ACETOKINASE REACTION IN SEVERAL PLEUROPNEUMONIA-LIKE ORGANISMS.*

Jaime Castrejon-Diez, Thelma N. Fisher** and Earl Fisher Jr.

Dept. of Microbiology and Dept. of Ophthalmology,
Tulane School of Medicine, New Orleans, Louisiana.

Received October 18, 1962

Recent studies on the lipid metabolism of pleuropneumonia-like organisms (PPLO) have shown the incorporation of C^{14} acetate into sterols. In particular, Mycoplasma laidlawii B was found to incorporate acetate into a carotenoid pigment (Smith and Rothblat, 1962). In addition to these observations several saturated and unsaturated fatty acids, 10 to 18 carbons in chain length as identified by gas chromatography (O'Leary, 1962), have been shown to accumulate in one PPLO strain. Furthermore, metabolic studies whereby gas exchange is measured in the Warburg respirometer have indicated that certain PPLO's oxidize organic substrates such as ethanol and short chain fatty acids (Lynn, 1960). Such data lend support to the hypothesis that lipid metabolism may be very important to these organisms. The present paper reports some work done in the study of the early steps in the incorporation of acetate into biosynthetic pathways by the Mycoplasma. Since phosphorylation of acetate is known to be the first step in the incorporation of acetate into lipids, it seemed reasonable to first inquire as to whether or not this reaction is operative or not in PPLO's. The study of such enzymatic activities by pleuropneumonia-like

* This investigation was supported by a United States Public Health Service Research Grant (E-2891).

** Research Career Development Awardee of the U.S.P.H.S.

organisms might provide additional information when comparing metabolic steps of these organisms with those of other microbial forms. Other observations to be published elsewhere regarding energy-yielding processes of saprophytic and pathogenic PPLO's suggest that certain well documented PPLO strains strongly resemble various bacterial types metabolically. Phosphorylation of acetate by ATP with kinase mechanisms has frequently been found in bacteria though its specificity varies. Rose et al. (1954) found that the enzyme from E. coli was specific for acetate, although propionate serves as substrate to a limited extent; the purified enzyme of Clostridium butyricum (Twarog and Wolfe, 1962) phosphorylates butyrate and propionate at the same rate, isobutyrate at a slower rate and acetate and valerate minimally. The presence of an acetokinase mechanism in several PPLO strains is presented in this paper.

Mycoplasma hominis, M. gallinarum, M. spumans and M. agalactiae obtained from Dr. D. G. Edward, Welcome Research Laboratories, England and M. laidlawii and M. gallisepticum provided by Dr. H. Adler, University of California at Davis were grown 36 hours at 37° in 2-liter batches of heart infusion broth (Difco) supplemented with 1% yeast extract (BBL) and 1% PPLO serum fraction (Difco). The cultures were centrifuged in the cold at 13,000 x g and washed once with saline. Cell-free extracts were prepared by adding 0.5% dodecyl sulfate (0.1 ml per 100 mg cells, wet weight) to cell suspensions; mixtures were held 15 minutes in an ice bath, then the extracts were dialyzed 24 hours in the cold using several changes of 0.02 M phosphate buffer pH 7. Acetokinase assays were measured over a 1 minute testing interval using 10 μmoles ATP, 300 μmoles acetate, 3 μmoles Mg^{++}, 700 μmoles neutralized hydroxylamine and 0.1 ml cell-free extract in a final volume of 1 ml. Hydroxamic

acids were determined by the method of Lipmann and Tuttle. The transfer of phosphate from acetyl-phosphate to ADP was studied using 8 μmoles acetyl-phosphate, 10 μmoles ADP, 3 μmoles Mg^{++}, tris buffer pH 7 and 0.1 ml cell-free extract. This reaction was stopped with 28% hydroxylamine and the hydroxamic acids measured as described above.

Of the 6 strains studied only M. hominis failed to form acetyl-phosphate while extracts from the other 5 species phosphorylated acetate at a rapid rate (Table I). The kinase reaction in most of these organisms

Table I.
ACETOKINASE REACTION IN SEVERAL PLEUROPNEUMONIA-LIKE ORGANISMS.

