Collateral Sensitivity to Methotrexate in Cells Resistant to Adriamycin

Terence S. Herman, Anne E. Cress, and Eugene W. Gerner

ABSTRACT

Chinese hamster ovary (CHO) cells, maintained and treated in log-phase growth, are extremely sensitive to Adriamycin but are very resistant to methotrexate-induced cell killing. The survival response to Adriamycin is biphasic, with the sensitive population showing a D0 of 0.08 μg/ml for 1 hr, while essentially no cell killing is produced by methotrexate treatments of up to 500 μg/ml for either 1 or 13 hr. Cells surviving Adriamycin treatment (5 μg/ml for 1 hr) and isolated in colony form were more resistant (D0 = 0.2 μg/ml) to subsequent treatments with Adriamycin than were the parental population, although the survival response was still biphasic. These Adriamycin-resistant cells (CHO-R/ADR), however, had become sensitive to methotrexate, with a nearly 50% cell killing achieved by treatment with 5 μg/ml for 1 hr. Uptake studies indicate that the acquired Adriamycin resistance is not due to a decrease in Adriamycin uptake, whereas the increase in methotrexate sensitivity is, in part, due to an increased uptake and a lower efflux of methotrexate. Levels of dihydrofolate reductase activity in the Adriamycin-sensitive methotrexate-resistant CHO cells are substantially higher (34%) than levels in Adriamycin-resistant (CHO-R/ADR) cultures. Titration of CHO cells with methotrexate doses of up to 500 μg/ml (for only 1 hr) reduced the dihydrofolate reductase activity to the level found in CHO-R/ADR cells treated with doses up to 5 μg/ml for 1 hr. Survival studies confirmed that Adriamycin resistance could be conferred to the CHO cells in a dose-dependent fashion by pretreating normal cultures with methotrexate. These and other data suggest that the collateral methotrexate sensitivity in the CHO-R/ADR cells is on the basis of both decreased levels of dihydrofolate reductase and increased net uptake of methotrexate. Dihydrofolate reductase levels seem to be a measure of Adriamycin sensitivity. These results may have important implications in the use of these two drugs, either alone or in combination, in cancer chemotherapy.

INTRODUCTION

ADR is currently one of the most widely used chemotherapeutic agents in cancer treatment. It exhibits a wide spectrum of activity in both solid and hematogenous cancers (8, 9). However, malignant clones resistant to this agent and to other chemotherapeutic drugs often emerge before complete eradication of disease can be accomplished. This resistance can be expressed as natural resistance, an inherent refractoriness of a set of tumor cells to a specific drug, or as acquired resistance, a shift from a response to chemotherapy to a lesser response (see Ref. 19 for a general review). Other investigators have reported the development of cross-resistance and collateral sensitivity for a number of drugs in colchicine-resistant cultured cells (4, 24). There is, however, essentially no information concerning collateral sensitivity in the case of ADR resistance.

Hill et al. (16, 17) have recently shown that cells resistant to MTX display collateral sensitivity to ADR. This group and others have demonstrated that some cell lines that are resistant to MTX have higher intracellular levels of DHFR (6, 17, 28), which is thought to be the target enzyme inhibited by MTX and other folate antagonists (1, 7). In addition, resistance to MTX may be the result of different mechanisms, since others have reported a lack of correlation between DHFR levels and MTX sensitivity. Alternative mechanisms of cellular resistance to MTX may include altered dissociation constants for the enzyme-MTX complex and altered levels of deoxyribonucleotide triphosphate pools (20, 21, 30). Transport of MTX into the cell is also an important determinant in the action of this drug (5, 31), so that alterations in normal uptake of MTX can lead to resistance to this drug (see Ref. 2 for review).

Because of the previous finding of collateral sensitivity to ADR in cells with MTX resistance (16, 17) and because of previous work suggesting that our CHO cells contained a component of ADR-resistant cells (3), we decided to investigate whether collateral MTX sensitivity was displayed in CHO cells resistant to ADR. We felt that this question was of clinical importance because these 2 drugs are used both concurrently and sequentially in cancer chemotherapy (8, 9).

