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4. The following abbreviations are used: ATP, CTP, GTP, and UTP for the ribonucleoside triphosphates of adenine, cytosine, guanine and uracil, respectively; RNA for ribonucleic acid; GMP for the ribonucleoside monophosphate of guanine; DNA for deoxyribonucleic acid; tris for *tris*-(hydroxymethyl)-aminomethane; and polyC for polycytidylic acid.
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Thalidomide: Effects on Ehrlich Ascites Tumor Cells in vitro

Abstract. *Thalidomide did not inhibit dehydrogenase activity or growth of Ehrlich ascites tumor cells in agar. When mixed with Ehrlich ascites tumor cells in vitro, thalidomide increased the mitotic activity. The effect of the thalidomide was not altered by the addition of nicotinic or folic acid, or by vitamin B_1 or B_6. Oxygen uptake by the tumor cells was not affected by thalidomide.*

An attempt was made to determine whether thalidomide (*1*) (DL-*N*-(2,6-dioxo-3-piperidyl)phthalimide) would produce any effect in vitro that would elucidate the metabolic pathway of this compound, which could then be validated in vivo. This study followed the demonstration that harmful effects are produced in the mouse embryo when the mother is given thalidomide prior to the formation of the limb buds (2). Our procedure is more efficient than testing in animals; because of intrauterine variability in mice, for example, several hundred pregnant females would have to be used for testing a specific pharmacological action of thalidomide.

Ehrlich carcinoma cells, which had been cultured as a cell strain for 33 months, were used. The cells were grown in a medium of Earle's salts (*3*) supplemented with 20 percent fetal calf serum and 20 percent bovine amniotic fluid. Cultured cells which had been placed in fresh medium 24 hours previously were counted, and 200,000 cells per 3 ml of fresh medium were transferred into screw-cap test tubes each containing a sterile 8 × 15-mm No. 2 coverslip. The tubes were incubated at 37°C for 6 hours at a 10-degree angle to allow attachment. The medium was removed and the cells were then exposed to 750 μg of thalidomide given in 3 ml of fresh medium. This concentration is approximately twice that which is soluble in saline at 37°C; however, after approximately 24 hours no thalidomide was visible and no change in the *p*H was noted. At 3-hour intervals, cells exposed to thalidomide and cells from the control tubes were fixed with methyl alcohol, dehydrated, and then stained with hematoxylin and eosin. The cells were still attached to the coverslips. The number of mitotic figures was recorded for the first 4000 cells counted on two coverslips with thalidomide-treated cells and two with control cells. Comparison of the incidence of mitotic figures in treated and control cells showed that thalidomide-treated cells had a maximum number of figures prior to the time required for control cells to reach their maximum. After 12 hours cells exposed to thalidomide averaged 66.75 ± 8.2 figures per 1000 cells while unexposed cells averaged 34.45 ± 5.9 figures per 1000 cells. This difference was statistically significant at the .01 level of probability. There was no change in the *p*H of the medium. At 24 hours the control cells exhibited the same incidence of mitotic figures as did the treated cells at 12 hours. No further difference was noted. This increased rate of mitosis was not reversed by the addition of 10 μg of nicotinic acid, folic acid, or pyridoxal hydrochloride per milliliter of medium, or by 2.5 μg of riboflavin per milliliter of medium.

Thalidomide was next tested for its ability to produce cytotoxicity or inhibition of dehydrogenase activity. Studies of growth inhibition were also conducted. Growth was indicated by the presence of a complete monolayer of Ehrlich cells overlayed with agar in the bottom of a petri dish, as described previously (*4*). Discs containing 25 to 100 μg of thalidomide did not inhibit the dehydrogenase activity of cells in an agar suspension, as indicated by the reduction of methylene blue. Formation of a complete monolayer of cells overlayed with agar was also uninhibited.

The effect of thalidomide on respiration and pyruvate oxidation of Ehrlich ascites tumor cells was also determined. Cells equivalent to 25 mg of dry-tissue weight were incubated for 100 minutes at 37°C in Krebs Ringer phosphate buffer free of calcium ions and containing 50 μmole of inorganic phosphate buffer, *p*H 7.4 in a total volume of 3.0 ml. Thalidomide added in amounts ranging from 30 to 6000 μg per 3 ml of medium had no inhibitory effect on oxygen uptake (determined manometrically) when pyruvate-1-C^{14} (in a final concentration of 0.01*M*) served as respiratory substrate. Nor did saturating concentrations of thalidomide produce a significant inhibition of pyruvate oxidation, as measured by the incorporation of C^{14} of pyruvate-1-C^{14} into the respiratory CO_2.

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