with the finding by Lotspeich[4] that pantothenic acid deficiency symptoms in rats may be accentuated on a high-fat diet, have prompted us to investigate the effect of pantothenate upon fatty acid oxidation.

Wistar rats at 28 days of age were placed on a purified diet[5] containing 9% fat. During the first two weeks on the diet, pantothenic acid was removed from all animals. Thereafter a division was made into deficient and control groups, with the latter receiving 20 mg. of pantothenic acid per kg. of diet. After three to five additional weeks on the above regimen, the deficient animals weighed 50 to 75 grams (about 30–40%) less than the controls, and often exhibited bloody whiskers and "whisky noses."

In the various experiments, livers from three deficient or two normal animals were homogenized with an equal weight of 0.9% cold KCl for 45 to 90 seconds at *p*H 6.8, and examined for their ability to oxidize fatty acids.

As may be seen from Table I, the oxidation of caproate in the deficient samples was less than half of that observed for the controls. Statistical treatment of the data showed that the oxidation of caproate in the controls was not significantly different from 100%, whereas in the deficients the mean value lay between 12 and 40% (confidence coefficient = 0.95). The difference between the two groups was found to be significant beyond the 1% level ($F = 29.97$; a value of 8 plus or higher is needed at this level). Preliminary experiments with rat liver mitochondria (cyclophorase at the third residue state[6]) revealed similar trends to those observed for homogenates, although the differences became pronounced only when higher levels of caproate (40 μ moles) were used per flask. This is probably due to the much higher mitrochondrial density in the cyclophorase preparations, so that with less than 20 μ moles of caproate the latter becomes the limiting factor for oxidation.

Preliminary results with butyrate oxidation were similar to those described for caproate. Extension of these studies is being made to include the effect of pantothenate deficiency upon oxidation within the citric acid cycle. Details of this and other aspects of oxidation by rat liver systems will be presented elsewhere.

TABLE I

OXIDATION OF CAPROATE BY RAT LIVER HOMOGENATES

Each flask contained 1 ml. of liver homogenate, 0.1 ml. of 0.1 *M* caproate at *p*H 7.2, 0.2 ml. of 0.1 phosphate buffer of *p*H 7.2, 0.1 ml. of 0.1 *M* adenylic acid, 0.2 ml. of 0.02 *M* $MgCl_2$, 0.1 ml. of 7×10^{-3} *M* cytochrome C. Final volume 3 ml.; alkali in center well; oxygen in gas phase; temperature = 37°.

	Caproate oxidized,[a] %	
	Normal animals	
Expt.	1[b]	2[c]
1	82	128
2	115	90
3	23	124
4	74	67
	Pantothenate deficient animals	
1	18	46
2	0	10
3	39	4
4	0	21
5	18	74
6	13	59
7	26	10

[a] Theoretical oxygen consumption by caproate = 8 atoms per mole.[7] [b] In presence of 1 mol α-ketoglutarate per flask. [c] In presence of 2 mols α-ketoglutarate per flask.

(4) W. D. Lotspeich, *Proc. Soc. Exptl. Biol. Med.*, **73**, 85 (1950).

(5) T. E. King, F. M. Strong and V. H. Cheldelin, *J. Nutrition*, **42**, 195 (1950).

(6) D. E. Green, W. F. Loomis and V. Auerbach, *J. Biol. Chem.*, **172**, 389 (1948).

(7) V. H. Cheldelin, I. S. Mirviss and D. E. Green, Abstracts, 118th Meeting of Am. Chem. Soc., Atlantic City, 5C (1950).

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RECEIVED AUGUST 13, 1951

A NEW METHOD FOR THE CONVERSION OF NITRILES TO ALDEHYDES[1]

Sir:

In the course of an investigation of a synthesis of methionine-α-C^{14}, it was desired to prepare β-methylmercaptopropionaldehyde from cyanide-labeled β-methylmercaptopropionitrile. The Stephen reduction[2,7] and direct reduction with lithium aluminum hydride[3] gave unsatisfactory results. This aldehyde was prepared in good yield from the corresponding nitrile by a procedure involving a hitherto unreported reaction of lithium aluminum hydride, the reduction of an ortho ester to an acetal.