MATERIALS AND METHODS

Method of Cell Culture. CHO cells, originally obtained in 1974 from Dr. Ronald M. Humphrey, University of Texas Cancer Center, M. D. Anderson Hospital, Houston, Texas, were grown in McCoy's Medium 5A supplemented with 20% (v/v) fetal calf serum, penicillin (100 units/ml), streptomycin (100 μg/ml), and amphotericin (0.25 μg/ml) (all from Grand Island Biological Co., Grand Island, N. Y.). Cells were maintained in logarithmic growth phase at 37°, in a 5% CO2-95% air atmosphere. Doubling time was 13 to 14 hr. Cell numbers were determined with an electronic particle counter (Coulter Electronics, Hialeah, Fla.).

Cell Viability Measurements. Cell viability was measured by the ability of single cells to form colonies in vitro. For this assay, known numbers of cells were plated in plastic Falcon No. 3002 Petri dishes (Falcon Plastics, Oxnard, Calif.) containing 5 ml of fresh medium. Three dilutions of the known cell number were made for each drug concentration, and each was plated in triplicate. After incubation for 7 or 8 days, colonies were fixed and stained with 0.5% crystal violet (Fisher Scientific Co., Fair Lawn, N. J.) in 95% ethanol. Viable cells were those
that produced colonies of more than 50 cells upon examination under a dissecting microscope. The plating efficiencies of control cultures were between 80% and 90%. The results from colony formation experiments, described in Charts 1, 2, 3, and 7, represent at least 3 replicate experiments per chart.

**Drug Treatments.** Exponentially growing cells were exposed to varying concentrations of ADR (Adria Laboratories, Wilmington, Del.) or MTX (Lederle Laboratories, Pearl River, N. Y.) in 60-mm Petri dishes for 1 hr at 37°C. Drug solutions were always prepared immediately prior to use by dissolving the drug in sterile water. Appropriate dilutions of drugs were added to fresh medium, with the volume of drug solution never exceeding 20% of the total volume of 5 ml. After treatment, the medium was removed, and the cultures were washed twice with warmed Puck’s Saline A (Grand Island Biological Co.) and trypsinized (0.05% for 2 to 3 min; known numbers of cells were plated into plastic Petri dishes for colony growth as described above.

**Production of ADR-resistant Cells.** CHO cells in exponential growth were exposed to ADR, 5 μg/ml, for 1 hr. After treatment, the drug was removed and cultures were washed with Puck’s Saline A. These treated cells were then trypsinized and replated in 100-mm plastic Petri dishes containing 15 ml of medium. These dishes were then incubated at 37°C for 7 days. Surviving colonies, designated as CHO-R/ADR cells, were then trypsinized and replated into plastic flasks. Thereafter, these CHO-R/ADR cells were continually maintained in ADR, 0.02 μg/ml, in addition to standard medium. This procedure was used because initial studies had shown that reverison to CHO ADR sensitivity occurred in CHO-R/ADR cells after about 45 days if these latter cells were maintained in growth medium without the drug. CHO-R/ADR cells, maintained in medium plus ADR, 0.02 μg/ml, grew exponentially with a doubling time similar to CHO cells in medium without ADR.

**Measurement of DHFR.** The activity of DHFR was measured by the method of Mathews and Huennekens (26); 2 × 10⁶ cells were used for each determination. Cells were removed from monolayer cultures by scraping with a rubber policeman. These cells were suspended in 0.1 M Tris-HCl buffer, pH 7.4, and were disrupted by sonication. The resulting cell homogenate was centrifuged at 12,000 × g for 10 min. The pellet was discarded, and the supernatant extract was placed on ice. The reaction mixture included 40 mM potassium phosphate (pH 7.4), 8 mM 2-mercaptoethanol, 0.08 mM dihydrofolate, 0.08 mM NADPH, and 0.20 ml of sample supernatant in a total volume of 1.00 ml. DHFR activity was then determined at 37°C by measuring the decrease in absorbance at 340 nm of the solution as NADPH was oxidized and dihydrofolate was reduced to tetrahydrofolate. Absorbance was measured with a temperature-controlled Beckman Model DU spectrophotometer. The results obtained, as described in Table 1, represent the mean of triplicate determinations and are confirmed by 2 replicate experiments.

