

(0.3 μ); m.p. 77–77.5°; $[\alpha]_D -10.5^\circ$, 5% in chloroform (calcd. for $C_9H_{17}NO_4$: C, 53.18; H, 8.43; N, 6.89; OCH_3 , 15.27. Found: C, 53.23; H, 8.52; N, 7.31; OCH_3 , 15.27).

The extreme ease of dehydration of Elaiomycin accompanied by a shift of λ_{max} from 237.5 m μ to 260 m μ , along with infrared spectral evidence of an O to N acyl migration during the evaporative distillation of II-acetate, indicated the –OH to be on a carbon atom adjacent to that containing the nitrogen in the C_5 moiety.

Since only one C–CH₃ group could be in the C_5 moiety and since Elaiomycin gave a positive iodoform test, the C_5 fragment was constructed as $CH_3-CHOH-CH(N=)CH_2OCH_3$, and this structure was confirmed by the synthesis of a diastereoisomeric mixture of the acetate-amide III, the infrared spectrum of which was essentially identical to that of III.

TABLE I

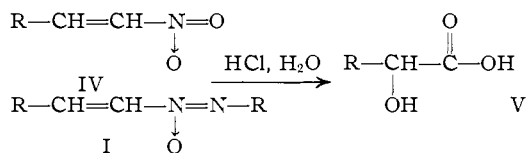
PURIFICATION OF MALATE SYNTHETASE

The complete assay system contained 10 μ moles of acetyl phosphate, 2 μ moles of cysteine, 10 μ moles of $MgCl_2$, 15 μ g. of coenzyme A, 25 μ moles of potassium glyoxylate, 10 units of transacetylase, 30 μ moles of TRIS buffer, pH 8.0 and enzyme, final volume, 3.0 ml.; incubation time, 15 min.; temperature, 33°.

Fraction	Total volume, ml.	Units ^a /ml.	Total units	Protein, mg./ml.	Specific activity, units/mg./protein	Recovery, %
1 Crude extract	50.0	21.6	1890	26.7	0.81	100
2 $(NH_4)_2SO_4$ fraction (0.35–0.70 saturation)	29.0	53.6	1555	27.9	1.92	82
3 Pr– SO_4 supernatant ^b	33.5	42.6	1426	11.7	3.64	76
4 Calcium phosphate gel ^c (supernatant)	68.0	10.7	728	0.52	20.58	38
5 $(NH_4)_2SO_4$ fraction (0.40–0.60 saturation)	33.0	17.9	581	0.47	37.90	31

^a A unit is defined as the amount of enzyme required to catalyze the disappearance of 1 μ mole of acetylphosphate per 15 min. under standard conditions. ^b 0.1 mg. protamine sulfate per mg. protein at pH 6.7. ^c 1.5 mg. $Ca_3(PO_4)_2$ gel added per mg. protein at pH 5.0.

The assignment of the N-oxide oxygen atom is based on aqueous acid hydrolysis of I to α -hydroxyoctanoic acid (V, R = $n-C_6H_{13}$) in 54% yield. We believe the carbon adjacent to the quaternary nitrogen to be oxidized⁴ as is the oxygen analog IV under the same conditions.⁵ Also, the ultraviolet curve of I (λ_{max} 237.5) is similar to that of IV⁶ (λ_{max} 235) while the alternate structure, $\text{>C=C-N=N(}\rightarrow\text{O)-}$, would be expected to absorb at a longer wave length.⁷ This work con-



clusively places the carbon to carbon double bond in the C_8 moiety, allows the assignment of the N-oxide oxygen to the nitrogen atom nearest the double bond, and completes that portion of the structure proof based on degradation experiments.

(4) J. N. Brough, B. Lythgoe and P. Waterhouse, *J. Chem. Soc.*, 4069 (1954).

(5) R. L. Heath and J. D. Rose, *ibid.*, 85 (1947).

(6) E. A. Braude, E. R. H. Jones and G. G. Rose, *ibid.*, 1104 (1947).

(7) J. N. Brough, B. Lythgoe and P. Waterhouse, *ibid.*, 4069 (1954).

