Sulfhydryl Variation in Bacterial Enzymes in Relationship to Chemotherapy with the Nitro-Furans.* (19360)

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The nitro-furans and furacin (5-nitro-2-furaldehyde semicarbazone) inhibit the growth of many bacteria(1), at least one filterable virus(2) and certain tumors(3). Recently it has been reported that furacin has the properties of a limited sulfhydryl (SH) inhibitor (4). In a further investigation of the relationship of furacin to SH enzymes in bacteria, furacin-resistant bacteria were found resistant to such SH inhibitors as p-chloromercuribenzoate (pCMB) and trivalent arsenicals. It was also observed that bacterial urease from *Micrococcus pyogenes var. aureus* was inhibited by SH inhibitors when the organisms were grown in media containing urea. When urea was omitted from the media, ureases were produced that were not inhibited by SH inhibitors or by furacin.

Methods. The basal medium for all experiments contained 2.5 g of peptone (Difco) and 1.5 g of beef extract (Difco) per liter. Four different laboratory strains of *Micrococcus pyogenes var. aureus* were tested. The inoculum (0.1 ml per liter) was prepared from a 24-hour culture grown in double strength basal medium. The inhibition experiments were carried out with organisms grown in basal medium alone, basal medium plus 2% urea and basal medium plus 1% glucose. The organisms were harvested after 14 hours and washed 3 times with small quantities of M/15 phosphate buffer pH 7.4. The weight of organisms was determined by turbidity measurements against a standard curve. Urease activity was determined by aeration and Nesslerisation in accordance with a procedure already described(4). The results of some typical experiments using whole organisms as the source of urease with various inhibitors are given in Table I. The cells were exposed to the inhibitor for 15 minutes, the urea substrate was then added and the reaction allowed to proceed for an additional 15 minutes, after which it was stopped with 1N HCl. In reactivation experiments, cysteine was added along with the substrate. The control experiments were identical in every respect except that water was substituted for the inhibitor.

Results. The total inhibition of urease activity with pCMB varied from 25% to 50% when the organisms were grown in the medium containing urea. However, no inhibition was noted when the organisms were grown in the absence of urea. The trivalent arsenicals showed the same pattern of inhibition as did furacin and pCMB. The urease of furacin-resistant organisms grown in the presence of urea was not inhibited by either pCMB or furacin. However, the same molar concentrations of the trivalent arsenicals p-carboxyphenylarsenoxide inhibited 6%, p-aminophenylarsenoxide 16%, and mapharsen 22% of the activity of urease derived from the furacin-resistant strain. Both the furacin and pCMB inhibitions of the susceptible organisms were reversed by cysteine (0.01 M). The furacin reactivation was 65% and the pCMB reactivation 82%.

Cell free extracts from organisms grown in the various media described above were prepared by the alumina method of Scott and Cohen(5). Results using such extracts are shown in Table I. The procedure used in measuring the activity of the cell free ureases was similar to the procedure with the whole cells. The cell free extracts behaved in exactly the same manner toward the inhibitors as the whole cell preparations. However, urease prepared from furacin-susceptible cells grown in the presence of urea was largely inactivated by the extraction procedure. A similar preparation from resistant cells was not inhibited.

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TABLE I. Effect of Various SH Inhibitors on Ureases from Whole Cell Preparations of *Micrococcus Pyogenes var. Aureus** and Cell Free Extracts from Organisms Grown in Various Media.

Source of urease	Inhibitor†	mg NH_3 nitrogen		% inhibition	
		Whole cells‡	Extract§	Whole cells	Extract
Basal medium + 2% urea	Furacin	.035	.014		
	—	.060	.019	41.6	‖
	pCMB¶	.029	.012		
	—	.060	.019	51.6	‖
	pCPA**	.034	—		
	—	.066	—	49.5	—
	pAPA††	.031	—		
	—	.066	—	53	—
	Mapharsen‡‡	.029	—		
	—	.066	—	56	—
Basal medium alone	Furacin	.044	.043		
	—	.043	.045	0	0
	pCMB	.043	.044		
	—	.043	.045	0	0
Basal medium + 1% glucose	Furacin	.087	.072		
	—	.088	.072	0	0
	pCMB	.086	.071		
	—	.087	.073	0	0

* Furacin susceptible cells used.
† All inhibitors 7×10^{-4} M.
‡ 4 mg washed cells used per tube.
§ Extract equivalent to 2.5 mg of whole cells used per tube.
‖ Activity too low for reliable measurement of % inhibition.
¶ p-chloromercurybenzoate.
** p-carboxyphenylarsenoxide.
†† p-aminophenylarsenoxide.
‡‡ 3-amino-4-hydroxyphenylarsenoxide HCl.