**Drug Uptake Studies.** ADR uptake was determined by measuring total fluorescence in a known number of cells. For 90 min, 10⁶ cells in exponential growth were exposed to ADR, 50 μg/ml. Cultures were washed twice with Puck’s Saline A, after which cells were scraped off the dishes and placed in Hanks’ balanced salt solution (Grand Island Biological Co.). The fluorescence of these cells was then assayed with a fluorescent-activated cell sorter (TPS-1; Coulter Electronics). This instrument measures fluorescent emission, due to the intracellular ADR, as cells pass through an argon laser beam (488 mm). Increasing fluorescence corresponds to increased ADR uptake on a per cell basis. This technique has been used previously by other investigators who demonstrated that increased drug uptake correlated with increased drug-induced cytotoxicity (14).

MTX uptake was measured using [³H]MTX (150 to 250 mCi/mm); Amersham/Searle, Arlington Heights, Ill.). CHO cells, growing exponentially in 60-mm plastic Petri dishes (10⁶ cells/dish), were exposed to 2 μCi of [³H]MTX (56 μg/ml) for varying times at 37°C. After treatment, cells were placed on ice, washed twice with warmed Puck’s Saline A, and scraped off the dishes. Cells were lysed in 0.1 ml of 1 M NaOH and solubilized in a toluene fluor containing PPO, 0.4 mg/ml, and POPPOP, 5 μg/ml, and then adjusted to 25% Triton X-100 (v/v). Samples were counted in a Searle Mark II liquid scintillation counter (65% efficiency for tritium).

**Efflux of MTX.** In these experiments, cells were exposed to 2 μCi of [³H]MTX (56 μg/ml) for 1 hr at 37°C. After this treatment, cells were washed twice with warmed Puck’s Saline A and placed in standard medium. At various times thereafter, cells were removed from the monolayer cultures by scraping, they were solubilized, and the remaining [³H]MTX was determined as described above.

**RESULTS**

The survival kinetics of CHO and CHO-R/ADR cells exposed to varying concentrations of ADR for 1 hr are shown in Chart 1. Both cultures display biphasic survival responses. Analysis of the initial slopes of each curve demonstrate that the CHO-R/ADR cells (D₀ = 0.02 μg/ml) are approximately 2.5 times less sensitive than the wild-type cells (D₀ = 0.08 μg/ml). The D₀, a standard radiobiological parameter used to describe the inverse of a log-linear survival curve slope, is the dose required to reduce survival by 1/e on the exponential portion of a survival curve. The survival differences between these 2 cell lines is greater in the middle range of ADR concentrations used (~20 times at 1.0 μg/ml and ~50 times at 2.0 and 5.0 μg/ml) than they are at the extremes of the dose response curve (~4 times at 0.2 μg/ml and ~15 times at 15 μg/ml). Results not shown demonstrated that CHO-R/ADR cells which were not maintained in ADR reverted to the CHO ADR sensitivity within 45 days. The reversion frequency was not determined.

Chart 2 shows the survival kinetics of CHO cells exposed to increasing concentrations of MTX for either 1 or 13 hr. These cells exhibit an extreme degree of resistance to MTX, and essentially no cell killing was achieved even with a concentration as high as 500 μg/ml for 13 hr. The doubling time of CHO cells growing exponentially in medium supplemented with MTX concentrations, from 5 to 500 μg/ml, was unaffected for 24 hr, after which the growth rate decreased (Table 1).

In order to determine whether collateral sensitivity to MTX exists in the CHO-R/ADR cells, the survival kinetics of the CHO-R/ADR cells, exposed to varying concentrations of MTX for 1 hr, was examined. These results are presented in Chart 3. In CHO-R/ADR cells, a 50% cell kill (equal to the percentage of cells in S phase during exponential growth) was achieved after 1-hr exposure to concentrations of MTX (ranging from 5
Effect of continuous exposure to MTX on the cell number increase of log-phase CHO cells

<table>
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<th>Time after addition of MTX (hr)</th>
<th>Cell no./dish \times 10^4 with following MTX concentration</th>
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HFR activity in log-phase cells treated with MTX in normal growth medium

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</table>

MTX Sensitivity in ADR-resistant Cells

Table 1

Table 2

Chart 1. Survival kinetics of CHO (ADR-sensitive (•)) and CHO-R/ADR (ADR-resistant (○)) cells, both treated in log-phase growth with increasing concentrations of ADR for 1 hr. Bars, S.E. of single-cell survival.

