was changed at hourly intervals during the experiment. A current of 10–20 milliamperes was maintained by suitable variation of the applied voltage (500–2000 volts).

In two typical experiments (Table I) 15–20% of the total nitrogen passed through the parchment membrane into the cathode cell. When the cathode fraction was assayed for ACTH potency by the ascorbic acid depletion method of Sayers, *et al.*,[5] it was found to contain almost all the hormone activity. The residue (77% of the total nitrogen) in the center compartment contained less than 1% of the original activity.

Incubation with pepsin[3a] in 0.01 *N* hydrochloric acid solution for 24 hours at 37° or heating a 1% solution in 0.2 *N* hydrochloric acid in a boiling water-bath[6] for one hour did not lead to inactivation of the cathode fraction. In preliminary ultracentrifugal studies on the cathode fraction, only one component with an $S_{20}$ value of approximately 1.0 was detected.

(5) M. Sayers, G. Sayers and L. A. Woodbury, *Endocrinology*, **42**, 379 (1948).

(6) C. H. Li, THIS JOURNAL, **73**, 4146 (1951).

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## THE PATHWAY OF INOSITOL FERMENTATION IN AEROBACTER AEROGENES[1]

*Sir:*

Previous work from this Laboratory[2] has indicated that *myo*-inositol[3] is metabolized by a pathway not involving conversion to glucose and degradation according to the Embden–Meyerhoff scheme. Further information has been obtained by the use of suspensions of *A. aerogenes* grown with strong aeration in a mineral medium containing glucose or *myo*-inositol as the sole source of carbon. Such cells are unable to split formic acid[4] or to produce acetoin. Thus, the fermentation of glucose, studied in a conventional Warburg apparatus in bicarbonate buffer in an atmosphere of 5% $CO_2$ and 95% $N_2$, resulted in the formation of acid, but not of $CO_2$ or $H_2$. In a typical experiment 0.32 mole of ethanol, 0.79 mole of lactate and 1.23 equivalents of other acids were formed per mole of glucose. When the phosphoclastic attack on pyruvate was inhibited by 0.0016 *M* arsenite, 1.95 mole of lactate were formed.

*myo*-Inositol, 2-keto-*myo*-inositol, L-1-keto-*myo*-inositol, and L-1,2-diketo-*myo*-inositol were fermented by cells grown on *myo*-inositol, but not by cells grown on glucose. All four substrates yielded acid and $CO_2$ but no $H_2$. 2-Keto-*myo*-inositol and L-1,2-diketo-*myo*-inositol were fermented two to three times as fast as *myo*-inositol or L-1-keto-*myo*-inositol. The fermentation of *myo*-inositol produced 0.68 mole of $CO_2$, 0.72 mole of ethanol and 1.54 equivalents of acid per mole. In the presence of arsenite, 0.72 mole of $CO_2$, 0.72 mole of ethanol, 0.68 mole of lactate, and 0.56 equivalent of unidentified acids were formed. These results show clearly that the $CO_2$ and the ethanol formed from *myo*-inositol are not derived from pyruvate.

On the basis of these and earlier experiments the following pathway of *myo*-inositol degradation may be tentatively suggested: *myo*-inositol − 2H→2-keto-*myo*-inositol − 2H→L-1,2-diketo-*myo*-inositol − 2H→$CO_2$ + acetate + pyruvate + 6H→ $CO_2$ + ethanol + lactate.

These end-products account for 70% of the *myo*-inositol. Since the first steps in this scheme are dehydrogenations, the greater rate of fermentation found for 2-keto-*myo*-inositol and L-1,2-diketo-*myo*-inositol may be explained by their ability to act as hydrogen acceptors.

Similar pathways in which dehydrogenations precede cleavage of the carbon chain may not be restricted to inositol. The recent demonstration[5] that fermentation of glucose by *Leuconostoc mesenteroides* yields equimolar quantities of $CO_2$, ethanol and lactate, and that the rate of fermentation may be increased by hydrogen acceptors indicates that a similar scheme may be the major glycolytic pathway in that organism.

(1) Supported by the William F. Milton Fund.

