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Induction of Malformations by Actinomycin D at Mid-Pregnancy in the Rat and Protection by L-Thyroxine[1] (40095)

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The antibiotic actinomycin D is a potent teratogenic agent in laboratory animals (1), for it blocks DNA-directed synthesis of messenger (m)RNA upon administration (2). When given by intraperitoneal injection to pregnant rats before mid-gestation, actinomycin D caused intra–uterine growth retardation and abnormal development of several organ systems (3–5); no malformations were produced, however, when treatment was begun after day 10 of pregnancy. The work of Philips *et al.* (6) suggested that subcutaneous conveyal of the drug might extend its range of action, and preliminary experiments showed indeed that actinomycin administration by the subcutaneous route on day 12 caused severe edema, club foot, crook tail, and exomphalos in the offspring. In subsequent experiments, reported below, actinomycin D was injected alone and in combination with thyroxine, in the hope that the latter treatment might ameliorate the teratogenic action of the drug: thyroxine has been shown to stimulate nucleic acid synthesis (7) and to prevent the growth-inhibitory effects of actinomycin D by combining with it (8). Some of the fetal rats, obtained by Cesarian section on day 21 of pregnancy, were kept for 6 months to study the long-range effects of the treatment.

Materials and methods. Thirty-six pregnant rats were separated randomly into four groups. Group I, 12 rats, received subcutaneously 200 μg/kg of actinomycin D[3] on day 12 of pregnancy; the day of finding sperm in the vagina was considered day 0. Group II, eight rats, received subcutaneously 200 μg/kg of thyroxine[4] on day 12 followed immediately by the same dose of actinomycin D. Group III, eight rats, was injected subcutaneously with 200 μg/kg of thyroxine on day 11 of pregnancy; the rats received the same amount of actinomycin D on day 12. Group IV consisted of eight uninjected rats. Other uninjected pregnant rats, scheduled to deliver at the same time as the rest, were kept in readiness to serve as foster mothers for some offspring of the experimental group.

The rats were housed under natural conditions of light and darkness in a temperature-controlled room and provided with stock diet[5] and distilled drinking water *ad libitum.* The young were removed under ether anesthesia on day 21 of pregnancy, weighed and examined for gross abnormalities. Some litters were killed, fixed and subsequently examined for abnormalities by razor blade sectioning. Selected members of other litters were placed with foster mothers and were raised to age 6 months.

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[3] Dactinomycin, obtained from Merck, Sharpe and Dohme, Rahway, NJ. The drug was dissolved in 10 ml of propylene glycol (1,2-propane diol) and brought with distilled water to final volume of 625 ml and concentration of 40 μg/ml. The final concentration of propylene glycol was 3 mg/ml, and the amount administered in this study was approximately one-tenth the dose having possible teratogenic effects in mice (9). No evidence exists to implicate propylene glycol as a teratogen in rats.

[4] L-Thyroxine, obtained as sodium salt, pentahydrate, from Aldrich Chemical Co., Inc., Milwaukee, WI, and administered as an alkaline solution. The hormone and actinomycin D were injected at adjacent sites between the shoulder blades.

[5] The diet consisted of 67.5% ground whole wheat, 15% casein, 7.5% skim milk powder, 0.75% NaCl (sufficient KI added to furnish 1 μg iodine per g of diet), 1.5% $CaCO_3$, 6.75% hydrogenated vegetable oil, and 1% fish oil. Fresh lettuce was supplied twice weekly.

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TABLE I. EFFECTS OF ACTINOMYCIN D ALONE AND IN COMBINATION WITH L-THYROXINE ON REPRODUCTIVE PERFORMANCE OF LONG-EVANS RATS AND ON SURVIVING YOUNG.