Chart 2. Survival kinetics of CHO cells treated while in exponential growth with increasing concentrations of MTX for either 1 (•) or 13 (○) hr. Following this treatment, cells were plated for colony growth as described in "Materials and Methods."

Chart 3. Survival kinetics of the ADR-resistant subline (CHO-R/ADR (○)) as compared with survival of normal CHO cells (•). Both cultures were treated, while in log-phase growth with increasing concentrations of MTX for 1 hr, as described in "Materials and Methods." Bars, SE of single-cell survival.

In an effort to explain the differential sensitivity to MTX in CHO and CHO-R/ADR cells, we examined the level of the target enzyme DHFR. Table 2 lists DHFR activity in the CHO and CHO-R/ADR cell lines and the decrease in activity of this enzyme after 1-hr incubations of whole cells with increasing concentrations of MTX. The level of DHFR activity (before exposure to MTX) is greater in CHO cells than in the CHO-R/ADR cells (7.23 versus 5.41 pmol NADPH oxidized per 10^7 cells per min). DHFR activity in CHO cells treated with MTX, 5 \mu g/ml, decreased only 13% relative to untreated controls, whereas similar treatment reduced DHFR activity nearly 50% in CHO-R/ADR cells. It should also be noted that 500 \mu g of MTX per ml for 1 hr (the maximal concentration which did not change medium pH) only reduced the DHFR activity in CHO.
cells to levels similar to those found in CHO-R/ADR cells exposed to 5 μg of MTX per ml for 1 hr. Thus, in cells sensitive to MTX (CHO-R/ADR), the initial DHFR activity is low and decreases to a greater extent, compared to cells resistant to MTX (CHO).

It is possible that the observed difference in inhibition of DHFR activity between the CHO and CHO-R/ADR cells, after exposure of whole cells to MTX, resulted from different DHFR isozymes within each cell population with different affinities for MTX. This question was addressed by measuring DHFR activity in a cell-free system after exposure to MTX. Cells were lysed by sonication. Incubation of the resulting 12,000 x g 10-min supernatant, from either cell line, with final MTX concentrations ranging from 1 to 500 μg/ml, in the reaction mixture, revealed no difference in the inactivation of DHFR activity (data not shown). Using extracts from both CHO and CHO-R/ADR cultures, MTX concentrations as low as 1 μg/ml inactivated more than 90% of the initial enzyme activity in this soluble fraction. This suggested that the differences observed in DHFR inactivation were not due to DHFR isozymes. A similar result has been reported previously (28).

Since the decrease in DHFR activity after exposure to low concentrations of MTX was more rapid in CHO-R/ADR than in CHO cells (Table 2), we postulated that the ADR-resistant cells might accumulate MTX more rapidly than do CHO cells. Because this suggested an altered membrane in ADR-resistant cells, we examined the uptake of both ADR and MTX into both cell populations, since the collateral sensitivity observed could have been explained by a reciprocal relationship between ADR and MTX uptake. Chart 4 demonstrates that the amount of ADR which entered cells after exposure to 50 μg/ml for 90 min (as measured by the integrated fluorescent histogram) was not different for the CHO (ADR-sensitive) and CHO-R/ADR (ADR-resistant) cells. This chart also shows that chronic exposure of the ADR-resistant cells to low doses of ADR contributed very little fluorescence as compared with exposure to 50 μg/ml. The data in Chart 4 represent total ADR uptake on a per cell basis and are not indicative of possible uptake rate differences between the 2 cultures.

Chart 5 shows the accumulation of [3H]MTX (56 μg/ml) in cultures after continuous exposure to the drug. By comparing Charts 5 and 6, net uptake of MTX can be derived. As can be seen, the CHO-R/ADR cells incorporate more MTX than do CHO cells. By 3 hr, the level of MTX found in the CHO-R/ADR cells was more than twice that found in the CHO cells (1.56 versus 0.71 μmol/10⁶ cells). Thus, the collateral sensitivity to MTX observed in ADR-resistant cells seems to be explained, at least partially, on the basis of an alteration in MTX uptake. However, the change in the CHO-R/ADR cells which confers ADR resistance does not correlate with an alteration of ADR uptake, although the rate of ADR uptake may be affected.

