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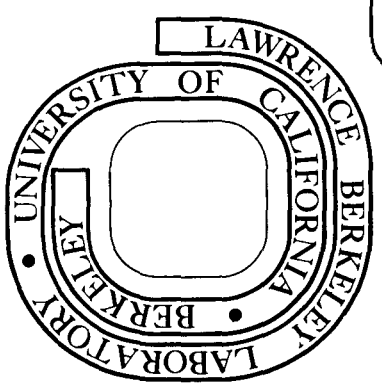

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DISCLAIMER

EXCRETION RATES OF ASCITES TUMOR CELL COMPONENTS AS A FUNCTION OF DRUG TREATMENT

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We have previously reported the chemotherapeutic effect of rifamycin derivatives on an ascites tumor, using increased life span as a criterion.[1] These derivatives inhibit (1) RNA-instructed DNA polymerase in crude viral extracts; (2) virus-induced transformation in tissue cultures; and (3) the growth of tumors *in vivo*.[2] One rifamycin derivative, rifazone-8_2 (R-8_2), not only inhibits transformation in chick fibroblasts but affects the growth of transformed cells.[3] The present study demonstrates that rifampicin and R-8_2 act as cytocidal (rather than cytostatic) agents against some ascites cell lines.

In order to distinguish between a cytocidal and a cytostatic effect on ascites cells, we have adopted a technique developed by Hofer *et al.*[4], in which cells are labeled with ^{125}I-iododeoxyuridine (^{125}IUDR). This is a convenient method for following the rate of cell death in individual live mice. Briefly, the technique consists of labeling tumor cells *in vivo* with ^{125}IUDR, which is incorporated exclusively into deoxyribonucleic acid.[5,6,7] The labeled cells are then inoculated into non-radioactive mice. The rate of cell death can be monitored by following the loss of radioactivity from the live mice, since the ^{125}I remains with the tumor cells until they die, at which time less than 3-5% of the isotope is reutilized in proliferating mouse tissue.[8]

If a cytotoxic drug acts by killing an additional fraction of tumor cells beyond the small percentage normally dying, this effect will be reflected in an increased excretion of ^{125}I. On the other hand, if the drug only prolongs the generation time of the tumor cells, this effect should be evident in an increased survival time of the host animal without an increase in the rate of ^{125}I excretion. Thus one can distinguish between a cytocidal and cytostatic effect.[9]

The ascites cell lines studied included TA3 and Ehrlich's (both maintained in LAF/J female mice), and L1210, MTX, and L5178Y (maintained in $C_3D_2F_1$/J female mice). The TA3 is an adenocarcinoma, the Ehrlich's is derived from a mammary carcinoma, and the L1210, MTX and L5178Y are all leukemic lines. All cell lines were carried by weekly i.p. transfer of 10^5 cells into the appropriate hosts. For experimental purposes, 10^6 cells were inoculated into the appropriate strain of donor mice. Four days later these mice received 2-4 μCi ^{125}IUDR i.p., divided into two doses, 6 hours apart. Twenty-four hours after the first dose, the labeled cells were harvested, washed twice with Earle's Balanced Salt Solution, and inoculated into recipient mice. All concentrations were adjusted so that the total inoculum was contained in 0.2 ml. All cell preparation was done at 4°C. For experiments involving TA3 cells, the inoculum was 10^6 cells/animal; for Ehrlich's, $4x10^6$; and for the three leukemic lines, 10^7. To avoid accumulation of ^{125}I in the thyroid, the drinking water of these mice was supplemented with 0.1% NaI, beginning 1-2 days before the experiment. In order to determine how fast the breakdown products of dead cells were being excreted, some animals were inoculated with cells which had been killed by heating in a waterbath at 65°C for 15 minutes. The radioactivity in individual mice was monitored immediately after inoculation and daily thereafter. The measuring equipment consisted of a pair of coaxially mounted, 3 mm thick NaI crystals, (12.5 cm diameter), equipped with a multichannel analyzer. The pulses from the 0.035 mev Te x-rays were counted. Individual mice were placed at the midpoint of the central axis of the crystals (set 5.5 cm apart), with the midline of the mouse at right angles to the crystals.

The rifamycin derivatives investigated were rifampicin (Rif) and R-8_2.[10] The drugs were suspended in 0.5% methylcellulose solution in 0.9% NaCl (MeC) at a concentration of 10 mg/ml. Control mice were injected with MeC. In

all experiments the first drug injection (4 mg) was made 12 hours after tumor inoculation. Subsequent injections of 2 mg were made 24 and 72 hours after cell inoculation. To determine whether the drugs affected the excretion of dead cells, in the experiments with Ehrlich's cells, drugs were injected into mice which had received killed cells (Fig. 1A). All injections were i.p.

