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

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SUMMARY

With the aid of a spectrophotometric method of assay for Miracil D, a comparison was made of the ability of cells of the parent sensitive line and of a Miracil D-resistant subline of leukemia L1210 to remove the drug from the suspending medium during incubation in vitro.

The uptake of Miracil D by the cells was proportional to the initial concentration of drug per cell in the suspending medium and to the length of the incubation period. The incubation temperature was varied between 0° and 37° C. without affecting the extent of drug uptake by the cells; this suggests that this process is nonenzymatic.

There was no difference, under these various conditions, between the sensitive and Miracil D-resistant cells in their permeability to this agent. This finding eliminates one of the possible mechanisms of resistance of this tumor to Miracil D.

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-thiaxanthenone (Miracil D, lucanthone, Nilodin) was based, with slight modifications, on the acetone-ether method of Newsome (5). 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 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 με 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.

Standard curves for Miracil D in 0.1 N HCl were established on various occasions. The optical densities followed Beer's law between 3 and 100 μg of drug/ml of acid. The recovery of drug after the acetone-ether extraction procedure averaged 90 per cent and was comparable for aqueous samples and ascites supernatants. The latter, moreover, yielded no materials absorbing at 443 με if extracted in the absence of Miracil D.

Analytical procedure.—In initial experiments, attempts were made to determine the Miracil D uptake by the two sublines of leukemia L1210 directly by extracting the drug from the cells following incubation in vitro or treatment in vivo. It was found, however, that the recovery of Miracil D from the cellular material, although always substantial, was neither complete nor reproducible despite repeated efforts to improve the extractability of the drug by homogenization and freeze-thawing of the leukemic...
BRINDLE et al.—Uptake of Miracil D by Leukemia Cells

1739

Chart 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.

Chart 2.—Percentage of Miracil D remaining in suspending medium after incubation of $5 \times 10^6$ 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.

cells. Degradation of Miracil D by contact with these living cells appeared to be a negligible factor.

It was therefore decided, in analogy to the procedure employed by Davidson (1) in studies on the permeability of cells of another subline of this leukemia to purine antagonists, to use the indirect approach of measuring the amount of Miracil D remaining in the suspending medium after incubation of the cells with the drug in vitro. All the results in this study will be expressed in this way.

In a typical experiment, ascitic fluid was withdrawn 7–9 days after implantation of the tumor from mice bearing either the sensitive parent line or the Miracil D-resistant subline of leukemia L1210. Aliquots were taken promptly for cell count, total nitrogen determination, packed cell volume, and, on occasion, morphologic observation for viability. After suitable dilution in ice-cold physiologic saline, 1.0-ml. aliquots of each cell suspension were incubated for 30 min. on a shaker at room temperature with 4.0 ml. of drug solution in physiologic saline. After removal of the cellular material by centrifugation, 2.5 ml. of the clear supernatant was assayed for Miracil D as outlined above.

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 $1 \times 10^4$ to $25 \times 10^4$, 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, $5-10 \times 10^4$ 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

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