REFERENCES

1. J. B. SCHENKMAN, J. A. BALL and R. W. ESTABROOK, *Biochem. Pharmac.* **16,** 1071 (1967).
2. A. RUBIN, T. R. TEPHLY and G. J. MANNERING, *Biochem. Pharmac.* **13,** 1007 (1965).
3. M. W. ANDERS and G. J. MANNERING, *Molec. Pharmac.* **2,** 319 (1966).
4. T. R. TEPHLY and G. J. MANNERING, *Molec. Pharmac.* **4,** 10 (1968).
5. N. E. SLADEK and G. J. MANNERING, *Molec. Pharmac.* **5,** 186 (1969).
6. O. H. LOWRY, N. J. ROSEBROUGH, A. L. FARR and R. J. RANDALL, *J. biol. Chem.* **193,** 265 (1951).
7. G. N. WILKINSON, *Biochem. J.* **80,** 324 (1961).
8. W. W. CLELAND, *Nature, Lond.* **198,** 463 (1963).
9. C. C. LI, Ed., *Introduction to Experimental Statistics*, p. 174. McGraw-Hill, New York (1964).
10. H. A. SASAME and J. R. GILLETTE, *Biochem. Pharmac.* **19,** 1025 (1970).
11. J. L. WEBB, in *Enzyme and Metabolic Inhibitors*, p. 59. Academic Press, New York (1963).
12. L. A. ROGERS and J. R. FOUTS, *J. Pharmac. exp. Ther.* **146,** 286 (1964).
13. M. W. ANDERS, A. P. ALVARES and G. J. MANNERING, *Molec. Pharmac.* **2,** 328 (1966).
14. F. KRATZ and H. STAUDINGER, *Hoppe-Seyler's Z. physiol. Chem.* 349, 455 (1968).

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Iodoacetate inhibition of lactate production and lipid, protein, ribonucleic acid [RNA] and deoxyribonucleic acid [DNA] synthesis in newborn rat brain cortex slices

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IODOACETIC ACID (IAA) has long been considered a classical inhibitor of glycolysis, exerting its effect primarily on glyceraldehyde-3-phosphate dehydrogenase.[1] Early studies with pigeon and rat brains concluded that lactate production was inhibited by IAA at a concentration which did not affect cell respiration.[2,3] Although other metabolic processes such as RNA[4] and phospholipid[3] synthesis are inhibited to some extent by IAA, the concentration needed is higher than that which is required for inhibition of glycolysis.

During the course of our studies concerned with the regulation of rat brain metabolism during ontogeny,[5–7] it was discovered that DNA synthesis *in vitro* from glucose-6-^{3}H in brain slices was more sensitive than lactate production to the inhibitory effects of IAA. To further characterize this effect, IAA was used as a probe to examine the degree of interdependence of macromolecular synthesis on glycolysis.

Four-week-old male Wistar rats were housed in separate cages and fed Purina laboratory chow and tap water *ad lib.* Animals were decapitated and cerebral cortices rapidly removed and placed in a Petri dish containing ice-cold saline. Brain slices were prepared according to the method of McIlwain and Rodknight[8] and lactate production and glucose-6-^{3}H (Amersham/Searle, 2·3 c/m-mole) incorporation into lipid, protein, RNA and DNA were assayed as previously described.[6] Iodoacetate was purchased from the Sigma Chemical Co. and adjusted to pH 7·4 before being added to the medium.

Iodoacetate effected a dose-dependent separation of the relative interdependence of lactate production and lipid, protein, RNA and DNA synthesis (Table 1). The approximate ID_{50} (IAA concentration resulting in 50 per cent inhibition) for each of the five pathways may be categorized into three groups: (1) lactate production and DNA synthesis with an ID_{50} of $2–4 \times 10^{-5}$ M, (2) lipid and protein synthesis with an ID_{50} of 2×10^{-4} M, and (3) RNA synthesis with an ID_{50} of $1·5 \times 10^{-3}$ M. Thus a separation of RNA and DNA synthesis was achieved based on their respective sensitivities to IAA inhibition, despite their dependence on glucose as common precursor.

In agreement with previous results examining the effects *in vitro* of IAA on glyceraldehyde-3-phosphate dehydrogenase and pigeon and rat brain glycolysis,[1–3] lactate production was inhibited at 5×10^{-5} M IAA. This concentration was lower than that which affects brain respiration. However,

TABLE 1. EFFECT OF IODOACETATE ON LACTATE PRODUCTION AND GLUCOSE-6-^{3}H INCORPORATION INTO LIPIDS, PROTEIN, RIBONUCLEIC ACID (RNA) AND DEOXYRIBONUCLEIC ACID (DNA) IN BRAIN CORTEX SLICES FROM 4-WEEK-OLD RATS*

