These resins are the first ones known to show a selectivity for sodium over potassium. This phenomenon and the subsequent inversion of the order of selectivity for the alkali cations as compared to sulfonic resins may be explained by a consideration of the polarizability of ionic groups and water as calculated by Teunissen and Bungenberg de Jong.[4] They found the order of polarizability to be: phosphate > water > sulfate. Since the order of polarizing ability of the alkali cations is Li > Na > K it would be expected that whereas in the sulfonic acid resins the volume sequence, as has been shown by Gregor, Gutoff and Bregman,[5] is Li > Na > K and consequently the order of preference for the alkali cations is K > Na > Li, in the case of the phosphonic resin the volume sequence should be K > Na > Li and the order of selectivity Li > Na > K. These volume and selectivity orders are in accord with values found in this laboratory for these resins and consequently the pressure-volume selectivity theory as expounded by Gregor[6] may be considered to apply to these systems when modified by the Teunissen–Bungenberg de Jong polarizability considerations. A detailed discussion of the experimental data together with the extension of this theory to carboxylic exchange resins will be given in a forthcoming paper.[7]

These resins show a volume increase of about 50% on going from the hydrogen to the sodium state. They are orange-yellow in the hydrogen state but show a striking color change to dark brown when placed in any of the alkali metal states.

Potential applications of phosphonous and phosphonic resins as a result of their unique properties include sodium depletion in physiological applications, rare earth separations, and use in mixed bed and reverse demineralization units.

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(5) H. P. Gregor, Fradelle Gutoff, and J. I. Bregman, *J. Colloid Science*, **6**, 3, 245 (1951).
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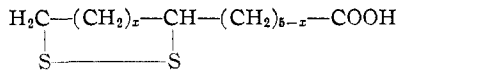
STRUCTURE OF PROTOGEN-A

Sir:

An unidentified growth factor for *Tetrahymena geleii* was first described in 1944 by Kidder and Dewey.[1] Using the test described by these workers it was found that several substances would produce a response in the organism.[2] These factors were called the "protogens."[2,3] The isolation of the sulfur-containing compound protogen-B from liver was described recently.[3] This compound (I) upon titration with sodium hydroxide was found to have a molecular weight corresponding to about 230 on the basis of one carboxyl group per molecule. Elementary analysis indicated the presence of 8 carbon and 2 sulfur atoms. When saponified with an excess of sodium hydroxide, I was converted to another compound (II) which gave a positive nitroprusside reaction and which contained one –SH group as indicated by titration with iodine. Reduction of I with sodium borohydride yielded a third compound (III) which contained two –SH groups as shown by iodine titration. Mild oxidation readily converted III to a disulfide (IV) as indicated by the disappearance of the nitroprusside reaction and by its reappearance on treatment with cyanide. Protogen-A[3] also gave a nitroprusside reaction after treatment with cyanide. A band at 1040 cm.$^{-1}$, present in the infrared absorption spectrum of protogen-B and indicating a sulfoxide group, was absent from the spectra of IV and of protogen-A. Protogen-A and IV appeared to be identical as shown by biological activity, liquid–liquid countercurrent distribution, paper chromatography and infrared absorption spectra. The absence of C-methyl groups in I was indicated by a negative Kuhn–Roth determination. Upon treatment of I with Raney nickel,[4] octanoic acid was obtained and identified by means of its infrared absorption spectrum, its melting point, and the X-ray powder photograph of its S-benzylthiuronium salt. These findings showed the probability of the following structure for IV, I being presumed to be a



sulfoxide. By the use of molecular models, a stable ring could be constructed for $x = 2$. The name "thioctic acid" is proposed for this structure ($x = 2$), a sulfur-containing organic acid with 8 carbon atoms. The synthesis of DL-thioctic acid with biological activity corresponding to that of the "protogens"[2] and the "lipoic acids"[5] is described in another communication.[6] Numerical prefixes indicating the position of the carbon atom to which the secondary sulfur is attached may be used to designate compounds in this series with different values for x.

(1) G. W. Kidder and V. Dewey, *Biol. Bull.*, **87**, 121 (1944).
(2) E. L. R. Stokstad, *et al.*, *Arch. Biochem.*, **20**, 75 (1949).
(3) E. L. Patterson, *et al.*, THIS JOURNAL, **73**, 5919 (1951).
(4) R. Mozingo, *et al.*, *ibid.*, **65**, 1013 (1943).
(5) L. J. Reed, *et al.*, *Science*, **114**, 93 (1951).
(6) M. W. Bullock, *et al.*, THIS JOURNAL, **74**, 1868 (1952).

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RECEIVED DECEMBER 26, 1951

SYNTHESIS OF *DL*-THIOCTIC ACID

Sir:

Thioctic acid,[1] a compound with the biological activity of protogen, was synthesized as follows: furylacrolein was hydrogenated to 2-tetrahydrofurylpropanol (I) over Raney nickel.[2] I was converted to 2-tetrahydrofurylpropyl chloride (II) with thionyl chloride and II was converted to γ-(2-tetrahydrofuryl)-butyric acid (III).[3] III was converted to a mixture of 5-hydroxy-8-iodocaprylic

(1) J. A. Brockman, Jr., *et al.*, THIS JOURNAL, **74**, 1868 (1952).
(2) A. Hintz, *et al.*, *Ber.*, **76**, 676 (1943).
(3) H. Gilman and H. P. Hewlett, *Rec. Trav. Chim.*, **51**, 93 (1932).

