4. H. B. Burch, C. A. Storvick, R. L. Bicknell, H. C. Kung, L. G. Alejo, W. A. Everhart, O. H. Lowry, C. G. King and O. A. Bessey, *J. biol. Chem.* **212**, 897 (1955).
5. R. M. Burton, N. O. Kaplan, A. Goldin, M. Leitenberg, S. R. Humphreys and M. A. Sodd, *Science* **127**, 30 (1958).
6. J. N. Williams, Jr., P. Feigelson and C. A. Elvehjem, *J. biol. Chem.* **187**, 597 (1950).
7. O. H. Lowry, N. R. Roberts and J. I. Kapphahn, *J. biol. Chem.* **224**, 1047 (1957).
8. D. W. Woolley, *Science* **128**, 1277 (1958).
9. A. Hoffer, H. Osmond, M. J. Callbeck and I. Kahan, *J. clin. exp. Psychopath.* **18**, 131 (1957).
10. J. J. Lewis and G. R. van Petten, *J. Pharmacol.* **136**, 372 (1962).
11. C. L. Kaul and J. J. Lewis, *J. Pharmacol.* **140**, 111 (1963).
12. J. J. Lewis and G. R. van Petten, *Brit. J. Pharmacol.* **20**, 462 (1963).
13. C. L. Kaul and J. J. Lewis, *Biochem. Pharmacol.* **12**, 1279 (1963).
14. H. McIlwain, *Metabolism of the Nervous System* (Ed. D. Richter) p. 341. Pergamon Press, London (1957).
15. C. L. Kaul, S. Livingstone and J. J. Lewis, *Biochem. Pharmacol.* In Press.

Biochemical Pharmacology, 1965, Vol. 14, pp. 638–640. Pergamon Press Ltd., Printed in Great Britain.

The inhibition of some glycolytic enzymes by chlorambucil*

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Chlorambucil {*p*[bis(2-chloroethyl)amino]phenylbutyric acid} has recently been the subject of investigations in antitumor studies.[1–3] Since chlorambucil increases blood glucose levels in rat,

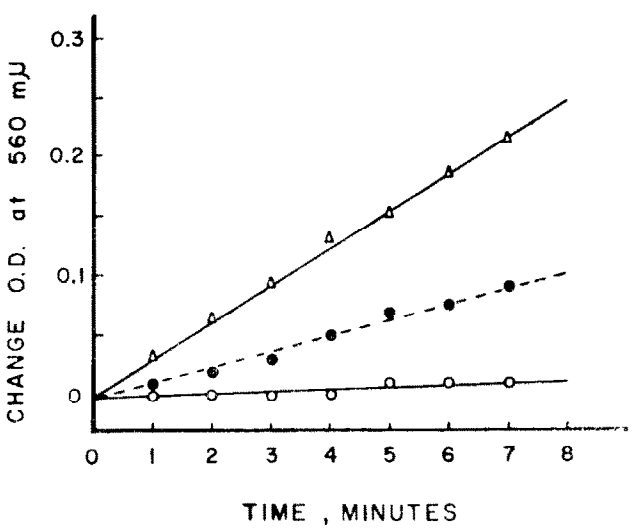

Fig. 1. The effect of chlorambucil on hexokinase. The final assay mixtures contained 4·47μg hexokinase; △, control hexokinase; ●, hexokinase preincubated with 2×10^{-3} M chlorambucil; ○, hexokinase preincubated with 4×10^{-3} M chlorambucil

inhibits respiration of yeast more significantly with glucose as an energy source than does citrate or succinate,[4] and lowers the glycogen content of lymphocytes,[5] its effect on several glycolytic enzymes was investigated in order to correlate the observed action on whole cells and organisms.

* Preliminary investigations were carried out at the Division of Pharmacology, Food and Drug Administration, Washington, D.C. The chlorambucil was kindly supplied by Dr. M. V. Nadkarni, Cancer Chemotherapy National Service Center, National Institutes of Health, Bethesda, Md.

