Intermediary Metabolism of Antibiotic Resistant and Antibiotic Sensitive Staphylococci. I. Pyruvate, Glucose and Acetate.* (22903)

MATTHEW H. FUSILLO AND DANIEL LEIGH WEISS
(Introduced by Monroe J. Romansky)
Laboratory Sections for Infectious Diseases, D. C. General Hospital, and George Washington University School of Medicine, Washington, D.C.

In an earlier study, Fusillo(1) noted that *Micrococcus pyogenes var. aureus* (staphylococci) which were naturally resistant to penicillin, streptomycin, and the tetracyclines appeared to exhibit a decreased exogenous requirement for thiamin and or nicotinamide. One might conclude from this study either that the organism is synthesizing its own vitamin requirement, compensating for this vitamin need, or that the enzyme complexes associated with thiamin and/or nicotinamide are no longer active. Padron and coworkers(2) in metabolic studies with *in vitro* selected chloramphenicol resistant organism supported the view that the latter enzymes were inoperable. However, in the former study(1) when thiamin and/or nicotinamide were added to the basal medium, stimulation of growth of resistant organism occurred. It was considered(1), that the vitamin associated enzyme systems were still operative. This report elaborates some observable differences in the metabolism of sensitive and naturally selected resistant organisms and presents evidence which reveals the continued existence of potentially active thiamin and/or nicotinamide associated enzymes in resistant cells.

Methods and materials. The sensitive strain used was *M. pyogenes var. aureus* (ATCC 9996) and the multiple resistant organism used was isolated from a burn wound, (Stickle). Stock cultures were carried on trypticase soy agar slants. Organisms for whole cell preparations were grown 16 to 18 hours at 37°C in trypticase soy broth. Organisms for frozen and thawed lysed cell preparations were grown on trypticase soy agar. Cells were harvested and washed 3 times in M/15 phosphate buffer, pH 6.8. Cell suspensions were adjusted to an optical density to contain 14 mg dry weight of cells per ml of suspension. Two ml of this suspension were used in each Warburg vessel. Oxygen uptake was determined with 10% KOH in the center wall, and air as the gas phase by conventional Warburg technics. Final volume in each flask was 2.6 ml. Whole cell preparations were refrigerated 18 hours at 4°C to reduce extremely high endogenous respiration.

TABLE I. Oxidation of Glucose, Pyruvate and Acetate by Whole Cell and Lysed Cell Preparations of Staphylococci.

	μl O_2 consumed/hr			
	Sensitive cells		Resistant cells	
	Whole	Lysed	Whole	Lysed
Glucose*	191	—	193	—
Pyruvate*	55	52	0	42
Acetate*	0	0	0	0

* Substrates 0.02 mM, dry wt cells 28 mg. All values corrected for endogenous respiration.

Results. Glucose was oxidized by both sensitive and resistant cells to the same extent. (Table I).

Whole cell preparations of sensitive cells oxidized pyruvate, whereas resistant cells did not. The resistant cells, when lysed, were found to oxidize pyruvate.

Cocarboxylase did not activate oxidation of pyruvate by whole resistant cells (Table II). Oxidation of pyruvate in lysed resistant

TABLE II. Effect of Co-factors on Pyruvate Oxidation by Whole and Lysed Resistant Cells.

	μl O_2 consumed/hr	
	Whole	Lysed
Pyruvate	0	42
" + cocarboxylase	0	43
" + Mg^{++}	0	70
" + " + cocarboxylase	0	101

Pyruvate and Mg^{++} 0.02 mM, cocarboxylase 2 μg. Dry wt of cells 28 mg. All values corrected for endogenous respiration.

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cells was not greater than the controls when cocarboxylase was added to the system. Magnesium ions stimulated oxidation of pyruvate in lysed resistant cell preparations but were without effect on whole resistant cells. In the presence of Mg^{++} and cocarboxylase, oxidation of pyruvate was further stimulated in the lysed resistant system. Acetate, malate, and acetaldehyde were not oxidized by any of the systems used.

Discussion. The inability of whole cell preparations of naturally selected multiple resistant staphylococci to oxidize pyruvate is similar to the findings of Ramsey and Padron(3) who used *in vitro* selected chloramphenicol resistant staphylococci. Ability of lysed preparations of these cells to oxidize pyruvate confirms the observations of Fusillo (1) that thiamin and/or nicotinamide enzyme complexes were still present.

The experiments reveal that Mg^{++}, which in sensitive cells would activate the carboxylase system, does not appear to be available for that purpose in resistant cells. Magnesium did not enhance pyruvate oxidation by whole cell preparations, but stimulation was evidenced in lysed resistant cell preparations. Weinberg(7) observed a reversal of inhibitory effect of oxytetracycline on growth of sensitive bacteria by Mg^{++} and other divalent cations. Also, he postulated that oxytetracycline might act as a chelating agent for the divalent ions. It follows from our experiments that in resistant cells, either Mg^{++} is not assimilated or that Mg^{++} is bound as it is assimilated. Whether Mg^{++} ions are bound as ribonucleate, as observed by Gale in penicillin resistance studies and whether this binding is reversible warrants further study. Chelation of Mg^{++} would explain inability of the tetracycline resistant organism to produce an anti-tetracycline enzyme comparable to penicillinase.

Conflicting views on acetate oxidation by staphylococci are apparent in the literature. Stedman and Kravitz(4) and Padron *et al.* (2) claim oxidation of acetate occurs while Wolin *et al.*(5) and the above studies noted the lack of oxidation.

Concerning the staphylococci, these and former findings further develop the concept that the steps involved in the dissimilation of pyruvate appear to be a major point of antibiotic attack, and that perhaps two(6) or more pathways are available for pyruvate dismutation. Multiple potential metabolic pathways that exist before the antibiotic block occurs in the normally favored pathway would explain the *a priori* existence of single and multiple antibiotic resistant variants of the staphylococci(8).

Summary. Evidence is presented that while whole cell preparations of antibiotic resistant staphylococci did not oxidize pyruvate, lysed cell preparations did. Magnesium ions and Mg^{++} plus cocarboxylase, but not cocarboxylase alone, stimulated pyruvate oxidation by lysed cells, but had no effect on the activity of whole cells. Activity of isolated cell free extracts, cell surface components, and chelating ability of tetracycline are being studied.

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