Product	Iodoacetate concn. (M) 0	5×10^{-6}	10^{-5}	5×10^{-5}	5×10^{-4}	10^{-3}	5×10^{-3}
Lactate production $ID_{50} = 35\ \mu M$	10·7 ± 0·4 (100)	11·4 ± 0·8 (113)	10·3 ± 0·8 (96)	4·0 ± 0·5 (37)†	0·9 ± 0·4 (8)†	0·4 ± 0·3 (4)†	0·7 ± 0·4 (7)†
DNA $ID_{50} = 20\ \mu M$	50,365 ± 2300 (100)	42,471 ± 5600 (84)	32,335 ± 2356 (64)†	15,652 ± 840 (31)†	2497 ± 440 (5)†	1772 ± 262 (4)†	2165 ± 456 (4)†
Lipid $ID_{50} = 0·2$ mM	116,983 ± 4100 (100)	143,005 ± 9338 (123)	116,146 ± 5478 (100)	118,411 ± 4622 (102)	20,667 ± 1165 (18)†	21,742 ± 2541 (19)†	13,076 ± 1368 (11)†
Protein $ID_{50} = 0·2$ mM	16,953 ± 1300 (100)	19,843 ± 2615 (117)	14,777 ± 2204 (87)	12,714 ± 821 (75)†	6232 ± 688 (37†)	5580 ± 712 (33)†	5766 ± 620 (34)†
RNA $ID_{50} = 1·5$ mM	37,650 ± 1400 (100)	44,240 ± 1900 (117)	45,607 ± 2286 (121)†	49,196 ± 4150 (131)†	19,691 ± 1908 (52)†	19,386 ± 3116 (51)†	17,272 ± 1254 (46)†

* Incorporation of glucose-6-^{3}H into lipid, protein, RNA and DNA is expressed as disintegrations per minute per gram of wet weight per hour and lactate production as micromoles lactate produced per gram wet weight per hour. Each value represents the mean ± S.E.M. of 6–16 animals. Numbers in parentheses are percentages with respect to controls without iodoacetate. Incubation flasks contained 100 mg wet weight of cerebral cortex slices in 2·5 ml Krebs–Ringer phosphate medium supplemented with 20 mM glycylglycine, pH 7·4, 1·0 mM glucose and 2·5 μc glucose-6-^{3}H. Assays were run under 100% oxygen in a Dubnoff shaker bath at 100 r.p.m. for 60 min at 37° and the brain tissue fractionated as previously described.[6] ID_{50} values were determined from semilogarithmic plots of per cent inhibition vs. log IAA concentration.

† Indicates statistical significance ($P < 0·05$) with respect to controls without iodoacetate.

the present study also clearly indicates that DNA synthesis from glucose was more sensitive to IAA inhibition than were the other metabolic processes. At 10^{-5} M IAA, a concentration not affecting glycolysis, 36 per cent inhibition of DNA synthesis occurred. Since RNA synthesis was significantly *elevated* at this level of IAA, it is possible that one site of action for this inhibitor on DNA synthesis involves some process concerned with the formation of DNA precursors, such as the sulfhydryl-sensitive ribonucleotide reductase complex.[9] The present results suggest the need for caution when interpreting experiments which utilize IAA as a "specific" glycolytic inhibitor. The effects of IAA vary in a dose-dependent manner and include more than one metabolic pathway.

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REFERENCES

1. G. T. CORI, M. W. SLEIN and C. F. CORI, *J. biol. Chem.* **173,** 615 (1948).
2. P. J. HEALD, *Biochem. J.* **55,** 625 (1953).
3. W. C. MCMURRAY, K. P. STRICKLAND, J. F. BERRY and R. J. ROSSITER, *Biochem. J.* **66,** 621 (1957).
4. M. FINDLAY, R. J. ROSSITER and K. P. STRICKLAND, *Biochem. J.* **55,** 204 (1953).
5. G. WEBER, *Adv. Enzyme Regulat.* **7,** 15 (1969).
6. G. WEBER, R. I. GLAZER and R. A. ROSS, *Adv. Enzyme Regulat.* **8,** 13 (1970).
7. R. I. GLAZER and G. WEBER, *Fedn. Proc.* **29,** 479 (1970).
8. H. MCILWAIN and R. RODKNIGHT, in *Practical Neurochemistry* (Eds. H. MCILWAIN and R. RODKNIGHT), p. 109. Little, Brown & Co., Boston (1962).
9. E. C. MOORE, P. REICHARD and L. THELANDER, *J. biol. Chem.* **239,** 3445 (1964).

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Phenylacetone oxime—An intermediate in the oxidative deamination of amphetamine*

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AXELROD[1] first demonstrated the conversion, by rabbit liver microsomes, of amphetamine to phenylacetone. No reports, to our knowledge, have appeared concerning the nature of possible intermediates in the reaction referred to as oxidative deamination. Human liver microsomes also apparently convert amphetamine to phenylacetone.[2] Phenylacetone and benzoic acid were shown by Dring *et al.*[3] to be major metabolites of amphetamine in rabbit, dog and human urine and these authors concluded that oxidative deamination was the predominant metabolic pathway for amphetamine in these species. The corresponding imine, a possible phenylacetone precursor, was not found in urine after amphetamine administration.

The present report is concerned with the nature of the intermediate formed during oxidative deamination of amphetamine by a rabbit liver microsomal system.

* A preliminary account of this work has appeared in *Pharmacologist* **12,** 255 (1970).