Based on the excretion rate of ^{125}I, Rif and R-8_2 are effective against Ehrlich's and TA3 ascites cells in LAF/J female mice, and the effect is cytocidal rather than cytostatic (Fig. 1 A&B). These drugs did not influence the rate of excretion of activity from killed cells (Fig. 1 A). Therefore, the differences in loss of activity in control mice (MeC) and drug-treated (Rif & R-8_2) is not due to an effect of the drugs on the ^{125}I excretion system itself. Rif is slightly more effective than R-8_2, probably because of its greater water solubility.[1] However, the drugs show little effect against the leukemic lines (Fig. 2 A,B&C). The latter observation is in agreement with the lack of therapeutic activity of these drugs against radiation-induced thymic lymphomas (unpublished data), and is supported by our observation of an increase of leukemia in rats injected with dimethylbenzanthracene and treated with rifamycin derivatives.[11]

In conclusion, Rif and R-8_2 are shown to be effective anti-tumor drugs against some cell lines, by selectively killing the tumor cells. The reason behind the resistance of leukemic cells to rifamycin derivatives is not understood at present and warrants further study. The existence of both susceptible and resistant cells provides an opportunity for further probing the mechanism of action of these drugs.

We are indebted to Dr. Patrica Durban for the use of the radioactivity counting equipment.

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LEGENDS FOR FIGURES

Fig. 1. Rate of loss of ^{125}I from mice inoculated with ^{125}IUDR-labeled ascites cells and treated with drugs. A = Ehrlich's, B = TA3. Data points marked with open symbols are for animals inoculated with live cells; with closed, animals inoculated with killed cells. Each point represents the average of 10 mice. Abbreviations: $R\text{-}8_2$ = rifazone-8_2, Rif = rifampicin, MeC = methylcellulose, DCC = killed cell control.

Fig. 2. Rate of loss of ^{125}I from mice inoculated with ^{125}IUDR-labeled ascites cells and treated with drugs. A = L1210, B = MTX, C = L5178Y. Data points marked with open symbols are for animals inoculated with live cells; with closed, animals inoculated with killed cells. Each point represents the average age of 10 mice. Abbreviations: $R\text{-}8_2$ = rifazone-8_2, Rif = rifampicin, MeC = methylcellulose, DCC = killed cell control.