(2) B. Magasanik, Am. Chem. Soc., Abst. of Papers, 119th Meeting, 20C (1951).

(3) Formerly *meso*-inositol. For the nomenclature of inositol derivatives see H. G. Fletcher, Jr., L. Anderson, and H. A. Lardy, *J. Org. Chem.*, **16**, 1238 (1951).

(4) J. L. Stokes, *J. Bact.*, **57**, 147 (1949).

(5) R. D. DeMoss, R. C. Bard and I. C. Gunsalus, *J. Bact.*, **62**, 499 (1951).

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## CRYSTALLIZATION OF A DERIVATIVE OF PROTOGEN-B

*Sir:*

The protozoan *Tetrahymena geleii* needs an unidentified fraction present in liver and other natural materials for growth.[1,2] The name "protogen" has been used to designate the substance or substances which are responsible for this growth effect. Concentrates with "protogen" activity have been shown to be needed for the growth of an unidentified *Corynebacterium*[3] and to have activity corresponding to the "acetate factor"[4,5] and the "pyruvate oxidation factor."[6]

Reed, *et al.*,[7] have reported the crystallization of a compound designated α-lipoic acid which appears to have biological properties similar to those of protogen.

The protogen activity of a papain digest of a water-insoluble fraction of liver was not extractable by organic solvents. However, after autoclaving with 3.3 *N* sodium hydroxide and acidifying with hydrochloric acid, the activity could be extracted with chloroform. Counter-current extraction by the method of Craig[8] using several solvent systems

(1) G. W. Kidder and V. Dewey, *Arch. Biochem.*, **8**, 293 (1945).

(2) E. L. R. Stokstad, *et al.*, *ibid.*, **20**, 75 (1949).

(3) E. L. R. Stokstad, *et al.*, *Proc. Soc. Exptl. Biol. Med.*, **74**, 571 (1950).

(4) E. E. Snell and H. P. Broquist, *Arch. Biochem.*, **23**, 326 (1949).

(5) B. M. Guirard, *et al.*, *ibid.*, **9**, 381 (1946).

(6) D. J. O'Kane and I. C. Gunsalus, *J. Bact.*, **56**, 499 (1948).

(7) L. J. Reed, *et al.*, *Science*, **114**, 93 (1951); *J. Biol. Chem.*, **192**, 851, 859 (1951).

(8) L. C. Craig and O. Post, *Anal. Chem.*, **21**, 500 (1949).

showed the presence of several forms of protogen. Most of the activity had a distribution coefficient of 0.4 ($C_{H_2O}/C_{CHCl_3}$) between chloroform and 0.5 *M* aqueous phosphate buffer at *p*H 7.2. As purification proceeded this form, called protogen-A, changed into another form, protogen-B, which had a distribution coefficient of 20 in this system. On Whatman No. 1 paper strip chromatograms developed with *n*-butanol saturated with 0.5 *N* ammonium hydroxide, protogen-A had an $R_f$ of 0.7 and protogen-B an $R_f$ of 0.33.

Counter-current extractions were continued in a 200-tube extraction apparatus until the distribution of organic solids as measured by wet combustion,[9] the biological activity, and the theoretical distribution curve of a single substance coincided. This highly purified material was then chromatographed on silicic acid to yield an acidic oil which gave a crystalline S-benzylthiuronium salt which could be crystallized from acetone–alcohol mixtures as small needles or rosettes melting at 132–134° (uncor.). Samples of this salt gave the following analyses: C, 48.66, 47.33, 48.68; H, 6.49, 5.62, 6.33; S, 23.84, 23.82; N, 7.57, 6.60.

The sulfur–nitrogen ratios show that protogen-B itself contains sulfur, and analyses of the free acid confirmed the presence of about 30% sulfur. The S-benzylthiuronium salt of protogen-B gave half-maximum growth for *Tetrahymena geleii* at 0.3 millimicrogram per ml. of culture medium; the potency of the salt thus corresponded to about 2.8 millimicrograms per "unit."[2] The salt also had biological activity corresponding to that of the "acetate factor" and reversed the inhibitory effect of propionate for *S. faecalis*.[10]

(9) M. J. Johnson, *J. Biol. Chem.*, **181**, 707 (1949).