The methyl and ethyl esters of ortho-β-methylmercaptopropionic acid were obtained from β-methylmercaptopropionitrile[4] according to McElvain's procedure.[5] Methyl ester, 57.6% yield, b.p. 51–52° (1 mm.), (calcd. for $C_7H_{16}O_3S$: C, 46.59; H, 8.89. Found: C, 46.98; H, 8.68.). Ethyl ester, 65.5% yield, b.p. 71–72° (0.8 mm.), (calcd. for $C_{10}H_{22}O_3S$: C, 53.98; H, 9.90. Found: C, 54.35; H, 9.89). The following method was then used to reduce the ortho esters to the corresponding acetals: One quarter of a molar equivalent of lithium aluminum hydride (1 *M* ether solution) was added to a boiling solution (0.33 *M*) of the ortho ester in benzene. The mixture was refluxed four hours. The complex was decomposed with Rochelle salt solution (30%) and the benzene extract was dried and distilled.

Both the methyl and ethyl acetals of β-methylmercaptopropionaldehyde were obtained in good yield. Dimethyl acetal, 97% yield, b.p. 73° (0.9 mm.), (calcd. for $C_6H_{14}O_2S$: C, 46.98; H, 9.33. Found: C, 47.11; H, 8.95). Diethyl acetal, 73% yield, b.p. 68–74° (0.7 mm.), (calcd. for $C_8H_{18}O_2S$: C, 53.84; H, 10.12. Found: C, 54.13; H, 9.81). The acetals are readily hydrolyzed to β-methylmercaptopropionaldehyde.[6] The 2,4-di-

(1) This work was supported by Contract N6ori-126, Task VIII-B with the Office of Naval Research, United States Navy.

(2) H. Stephen, *J. Chem. Soc.*, **127**, 1874 (1925).

(3) L. Friedman, Abstracts, 116th Meeting American Chemical Society, Atlantic City, N. J., 1949, p. 5 M.

(4) S. Akabori, T. Kaneko and S. Matizuki, *J. Chem. Soc. Japan*, **59**, 1136 (1938).

(5) S. M. McElvain and J. W. Nelson, THIS JOURNAL, **64**, 1824 (1942).

(6) G. Barger and F. P. Coyne, *Biochem. J.*, **22**, 1417 (1928).

nitrophenylhydrazone (m.p. 119.5–120.5°) was prepared from each acetal. Mixed melting points with an authentic sample[7] showed no depression.

Apparently this reduction is of general applicability. The orthoformic, orthoacetic, and orthovaleric ethyl esters, prepared from the nitriles, were converted to the corresponding acetals in good yields.

An article giving detailed results of our experiments will be submitted in the near future.

(7) *Cf.* C. D. Hurd and L. L. Gershbein, THIS JOURNAL, **69**, 2328 (1947).

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ACID TRANSFORMATION PRODUCTS OF LEUCOVORIN

Sir:

Communications from these laboratories[1] have described the synthesis of a formyltetrahydropteroylglutamic acid, leucovorin (I), with activity for *Leuconostoc citrovorum* 8081.[2] From our chemical studies on the pure crystalline vitamin, we have concluded that the probable structure of leucovorin is 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid.[3] During this investigation we have isolated and characterized several acid transformation products of I.

