Synthesis of (±) Methyl Fluoromalate

THE observations of Peters *et al.*[1] that the administration of fluorocitrate resulted in the inhibition of the tricarboxylic acid cycle suggested the synthesis of other fluorinated acids of biochemical interest. In this preliminary communication, we wish to report the synthesis of (±) methyl fluoromalate and fluoromalamide.

Ethyl ethoxalylfluoroacetate, prepared according to Rivett's method[2], was reduced with potassium borohydride (two equivalents) in absolute methanol with vigorous stirring. The temperature of the reaction mixture rose to 50° during the reaction, which was complete after 90 min. Excess dry methanolic hydrogen chloride was added and the filtered solution distilled with repeated addition of methanol to remove methyl borate. The remaining solution was neutralized with lead carbonate, filtered and dried (sodium sulphate). Removal of the solvent furnished (±) methyl fluoromalate (transesterification having taken place during the reactions) as a colourless viscous liquid. The ester gave negative tests for carbonyl groups, a positive test for hydroxy groups and had the following microanalytical composition: C, 40·0; H, 5·8 per cent. $C_6H_9O_5F$ requires C, 40·1; H, 5·1 per cent.

The reduction of ethyl ethoxalylfluoroacetate with lithium aluminium hydride and aluminium *iso*propoxide was also attempted; but preliminary experiments gave much poorer yields of the ethyl fluoromalate.

(±) Methyl fluoromalate was identified by converting it into the diamide with saturated methanolic ammonia for 48 hr. The fluoromalamide was recrystallized from *iso*propyl alcohol (m.p. 160° uncorr.). A mixed melting point with malamide[3] (m.p. 162° uncorr.) gave a depression (m.p. 130° uncorr.). Fluoromalamide exhibited an absorption band at 1,050 cm.$^{-1}$ which was attributed to the C–F bond and gave the following micro-analytical results: found: C, 32·1; H, 4·4; N, 18·8 per cent. $C_4H_7O_3N_2F$ requires C, 32·0; H, 4·65; N, 18·7 per cent.

The results of biochemical assay[4] and details of this work will be published elsewhere. We thank Dr. E. M. Gal for his advice and interest in these experiments.

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[1] Peters, R. A., Wakelin, R. W., and Buffa, P. *Biochem. J.*, Proc. xiii, **50** (1952).
[2] Rivett, D. E., *J. Chem. Soc.*, 3710 (1953).
[3] Claisen, *Ber.*, **47**, 2031 (1914).
[4] Gal, E. M., Peters, R. A., and Wakelin, R. W., *Proc. Biochem. Soc.* (to be published).

Reversal and Mechanism of Oxidative Phosphorylation

THE importance of reactions in which electron transport from reduced pyridine nucleotides to oxygen is coupled with the formation of adenosine triphosphate from adenosine diphosphate and inorganic phosphate is well recognized by biochemists. Although much pertinent information has been accumulated, no clear picture of the sites and mechanism of uptake of inorganic phosphate coupled with electron transport has yet been obtained. This communication gives some results of exchange reactions of inorganic phosphate labelled with phosphorus-32 and oxygen-18 which may give some additional insight concerning oxidative phosphorylation, together with a brief presentation of a hypothesis for the mechanism of coupling of electron transport with inorganic phosphate uptake.

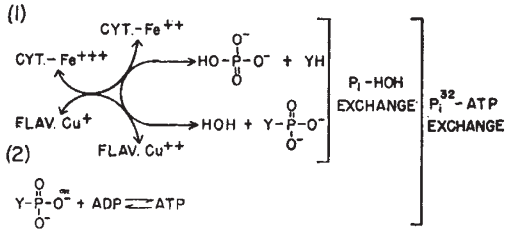
EXCHANGE REACTIONS CATALYSED BY LIVER MITOCHONDRIA IN THE ABSENCE OF ADDED SUBSTRATE

Oxygen uptake	<0·05	μM
Inorganic phosphate (phosphorus-32) – ATP exchange		
Total phosphorus-32 added	3,670	c.p.m.
Total phosphorus-32 found in ATP	820	c.p.m.
Amount of reaction, $^{32}Pi \rightleftharpoons$ ATP	14	μmoles
Inorganic phosphate – oxygen-18 exchange		
Initial atom % excess oxygen-18 in inorganic phosphate	0·278	
Final atom % excess oxygen-18 in inorganic phosphate	0·063	
Amount of reaction, $Pi - {}^{18}O \rightleftharpoons$ HOH	280	μ atoms

Each Warburg flask contained 21 μM K^+ – ATP, 21 μM $MgSO_4$, 150 μM KCl, 150 μM sucrose, 45 μM inorganic phosphate (phosphorus-32 or oxygen-18) and washed liver mitochondria from fasted rats from 0·5 gm. fresh liver. Flasks were incubated 5 min. at 23° before addition of the inorganic phosphate-32 and measurement of oxygen uptake. Reaction time, 15 min.

Various considerations suggested that oxidative phosphorylation under suitable conditions might be a demonstrably reversible process. Prominent among these was the unexplained observation of Cohn[1] that in the presence of tissue preparations carrying out oxidative phosphorylation the oxygen of phosphate was rapidly exchanged with that of water. Subsequent experiments reported briefly herein have shown that liver mitochondria preparations in the absence of added substrate and net oxygen uptake will catalyse a rapid exchange of inorganic phosphate–^{32}P with adenosine triphosphate accompanied by a very extensive exchange of the oxygen atoms of inorganic phosphate as measured with oxygen-18. Results of a typical experiment are shown in the accompanying table. Although there was little net change in the concentrations of adenosine triphosphate and inorganic phosphate, extensive exchange had occurred and the two substances appeared to be in equilibrium. The total micromoles of inorganic phosphate which was incorporated into and released from adenosine triphosphate was calculated on the basis of the expected exponential approach to isotopic equilibrium, with the assumption that two phosphate groups of the adenosine triphosphate were available for equilibration with inorganic phosphate.

The exchange of approximately twenty atoms of phosphate oxygen for each atom of inorganic phosphate incorporated into adenosine triphosphate shows clearly that reversal of the overall reaction $Pi + ADP \rightleftharpoons ATP + H_2O$ could account for only one-twentieth of the oxygen exchange observed. The demonstration of the reversibility of the overall reaction does suggest, however, a satisfactory explanation for the observed oxygen exchange. If, as depicted in the accompanying scheme, the initial reaction or reactions of oxidative phosphorylation accompanying electron transport involve loss of an oxygen from inorganic phosphate, reversal of these



An explanation of ^{32}Pi—ATP and Pi—HOH exchange. Overall: ADP + $^{32}Pi \rightleftharpoons$ ATP + HOH

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