group is not significant, it is interpreted as an indication that irradiation of the male prior to mating may increase the frequency of resorption of whole litters in rabbits.

The difference in numbers of young stillborn, 4.3 and 11.1%, respectively, in the two groups is highly significant ($P < .01$). The litters of 2 control and 3 test females which did not prepare a nest prior to parturition were excluded from the summary, because of the possibility that the young may have died from exposure. The observed difference in viability of the young at birth is believed to be due to the exposure of the male parent to irradiation prior to mating.

The average number of young born was 7.7 and 6.4, respectively, in the control and test groups (Table 2). The over-all reduction in fertility is further emphasized in the average number born alive per mating (Table 2).

Since it has been suggested that genetic damage would more likely be apparent in offspring conceived immediately after irradiation of the parent than in later births (*15*), the data on fetal mortality (Table 1) and numbers of young born (Table 2) for all females mated to irradiated males were tabulated so that any differences in fertility, zero to 35 days and 36 to 108 days post-treatment, could be studied. The data fail to show any difference; consequently, the data for the two periods were pooled for analysis. Admittedly the two intervals were somewhat arbitrarily selected, but it has been reported that in rabbits, after ligation of the epididymis, the sperm contained in the epididymis were in no case capable of effecting fertilization longer than 38 days (*16*).

No information regarding the viability or growth of the young which were born alive was obtained from this study. It is conceivable that not all the defective offspring died before or at the time of birth. A study of the growth rates and other criteria of viability in such young would seem desirable.

The pooled fertility data for all treated males indicate that deleterious effects on the prenatal viability of the offspring occur when the male has been exposed to the relatively low levels of radiation (100, 200, or 300 r) used in this study. In view of this, extreme caution should be exercised in the voluntary exposure of humans to ionizing radiation approaching this order of magnitude until further experimental evidence is available.

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Electron Microscopy of Isolated Chromosomes[1]

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Electron microscopical studies of isolated chromosomes have been made by a number of investigators (*1–3*). In the present work, the organization of chromosomes isolated from turkey and chicken erythrocytes and from calf thymus by the methods of Mirsky and Ris (*4, 5*) has been studied in the electron microscope, which was used in conjunction with certain chemical and enzyme treatments. Special emphasis has been placed on the use of an electron staining procedure for desoxyribose nucleic acid, which has been described by Bretschneider (*6*).

Since the procedure used for the isolation of fowl erythrocyte chromosomes has been slightly modified from that described by Mirsky and Ris (*4*), it is outlined briefly here. Oxalated turkey or chicken blood was frozen at −40° C immediately upon withdrawal from the fowl. The thawed blood was then used for the isolation of erythrocyte nuclei, which were washed with 0.14 *M* NaCl until practically colorless. Four successive 5-min treatments of dilute suspensions of the nuclei in the Waring blendor produced a chromosome suspension almost free of unbroken nuclei (microscopic examination of aceto-orcein stained specimens). The chromosomes, washed twice by dispersion in saline in the blendor, were stored in plastic tubes in a cold room at 4° C. The use of blood that had been frozen prior to use for isolation of nuclei was found to greatly facilitate their rupture in the subsequent Waring blendor treatment.

The staining procedure described by Bretschneider (*6*) for the demonstration of desoxyribose nucleic acid in bull sperm has been applied to whole chromosomes from fowl erythrocytes and from calf thymus. "Residual chromosomes" were prepared from fowl erythrocyte chromosomes by 1 *M* NaCl extraction (*2*), in order to remove the outer nucleohistone layer, and were similarly stained. The staining procedure applied to concentrated suspensions of chromosome preparations in saline comprised mercuric chloride fixation,

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washing with alcoholic iodine solution, treatment with alcoholic dimedon solution followed by washing with a water-ethanol sequence of 70–0% ethanol, hydrolysis with HCl, silver nitrate staining, and pepsin digestion.

Chromosome preparations, mounted on Formvar films, were examined in an RCA Model EMU electron microscope. Certain specimens were shadowed with chromium by standard techniques. Aceto-orcein staining and the application to the mounted specimens of electron stains such as 0.1% phosphotungstic acid, saturated molybdic acid solution, and 2% osmic acid were also carried out.

