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Effect of Cholesterol Activated by Ultra-violet Irradiation on Growth of Tubercle Bacilli in Vitro.

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The beneficial effects of ultra violet light in the treatment of tuberculosis have been known since the pioneer work of Finsen (Bie).[1] More recently the curative effect in intestinal tuberculosis has attracted attention. Experimental proof of the nature of this action, whether a general constitutional effect or a specific inhibitory influence on the growth of the organisms has not been offered.

The bactericidal action of direct ultra-violet irradiation is well known. Eidinow[2] states that it has been shown that an "erythema dose" of ultra violet light applied to the skin of animals increased the hemo-bactericidal power of the blood. Ultra violet light then appears to have both a direct and an indirect bactericidal action, but whether this bactericidal action is possessed by irradiated photosensitive substances is not known. It has been shown that cholesterol irradiated by ultraviolet light and ingested produces some of the biological effects which are produced by direct irradiation. More recently the effect of the cholesterol has been attributed to a contamination, ergosterol, which is usually present in unpurified cholesterol.

It seemed of interest to test the effect of biologically active (irradiated) impure cholesterol upon the growth of tubercle bacilli *in vitro*.

Experimental: Cholesterol (Merck) was biologically activated by exposure to ultra violet light for one hour at a distance of 50 cm. The source of ultra violet light was a Hanovia, mercury vapor quartz tube. The cholesterol was dissolved in ether, poured into flat dishes and allowed to dry. In this manner a thin film of cholesterol was exposed to the light. This cholesterol was shown to be biologically active because 15 mg. daily prevented the development of rickets in 3 rats on a rickets-producing diet (Steenbock No. 2965), while 3 rats on the same diet that received the same amount of non-irradiated cholesterol developed severe rickets (histological).

Culture medium was then prepared to test the effects of the biologically activated cholesterol on the growth of tubercle bacilli *in vitro*. Twelve tubes of glycerine agar containing a 0.5% suspension

[1] Bie, U., *Brit. Med. J.*, 1899, ii, 825.

[2] Eidinow, Albert, *Proc. Roy. Soc. Med.*, 1926, xix, 50.

of irradiated cholesterol and 12 tubes containing a 0.5% suspension of non-irradiated cholesterol were prepared. The cholesterol suspension was obtained by dissolving the required amount of cholesterol in ether and vigorously stirring the ether suspension into the hot medium. In this manner a finely dispersed suspension was obtained and the medium had a milky opalescent appearance. Each of the 24 tubes was then inoculated with human tubercle bacilli. The tubercle bacilli were obtained from the American Type Culture Collection. Inoculation of the glycerine agar tubes was made evenly by suspending the tubercle bacilli in a small amount of normal saline and inoculating the culture medium with equal amounts by means of a capillary pipette. The tubes were then plugged, corked, incubated and examined at 7-day intervals up to 4 weeks. A luxuriant growth of tubercle bacilli was obtained on all tubes inoculated save one which was overgrown by contaminating yeast. There was no appreciable difference in either rate or amount of growth between those cultures growing on medium containing a suspension of non-irradiated and those growing in medium containing a suspension of irradiated cholesterol.

A culture medium was then employed in which the cholesterol was in solution rather than in suspension. For this purpose Dorset's egg medium was chosen and 24 tubes were prepared as follows: Twelve tubes were filled with ordinary egg medium and 12 with egg medium which had previously been irradiated for one hour at a distance of 50 cm. from the lamp. These 24 tubes were inoculated and incubated in the same manner as the glycerine agar tubes and again no appreciable difference was observed in either the rate or the amount of growth at the end of 4 weeks' incubation.

Conclusions: The growth of human tubercle bacilli was not affected by a medium containing a suspension of cholesterol irradiated by ultra violet light or by an irradiated medium containing cholesterol in solution.

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Development of Vitamin A During Ripening of Tomatoes.

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In the progress of an inquiry into the conditions under which vitamin A is developed in plants a comparison was made of the vitamin