Chromosome changes in embryos treated with various teratogens

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INTRODUCTION

The association of chromosome changes with certain syndromes of congenital malformations has raised new questions as to the possible role of abnormal chromosome complements in teratogenesis. For example, mosaics are thought to originate in the first divisions of the fertilized ovum, and one may ask how late in embryogenesis permanent chromosome changes can be induced. Investigations have been undertaken in this laboratory to ascertain whether teratogens administered to pregnant rats on the 11–13th days of gestation, when many malformations can be induced, will result in embryos with chromosomal aberrations as well as malformations.

In a previous report (Soukup, Takacs & Warkany, 1965) we have described experiments in which pregnant rats were treated with teratogenic doses of X-ray followed by examination of embryos for detectable chromosome abnormalities.

In embryos irradiated on the 13th day with 400 r and examined 6–12 h later, approximately 60% of the countable metaphase figures revealed chromosome aberrations. Detectable chromosome damage dropped sharply within 24 h after irradiation, and by 72 h it approached control levels. It was concluded that chromosome aberrations induced by X-rays during organogenesis disappeared rapidly and almost completely even in grossly abnormal embryos. When the X-ray dose was reduced to 200 r, which is still a teratogenic dose, only 7% abnormal metaphase figures were observed 6 h after irradiation.

In the present investigation results obtained from embryos exposed to some teratogens other than X-rays are reported. The known chemical teratogens, nitrogen mustard, chlorambucil, streptonigrin and hydroxyurea, were injected into pregnant rats; thalidomide was fed to pregnant rabbits. In addition, embryos from rats exposed to a teratogenic riboflavin-deficient diet were examined for chromosome changes.

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MATERIALS AND METHODS

For parenteral use nitrogen mustard was available (Mustargen, Merck); streptonigrin (a generous gift from Pfizer) was dissolved in alcohol and water; chlorambucil (Leukran, Burroughs-Wellcome) was suspended in sesame oil; hydroxyurea (Squibb) was dissolved in saline. Female rats of a commercial strain, 200–270 g in weight, were bred; the day in which sperm were found in the vagina was counted as the first day of pregnancy. The chemical teratogens were injected into pregnant rats subcutaneously or intraperitoneally in a single dose. Embryos were removed at various times after injection of the teratogen, examined under the dissecting microscope for gross abnormalities, and then processed for chromosome analysis. Some animals were permitted to continue gestation to the 20th or 21st day when the fetuses were removed and examined for malformations.

Dutch-belted rabbits were force-fed thalidomide 200 mg/day, on the 8th to the 12th or 13th day of pregnancy, or fed one single massive dose of 1000 mg on the 13th day.

A riboflavin-deficient diet (Nutritional Biochemicals Corporation) was used as a teratogenic regime in an inbred strain of Wistar rats, previously shown to be highly susceptible to this treatment. The rats were on this diet from the first day of pregnancy to the 13th or 14th day when they were sacrificed. Embryos were processed for chromosome analysis by a ‘direct’ method in most experiments. According to this method embryos were first homogenized by pipetting. The resulting cell suspension was treated briefly with Hanks-calcicine, then with a hypotonic solution, and then fixed. After air-drying, the cells were stained with acetic orcein. This method, a modification of the bone marrow procedure of Tjio & Whang (1962), has been previously described in detail (Soukup et al. 1965). In the chlorambucil, hydroxyurea and streptonigrin series of experiments, the direct method was supplemented with a squash method, modified from the procedures of T. C. Hsu (personal communication) and Welshons, Gibson & Scandlyn (1962). Before squashing, embryos or parts of embryos were allowed to stand in 0.9 % sodium citrate for 20–30 min. This hypotonic solution was pipetted off, the tissues were washed in 45 % acetic acid and stained for 10–15 min in 2 % orcein dissolved in 65 % acetic acid. Small pieces of embryonic tissue were then placed on slides with a drop of fresh stain and a coverslip was laid on top. The preparation was tapped gently to spread the tissue, then pressed lightly several times between absorbent paper and finally squashed hard. When coverslips were sealed with Kronig cement it was possible to preserve these chromosome preparations for 1–3 days.