Whole chromosomes from fowl erythrocytes, when stained with aceto-orcein or with the electron stains just mentioned, were found to be moderately kinked strands with diameters ranging from 0.06 to 0.5 μ and lengths from 3 to 8 μ. The light micrographs of Mirsky and Ris (*4*) show a range of diameters from 0.3 to 0.8 μ and lengths from 3 to 5 μ. There was no definite evidence for chromomeres or multiple strands within the whole chromosomes when studied by the above techniques.

"Residual chromosomes" from turkey erythrocytes when shadowed with chromium, with and without chromic acid fixation, gave structures similar to those reported by Denues (*2*). The reduction in diameter caused by 1 *M* NaCl treatment is notable, and the "residual chromosomes" were thus found to be kinked strands with an average diameter of about 0.05 μ. There was no evidence for the association of two or more strands in most of the structures observed.

Typical results of the silver-staining procedure of Bretschneider (*6*) applied to whole chromosomes from fowl erythrocytes and from calf thymus are illustrated in Fig. 1. In Fig. 1 *A* it can be seen that material of high electron scattering power, probably silver, is uniformly distributed along a single chromosome strand. Chemical analysis of the strands indicated 7.17% of silver present. Chromium shadowing indicated that the strands were not flat ribbons but had cross sections that were approximately circular. The dark projections protruding from the strand were present before exposure to the electron beam, since it was observed that the projections cast shadows. Exposure of the strands observed at low electron beam intensities (Fig. 1 *A*) to beams of higher intensity caused staining material to leave certain regions either by migration or evaporation and produced the structure seen in Fig. 1 *B*. The principal feature of chromosome structure revealed by this figure is the long thin cylindrical casing which will be called a tubule. Completely identical structures were obtained by the Bretschneider staining procedure, regardless of whether the starting material was whole chromosomes

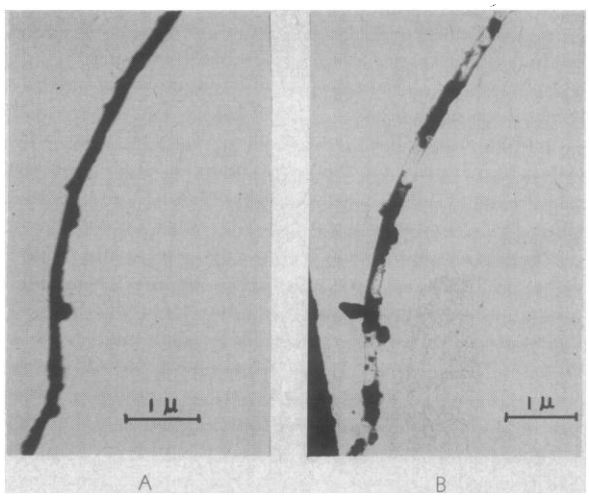

FIG. 1. Calf thymus chromosome after Bretschneider staining procedure: *A*, observation at low beam intensity; *B*, after exposure to higher beam intensity.

from fowl erythrocytes and from calf thymus or "residual chromosomes" from turkey erythrocytes. This tubule is the key structural element of the chromosomes studied in this investigation.

As a control, sheep erythrocytes were carried through the same isolation and staining techniques as fowl erythrocytes. Although occasional strandlike structures were seen in the specimens stained with aceto-orcein, no strands were seen after staining with silver in the usual manner.

In a study of the various steps in the Bretschneider staining procedure applied to whole chromosomes it was found that, until the digestion with pepsin in 0.1 *N* HCl, the major part of the desoxyribose nucleic acid, and some of the histone of the outer sheath, are still attached to the central tubule, and the great reduction in diameter occurs when these constituents are removed. The complete absence of ribose nucleic acid in the tubule was shown by chemical analysis.

A more detailed account of the present work will be published elsewhere. Studies carried out on chromosomes in smears from *Drosophila melanogaster* and *Rhoeo discolor* are reported in a separate paper.

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