In order to investigate whether an altered rate of efflux of MTX in CHO-R/ADR cells could account for the higher concentration of MTX in these cells after treatment, we exposed both cell populations to [3H]MTX, 56 μg/ml, for 1 hr, after which cells were washed and placed in drug-free medium. Chart 6 shows the amount of [3H]MTX remaining over time in each cell line after cessation of drug exposure. Over the 5 hr of sampling after removal of drug, the decrease in [3H]MTX in CHO cells was approximately 3 times greater than that in CHO-R/ADR cells (0.15 versus 0.05 μmol/10⁶ cells). The data obtained suggest that the uptake and efflux studies reflect both free and DHFR-bound MTX, since the CHO cells display higher DHFR activity, compared to CHO-R/ADR cells; however, they show only one-half the MTX level after incubation times of more than 1 hr. If our MTX uptake and efflux studies reflected only DHFR-bound MTX, then the enzyme levels (Table 2) would be proportional to the uptake results.

Since it appeared that the difference in sensitivity to ADR of the 2 cell populations could not be explained on the basis of a different uptake of ADR and different levels of DHFR activity were observed, we undertook to determine whether the decreased level of DHFR activity in the CHO-R/ADR cells was related to the ADR resistance. Chart 2 showed that MTX doses up to 500 μg/ml for 1 or 13 hr caused no killing of CHO cells. These treatments also did not affect the rate of semiconservative DNA replication, measured by CsCl centrifugation of density-labeled DNA as previously described (27), in treated S-phase CHO cells (data not shown), nor did it affect the doubling time for up to 24 hr when these cells were grown in medium containing MTX (Table 1). However, incubation of CHO cells with increasing concentrations of MTX decreased DHFR activity in a predictable manner (see Table 2). Chart 7 shows the

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**Chart 4.** Uptake of ADR into CHO cells (○-○) and CHO-R/ADR cells (△-△). CHO cells after pretreatment of cells with MTX, 500 μg/ml (△-△), and CHO-R/ADR cells maintained in ADR, 0.02 μg/ml, during exponential growth (○-○). Increasing fluorescence is taken as a measure of increased ADR uptake into cells, as described by others (14). Points, means of 2 experiments.

**Chart 5.** Uptake of MTX into CHO-R/ADR (○) and CHO (●) cells over time. Cells in log-phase growth from each culture were exposed continuously to [3H]MTX, 2 μCi/ml, (150 to 250 mCi/mmol) for up to 6 hr. Samples were taken each hr and processed as described in "Materials and Methods." Points, means of 2 experiments.
effect of 1-hr pretreatment of CHO cells with MTX on subsequent sensitivity to ADR. After pretreatment with MTX, 5 \( \mu \)g/ml, for 1 hr, the cytotoxic effect of 1-hr exposures to ADR is essentially the same as for the normal CHO cells not pretreated with MTX. After pretreatment with MTX, 500 \( \mu \)g/ml, for 1 hr, however [a dose that decreases DHFR activity to the level found in CHO-R/ADR cells (Table 2)], the survival curve was not significantly different from that of CHO-R/ADR cells. In addition, after pretreatment of CHO cells with MTX, 250 \( \mu \)g/ml, for 1 hr, which produced DHFR activity between that found in the CHO and CHO-R/ADR cells, we observed intermediate sensitivity of these cells to ADR.

**DISCUSSION**

The data presented in this report suggest a relationship between the levels of DHFR activity and sensitivity to ADR. We have shown that decreasing the activity of DHFR by 1-hr treatment with MTX will result in cells resistant to ADR.

The possibility existed that pretreatment of the ADR-sensitive cells with MTX altered the cell cycle kinetics or the distribution of cells in the cell cycle. If the cell cycle kinetics and/or distribution were changed, the ADR target in these cells might be altered and might result in the relative resistance of these cells to ADR.

To exclude this possibility, the total number of CHO cells was measured in the presence of various concentrations of MTX for up to 60 hr. If MTX has an effect on cell cycle kinetics or arrests cells in a particular cell cycle compartment, one would expect a change in total cell numbers with time. Table 1 shows that the 2 MTX concentrations used do not effect the increase in cell number, as compared to controls, for 24 hr. From these data, it is unlikely that the 1-hr pretreatment would cause a major effect on cell cycle kinetics and/or distribution and result in the significant ADR resistance shown in Chart 7.