Metaphases with thirty-seven or more chromosomes were chosen for analysis. If specific aberrations such as breaks, exchanges, fragments, dicentrics or rings were seen, the metaphase figure was considered abnormal, irrespective of the number of chromosome abnormalities found. Achromatic breaks or non-
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staining gaps were not scored as aberrations. Although note was taken of specific chromosome aberrations, the analysis was actually based on the percentage of abnormal metaphase figures.

Some of the chemical teratogens used in vivo were also tested on rat embryonic cells growing in tissue culture. The usual procedure was to subculture the cells and to add the teratogen 6 h later. Twenty-four hours after exposure to a teratogen, cells were processed and examined for abnormal metaphase figures.

RESULTS

Nitrogen mustard

Nitrogen mustard and the chemically closely related chlorambucil are classified as polyfunctional alkylating agents, capable of reacting with proteins and nucleic acids in the living cell. These agents are said to be radiomimetic because they produce biological end-effects similar to those of X-rays, although the mechanisms leading to the end-effects may be very different (Alexander & Stacey, 1958; Koller, 1958; Stacey, Cobb, Cousens & Alexander, 1958; Marin & Levis, 1964).

Teratogenic effects of nitrogen mustard administered to the rat on the 12th–13th days of pregnancy have been demonstrated by Haskins (1948) and Murphy, DelMoro & Lacon (1958). After injecting seven pregnant rats subcutaneously with 1 mg/kg nitrogen mustard on the 12th or 13th day of pregnancy we found external malformations in 90% of the fetuses on the 20th or 21st day of gestation. The most common abnormalities were general growth retardation, small meningocele, short mandible, syndactyly and short kinky tail.

Embryos from twenty other pregnant rats receiving the same dose of nitrogen mustard were examined for chromosome changes 6, 12, 18 and 24 h after injection. Data from embryos injected on the 12th or 13th day did not differ from each other and were therefore pooled (Table 1). Six hours after injection of nitrogen mustard there was some indication of mitotic inhibition but no increase in abnormal metaphase figures. Twelve and 18 h after injection abnormal metaphase figures averaged 15% and 14% respectively. In two additional experiments in which analysis was made 15 h after injection, a peak of 18% abnormal metaphases was seen (Text-fig. 1).

The types of aberrations were typically chromatid breaks and exchanges as shown in Plate 1, fig. A. In about one-fourth of the abnormal metaphase figures the chromosomes showed various degrees of severe damage, as seen in Plate 1, fig. B. Fragmentation of chromosomes, produced by nitrogen mustard injected into rats, has also been observed by Koller (Koller & Casarini, 1952; Koller, 1958). Previous evidence from studies of the action of nitrogen mustard on synchronized cells in culture points to a block in DNA synthesis as the primary effect (Walker & Helleiner, 1963; Crathorn & Roberts, 1966). Other cells may show chromosome fragmentation and die (Levis, Danielli & Piccinni, 1965).
The effect of nitrogen mustard on rat embryonic cells in tissue culture is shown in Table 2. *In vitro*, a concentration of $1 \mu g/ml$ nitrogen mustard resulted in mitotic inhibition and death of cells. At $0.1 \mu g/ml$ the drug produced abnormal metaphase figures in a percentage comparable to the maximal effect observed *in vivo*.