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CONVERSION OF ACETATE AND GLYOXYLATE TO MALATE¹

Sir:

An enzyme has been obtained from acetate-grown *Escherichia coli*, strain E-26, which converts equimolar concentrations of acetate and glyoxylate to malic acid. This enzyme has been tentatively designated as *malate synthetase*.

Purification of malate synthetase by several ammonium sulfate precipitations, calcium phosphate gel and protamine sulfate treatments, resulted in a 50-fold purification with an approximate 30% yield (Table I). These preparations were found to be free of fumarase as measured by the sensitive spectrophotometric method of Racker.² Experiments conducted with these fumarase-free preparations revealed that for each μ mole of acetyl phosphate and glyoxylate disappearing, one μ mole of malate is formed (Table II). In addition

to the substrates, transacetylase,³ coenzyme A, magnesium ions and cysteine were added. In the absence of acetyl phosphate, coenzyme A, glyoxylate, transacetylase, malate synthetase or boiled enzyme controls, no malate is formed.

TABLE II

STOICHIOMETRY OF THE MALATE-SYNTHETASE REACTION

The reaction mixture contained 15 μ moles of acetyl phosphate, 15 μ moles of potassium glyoxylate, 30 μ moles of TRIS buffer, pH 8.0, 20 μ moles of $MgCl_2$, 2 μ moles of cysteine, 25 μ g. of coenzyme A, 10 units of transacetylase, and 260 μ g. of enzyme; final volume, 2.0 ml.; temperature of reaction 33°; incubation time, 30 min.

Expt.	Acetyl phosphate, μ moles			Glyoxylate, μ moles			Malate formed, μ moles
	Initial	Final	Δ	Initial	Final	Δ	
1	15.00	7.72	7.28	15.00	6.45	8.55	7.56
2	15.00	7.84	7.16	15.00	6.85	8.15	7.33

Malate was routinely estimated by the fluorometric method of Hummell.⁴ In large scale ex-

(1) A preliminary report of part of this investigation was presented at the national meetings of the Society of American Bacteriologists at Houston, Texas, and a summary published in *Bact. Proc.*, pp. 111 (1956).

(2) E. Racker, *Biochim. et Biophys. Acta*, **4**, 20 (1950).

(3) The source of transacetylase used in the assay system was a partially purified preparation from *E. coli*, American Type Culture Collection No. 4157, free of fumarase and malate synthetase enzymes.

(4) J. P. Hummell, *J. Biol. Chem.*, **180**, 1225 (1949).

periments, the product of the malate synthetase reaction was isolated by paper chromatography and shown to have identical R_f 's in several solvent systems as authentic malic acid. Malate was further characterized by use of the malate decarboxylating enzyme obtained from *Lactobacillus casei*⁵ and by partially purified preparations of fumarase obtained from *E. coli*.

The precise mechanism of the reaction is under investigation.

(5) M. L. Blanchard, S. Korke, A. del Campillo and S. Ochoa, *J. Biol. Chem.*, **187**, 875 (1950).

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BOOK REVIEWS

Progress in Low Temperature Physics. Volume I. Edited by C. J. GORTER, Professor of Experimental Physics, Director of the Kamerlingh Onnes Laboratory, Leiden. Interscience Publishers, Inc., 250 Fifth Avenue, New York 1, N. Y. 1955. xii + 418 pp. 16 × 23 cm. Price, \$8.75.

When the scale of absolute temperature was first proposed by Kelvin, the concept of an absolute zero as the ultimate point of zero thermal energy implied, superficially at least, that the region of very low temperatures was a kind of grave-yard where nothing happened. Thus, it was quite a shock to learn through the discovery by Kamerlingh Onnes of superconductivity with its frictionless circulation of electrons that in this realm of eternal atomic rest there appeared the closest thing yet found to perpetual motion. This initial paradox has been followed in the course of years by a series of others in some ways even more startling; such as, absolute diamagnetism and the superfluidity of helium. So today we see clearly that in the region of very low temperature we have one of the great frontiers of science across which there is a vista of one of the strangest domains of phenomena known to man. How far removed it is from familiar ground can be seen by considering that high temperatures commonly used in the laboratory lie above room temperature only by a factor of ten, while the lowest temperatures now available through adiabatic demagnetization represent a decrease on the absolute scale by a factor of ten thousand.

