Complexing of Fatty Acids by Triton WR1339 in Relation to Growth of Mycobacterium tuberculosis

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Mycobacterium tuberculosis grew in the presence of toxic concentrations of free fatty acids when an adequate amount of triton WR1339 was added to the medium. Triton produced a solubilizing effect on turbid suspensions of fatty acids, indicating the formation of a detergent-lipid complex. Oleic acid complexed with triton served as a source of carbon for growth of M. tuberculosis.

Triton WR1339, an arylalkyl polyether of phenol, is a nonionic surface active agent that suppresses experimental tuberculosis in mice and guinea pigs (1, 4). The suppression appears to be due to the inability of tubercle bacilli to proliferate in macrophages of triton-treated animals where the detergent accumulates in the lysosomes (2, 6). The detergent is not, however, bacteriostatic for tubercle bacilli under normal in vitro cultural conditions. Triton has been incorporated into 7H10 medium employed for primary isolation of Mycobacterium tuberculosis (5). In the present report, a study has been made of the ability of triton WR1339 to bind free fatty acids and thereby render the lipids nontoxic to tubercle bacilli.

The H37Rv strain of M. tuberculosis was employed in the study. The organism was cultured in modified enriched Kirchner medium (3) to which 0.5% glucose was added. The basal medium contained the following ingredients: 3 g of dibasic sodium phosphate; 4 g of monobasic potassium phosphate; 3 g of ammonium sulfate; 2.5 g of sodium citrate; 0.6 g of magnesium sulfate; 0.05 g of ferric ammonium citrate; and water to a final volume of 1,000 ml. The medium was enriched by the addition of thiamine, riboflavin, pyridoxine, nicotinic acid, pantothenic acid, and inositol at a concentration of 0.5 μg/ml; folic acid and biotin at 0.1 μg/ml; and B12 at 0.001 μg/ml. The following amino acids were also added in a concentration of 10 μg of the Dl-form per ml and 5 μg of the L-form per ml: L-alanine, L-arginine, DL-aspartic, DL-cystine, L-glutamic acid, glycine, L-histidine, DL-isoleucine, DL-leucine, L-lysine, DL-methionine, DL-phenylalanine, L-proline, DL-serine, DL-threonine, DL-tryptophan, L-tyrosine, and DL-valine.

The fatty acids were employed as sodium salts. Stock solutions of 1% unsaturated fatty acids were sterilized by passage through bacteriological fritted glass filters, whereas solutions of saturated fatty acids were autoclaved for 15 min at 121 C. Triton was prepared as a 10% solution and also sterilized by autoclaving. Appropriate amounts of sterile solutions of triton and fatty acids were added to sterile double-strength medium which was then adjusted to single strength by the addition of distilled water. The media were inoculated with 0.05 ml of a washed suspension of a 5- or 7-day culture of tubercle bacilli diluted to an optical density of 0.05. This inoculum contained approximately 6 × 10⁴ viable cellular units. After incubation at 37 C for 21 to 28 days, growth was determined turbidimetrically, by using a Roux Photometer equipped with a 640 nm light filter. When necessary, 1 drop of a 10% solution of Tween 80 was added to the cultures before measuring growth, after which the organisms were agitated vigorously on a Vortex mixer.

The effect of varied levels of triton on growth of M. tuberculosis is recorded in Table 1. Concentrations of triton up to 0.1% produced a minimal effect on growth of M. tuberculosis. Growth of the organism was reduced progressively as the concentration of triton was increased beyond 0.1%. Growth in the presence of 1% triton was reduced approximately 38%.

Preliminary to examination of the relationship between triton and free fatty acids, the minimal concentrations of oleic, stearic, palmitic, myristic, lauric, and capric acids that effected complete inhibition of growth were determined. Oleic and palmitic acids inhibited growth of the tubercle bacilli at a concentration of 2 μg/ml and myristic and lauric acids were inhibitory at a level of 4 μg/
TABLE 1. Effect of Triton WR1339 on growth of the H37Rv strain of Mycobacterium tuberculosis

<table>
<thead>
<tr>
<th>Triton WR1339 (mg/ml)</th>
<th>Optical density of cultures*</th>
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<tbody>
<tr>
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<tr>
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</tr>
<tr>
<td>20.00</td>
<td>0.330</td>
</tr>
</tbody>
</table>

* Recorded after incubation of the cultures for 21 days at 37 C in modified enriched Kirchner medium containing 0.5% glucose.

ml. The minimal inhibitory concentrations of capric and stearic acids were 8 and 20 μg/ml, respectively.

To determine the effect of triton on fatty-acid toxicity, various levels of the detergent were added to media containing inhibitory amounts of oleic, palmitic, myristic, and lauric acids. The media were then inoculated with M. tuberculosis. After incubation of the media, the minimal amount of triton that permitted complete growth of the organism at each concentration of fatty acid was ascertained (Fig. 1). The weight ratios of triton to fatty acid which were required for optimal growth were as follows: oleic acid, 50:1; palmitic acid, 25:1; myristic acid, 200:1; and lauric acid, 2,000:1. The organism did not produce growth in the presence of the minimal inhibitory concentration of capric acid and the maximal amount of triton employed (2%).

Further evidence of binding of fatty acids by triton was obtained by adding the detergent to turbid suspensions of fatty acids and observing clarification of the medium. The weight ratio of triton to palmitic and myristic acids required for clarification was 25:1. The corresponding value for stearic acid was 50:1.

The ability of M. tuberculosis to utilize oleic acid complexed with triton as a source of carbon for growth was determined (Fig. 2). A 2.5% solution of triton containing 250 μg of oleic acid per ml was prepared and incorporated into the basal medium. A control medium containing an equivalent amount of oleic acid and bovine albumin, fraction V, was similarly prepared. The media were inoculated with M. tuberculosis and incubated at 37 C. The optical density of each of the cultures was determined periodically for 17 days. The rate of growth of the organism was similar in the triton-oleate and albumin-oleate media, but growth appeared earlier in the latter medium.

Although conclusive evidence of binding of fatty acids by triton was not obtained, the solubilizing effect on fatty acids, as well as elimination of toxicity of the lipids with regard to growth of M. tuberculosis indicated interaction between the detergent and free fatty acids. Scanu and Oriente (7) reported that triton formed a complex with the lipid moiety of lipoproteins. It was postulated that the lipids passed into the micelles of triton, remaining enclosed and possibly located between the hydrocarbon chains of the detergent. The
finding that fatty acids can be taken up by *M. tuberculosis* from the triton-fatty acid complex and utilized as a source of carbon for growth suggests a loose association between the detergent and lipid.

The data concerning toxicity reduction and the solubilizing effect on fatty acids show that, although similar amounts of triton were required to "solubilize" palmitic and myristic acids, a severalfold greater amount of the detergent was necessary for elimination of toxicity of the latter fatty acid. The increased amount of triton may have been required for complexing inhibitory amounts of fatty acid released from the initial triton-lipid complex as a result of the lesser nonpolarity of myristic acid.

The approximate average molecular weight of triton WR1339 has been estimated to be in the range of 2,000 to 3,000 (I. S. Shupe, personal communication). By using this estimated value, the data suggest that the approximate molar ratio of triton to fatty acid required for optimal binding is 5:1 for oleic and stearic acids and 2.5:1 for palmitic and myristic acids.

**ADDENDUM IN PROOF**

P. D. Hart, J. E. Lovelock, and T. Nash (J. Hyg. 60:509–525, 1962) reported that triton WR1339 abolished the bactericidal effect of C_{12}-C_{18} fatty acids on *Mycobacterium tuberculosis*.

**LITERATURE CITED**