**Table 1.** Chromosome changes in rat embryos whose mothers were treated with various teratogens

<table>
<thead>
<tr>
<th>Hours after treatment</th>
<th>No. of metaphase figures counted</th>
<th>Abnormal metaphase figures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Controls</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Direct method</td>
<td>---</td>
<td>1626</td>
</tr>
<tr>
<td>Squash method</td>
<td>---</td>
<td>1111</td>
</tr>
<tr>
<td><strong>Nitrogen mustard</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>1 mg/kg injected 12th or 13th day</td>
<td>6</td>
<td>248</td>
</tr>
<tr>
<td>Direct method</td>
<td>12</td>
<td>254</td>
</tr>
<tr>
<td></td>
<td>15</td>
<td>224</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>489</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>403</td>
</tr>
<tr>
<td><strong>Chlorambucil</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>8 mg/kg injected 13th day</td>
<td>6</td>
<td>341</td>
</tr>
<tr>
<td>Direct and squash methods pooled</td>
<td>18</td>
<td>759</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>751</td>
</tr>
<tr>
<td></td>
<td>30</td>
<td>302</td>
</tr>
<tr>
<td></td>
<td>48-72</td>
<td>438</td>
</tr>
<tr>
<td><strong>Streptonigrin</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>0.25 mg/kg injected 11th day</td>
<td>6</td>
<td>185</td>
</tr>
<tr>
<td>Direct method</td>
<td>12</td>
<td>197</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>888</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>222</td>
</tr>
<tr>
<td>Squash method</td>
<td>6</td>
<td>645</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>455</td>
</tr>
<tr>
<td><strong>Hydroxyurea</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>750 mg/kg injected 13th day</td>
<td>6</td>
<td>181</td>
</tr>
<tr>
<td>Direct and squash methods pooled</td>
<td>18</td>
<td>396</td>
</tr>
<tr>
<td></td>
<td>24</td>
<td>248</td>
</tr>
<tr>
<td>1500 mg/kg injected 13th day</td>
<td>18</td>
<td>273</td>
</tr>
<tr>
<td>Direct and squash methods pooled</td>
<td></td>
<td></td>
</tr>
<tr>
<td><strong>Thalidomide (rabbit embryos)</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>200 mg/kg fed 8–12th day (total 1000 mg)</td>
<td>---</td>
<td>192</td>
</tr>
<tr>
<td>200 mg/kg fed 8–13th day (total 1200 mg)</td>
<td>---</td>
<td>489</td>
</tr>
<tr>
<td>1000 mg fed 13th day</td>
<td>6</td>
<td>86</td>
</tr>
<tr>
<td></td>
<td>18</td>
<td>113</td>
</tr>
<tr>
<td><strong>Riboflavin-deficient diet</strong></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Fed 0 to 13th–14th day</td>
<td>---</td>
<td>377</td>
</tr>
<tr>
<td>Direct method</td>
<td>---</td>
<td></td>
</tr>
</tbody>
</table>


Chlorambucil

Murphy tested chlorambucil and four other alkylating agents on day 12 (our 13th day), and found the teratogenic end-effects of these agents to be similar; chlorambucil was the most teratogenic of the group in her series of experiments (Murphy et al. 1958). We injected three pregnant rats with 8 mg/kg

Text-fig. 1. Percentages of abnormal metaphase figures from embryos of pregnant animals treated with different teratogens. Curves obtained in a previous study in which rats were treated with 400 r and 200 r X-rays are included for comparison. ○—○, 400 r X-ray; ●—●, 200 r X-ray; △—△, nitrogen mustard; ▲—▲, chlorambucil; □—□, streptonigrin; ---, hydroxyurea, thalidomide, and riboflavin-deficient.

Table 2. Effect of teratogen on rat embryonic cells in tissue culture

Chlorambucil and thalidomide were also added to cells in vitro but these compounds are insoluble in water or salt solution. No abnormal metaphase figures were seen.

<table>
<thead>
<tr>
<th>Teratogen</th>
<th>Concentration in vitro (μg/ml)</th>
<th>Countable metaphase figures</th>
<th>Abnormal metaphase figures (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Nitrogen mustard</td>
<td>1.0</td>
<td>10</td>
<td>Mitotic inhibition</td>
</tr>
<tr>
<td></td>
<td>0.1</td>
<td>211</td>
<td>14</td>
</tr>
<tr>
<td>Streptonigrin</td>
<td>0.01</td>
<td>238</td>
<td>60</td>
</tr>
<tr>
<td></td>
<td>0.001</td>
<td>692</td>
<td>25</td>
</tr>
<tr>
<td>Hydroxyurea*</td>
<td>0.5</td>
<td>60</td>
<td>0</td>
</tr>
<tr>
<td></td>
<td>1.0</td>
<td>72</td>
<td>14</td>
</tr>
<tr>
<td></td>
<td>10.0</td>
<td>100</td>
<td>7</td>
</tr>
</tbody>
</table>

* Six other experiments in which 0.1 to 10.0 μg/ml concentration of hydroxyurea was tested on rat embryonic cells for varying lengths of time showed only mitotic inhibition.
chlorambucil on the 13th day and opened them at various stages from the 16th to 19th day. In 79% of the embryos malformations of the limb and tail were seen.

Twenty-two pregnant rats were treated in the same way and their embryos were removed 6, 18, 24, 30, 48 and 72 h later for chromosome analysis. Abnormal metaphase figures found at 18 h after injection were somewhat fewer than those seen with nitrogen mustard; however, they were more numerous at 6 and 24 h. Differences are illustrated by the curves in Text-fig. 1. No evidence of mitotic inhibition was noted after treatment with chlorambucil. Chromosome abnormalities noted were chromatid breaks and exchanges.

