Streptonigrin: Effect on the First Meiotic Metaphase of the Mouse Egg

Abstract. *Streptonigrin, an antibiotic and antitumor agent, alters the chromosomes of the mouse ovum during meiosis. Agglutination of bivalents or achromatic gaps and breaks occurred in the larger pairs both in vitro and in vivo. This newly detected cytogenetic effect suggests that such agents can gain access to developing mammalian ova and destroy the normal progress of meiosis.*

Much interest currently attaches to theoretical errors in female mammalian meiosis as mechanisms underlying the production of abnormal zygotes, but very few data are available in this area of reproductive pathology as studied by cytogenetic techniques of the type applied to lower forms. Radiation, radiomimetic drugs, and similar agents have been used in study of histologic or functional changes in the mammalian ovary, without the additional information obtainable by cytogenetic analysis of the number and morphology of the chromosomes of the ovum as it proceeds through meiosis from dictyate to first meiotic metaphase and beyond.

As part of a broad investigation of agents that may constitute a potential environmental hazard to female oocyte development, I investigated Streptonigrin (*1*), the antibiotic and antitumor agent [already shown to be very destructive to mitotic chromosomes in human leukocyte cultures (*2*)], for its effect on the early stages of the dividing chromosomes of the egg of the female mouse.

Using a modification of the technique in vitro of Edwards (*3*) I observed that, at concentrations of Streptonigrin very similar to those that were active in the human leukocyte system, varying degrees of abnormality could be produced in the first meiotic metaphase bivalents of mouse eggs. Incubations, each of about ten ova, were established from pooled ova obtained from cycling of adult female mice of a Strong-A derived strain; the ova were maintained at p*H* 7.2 to 7.4 in an atmosphere of air containing 5 percent CO_2 at 37°C for 6.5 to 7.0 hours. This period is correct for production of first meiotic metaphase figures (*3*).

Control tubes, containing either no Streptonigrin or dimethylacetamide (used for dissolving the Streptonigrin), were incubated simultaneously with each set of experimental ova. After being harvested, the ova were treated with hypotonic solution of citrate (0.7 percent) for 10 minutes and fixed with 50 percent acetic acid. Phase examination of bivalents was carried out on unstained preparations before ova were lightly squashed stepwise, with microscopic control. Slides were stained with lacto-aceto-orcein and mounted.

At a Streptonigrin concentration of 1.0 μg per milliliter of incubation media, the ova had proceeded from the dictyate stage to a relatively organized state of pairing, but definition of individual bivalents was impossible because of agglutination of the chromosomes (Fig. 1). Such morphology could result from the coalescence of chromatin material observed with high concentrations of Streptonigrin in a study of nucleic acids (*4*). In approximately 40 percent of the ova, concentrations of 0.1 μg/ml produced achromatic gaps and breaks—most easily recognized in the longer pairs (Fig. 1). Concentrations below 0.1 μg/ml produced no cytogenetic abnormality detectable by my microscopy.

The possibility that the changes observed in vitro could be induced in vivo was then studied in the immature mouse by use of the superovulation technique (*5*). The minimum effective dose of freshly made Streptonigrin proved to be 1.25 μg per gram of body weight administered subcutaneously daily during hormone treatment. This dose produced some edema of dorsal subcutaneous tissues in the animals, but they remained active and apparently healthy. Ova were harvested from the ovaries 6 to 7.5 hours after administration of human chorionic gonadotrophin (HCG) and then prepared for cytologic examination like the incubated eggs; approximately 20 ova were obtained from each of 117 mice.

The findings were very similar to the effects in vitro; again the abnormalities were located in the longer bivalents (Fig. 1). Examination of the oviducts 14 to 18 hours after administration of HCG revealed the absence of ovulated eggs. Large intrafollicular ova contained an abstricted polar body I and a distorted second metaphase configuration in the nearby cytoplasm, or an irregular dark mass that was recognizable only with difficulty as nuclear material.

These observations detected a hitherto unreported ability of an antibiotic, chemically related to several in general use, to gain access to and alter

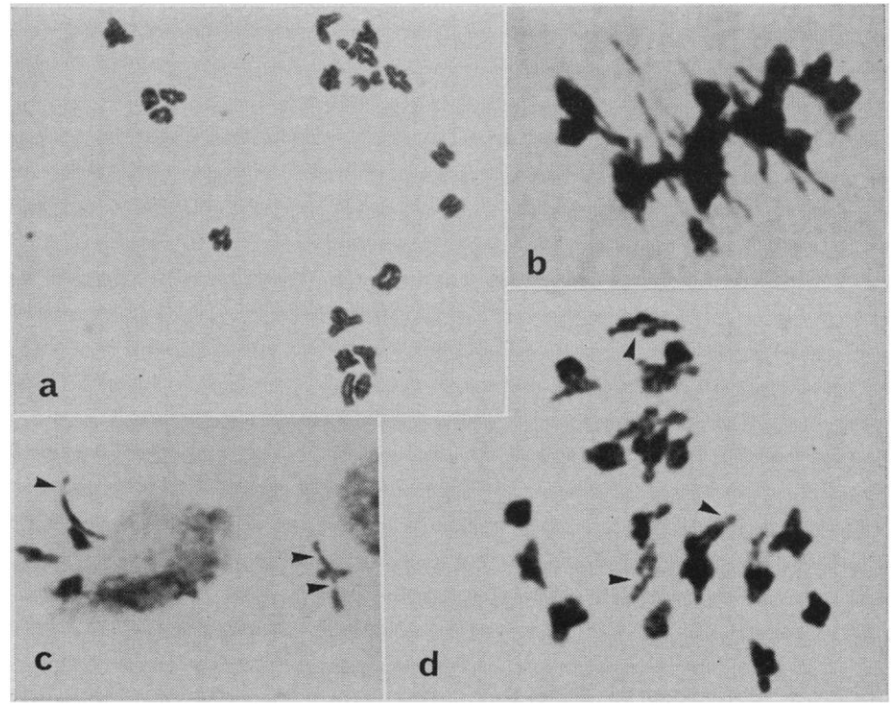

Fig. 1. Meiotic metaphase-I configurations: (a) normal, (b) 1.0 μg/ml in vitro, (c) 0.1 μg/ml in vitro, (d) in vivo. Arrows indicate gaps and breaks.

