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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