. DE BRAGANZA and D. NACHMANSOHN, *J. biol. Chem.* **189,** 705 (1951).
2. A. S. V. BURGEN, G. BURKE and M. L. DESBARATS-SCHONBAUM, *Br. J. Pharmac. Chemother.* **11,** 308 (1956).
3. W. C. DAUTERMAN and K. N. MEHROTRA, *J. Neurochem.* **10,** 113 (1963).
4. B. A. HEMSWORTH and D. MORRIS, *J. Neurochem.* **11,** 793 (1964).
5. A. HEMSWORTH and J. C. SMITH, *J. Neurochem.* **17,** 171 (1970).
6. R. E. MCCAMAN and J. M. HUNT, *J. Neurochem.* **12,** 253 (1965).
7. A. H. BECKETT, N. J. HARPER and J. W. CLITHEROW, *J. Pharm. Pharmac.* **15,** 349 (1963).
8. R. S. CAHN, *J. chem. Education* **41,** 116 (1964).
9. L. T. POTTER, V. A. S. GLOVER and J. K. SAELENS, *J. biol. Chem.* **243,** 3864 (1968).
10. H. LINEWEAVER and D. BURK, *J. Am. chem. Soc.* **56,** 658, (1934).
11. A. H. BECKET, N. J. HARPER and J. W. CLITHEROW, *J. Pharm. Pharmac.* **15,** 362 (1963).

* The preliminary work of this study was carried out at the Montefiore Hospital and Medical Centre, Bronx, New York 10467.

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Antagonism of the anti-tumour effects of asparaginase by methotrexate

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A RECENT publication has reported that the inhibitory effects of methotrexate against the L5178Y mouse leukaemia can be completely antagonised by pretreatment with asparaginase, which is also tumour inhibitory in this system.[1]

In experiments on the mouse R_1 lymphoma we have shown that combinations of asparaginase and methotrexate given simultaneously, are significantly less effective than if asparaginase is given prior to methotrexate treatment, or vice versa.

Materials and methods

The R_1 lymphoma was induced by irradiation of a CBA mouse and has, since induction, been maintained in the same strain, by routine transplantation of ascites cells. A sub-line of the tumour, showing partial resistance to asparaginase, was used in these experiments. The optimum tumour

inhibitory dose of methotrexate (3 mg/kg i.p. in water for 5 days, commencing 3 days after subcutaneous injection of the tumour) was previously determined. Asparaginase (Bayer: Crasnitin) was previously shown to be maximally effective against the tumour at doses above 40 i.u./kg i.p. in water for 5 days. The R_1 lymphoma is injected subcutaneously (10^6 ascites cells) where it forms a small primary tumour and rapidly disseminates to kill the animals at about 10 days. Survival time of the animals following subcutaneous injection of tumour cells is directly related to the number of cells injected.

Groups of 10 animals were used and effectiveness of the drugs and drug combinations was measured by survival time. Table 1 shows that the control animals die within 9–10 days following subcutaneous injection of 10^6 cells. Daily doses of methotrexate have a considerable effect on the tumour, extending

TABLE 1

Treatment	*Days of treatment	Range of survival times (days)	Mean survival time	Probability
Control	—	9–10	9·8	
Methotrexate 3 mg/kg i.p. × 5	3, 4, 5, 6, 7	17	17·0	< 0·001
Asparaginase 4000 i.u./kg i.p. × 5	3, 4, 5, 6, 7	12	12·0	< 0·001
Methotrexate 3 mg/kg i.p. × 5 + Asparaginase 4000 i.u./kg i.p. × 5	3, 4, 5, 6, 7	15–17	16·2	< 0·001
Methotrexate 3 mg/kg i.p. × 5 followed by Asparaginase 4000 i.u./kg i.p. × 5	3, 4, 5, 6, 7 10, 11, 12, 13, 14	19–21	19·8	< 0·001
Asparaginase 4000 i.u./kg i.p. × 5 followed by Methotrexate 3 mg/kg i.p. × 5	3, 4, 5, 6, 7 10, 11, 12, 13, 14	19–21	19·8	< 0·001

* 1×10^6 tumour cells transplanted subcutaneously on day 0.

the survival time to 17 days. Asparaginase is less effective but causes a significant 2-day extension of survival time. When the two drugs are administered simultaneously, the extension of survival time is not significantly different from that seen with methotrexate alone, implying that the anti-tumour effect of asparaginase is completely antagonised by methotrexate. However, when the two agents are administered consecutively, by either of the schedules shown in Table 1, the effects of the two drugs are additive.