Crystalline yeast hexokinase, rabbit muscle lactic dehydrogenase, aldolase, and enolase were treated in the manner described by Dixon and Needham.[6] The enzymes were preincubated at 0° with 20% ethanol or ethanolic chlorambucil for up to 110-min periods, and assayed for enzyme activity.[7–10] Determination of the concentration of hexokinase was performed by measurement of the absorption at 278 mμ in the Beckman DU spectrophotometer. Lactic dehydrogenase and aldolase concentrations were determined by measurement of the absorption at 280 mμ. Enolase concentrations were estimated by the method of Lowry *et al*,[11] with crystalline bovine albumin as a standard.

Chlorambucil at a concentration of 4×10^{-3} M effects a 96% inhibition of hexokinase activity, whereas 2×10^{-3} M chlorambucil exerts a 57% inhibition (Fig. 1).

Lactic dehydrogenase is fully inhibited by chlorambucil at a concentration of 5×10^{-4} M. It is inhibited to the extent of 47% by 2×10^{-4} M chlorambucil and is not affected by 1×10^{-4} M inhibitor (Fig. 2).

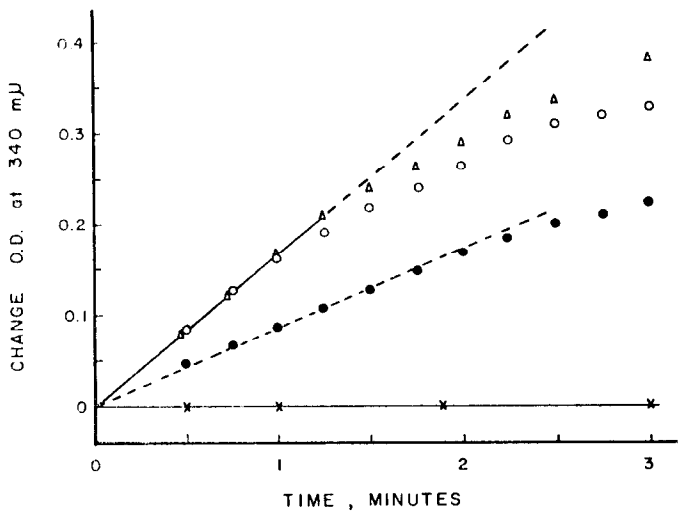

FIG. 2. The effect of chlorambucil on lactic dehydrogenase (LDH). The final assay mixtures contained 10·1 μg LDH; △, control LDH; ×, LDH preincubated with 5×10^{-4} M chlorambucil; ●, LDH preincubated with 2×10^{-4} M chlorambucil; ○, LDH preincubated with 1×10^{-4} M chlorambucil.

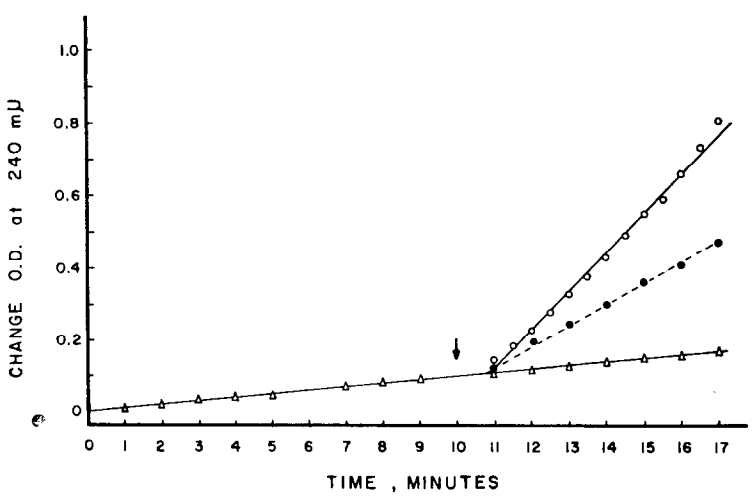

FIG. 3. The effect of chlorambucil on aldolase. The final assay mixtures contained 14·95 μg aldolase; ○, control aldolase; ●, aldolase preincubated with 1×10^{-3} M chlorambucil; △, aldolase preincubated with 2×10^{-3} M chlorambucil.