Group[a]	Treatment schedule (dose/kg of body weight)	Treatment schedule (day of pregnancy)	Average no. of implants	Surviving young (%)	Average wt of survivors (g)	Abnormal survivors (%)
I (12)	200 μg actinomycin	12	13	63	5.5	14[b]
II (8)	200 μg actinomycin + 200 μg thyroxine	12	12	86	5.8	2[c]
III (8)	200 μg actinomycin + 200 μg thyroxine	12 11	11	85	5.9	0
IV (8)	—	—	12	92	5.7	0

[a] Number of animals in parenthesis. They were killed on day 21 or pregnancy (day of finding sperm = day 0) and delivered of their young by Cesarean section.

[b] Eleven survivors had club feet, one had exomphalos, and two were severely stunted and weighed less than 4 g at delivery.

[c] Mild edema.

Results. All rats gained weight in the course of the experiment, but only those in Group III matched the growth rates of Group IV animals. All pregnant rats had live young. None of the treatments affected the average number of implantation sites or the average weight of surviving young at term (Table I), but only 63% of the implants in Group I survived to day 21 of pregnancy. Survival in Groups II and III was about equal and much improved over that in Group I. Fourteen percent of survivors were abnormal in Group I, whereas only 2% were abnormal in Group II, and there were no malformed young in Group III. Of 14 abnormal young in the first group, 11 had club foot, one had exomphalos and two were severely stunted. The only abnormality noted in some Group II animals was slight edema which disappeared within a day.

All of the male and female offspring in Groups II, III, and IV selected for observation survived to age 6 months, when they were killed. On the other hand, only 70% of males and 50% of females in Group I survived that long. Deaths in this group occurred within the first 90 days of life; no cause of death was found at autopsy. Pregnancy was uneventful in the four Group I females that were mated with littermates, and newborn appeared normal in all respects.

Discussion. The data presented in this study demonstrate that choice of a different route of administration can extend the teratogenic time range of actinomycin D to day 12 or beyond. Abnormalities most often encountered were club foot, exomphalos, generalized edema, and overall stunting of growth.[6] There was also a significant elevation in fetal mortality, but survivors were of normal size and proportions, except as noted. The teratogenic effects were completely suppressed by prior treatment of the mothers with thyroxine, and embryolethal effects were reduced significantly. Concurrent injection of thyroxine and actinomycin D caused a similar reduction of lethal effects and prevented permanent defects. Actinomycin D seems to bind more readily to differentiating cells than to cells which have already differentiated (11), and it may be conjectured that thyroxine administered on day 11 of development prevented the teratologic action of the drug by accelerating development of the embryo past the state of susceptibility. Alternatively, the hormone by augmenting available nuclear binding sites may have neutralized the cytotoxic and cytostatic action of the drug; both in the chick (12) and hamster (13), DNA administered together with actinomycin D reduced the embryolethal and teratogenic effects of the antibiotic. However, neither hypothesis can explain adequately our finding that thyroxine given concurrently with actinomycin was almost as effective in protecting mother and fetus as was its injection the day before. Kim *et al.* (8) reported that thyroxine formed a complex with actinomycin D similar to that

[6] Spontaneously occurring congenital malformations in Long-Evans rats of our colony are exceedingly rare (10), and none was seen in control rat litters of this study.

formed by the antibiotic with deoxyguanosine. Whereas actinomycin alone completely inhibited growth of bacterial cultures, growth was normal when hormone was added to the medium. Formation of such a complex may also have resulted from an interaction of actinomycin D and thyroxine in this study, and its inability to enter the cell in toxic quantities could account for the minimal teratogenic effect of the drug.

Summary. The embryolethal and teratogenic effects of actinomycin D were studied in Long-Evans rats. Subcutaneous administration extended the teratogenic time range of the drug beyond that previously identified by intraperitoneal injections; a single dose (200 μg/kg body weight) given on day 12 of pregnancy caused club foot, generalized edema, exomphalos, and growth retardation in 14% of surviving young. When equal amounts of L-thyroxine were injected concurrently with actinomycin D on day 12 of development, the effects of actinomycin on the mothers were ameliorated and abnormalities in the young were strikingly reduced. When injected one day previous to treatment with actinomycin D, thyroxine prevented fetal malformations. These results may be interpreted on the hypothesis that the hormone forms a harmless complex with actinomycin D.

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