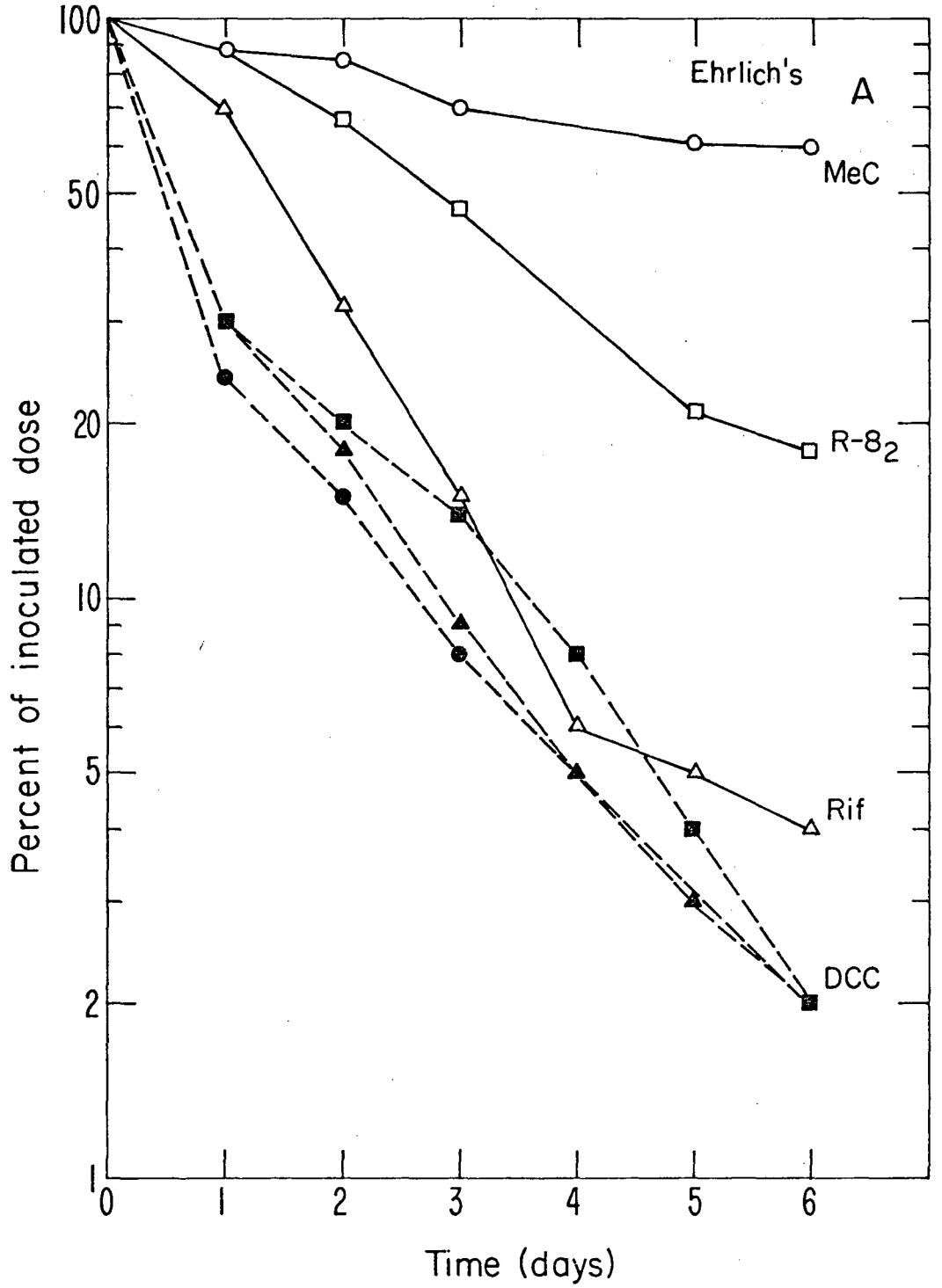

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Fig 1. - Hughes + Calvin

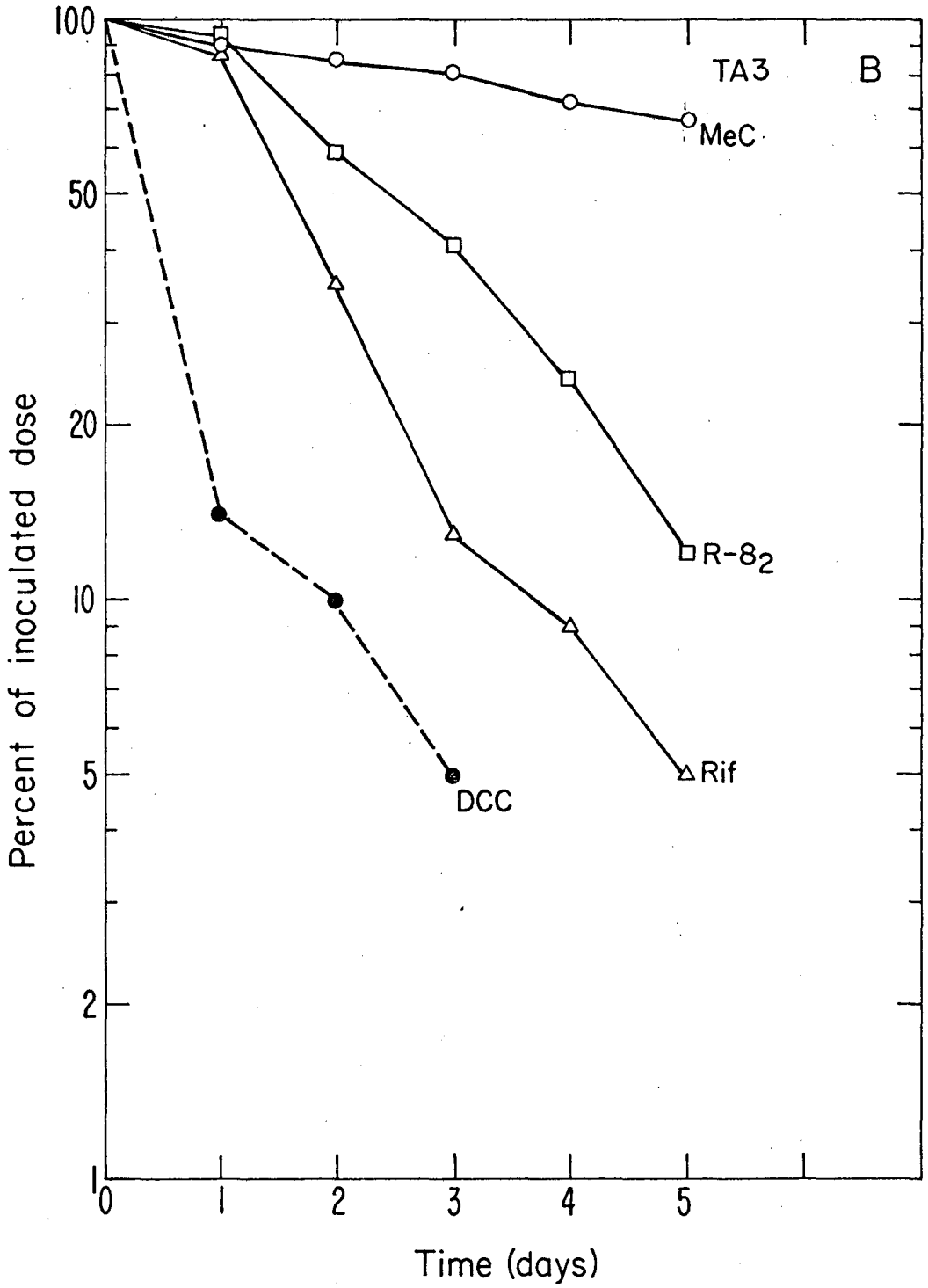