In these chlorambucil experiments it was possible to compare results obtained by squashing individual organs of the embryo with results from whole embryos processed by the direct method. A total of 1318 metaphase figures were counted from preparations obtained with the squash method and a total of 1269 metaphase figures were analysed with the direct method. The results obtained with the squash method and direct method showed close agreement in seventeen of nineteen separate experiments. These findings suggest that chromosome damage is random throughout the embryo. There was no evidence that chromosome damage in organs which are usually deformed by chlorambucil was different from that in the whole embryo.

*Streptonigrin*

Streptonigrin, an antibiotic and antitumor agent, is known to inhibit DNA synthesis (Sinclair, 1965; Rosenkranz, Rose, Morgan & Hau, 1966). Cohen, Shaw & Craig (1963) have shown that the compound in low concentrations of 0.01–0.001 μg/ml produces non-random breaks in chromosomes of cultured human leucocytes. Extensive chromosome breakage was also observed by us after treatment of rat embryonic cells in culture with streptonigrin (Table 2). An example of a mitotic figure of rat embryonic cell from a tissue culture treated with streptonigrin is shown in Plate 1, fig. C.

Streptonigrin has been shown in this laboratory to be a potent teratogen in rats (Warkany & Takacs, 1965). When administered in doses of 0.25 mg/kg on the 9th or 10th day of pregnancy it produced many severe gross malformations.

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**Plate 1**

Fig. A. Metaphase figure showing breaks and exchanges from an embryo examined 18 h after injection of the pregnant rat with 1 mg/kg nitrogen mustard.

Fig. B. Metaphase figure showing severe chromosome damage from an embryo examined 18 hr after injection of the pregnant rat with 1 mg/kg nitrogen mustard.

Fig. C. Metaphase figure showing breaks and exchanges from rat embryonic cell grown in culture, treated with 0.01 μg/ml streptonigrin and processed 24 h later.

Fig. D. Metaphase figure showing breaks and exchanges from an embryo examined 6 h after treatment of pregnant rat with 0.25 mg/kg streptonigrin.
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including exencephaly, short trunk, omphalocele, malposition of feet, and absence of tail and eyes. However, embryos 9 or 10 days old are so small that only minute amounts of tissue can be obtained for chromosome analysis. Therefore, pregnant rats were injected on the 11th day when malformations were less severe but gross abnormalities of the eyes, limbs, trunk and tail can still be produced in 75% of the fetuses.

In one series of experiments embryos were processed by the direct method. A maximum of only 3-6% abnormal metaphase figures was seen 6 h after injection with streptonigrin. In another series of experiments the squash method was used to examine embryos for chromosome changes. At 6 h after injection 7% abnormal metaphases were observed (Table 1). Breaks and exchanges were the common aberrations (Plate 1, fig. D). There was no evidence of mitotic inhibition. The low number of chromosome aberrations was noteworthy since the concentration of streptonigrin in the embryo theoretically should be much larger than the amount of streptonigrin which produced chromosome aberrations in cells growing in tissue culture.

Hydroxyurea

Hydroxyurea is a compound of considerable interest to the teratologist and to the oncologist. Interference with DNA synthesis has been claimed (Sinclair, 1965; Swartz, Garofalo, Sternberg & Philips, 1965; Bose, Gothoskar & Rana-dive, 1966). Fragmentation and breakage of mammalian chromosomes in vitro have been observed after hydroxyurea treatment (Borenfreund, Krim & Bendich, 1964; Oppenheim & Fishbein, 1965). The possibility that hydroxyurea acts to block many enzymes and thereby indirectly produces chromosome damage has also been suggested (Fishbein & Carbone, 1963; Oppenheim & Fishbein, 1965). In nine experiments in which we treated rat embryo cells in vitro with 0.5-10 μg/ml hydroxyurea for 1-3 days a maximum of 14% abnormal metaphase figures was seen (Table 2), but it should be mentioned that in six of the nine experiments mitosis was almost completely inhibited.