Enolase is inhibited to the extent of 99·5% by 1×10^{-4} M chlorambucil and to the extent of 30% by $3{\cdot}3 \times 10^{-5}$ M inhibitor (Fig. 4).

Aldolase is fully inhibited by 2×10^{-3} M chlorambucil and is inhibited to the extent of 43% by 1×10^{-3} M inhibitor (Fig. 3).

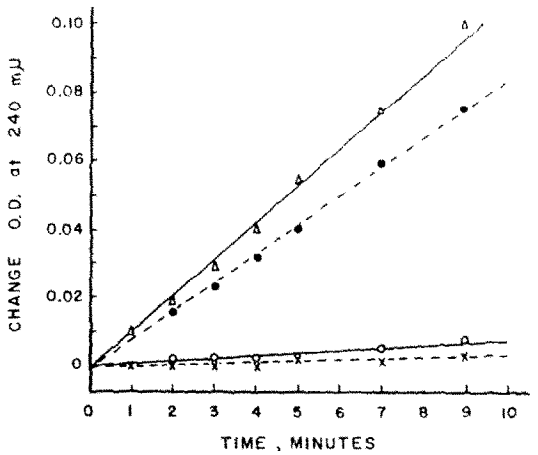

FIG. 4. The effect of chlorambucil on enolase. The final assay mixture contained 0·445 μg *enolase*; △, control enolase; ●, enolase preincubated with $3{\cdot}3 \times 10^{-5}$ M *chlorambucil*; ○, enolase preincubated with 1×10^{-4} M chlorambucil; ×, enolase preincubated with 5×10^{-4} M chlorambucil.

These results suggest that chlorambucil may inhibit glycolysis at several points, thereby impairing glucose mobilization and energy production. The nature of the inhibition is not yet known, although methyl-bis(2-chloroethyl)amine and such derivatives as chlorambucil have been reported to alkylate proteins by reaction with thiols, carboxylate ions, imidazole groups, and amino groups,[12-15] and may also bind to protein without alkylation.[16]

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REFERENCES

1. J. L. EVERETT, J. J. ROBERTS and W. C. J. ROSS, *J. chem. Soc.* 2386 (1953).
2. D. A. G. GALTON, L. S. ISRAELS, J. D. N. NABARRO and M. TILL, *Brit. med. J.* **7**, 1172 (1955).
3. A. I. KRAVCHENKO and A. A. GRUSHINA, *Vop. Onkol.* **7**, 72 (1961).
4. R. S. YOUNG, L. HURWITZ and E. I. GOLDENTHAL, *J. cell. comp. Physiol.* **52**, 353 (1958).
5. G. MEARDI, *Proc. 7th Internatl. Congr. Intern. Soc. Hematol.*, Rome, **2**, 53 (1958).
6. M. DIXON and D. M. NEEDHAM, *Nature, (Lond.)* **158**, 432 (1946).
7. R. A. DARROW and S. P. COLOWICK, in *Methods in Enzymology*, vol. 5, p. 226, S. P. COLOWICK and N. O. KAPLAN, Eds. Academic Press, New York (1962).
8. W. E. C. WACKER, D. D. ULMER and B. L. VALLEE, *New Engl. J. Med.* **255**, 449 (1956).
9. V. JAGANNATHAN, K. SINGH and M. DAMODARAN, *Biochem. J.* **63**, 94 (1956).
10. A. HOLT and F. WOLD, *J. biol. Chem.* **236**, 3227 (1961).
11. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193**, 265 (1951).
12. F. S. PHILIPS, *Pharmacol. Rev.* **2**, 281 (1950).
13. W. C. J. ROSS, *Ann. N. Y. Acad. Sci.* **68**, 669 (1958).
14. K. A. STACEY, M. COBB, S. F. COUSENS and P. ALEXANDER, *Ann. N. Y. Acad. Sci.* **68**, 682 (1958).
15. P. ALEXANDER and S. F. COUSENS, *Biochem. Pharmacol.* **1**, 25 (1958).
16. J. H. LINFORD, *Canad. J. Biochem.* **41**, 931 (1963).