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Fig. 1 - Hughes & Calvin

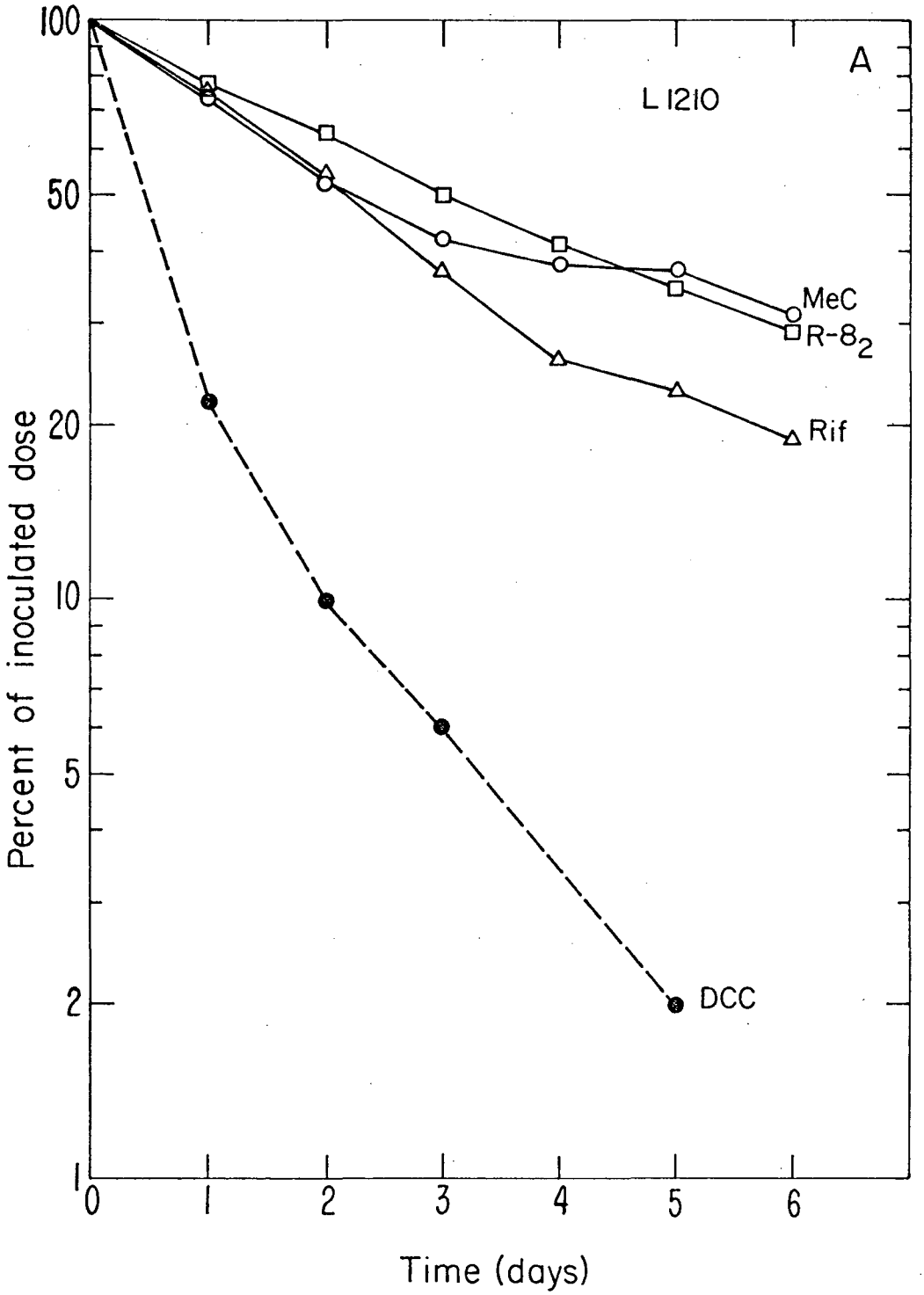

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Fig. 