We have seen that lowering DHFR levels in our CHO cells by a pretreatment with MTX will result in cells with an increased resistance to ADR. In addition, selected cells that are relatively ADR resistant (CHO-R/ADR) have a decreased level of DHFR activity, compared to controls, and show a marked increased sensitivity to MTX. These cells show an increased uptake of MTX partially due to a slower rate of MTX efflux. Thus, the increased sensitivity of CHO-R/ADR to MTX is probably due to the increased intracellular level of the lethal drug and the lowered activity of the target enzyme (DHFR). The resistance of MTX in our CHO cells, compared to CHO-R/ADR cells which are sensitive to MTX, is similar to the acquired MTX resistance described previously by others (6, 17, 28), in that these cells show increased levels of DHFR and decreased uptake of MTX. This type of resistance may be different from the intrinsic resistance to MTX, as described by Tattersal et al. (30), which did not correlate with DHFR levels in their studies. The similar inactivation of DHFR, derived from both cell lines, by MTX is consistent with other data concerning the interaction of MTX with this enzyme purified from cells with acquired MTX resistance (12, 13, 32). Our results are also consistent with the work of other laboratories, demonstrating the importance of MTX transport in determining MTX sensitivity (5, 31).

In contrast, the relationship between ADR sensitivity and DHFR activity is not immediately obvious. Several laboratories have demonstrated that ADR affects DNA and RNA synthesis (3, 22) and interferes with normal cell cycle progression (3, 11, 23). Genome damage correlates with these cell cycle blocks (18), and it has been shown that ADR decreases the activity of both DNA-dependent DNA and RNA polymerases (15, 25). It has been suggested that a specific target for ADR cytotoxicity may be rRNA synthesis in the nucleolus (11).

Recently, Spandidos and Siminovitch (29) reported that the genes controlling the level and structure of DHFR are linked in CHO cells. These authors suggest that, in general, genes controlling enzymes in a common metabolic pathway may be linked, or syntenic (29). Cheillo et al. (10) have reported that DHFR levels are related to the levels of thymidylate synthetase and thymidine kinase in cultured mammalian cells. Thus, it may be plausible that the structural genes controlling the level of DHFR activity may be linked to genes controlling the target(s) for ADR cytotoxicity. Our results, indicating that ADR resistance can be produced by simply titrating with MTX, suggest either a protective effect of MTX on the ADR target or that the target for ADR cytotoxicity is related to DHFR levels. It is
interesting to note that the CHO-R/ADR response to ADR reported here is similar to that reported previously for CHO cells (3). The latter cells (3) were MTX sensitive and quite stable in their response to both MTX and ADR, whereas our CHO-R/ADR cells revert to an ADR-sensitive, MTX-resistant state within 45 days. Our current CHO cells have proven to have a very constant response to both ADR and MTX over a 2-year period.

Another possible explanation of the results in this report may pertain to the intracellular metabolism of ADR. Since the uptake of ADR is similar in both CHO and CHO-R/ADR cells, it is possible that DHFR may be involved, through its role in protein synthesis, in differential metabolism of ADR in these 2 cell lines. This may be consistent with the effect of pretreatment of CHO cells with MTX, since subsequent ADR sensitivity appears to be a function of acutely altered DHFR activity. Thus, altered DHFR levels may result in different rates of metabolism of ADR to inactive metabolites.

The extreme ADR sensitivity and MTX resistance of this CHO line is undoubtedly unusual and may well not reflect a clinically applicable situation. We are now seeking to correlate the decreased ADR sensitivity, in other cell lines and in biopsible human tumors, with DHFR activity. If a relationship similar to that reported here is found, then treatment of tumors resistant to ADR with high-dose MTX would be reasonable. Conversely, the use of ADR in tumors which have become highly resistant to MTX because of high intracellular levels of DHFR activity should be effective. In addition, our data suggest that concurrent treatment with MTX and ADR may be counterproductive after tumors have become highly resistant to MTX due to increased levels of DHFR activity.

REFERENCES


* E. W. Gerner, unpublished results.
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