Discussion. These experiments suggest that *Micrococcus pyogenes var. aureus* produces ureases with different affinities for SH inhibitors. In the absence of urea, a urease is produced which is less susceptible than jack bean urease to SH inhibitors(4). This may indicate that these ureases either do not contain SH groups or have SH groups that are slowly reactive or relatively inaccessible to SH inhibitors such as pCMB. Previous experiments with dehydrogenases(4) have shown that in the processes of the development of furacin resistance, SH dehydrogenases become resistant to pCMB and trivalent arsenicals. A similar phenomenon seems to occur with ureases from *Micrococcus pyogenes var. aureus,* although these organisms synthesize urease, which may not be dependent for its function on the presence of free SH groups when the bacteria are grown in media free of urea. These experiments also indicate that in bacteria the same enzyme may function either as a SH or non-SH enzyme. The exact nature of the transformation from SH to non-SH is open to conjecture. Two substrains may exist, one carrying the SH urease and the other the non-SH enzyme. This transformation may be the result of adaption or may be genetically controlled. Both enzymes may be simultaneously present in the same organism; the type of enzyme predominating in a given reaction may be dependent on the substrate.

Several theories have been advanced to explain the phenomenon of resistance to antibacterial agents such as alternate metabolic pathways, formation of antagonists or changes in permeability(6). It does not appear likely that the above result with bacterial ureases or the previous experiments with dehydrogenases showing cross-resistance with various SH inhibitors, can be explained solely on the basis of the above theories. The possibility of another mechanism should be considered in explaining resistance to the nitrofurans. Bacteria may be able to transform their enzymes into either the SH or non-SH form as circumstances may require. This phenomenon may be designated as SH shift. A definitive proof of the theory of SH shift will have to await the actual isolation from bacteria of the postulated SH and non-SH forms with a single

enzyme. The possibility should also be considered that changes can occur with other labile or reactive groups, such as free amino or hydroxy groups on the surface of enzyme proteins, in connection with other types of resistance, especially where the usual theories are not entirely applicable.

Results which are analogous to the postulated phenomenon of SH shift have been described. Ingbar and Kass(7) have shown that normal hemoglobin has two moles of SH per mole of hemoglobin while hemoglobin from sickle cell anemia has three moles of SH per mole. Hughes(8) has isolated two fractions of serum albumin, one fraction having a SH group and the other appearing to have no SH group. There are some instances where the same enzyme will function either in SH or in an apparent non-SH form depending on the tissue from which it is derived. The α-glycerophosphate dehydrogenase of rabbit thigh muscle is apparently non-SH and is not inhibited by furacin(4). The same enzyme from *E. coli* is inhibited by furacin(4). The presence or absence of a SH group in a given enzyme may not affect its ability to function on a given substrate. The presence of the SH group, however, may make the enzyme liable to inhibition by SH inhibitors.

Summary. In the presence of urea, *Micrococcus pyogenes var. aureus* produces a urease that is inhibited by furacin, p-chloromercuribenzoate and trivalent arsenicals. When the organisms are grown in the absence of urea or when furacin-resistant organisms are grown in media containing urea, ureases are obtained that are not affected by the above inhibitors. In explanation of these results as well as those previously reported involving the inhibition of dehydrogenases, it is suggested that some aspects of resistance to the nitrofurans can be explained by a shift of certain enzymes from the SH to a non-SH form.

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A Cofactor for the Formic Hydrogenlyase Enzyme System.* (19361)

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A survey of the literature concerning the effects of oleic acid on the growth of microorganisms reveals 3 major findings: 1) this fatty acid is essential for the optimal growth of some species of the genera *Lactobacillus, Corynebacterium,* and *Mycobacterium*(1-4); 2) oleic acid is required for certain strains of lactobacilli only in the absence of biotin which it apparently replaces(3,5); and 3) in sharp contrast to the growth stimulating effect, the toxic action of this fatty acid for various microorganisms has been recognized(2-4,6). These actions are not strictly limited to oleic acid, since some of these effects have been reported for other unsaturated fatty acids, chief-

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