Hydroxyurea has been shown to be teratogenic in the pregnant rat (Murphy & Chaube, 1964; Chaube & Murphy, 1966). We injected pregnant rats with 750 mg/kg on the 13th day and permitted them to continue gestation until the 21st day. Ninety-two percent of the fetuses showed gross malformations. All were small and abnormalities such as cleft palate, abnormal head and tail, syndactyly, malposition of limbs and short mandible were seen. Embryos of other pregnant rats treated in the same way were removed 6, 18, and 24 h after injection and analysed for chromosome abnormalities. In ten experiments no chromosome changes were found in 825 metaphase figures analysed. In two additional experiments pregnant rats were injected with a single dose of 1500 mg/kg, and embryos were examined 18 h later. One abnormal metaphase was seen out of 273 examined.
Thalidomide

The rat embryo usually has been found resistant to the teratogenic effect of thalidomide but Somers (1962) produced malformations in the young of thalidomide-treated rabbits, results confirmed by Felisati (1962), Giroud, Tuchmann-Duplessis & Mercier-Parot (1962), Spencer (1962), and Staples & Holtcamp (1963).

Pregnant Dutch-belted rabbits fed thalidomide, 200 mg/day, on the 8th to 13th days of pregnancy may have 50% or more malformed fetuses. We examined embryos of eight does after the same treatment on the 12th or 13th day of pregnancy for chromosome abnormalities. The total dose administered to a mother was 1000 or 1200 mg of thalidomide. No chromosomal abnormalities were seen in 681 countable metaphase figures in embryonic tissues. In two additional experiments a single massive dose of 1000 mg was given on the 13th day and embryos were examined 6 or 18 h later. No abnormal chromosomes were seen in 199 countable metaphase figures.

Normal chromosomes have been found in tissues of children and fetuses deformed by thalidomide (Hughes et al. 1962; Tsuda et al. 1963) although some heteroploidy has also been reported (Hirsch, 1963). Using apparently insoluble thalidomide on rat embryonic cells grown in culture, we did not find any chromosome aberrations. Whether such in vitro investigations simulate changes in the living mammalian embryo is debatable. In at least two studies chromosome breakage produced by thalidomide in vitro has been reported. In one of these the thalidomide was hydrolysed with HCl to dissolve it (Natarajan & Nilsson, 1966); in the other it was dissolved in dioxane (Jensen, 1965).

Riboflavin-deficient diet

In this experiment no positive teratogen was given. In previous work it was found in this laboratory that in an inbred strain of Wistar rats malformations can be induced by a riboflavin-deficient diet in 75% of the fetuses if the diet is given from the first to the 16th day of pregnancy. The abnormalities seen on the 21st day are limited to the skeleton. On gross examination cleft palate, micrognathia, shortness of extremities, syndactylysm and tail defects were found. We used the same strain of rats and the same diet and removed the embryos on 13–14th day for chromosome analysis, i.e. the time at which the malformations originate (Warkany, 1954). In 377 metaphase figures obtained with the direct method not a single chromosome aberration was seen.

DISCUSSION

In separate experiments pregnant animals have been treated with different chemical compounds and in one case with a riboflavin-deficient diet. All these agents and the dietary regime have been shown to be teratogenic while organo-
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genesis is still in progress. Most of the teratogens were administered to pregnant rats on the 11th, 12th or 13th day. This period was selected because (1) enough embryonic material is available for chromosome preparations and (2) mitotic rate is still very high throughout the embryo so that a large number of metaphase figures can be analysed for chromosome aberrations.

A summary of chromosome changes found in embryos from pregnant animals treated with various teratogenic agents is seen in Text-fig. 1. For comparison, results obtained in previous investigations from 13th-day embryos irradiated with 400 r and 200 r are included. It should be noted that a 400 r dose is not only highly teratogenic, but also eventually lethal in almost every case. Thus 60% abnormal metaphase figures probably represent severe cellular damage to these embryos not compatible with life. Irradiation with 200 r would seem to be a more appropriate treatment to study the relationship of congenital malformations and chromosome changes, since after exposure to that dose almost all the embryos survive to birth and all show malformations. In such embryos only 7% of the metaphase figures examined were abnormal.

Comparison of results obtained with various teratogens presents certain problems. Whereas X-rays reach the embryo directly, the chemical teratogens used in the present study reach the embryo indirectly at different time intervals after administration, and in different concentrations. This is due in part to the differing physico-chemical properties of the agents themselves and to variations in disposition of the compounds in the body. In our experiments it was not possible to equalize dose, time and method of administration for all the teratogens used, since in every experiment borderline conditions had to be created to obtain survival of fetuses with malformations and to avoid toxic or lethal consequences to the mother. Nevertheless, results presented in Text-fig. 1 compare chromosome changes in embryos with similar teratologic effects and are therefore meaningful.