At *p*H 1.3 or below, leucovorin yielded isoleucovorin chloride (II), repeatedly crystallized by solution in 12 *N* hydrochloric acid and dilution to 2 *N;* boat-shaped crystals; n_L, 1.508 ± 0.004; n_S, 1.84 ± 0.01; m.p. 250–251° (dec.). *Anal.* Calcd. for $C_{20}H_{22}N_7O_6Cl$: C, 48.8; H, 4.51; N, 19.9; Cl, 7.21; CHO, 5.90.[4] Found: C, 49.4; H, 4.73; N, 20.2; Cl, 7.32; CHO, 5.74 (corrected for 6% water, Karl Fischer titration). Crystallization of II at *p*H 2 yielded anhydroleucovorin-A (III), hair-like needles from 0.01 *N* hydrochloric acid; parallel extinction: n_S, 1.63; n_L, 1.87; oblique extinction (30°): n_S, 1.48; n_L, >1.90; m.p. (anhydrous form) 250–257° (dec.). *Anal.* Calcd. for $C_{20}H_{21}N_7O_6 \cdot 4H_2O$: C, 45.5; H, 5.42; N, 18.6; CHO,[4] 5.50; H_2O, 13.7. Found: C, 45.4; H, 5.44; N, 18.6; CHO, 5.68; H_2O, 13.4. II or III at *p*H 4 (hot) gave anhydroleucovorin-B (IV), tablets; n_S, >1.90; n_L, 1.57; anhydrous form, non-melting below 330°. *Anal.* Calcd. for $C_{20}H_{21}N_7O_6 \cdot 1/2H_2O$: C, 51.7; H, 4.78; N, 21.1; CHO,[4] 6.25; H_2O, 1.94. Found: C, 51.7; H, 4.68; N, 21.1; CHO, 6.22; H_2O, 1.6 (by Karl Fischer titration). In 0.1 *N* hydrochloric acid II, III and IV exhibit a maximum at 355 mμ (T = 35 ± 1%, 10 mg./liter); in anaerobic sodium hydroxide solution each is converted to leucovorin in high yield.

(1) J. A. Brockman, Jr., *et al.*, THIS JOURNAL, **72**, 4325 (1950); B. Roth, *et al.*, *ibid.*, in preparation.

(2) H. E. Sauberlich and C. A. Baumann, *J. Biol. Chem.*, **176**, 165 (1948).

(3) W. Shive, *et al.*, proposed the structure 5-formyl-5,6,7,8-tetrahydropteroylglutamic acid for "folinic acid-SF"; 119th meeting, American Chemical Society, Boston, Mass., April, 1951.

(4) Formic acid is liberated by the drastic acid hydrolysis in the usual analysis for the formyl group.

Chemical and physical evidence indicate that II, III and IV are representatives of a new class of tetrahydropteroylglutamic acid derivatives, in which an imidazoline or imidazolidine ring has been formed linking the N^5- and N^{10}-positions by a single carbon bridge derived from the formyl group. Such ring compounds may be intermediates involved in the synthesis of leucovorin.[1]

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CATION EXCHANGE EQUILIBRIA

Sir:

In a recent valuable paper on cation exchange equilibrium, Lowen and associated authors[1] discuss a paper by Krishnamoorthy, Davis, and Overstreet.[2] Apparently two misconceptions are involved in this discussion. (a) The authors state "It should be mentioned, however, that the arbitrary substitution of equivalent fraction for mole fraction in the resin phase, in the equilibrium expression given by these authors (which may possibly be justified on the basis of the statistical nature of the exchange of ions among resin sites), results in decreased apparent hysteresis and more nearly constant values of K_a."

It should be pointed out that the terms used in the equation derived by Davis for the paper in *Science* represent moles (or, statistically, ions) and not equivalents (or, statistically, individual charges). This situation is more clearly represented in a detailed paper by Davis.[3]

(b) The work reported in THIS JOURNAL involved basic cation–hydrogen ion exchanges. Both in the *Science* paper cited and in another paper by Davis[4] it is shown that the exchange "constant" is probably not invariant for exchanges involving hydrogen ions. The statistical theory applies only to completely dissociated ions, so that the ion-exchanger bond strength is not a function of the concentration (at least to a first approximation).

I should like to comment that probably all successful attempts to formulate theoretical ion exchange equations represent only approximately adequate working hypotheses, which may have considerable heuristic value in relation to physical intuition. It is doubtful that any such equations are completely adequate. We may except, of course, those expressed in terms of activities, but, as Lowen and his group point out, this result is unavoidable in view of the circular dependency of K_a and the a values.

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(1) W. K. Lowen, R. W. Stoenner, W. J. Argersinger, Jr., A. W. Davidson and D. N. Hume, THIS JOURNAL, **73**, 2666 (1951).

(2) C. Krishnamoorthy, L. E. Davis and R. Overstreet, *Science*, **108**, 439 (1948).

(3) L. E. Davis, *J. Colloid Science*, **5**, 71 (1949).

(4) L. E. Davis, *ibid.*, **5**, 107 (1950).