In the preceding paper, the development of a Miracil D-resistant subline of mouse leukemia L1210 has been described (3). In view of its biologic stability and other characteristics, it was of interest to inquire into reasons for the failure of this subline to respond to this agent. The present experiments were designed to affirm or eliminate one possible mechanism of resistance (cf. Ref. 4), i.e., reduced uptake of Miracil D by the resistant cells upon exposure to the drug. A preliminary report of some of the results has been made (2).

### MATERIALS AND METHODS

**Leukemia L1210 ascites.**—The sensitive and Miracil D-resistant sublines of this tumor are described in the preceding paper (3) and were carried in the same way as in that concomitant investigation.

**Determination of Miracil D.**—A spectrophotometric assay method for 1-diethylaminoethylamino-4-methyl-10-thiaxanthone (Miracil D, lucanthone, Nilodin) was based, with slight modifications, on the acetone-ether method of Newsome (5).2 Briefly, a 2.5-ml. aliquot of Miracil D-containing samples was treated with 3.5 ml. of acetone, and the resulting precipitate was centrifuged off. After addition of 5 ml. of n NaOH to the supernatant, the mixture was shaken twice with ether (total volume, 25 ml.). The combined ether layers were extracted with 7.5 ml. of 0.1 N HCl, and the optical density of this extract was measured at 443 m\(\mu\) in a Beckman DU spectrophotometer equipped with a tungsten lamp, against a water blank which had been carried through the same double extraction procedure. A Miracil D standard was included in each experiment and was also carried through the extraction procedure.

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CBART 1.—Percentage of Miracil D remaining in suspending medium after incubation with different numbers of cells. The open bars represent the sensitive, and the filled bars the Miracil D-resistant, cells of leukemia L1210. The means of two to eight experiments are given.

CBART 2.—Percentage of Miracil D remaining in suspending medium after incubation of $5 \times 10^4$ cells with different initial amounts of drug. The open bars represent the sensitive, the filled bars the Miracil D-resistant, cells of leukemia L1210. The means of two experiments are given except for the values at 600 and 1200 µg. of Miracil D, which were obtained in a single experiment.

RESULTS AND DISCUSSION

This investigation had two related objectives: (a) to assess the ability of cells of leukemia L1210 to take up Miracil D from the suspending medium in vitro under various conditions, and (b) to compare cells of the sensitive parent line and of the Miracil D-resistant subline in this regard. In the presentation of the detailed results, it
will be apparent that there was no difference between the sensitive and Miracil D-resistant cells of this tumor in permeability to the drug under any of these conditions, and that therefore the mechanism of resistance to this agent will have to be explained in another way.

Dependence of uptake of Miracil D on outside drug concentration.—Two series of experiments were carried out to assess this factor. Chart 1 summarizes the results obtained when different numbers of cells were incubated for 30 min. at room temperature in a constant volume of suspending medium containing a total of 400 μg. of Miracil D.

As the number of cells was increased from $1 \times 10^3$ to $25 \times 10^3$, there was an almost linear fall in the amount of Miracil D remaining in the suspending medium; at higher cell populations, there was little additional removal of the drug by the cells.

Chart 2 presents the evidence obtained when a constant number of cells were exposed for 30 min. at room temperature to different amounts of the drug in a constant volume of suspending medium. The percentage of Miracil D remaining in the latter was essentially constant over a 10-fold range of initial Miracil D concentrations.

The data obtained in these two series of experiments were recalculated on a logarithmic basis, relating the initial concentration of Miracil D in the suspending medium to the amount of drug associated with the cellular fraction (i.e., not remaining in the suspending medium after incubation for 30 min.). Chart 3 demonstrates that the amount of drug taken up or adsorbed on the average cell was directly proportional to the initial concentration of drug per cell in the suspending medium.

Effect of length of incubation period on uptake of Miracil D.—To assess this variable, cells of either line of leukemia L1210 were shaken with 400 μg. of Miracil D at room temperature for periods shorter and longer than the usual 30-min. incubation step. Table 1 shows that the amount of drug remaining in the suspending medium decreased with increasing incubation time, particularly at the less dense cell populations, where the initial drug concentration per cell was higher.

Effect of incubation temperature on uptake of Miracil D.—In a series of experiments, $5-10 \times 10^3$ cells of either line of leukemia L1210 were incubated for 30 min. at different temperatures with 400 μg. of Miracil D. Their ability to remove the drug from the suspending medium was the same at 0°C, 22°—25°C, and 37° C., as shown in Table 2. This finding supports the conclusion that the uptake of this drug by these cells is nonenzymatic in nature.

In this regard, as in all others summarized in this paper, cells of the Miracil D-resistant subline of leukemia L1210 were indistinguishable from cells of the sensitive parent line.

REFERENCES
Uptake of Miracil D by Cells of Sensitive and Miracil D-resistant Lines of Mouse Leukemia L1210

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