	Specific activity				
	M. laidlawii	M. gallinacum	M. gallisepticum	M. agalactiae	M. spumans
Optimum pH	7-7.5	6-6.5	6.0	6.5	6.0
Specific activity at optimum pH	5.7	6.6	2.4	6.7	4.4
Without enzyme	0	0	0	0	0
Without Mg^{++}	0	0	0	0	0.5
Mn^{++} instead of Mg^{++}	4.1	5.7	1.3	7.0	4.3

Specific activity = μmoles hydroxamic acid formed /min/mg protein
Assays were performed as described in the text.

was found to require Mg^{++} although this requirement could be replaced by Mn^{++}, while in the case of M. agalactiae activity with Mn^{++} was slightly higher than with Mg^{++}. The optimum pH for the reaction for the different strains varied from 6 to 7.5, but in all cases the range was broad. Extracts were tested from pH 3.5 to 9.8 and for all the pH values studied, the reaction could be demonstrated. The transfer of phosphate from

acetyl-phosphate to ADP was observed with the 5 strains (Table II) but not with M. hominis. The presence of CoASH did not increase the rate of disappearance of acetyl-phosphate from the reaction mixture. The disappearance of acetyl-phosphate was shown to be dependent on the presence of ADP, for in its absence the substrate did not decrease with the exception of M. laidlawii and M. gallinarum preparations where acetyl-phosphate did diminish slightly even though ADP was not added. In addition to the acetokinase enzyme it appeared that a non-specific phosphatase might be present in cell-free extracts of M. laidlawii and M. gallinarum.

Table II
TRANSFER OF PHOSPHATE FROM ACETYL-PHOSPHATE TO ADP.

	M. laidlawii	M. gallinarum	M. gallisepticum	M. agalactiae	M. spumans
	Specific activity				
Complete system	6.9	6.1	2.9	6.6	4.2
Minus enzyme	0.0	0.0	0.0	0.0	0.0
Minus ADP	2.4	0.3	0.0	0.0	0.0
Complete + CoASH	7.8	6.1	2.9	6.6	4.5

Results expressed as specific activity or μmoles acetyl-phosphate disappearing per min per mg protein.

Several acids besides acetate, namely propionate, butyrate, isobutyrate, beta-hydroxy-butyrate, valerate, isovalerate, hexanoate, heptanoate, succinate and malonate were tested to study the specificity of the reaction as carried out by these organisms. No reaction with the above substrates was demonstrated with extracts of M. laidlawii, M. gallisepticum, M. agalactiae and M. spumans. However extracts of M.

gallinarum showed less specificity and were able to phosphorylate propionate, acetate and butyrate effectively, in this order (Table III). From our experiments it is not possible at the present time to say whether these reactions are due to one or to several enzymes. Because of the difficulties in obtaining large yields of PPLO's, these studies have been made with crude cell-free extracts. The C. butyricum enzyme purified by Twarog and Wolfe (1962) is capable of phosphorylating several short chain fatty acids.

Table III
ENZYMATIC PHOSPHORYLATION OF ORGANIC ACIDS
BY EXTRACTS OF M. gallinarum.

Substrate	μmoles hydroxamic acid formed/min/mg protein
Acetate	5.3
Propionate	6.9
Butyrate	5.1
Valerate	4.0
Beta-hydroxy-butyrate	1.1
Isobutyrate	3.6
Isovalerate	2.1
Control (no substrate)	0.0

Observations regarding differences in biochemical activities of PPLO's may prove to be a useful approach in the study of the Mycoplasmatales, not only as a means of separating organisms taxonomically, but also to suggest ideas as to their relationship with bacteria and other microbial agents.

REFERENCES

Lipmann, F., and Tuttle, L. C. J. Biol. Chem. 159, 21 (1945).
Lynn, R. J. Ann. N. Y. Acad. Sci. 79, 538 (1960).
O'Leary, W. Biochem. Biophys. Research. Communs. 8, 87 (1962).
Rose, I. A., Grunberg-Manago, M., Corey, S. R., and Ochoa, S. J. Biol. Chem. 211, 737 (1954).
Smith, P. F., and Rothblat, G. H. J. Bact. 83, 500 (1962).
Twarog, R., and Wolfe, R. S. J. Biol. Chem. 237, 2474 (1962).