2 - Hughes + Calvin

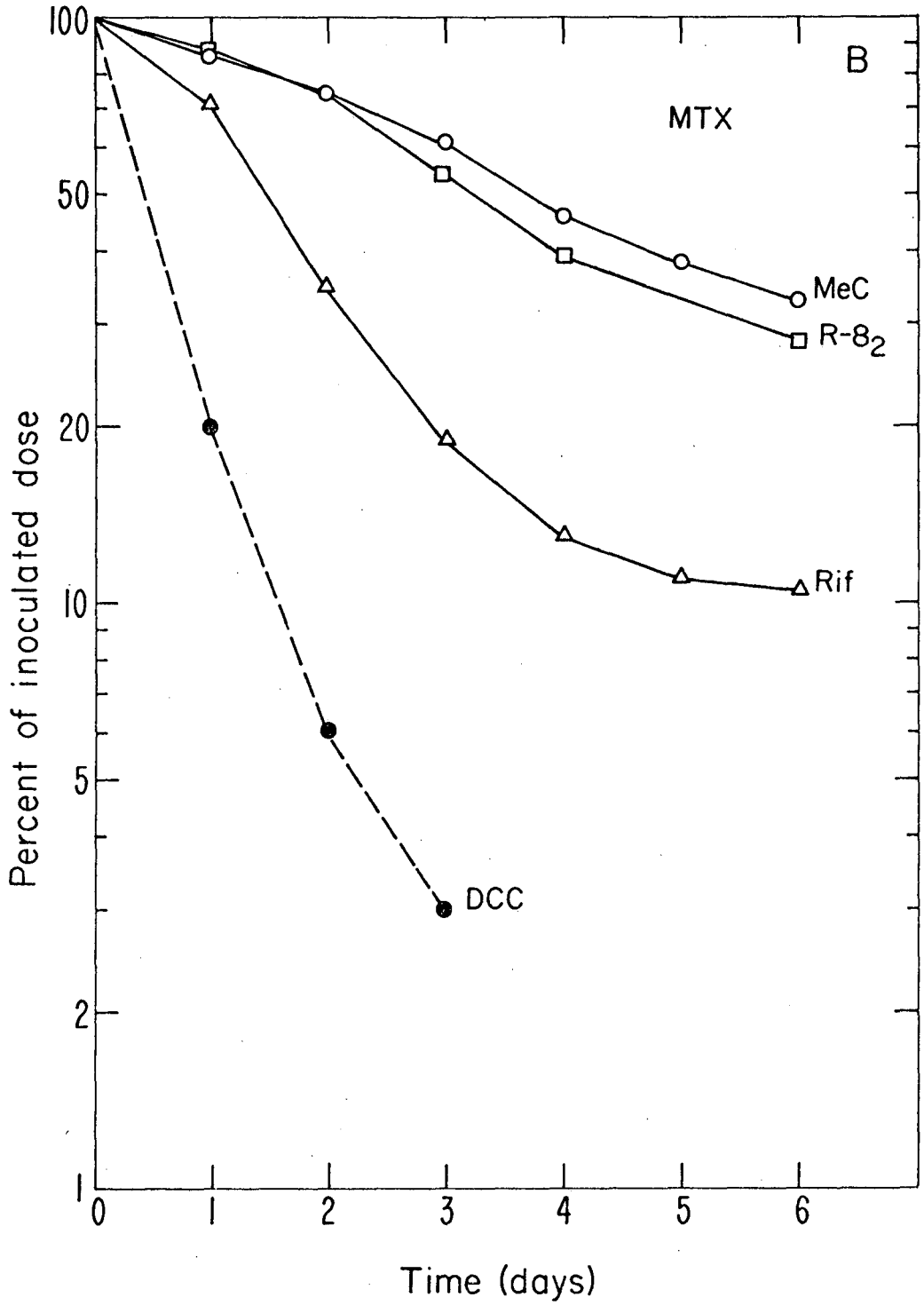

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Fig. 2 - Hughes + Calvin