It is fitting that this review of low temperature progress should be initiated at Leiden and edited by Professor Gorter, the present director of the Kamerlingh Onnes Laboratory where the first great spear-head of exploration in this field was launched; and the quality of the book is on a par with the quality of the work which has made Leiden the capital of the cryogenic realm. There are eighteen articles, each by an outstanding authority, with the topics selected to provide discussion of the major fields of interest, and the treatment planned to give an integrated presentation with a minimum of duplication. This, of course, brings forth summaries, some aimed almost exclusively at theory, some almost entirely on experimental developments, some in between, and all filling an unusual need, because the activity in both the theoretical and experimental phases of low temperatures is intense; and there has been no adequate general review for a long time.

The titles and authors of the articles give the best brief summary possible of the scope of the book:

- I. C. J. Gorter, The Two Fluid Model for Superconductors and Helium II
- II. R. P. Feynman, Application of Quantum Mechanics to Liquid Helium
- III. J. R. Pellam, Rayleigh Disks in Liquid Helium II
- IV. A. C. Hollis Hallett, Oscillating Disks and Rotating Cylinders in Liquid Helium II
- V. E. F. Hammel, The Low Temperature Properties of Helium Three
- VI. J. J. M. Beenakker and K. W. Taconis, Liquid Mixtures of Helium Three and Four
- VII. B. Serin, The Magnetic Threshold Curve for Superconductors
- VIII. C. F. Squire, The Effect of Pressure and of Stress

- IX. T. E. Faber and A. B. Pippard, Kinetics of the Phase Transition in Superconductors
- X. K. Mendelssohn, Heat Conduction in Superconductors
- XI. J. G. Daunt, The Electronic Specific Heat in Metals
- XII. A. H. Cooke, Paramagnetic Crystals in Use for Low Temperature Research
- XIII. N. J. Poulis and C. J. Gorter, Antiferromagnetic Crystals
- XIV. D. DeKlerk and M. J. Steenland, Adiabatic Demagnetization
- XV. L. Neel, Theoretical Remarks on Ferromagnetism at Low Temperatures
- XVI. L. Weil, Experimental Research on Ferromagnetism at Very Low Temperatures
- XVII. A. Van Itterbeek, Velocity and Absorption of Sound in Condensed Gases
- XVIII. J. De Boer, Transport Phenomena in Gases at Low Temperatures

Considering the current interest in the theory (superconductivity and superfluidity are still unsolved riddles) it is a pity that a few of the exceptionally good articles like Feynman's could not have been made two or three times as long. It would have been good to include also at least one article on the basic meaning of low temperature *per se*. It would also have been good to have a special discussion of the bearing of recent cryogenic work on quantum statistics, especially that part closely associated with the Third Law of Thermodynamics and of special interest for physical chemistry. But, since as Gorter says, low temperature is concerned with at least some paragraphs in every chapter of physics, one cannot ask for completeness short of a multi-volume handbook. For that reason it is to be hoped that future volumes of this new series will appear regularly and frequently.

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Small-Angle Scattering of X-Rays. By ANDRÉ GUINIER, PROFESSOR, Université de Paris (France), and GÉRARD FOURNET, LECTURER, Ecole Supérieure de Physique et Chimie, Paris. Translation by Christopher B. Walker, Institute for the Study of Metals, University of Chicago. Followed by a bibliography by Kenneth L. Yudowitch, Johns Hopkins University. Structure of Matter Series. Maria Goeppert Mayer, Advisory Editor. John Wiley and Sons, Inc., 440 Fourth Avenue, New York 16, N. Y. 1955. vii + 268 pp. 15.5 × 23.5 cm. Price, \$7.50.

This is the first book in a recently developed branch of X-ray analysis which promises to be of considerable interest, particularly to chemists, biologists and metallurgists. The earliest quantitative work was done about 1939 by Professor Guinier, the senior author. Since then several hundred papers have appeared and instrumental and interpretive techniques have developed to the point where attention can