Discussion

Methotrexate is toxic for cells in cycle, and asparaginase for cells that cannot synthesise their own asparagine. If a further requirement for asparagine dependent cells is that they must also be dividing for cytotoxicity to occur, then the antagonism of the antitumour effect of asparaginase by methotrexate may be explained. The dose level of methotrexate employed may be sufficient to kill all cells in cycle with the result that, although cells without the ability to synthesise asparagine may be in the surviving fraction, these are not in division and are, therefore, not sensitive to asparaginase. When the drug combinations are given consecutively there is addition since, where asparaginase is given first, there are obviously asparagine-deficient cells in cycle while, when given after methotrexate, 3 days elapse

between the end of the methotrexate injections and the commencement of asparaginase, probably enabling the dividing fraction of the tumour to be reconstituted from the resting cells.

The R_1 lymphoma, in its response to anti-tumour agents, appears to be a suitable model for studying drug combinations likely to be effective in the treatment of acute lymphoblastic leukaemia. In view of the use of both methotrexate and asparaginase in the treatment of this disease, it would seem important that these two agents should always be administered consecutively and not simultaneously.

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REFERENCE

1. R. L. CAPIZZI, W. P. SUMMERS and J. R. BERTINO, *Proc. Am. Ass. Cancer Res.* **11**, 14 (1970).

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Inhibition of monoamine oxidation in brain by monoamine oxidase inhibitors

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MANY data have accumulated in recent years on the inhibition of monoamine oxidase (MAO) by hydrazide and non-hydrazide inhibitors resulting in an elevated level of biogenic amines in brain.[1–7] Contrary to these, observations were also made indicating that trans-phenylcyclopropylamine (trans-PCP) and iproniazid failed to elevate brain level of tryptamine.[8,9] The earlier work on enzymatic reduction of tetrazolium salts or other redox indicators in the presence of amines[10–12] has been extended by others,[13–15] although it is not known whether the amine dehydrogenating system (MADH) is identical with MAO or not. However, histochemical studies using tetrazolium salt reduction in the presence of monoamines have always been described as MAO activity in the literature.[16–21] A preliminary account of certain differences observed in the reaction mechanism of monoamine oxidation catalysed by rat brain MAO and MADH systems and their response to inhibitors like iproniazid, trans-PCP and pargyline is described in this communication.

Whole brain homogenates of adult albino rats were prepared as a 10 per cent suspension in 0·25 M sucrose. The reaction mixture for MAO assay contained 0·02 M phosphate buffer pH 6·5, 0·0125 M semicarbazide pH 6·5, 0·01 M tyramine and 50 mg of tissue homogenate in a final volume of 2 ml. Aldehyde formed was measured at 420 mμ by the method of Green and Haughton[22] as described previously.[23] The reaction mixture for MADH assay consisted of 0·025 M phosphate buffer pH 7·0, 0·5 mg neo-tetrazolium chloride (NTC), 0·01 M tyramine and 100 mg of tissue homogenate in a final volume of 2 ml. NTC reduction was measured at 520 mμ according to the method of Lagnado and Sourkes.[14] Air was used as the gas phase in aerobic experiments and anaerobic experiments were carried out *in vacuo* in Thunberg tubes.[24] Incubations were carried out at 38° for 30 min with a preincubation period of 5 min unless stated otherwise. When the inhibitors were added, they were allowed to incubate with the enzyme for 20 min prior to addition of the substrate. Ammonia formed in these systems was determined by the manometric method of Braganca *et al.*[25] followed by nessler-isation. All values are corrected for appropriate blanks without any added substrate. *In vivo* experiments were performed by administering daily to groups of six rats intraperitoneal injections of MAO