Certain general impressions can be gained from these experiments. Radio-mimetic agents such as nitrogen mustard and chlorambucil produce as an average not more than 20% abnormal metaphase figures in the embryo. Streptonigrin, like 200 r X-irradiation, produces fewer than 10%. If we compare these results with those from tissue culture, in which chromosome injuries are much more evident, it would appear that we detected only a small part of the potential chromosome damage of these agents in the embryo. Perhaps this is because many cells in the embryo after exposure to a teratogen die or are stopped in interphase, whereas synchronized cells in culture may progress to metaphase and reveal chromosome damage. Chromosome protective and repair mechanisms may be more efficient in the embryo than in tissue culture. It is also possible that our methods are not sufficiently developed to detect all metaphase figures with damaged chromosomes. The findings with streptonigrin, in particular, illustrate the difficulty of extrapolation of in vitro to in vivo results. Since streptonigrin is extremely effective in producing chromosome breakage in vitro,
it seemed reasonable to expect that it would also produce extensive chromosome breakage in the embryo. This was not the case. However, it is difficult to compare concentration of teratogens in vitro to concentrations in vivo and conclusions from such comparisons must be guarded.

The types of chromosome abnormalities produced in this study by the teratogens were mostly breaks and exchanges similar to those seen in embryos after X-irradiation. It was concluded that these results indicate early cell damage, occurring in the first division after teratogenic treatment. There was no indication of non-random breakage of specific chromosomes, or of persistence of permanent chromosome abnormalities. Such non-specific chromosome changes are in many cases a reflexion of general cell damage and may not necessarily be considered a direct effect of the teratogen upon the chromosome. On the other hand, chromosome breakage is one of the few conspicuous and detectable effects of early cell damage and for this reason it may serve as an important indicator of teratogenic action in the embryo.

As pointed out before, the methods used by us for chromosome analyses could not be successfully applied to older fetuses and the newborn, because only relatively few metaphase figures are obtained. We have not ruled out the possibility that permanent chromosome changes on a very low level persist in late fetal or postnatal life.

SUMMARY

Pregnant animals were treated with five chemical teratogens and a teratogenic dietary regime. Embryos were examined for chromosome changes in squash or 'direct method' preparations at various times after administration of the teratogen.

Embryos from rats treated with a teratogenic dose of nitrogen mustard or chlorambucil showed a peak of less than 20% metaphase figures with chromosome abnormalities. Streptonigrin produced less than 10%. The maximum chromosome abnormalities appeared within 24 h after injection and almost all abnormal metaphase figures disappeared within 48 h after injection.

Other chemical teratogens such as hydroxyurea injected into pregnant rats and thalidomide fed to pregnant rabbits produced no detectable chromosome abnormalities in the embryos. There were also no abnormal metaphase figures detectable in rat embryos after maternal exposure to a riboflavin-deficient diet.

RÉSUMÉ

*Modifications chromosomiques observées dans les embryons traités avec différentes substances tératogènes*

Les animaux en gestation ont été traités avec cinq substances tératogènes et ont été soumis à un régime diététique tératogène. Les modifications des chromosomes ont été observées sur des préparations par la méthode directe ou par
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écrasement des noyaux chez des embryons à différents moments après administra-

tion de la substance tératogène.

Des embryons de ratte soumis à une dose tératogène d’ypérite azotée ou de chlorambucil présentent moins de 20 % de figures métaphasiques avec des anomalies chromosomiques. La streptonigrin en produit moins de 10 %. Le maximum d’anomalies chromosomiques apparaît au cours des 24 h qui suivent l’injection et presque toutes les figures métaphasiques anormales disparaissent dans les 48 h après l’injection.

D’autres substances tératogènes telles que l’hydroxyurée injectées aux rattes gestantes et la thalidomide administrée aux lapines gestantes ne provoquent pas d’anomalies des chromosomes visibles chez les embryons. On n’observe en outre aucune figure métaphasique anormale dans l’embryon de ratte après exposition des mères à un régime déficient en riboflavine.

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REFERENCES


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