acid (IV) and its lactone by cleavage of the tetrahydrofuran ring with a solution of potassium iodide in 95% phosphoric acid. The crude mixture containing IV and its lactone was heated at reflux for sixteen hours with 2 moles of thiourea and 1.5 moles of hydrobromic acid (34%) and the resultant reaction mixture was hydrolyzed with 0.5 *N* sodium hydroxide at 100° for 15 minutes. The solution was acidified and extracted with chloroform. The chloroform solution was then treated with a slight excess of aqueous potassium iodide–iodine solution, chloroform was removed by distillation and the residue was purified by chromatography on silicic acid to yield a yellow oil, neutralization equivalent = 208. The biological activity of the oil was approximately 20% that of protogen-A for *Tetrahymena geleii* and a species of *Corynebacterium*.[4] Oxidation of the oil with *t*-butyl hydroperoxide yielded a second biologically active compound with properties closely similar to those of protogen-B as measured by paper chromatography, solvent distribution and infrared studies. This compound gave a crystalline S-benzylthiuronium salt, m.p. 143 to 144°, calculated for $C_{16}H_{24}N_2S_3O_3$: C, 49.45; H, 6.23; N, 7.21; S, 24.76; found C, 49.81; H, 6.30; N, 7.31; S, 25.39; C-methyl, negative. The position of the secondary sulfur atom cannot be stated unequivocally, as migration of the hydroxyl group has been shown to occur in aliphatic hydroxy-acids when treated with heat and acid.[5]

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

(5) E. E. Blaine and A. Kohler, *Compt. rend.*, **148**, 1772 (1909).

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THE BIOSYNTHESIS OF SQUALENE AND CHOLESTEROL[1]

Sir:

Squalene from shark liver oil was shown to be a dihydrotriterpene in 1926.[2,3] Although the structure of cholesterol was then only incompletely known, the suggestion was made that squalene might be an intermediate in steroid biosynthesis.[2,4] Balance studies which were carried out gave conflicting results.[5,6] Work carried out with isotopic tracers during recent years has demonstrated that acetate is the principal carbon source of cholesterol.[7,8] The distribution of acetate carbon which was found in the cholesterol molecule, led to the suggestion that cholesterol biosynthesis might proceed via the condensation of isoprenoid units.[9] The data were also compatible with a cyclization of squalene to cholesterol as proposed by Robinson.[10]

It has now been shown that squalene is synthesized biologically from acetate, that squalene is absorbed from the gut, and that carbon from labeled squalene is efficiently incorporated into cholesterol. Rat tissues do not contain detectable quantities of squalene. However, when the hydrocarbon is fed, a small amount can subsequently be recovered from the liver and intestinal tract. Rats received in their diet 0.5 g. of squalene and 0.54 millicurie of 1-C^{14} acetate (0.125 g.) per 100 g. rat per day for two days. The combined nonsaponifiable fractions of the livers and intestinal tracts were chromatographed on alumina and "washed out" with normal cholesterol. This yielded 35 mg. of hydrocarbon, having a specific activity of 2080 c.p.m. A portion was diluted with purified natural squalene[11] and two isomeric hexahydrochlorides,[2] m.p. 108° and 144° were prepared. Corrected for dilution, these derivatives had specific activities of 2120 c.p.m. and 2040 c.p.m., respectively. This demonstrated that all of the radioactivity of the hydrocarbon fraction resided in the squalene. The remainder of this C^{14} squalene was fed to mice at a level of 10 micromoles of squalene per animal per day for two days. Cholesterol and fatty acids were isolated from tissues. Data from one of two identical experiments are shown in Table I.

TABLE I

FEEDING OF C^{14} SQUALENE, 2080 C.P.M.,[a] TO MICE

1. Liver and gut	C^{14}, c.p.m.	% of squalene C recovered	RIC[b]
Cholesterol digitonide	132[c]	4.2	6.4
Cholesterol dibromide	131		
Fatty acids	<2		
2. Carcass and viscera			
Crude steroids	34		2.1
Cholesterol digitonide	43[c]	3.9	
Cholesterol dibromide	42		
Fatty acids	0		
		8.1	

[a] All C^{14} values expressed as c.p.m. of infinitely thick $BaCO_3$ samples. [b] RIC = (c.p.m. of cholesterol/c.p.m. of squalene fed) × 100. [c] Calculated for free cholesterol.

Comparison with earlier results indicates that the utilization of squalene carbon for cholesterol formation is 10–20 times as efficient as that of acetate.[7,8] It is also more than three times as efficient as that of isovaleric acid,[12] until now the most efficient carbon source of cholesterol. The percentage recovery of squalene carbon in cholesterol is based on the total amount fed. Since squalene is not quantitatively absorbed from the gut,[5] this figure (8%) represents a minimal value. The insignificant isotope concentration in the fatty acids precludes the possibility that squalene was

(1) Supported by a grant from the Life Insurance Medical Research Fund.

(2) I. M. Heilbron, E. D. Kamm and W. M. Owens, *J. Chem. Soc.*, 1630 (1926).

(3) I. M. Heilbron, T. P. Hilditch and E. D. Kamm, *ibid.*, 3131 (1926).

(4) H. J. Channon, *Biochem. J.*, **20**, 400 (1926).

(5) H. J. Channon and G. R. Tristram, *ibid.*, **31**, 738 (1937).

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(9) J. Würsch, R. L. Huang and K. Bloch, *ibid.*, in press.

(10) R. Robinson, *J. Soc. Chem. Ind.*, **53**, 1062 (1934).

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