(10) F. P. Day, et al., *Bacteriological Proceedings*, Soc. of Am. Bacteriologists, p. 136 (1951).

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## CHEMICAL NATURE OF α-LIPOIC ACID

*Sir:*

α-Lipoic acid, which has been obtained in crystalline form from processed liver, will in catalytic amount replace the growth-stimulating activity of acetate for certain lactic acid bacteria, and is required for the oxidative decarboxylation of pyruvate by these bacteria.[1]

Analytical data on the α-lipoic acid obtained by one of us (L.J.R.) follows. Sample 1 (3 mg.): monocarboxylic acid, *pKa* 4.7, equivalent weight 224; m.p. 47.5–48.5°. Ultraviolet spectrum indicated absence of conjugated double bonds. X-Ray characterization has been reported.[1] Sample 2 (10 mg.): m.p. 47.5°; *pKa* 4.8; equivalent weight 220. X-Ray diffraction pattern identical with that of first sample. Spot tests[2] for sulfur positive, for nitrogen negative. Nitroprusside test for thiol negative, but positive after treatment with sodium cyanide, suggesting a disulfide linkage. *Anal.* C, 46.35; H, 6.79; S, 31.21. Calcd. for $C_8H_{14}S_2O_2$: C, 46.57; H, 6.84; S, 31.08. Polarographic determination revealed presence of easily reducible group which undergoes two electron change on reduction. The half wave potential at *p*H 6.7 was −0.59 v. *versus* saturated calomel electrode and −0.31 v. at *p*H 1.3.

Bioautographic[3] and countercurrent distribution[4] studies showed that several distinct compounds possessing acetate-replacing and pyruvate oxidation factor activity, in addition to α-lipoic acid, exist in biological preparations. That one or more of these compounds may be mixed disulfides formed from reduced α-lipoic acid and naturally occurring thiols has been demonstrated. Treatment of α-lipoic acid with glutathione, cysteine or several other thiols, followed by oxidation with iodine,[5] resulted in formation of a series of new compounds, as demonstrated by bioautographs,[3] possessing acetate-replacing factor activity.

Acid hydrolysates of natural materials contain, in addition to α-lipoic acid, a chemically related acidic substance which migrates less readily than α-lipoic acid both on paper chromatograms and from a buffered aqueous phase in countercurrent distributions.[3,4,6] In the preparation of bioautographs α-lipoic acid always is partially converted to this more polar substance, but the reverse transformation does not occur. This suggested that the more polar acid is an oxidation product of α-lipoic acid. It has been demonstrated that α-lipoic acid is converted to the former acid by means of hydrogen peroxide under conditions which would convert a sulfide to a sulfoxide.[7] Furthermore, the more polar acid is converted to α-lipoic acid by treatment with hydriodic acid under conditions used for conversion of a sulfoxide to a sulfide.[8]

(1) L. J. Reed, B. G. DeBusk, I. C. Gunsalus and C. S. Hornberger, Jr., *Science*, **114**, 93 (1951).

(2) F. Feigl, "Manual of Spot Tests," Academic Press, Inc., New York, N. Y., 1943, pp. 163, 172.

(3) L. J. Reed, *et al.*, *J. Biol. Chem.*, **192**, 851 (1951).

(4) I. C. Gunsalus, *et al.*, *ibid.*, in press.

(5) G. M. Brown and E. E. Snell, *Proc. Soc. Exptl. Biol. Med.*, **77**, 138 (1951).

(6) L. J. Reed, *et al.*, *J. Biol. Chem.*, **192**, 859 (1951).

(7) M. Gazdar and S. Smiles, *J. Chem. Soc.*, **93**, 1833 (1908).

(8) G. Toennies and T. F. Lavine, *J. Biol. Chem.*, **113**, 571 (1936).

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## THE LOWER OXIDATION STATES OF ALUMINUM[1]

*Sir:*

Evidence for the existence of the +2 and +1 oxidation states of aluminum includes demonstration of the existence of certain compounds prepared

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