Formation in a Mycobacterium of an Adaptive Enzyme for Oxidation of Phloroglucinol. (22414)

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The rate of adaptive enzyme formation in micro-organisms is influenced by a number of conditions. Provided that permeability is not a limiting factor, the metabolic and nutritional state of the cell may be the most important in determining the rate. Washed suspensions of a species of mycobacterium form enzymes for the oxidation of benzoic acid and inositol(1). Under the same conditions the formation of the inositol enzyme takes a much longer time. It appears that it is more difficult for the cell to make this enzyme because the age of the culture, the presence of assimilable nitrogen, and certain drugs affect inositol enzyme formation much more than they do benzoate enzyme formation. It has now been shown that this organism makes an enzyme which oxidizes phloroglucinol and which is different from the other two in that it is formed more slowly and the factors which affect the inositol enzyme formation, with exception of the drugs, have little effect on the formation of the phloroglucinol enzyme. Thus, under conditions which are apparently optimal for the formation of 2 enzyme systems, the formation of the third is unaltered from the original basic rate.

Methods. Mycobacterium BCG ATCC No. 8420 was grown for 3 to 7 days on 20 ml of Long's synthetic medium(2). The cell mass was broken up, washed twice with water by centrifugation in Hopkin's tubes, and then taken up in 0.05 M Na-K phosphate buffer pH 7.8 so that 0.1 ml of packed cells was suspended in 1 ml of buffer. Each Warburg vessel, which had a fluid volume of 2 ml, contained 0.5 ml of this suspension.

Results. Two criteria were used to show that the delay in the oxidation of phloroglucinol was the result of slow enzyme formation rather than slow penetration into the cell. Low concentrations of antibiotics inhibit enzyme formation in this organism(1) but have no effect on the oxidation once the enzyme is formed. In this case, 0.05 γ/ml of aureomycin completely prevented the oxidation of phloroglucinol when added with it but was without effect on the rate when the drug was added after the oxidation was proceeding. Then, pre-incubation of the cells for 90 minutes with 50 γ of phloroglucinol decreased the latent period when 0.5 mg of phloroglucinol was added. On the other hand, pre-incubation with benzoate or inositol had no effect. Further, cells grown in benzoate or inositol did not oxidize phloroglucinol more rapidly. Phloroglucinol reacts with the medium to produce a colored complex and this may explain why the enzyme is not formed when grown in the presence of phloroglucinol.

Phloroglucinol is toxic. This is seen from the following figures for the oxygen uptake in a 2-hour period with addition of 0.1, 0.5, 1.0, and 2.0 mg of phloroglucinol per vessel. In cmm of oxygen these were, respectively, 54, 87, 72 and 53. This toxicity makes it difficult to assess the effect of various factors on enzyme formation since different conditions may alter the resistance of the cells to the toxic effect. In general, it can be stated that the age of the culture from which the cells were taken had no consistent influence on the length of the latent period. This is in contrast to the effect of age on the rate of formation of the benzoate and inositol enzymes. Pre-incubation of the cells with $(NH_4)_2SO_4$, which has a marked effect on the formation of the inositol enzyme, had little or no effect on the formation of the phloroglucinol enzyme. Pre-incubation with trehalose, pyruvate, fatty acids, asparagin, catechol, and pyrogallol was also without effect.

Neither resorcinol nor phenol was oxidized even if the cells were pre-incubated with phloroglucinol. Cells grown in 50 γ/ml of resorcinol or phenol were unable to oxidize them nor were these cells able to oxidize phloroglucinol more rapidly. Normal cells,

TABLE I. Oxidation of 0.4 mg Each of Phloroglucinol and Orcinol. Pre-incubation period was 90 min., pH 7.8, 37°. Figures are mm³ oxygen uptake. Autorespiration has been subtracted.

Compound	Pre-incubated with	Min. 30	60	90	120	150	180
Phloroglucinol	—	−4	10	23	53	77	98
"	50 γ phloroglucinol	10	24	38	72	99	129
"	50 γ orcinol	−4	12	25	56	81	108
Orcinol	—	−9	−9	−6	−5	15	34
"	50 γ phloroglucinol	−1	14	39	72	108	143

however, oxidized orcinol after a long latent period which could be shortened by pre-incubation with phloroglucinol. This is shown in Table I in comparison with the oxidation of phloroglucinol. Pre-incubation with orcinol had only a slight effect on the latent period for phloroglucinol. For the formation of the enzyme, therefore, 3 hydroxy groups meta to one another on the ring are necessary. If one was substituted by a methyl group as in orcinol, the enzyme was formed much more slowly, but the enzyme once formed was able to oxidize orcinol fairly rapidly. The substitution of a carboxyl group as in 3, 5, dihydroxybenzoic acid caused complete loss of activity. It did not cause formation of the enzyme, was not oxidized by it, nor did it inhibit formation or oxidation. This was also true of phloridzin which is a glucoside of phloroglucinol. Enzyme formation occurred at the normal rate in the absence of added phosphate.

The oxygen uptake varied between 4 and 6 atoms per molecule of phloroglucinol and 2 molecules of carbon dioxide per molecule were produced. The ring was therefore split and some assimilation of the end products, if it occurred, could account for the variability of the oxygen uptake. It is probable that more than one enzyme is involved in the overall reaction.

Summary. The rate of formation of the enzyme which oxidizes phloroglucinol is not affected by factors which alter the rate of formation of the enzymes which oxidize benzoic acid and inositol. Some of the characteristics of the phloroglucinol oxidation are described.

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Effect of Adrenocorticotropin on Uptake of S^{35}-Labelled Cysteine by Rat Adrenal Glands.* (22415)

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Reports of the effects of the adrenocorticotropic hormone (ACTH) upon sulfur distribution in the animal body have been largely confined to extra-adrenal tissues and fluids. Kinsell *et al.*(1) demonstrated a 50% rise in urinary cystine and cysteine after the administration of the hormone. Hess *et al.* (2) found a marked fall in the concentration of reduced glutathione in blood but no change in total glutathione levels after administration of ACTH to human patients. Ingbar *et al.* (3) found that the hormone produced no changes in the blood glutathione of rats although it did cause a reduced non-protein

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