the chromosomes of the developing female mammalian germ cell. The larger bivalents are altered most consistently, but no definite parallel with the work on human leukocytes, in which the pericentric regions and secondary constrictions of group-A chromosomes were broken (*2*), has been demonstrated. Bennett (*6*) has confirmed the presence of secondary constrictions in mouse chromosomes 7, 18, and 20, and it is conceivable on morphologic grounds that one of the frequently altered pairs in the experiments that I have described is No. 7. How Streptonigrin achieves discontinuity and disorganization of female meiotic chromosomes, that have already completed synthesis of DNA (*7*), is not elucidated by these experiments, but, as a tool for studying agents that may produce mutations by acting on intrafollicular ova, the compound and the techniques seem very promising.

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References and Notes

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Deficient Complement Fixation by Aggregated Gamma Globulin from Hypogammaglobulinemic Patients

Abstract. *We found that several normal samples of aggregated gamma globulin consistently fixed essentially equal amounts of guinea pig complement, while samples of gamma globulin from three of four hypogammaglobulinemic patients were markedly deficient in this biologic function. Thus, the previously observed limited heterogeneity of gamma globulin in patients with hypogammaglobulinemia is also associated with inefficiency in at least one biological function.*

Hong and Good (*1*) demonstrated that the gamma globulin (IgG) isolated from patients with hypogammaglobulinemic syndromes showed less electrophoretic heterogeneity, variable mobilities, and differences in dye-binding capacity, compared to IgG from normal serum. We find significant deficiency in the complement-fixing capacity of heat-aggregated IgG obtained from the serum of hypogammaglobulinemic patients. Thus, these patients have qualitative as well as quantitative abnormalities of IgG.

By column chromatography (*2*), IgG was isolated from four patients with hypogammaglobulinemia and from 11 normal individuals. Samples of IgG (0.25 and 0.5 mg) in 0.5 ml of 0.15*M* sodium chloride were aggregated by heating at 63°C for 20 minutes (*3*). Complement studies were made with only slight modification of the methods of Mayer (*4*). Guinea pig complement was diluted in barbital-buffered saline containing calcium ($1.5 \times 10^{-4}M$) and magnesium ($5 \times 10^{-4}M$) to a concentration which would provide approximately 30 hemolytic units of complement ($C'H_{50}$) units in 1.0 ml. Samples of 0.5 ml of this complement preparation were added to tubes containing 0.5 ml of the aggregated IgG, thereby making a final volume of 1.0 ml. The mixtures were then incubated at 37°C for 90 minutes, and the remaining complement activity was determined (*4*). The number of units of complement fixed was obtained by subtracting the residual units in the samples reacted with IgG from the number of units in controls incubated at 37°C for the same period of time with 0.5 ml of 0.15*M* sodium chloride lacking IgG. Determinations were done in duplicate.

In repeated examination of normal serums, we found that IgG from individual samples, heat-aggregated in the manner described, showed consistent complement-fixing ability. Results of our study of normals are shown in Table 1. In all instances the unaggregated IgG fixed less than 1.7 units.

In contrast to observations with normal IgG, studies of the complement-fixing ability of aggregated IgG from patients with hypogammaglobulinemia revealed clear differences. The serums of three of the four patients consistently showed inadequate complement-fixing capacity. The aggregated IgG from patient S on two occasions fixed 3.6 and 5.0 $C'H_{50}$ units out of 16, while normal aggregated IgG (studied simultaneously) fixed 11.4 to 13.4 units. Samples of IgG from two other patients fixed 5.5 to 7.5 $C'H_{50}$ units. The IgG from the fourth patient on several occasions fixed amounts of complement equal to or slightly less than normal. In contrast to the others, this individual has never experienced undue susceptibility to infection. His quantitative immunoglobulin abnormality was discovered in a family study in which the propositus was an acquired agammaglobulinemic. The data also show that IgG preparations from these hypogammaglobulinemic patients, unlike similar preparations from normal serum, vary widely from individual to individual in complement-fixing ability (Fig. 1).

Investigations by Ishizaka and Ishizaka (*3*, *5*) have shown conclusively that the complement-fixing (or inactivating) and skin-reactive properties of aggregated human γ-globulin, regardless of the method of aggregation, are essentially identical with those of antigen-antibody complexes. For this reason, we used heat aggregation of our IgG samples to study one aspect of their biologic activity.

Our study revealing that the isolated IgG from some patients with hypogammaglobulinema differs from normal IgG in at least one biologic function and the finding of immunochemical variations from normal in the same IgG samples (*1*) support the thesis that there is a deficient gene product in some of these patients. The data suggest that at least three of the patients we studied are deficient in ability to synthesize a portion or portions of the total IgG population which provides complement-fixing sites when heat-aggregated. The electrophoretic study of the IgG from these same patients shows a limited heterogeneity; this also suggests the presence of only limited segments of the total normal IgG population (*1*). The limited complement-fixing ability of the IgG from our patients might be explained by the absence of a group or groups of IgG molecules which carry complement-fixing sites