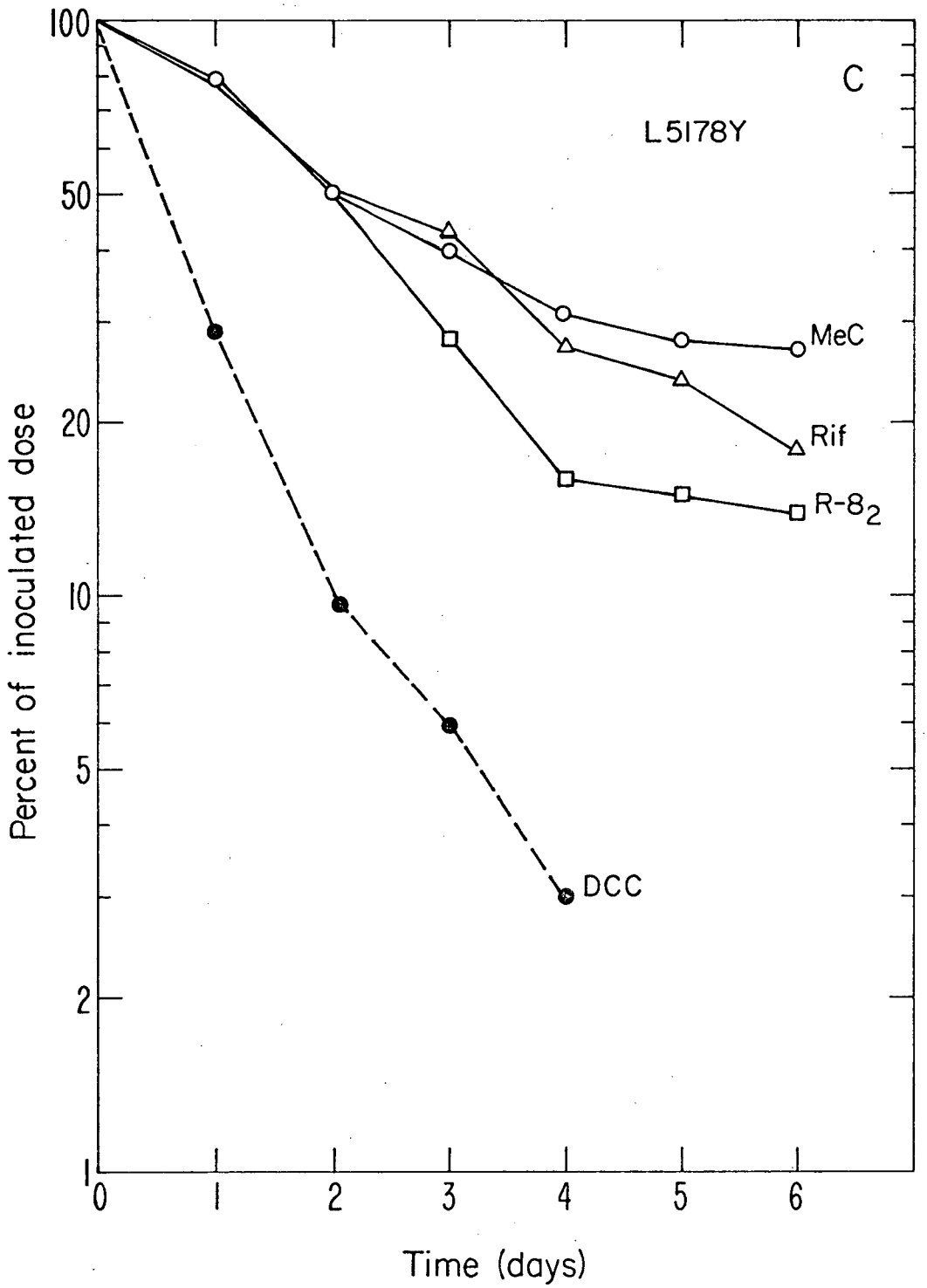

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Fig. 2 - Hughes & Calvin

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