Teratogenic Effects of Trypan Blue on Hamster Embryos

by Vergil H. Ferm

From the Department of Anatomy, University of Wisconsin, Madison

WITH ONE PLATE

Little information is available in the literature on the susceptibility of the hamster embryo to teratogens. Desoxycorticosterone acetate apparently has some teratogenic activity (Tedford & Risely, 1950), but the degree and type of abnormality produced by this agent in the hamster have not been recorded. Orsini (1952) has described naturally-occurring urogenital anomalies which appear in females of a piebald strain of the golden hamster.

The short gestation period, the accuracy of timed matings (Ward, 1946), and the large litter size make this animal an excellent one for embryological research on teratogens. The purpose of the present study was to determine the susceptibility of the hamster to the teratogenic effects of trypan blue and to learn what effects this dye had on other aspects of pregnancy and gestation. Neither the site nor the mode of action of this compound has been established. Failure to detect the dye in the embryonic tissues proper has led to hypotheses suggesting that some derangement of maternal physiology (Gillman et al., 1948) or alterations in the permeability of the yolk-sac placenta, because of its peculiar affinity for the dye (Hamburgh, 1954), was responsible for the teratogenic activity. Observations on the placental transfer of trypan blue into the fluid of the young rabbit blastocyst (Ferm, 1956) introduce the possibility that the dye may have a direct teratogenic effect on the developing rabbit embryo.

MATERIALS AND METHODS

The variables of pregnancy measured in this experiment included: gross foetal weights, maternal weight gain, placental weights, placental-foetal weight ratios, total litter size, percentage of litter resorption, and the survival sex ratio.

Adult female golden hamsters (Cricetus auratus), from a colony maintained in this laboratory by Dr. Margaret Orsini, were used in this experiment. At least two estrous cycles were followed in all the animals. Sixty hours prior to the

1 This investigation was supported by a grant from the Wisconsin Alumni Research Foundation.
2 Present address: Department of Anatomy, College of Medicine, University of Florida, Gainesville, Florida, U.S.A.

estimated time of the next succeeding ovulation, the animals received subcutaneously one-half of their total dose of trypan blue (obtained from Matheson Coleman & Bell, Co., Norwood, Ohio) as a 1 per cent. solution in saline. This preovulation injection was given to observe the effect of the dye on ovulation. Forty-eight hours after injection the females were put with healthy, rested males from 4 p.m. to 8 a.m. The vaginal discharge was examined the morning after mating for the typical post-estrous appearance (Deanesly, 1938). On the fifth day of gestation (about 130 hours after the estimated time of ovulation) the females received the remaining half of the dose in an identical manner. One-half of the experimental group received a total of 10 mgm. of the dye (Series X), the other half received 15 mgm. (Series XV). Control animals received \( \frac{1}{2} \) c.c. of normal saline at corresponding times before and during pregnancy. On the fifteenth day of gestation (developmental age of 14 days, 8–9 hours), the females were sacrificed and the foetuses and their placentae removed, examined, and weighed on a Roller-Smith torsion balance. Resorption sites were carefully sought and included in the record of total litter size. Maternal weights were recorded at the time of the first injection and again at the time of sacrifice. All animals were individually caged from the time of the first injection and were handled minimally. The diet consisted of Purina laboratory chow supplemented twice weekly with fresh greens.

**RESULTS AND DISCUSSION**

The results of this experiment are summarized in Table 1 and the evidence of anomalies may be found in figs. 1–4 of the Plate.

Under the conditions of this experiment, trypan blue is a teratogenic agent in

<table>
<thead>
<tr>
<th>Table 1</th>
</tr>
</thead>
<tbody>
<tr>
<td><strong>Effect of trypan blue on hamster embryos</strong></td>
</tr>
</tbody>
</table>

<table>
<thead>
<tr>
<th>Series</th>
<th>No. of animals</th>
<th>Maternal weight gain (gm.)</th>
<th>No. of living foetuses</th>
<th>Per cent. resorption</th>
<th>Mean foetal weight (mgm.)</th>
<th>Mean placental weight (mgm.)</th>
<th>Placental-foetal weight ratio</th>
<th>Sex ratio</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls</td>
<td>14</td>
<td>28-6</td>
<td>132</td>
<td>9-4</td>
<td>1,425 ( \pm 22-5^* )</td>
<td>446 ( \pm 13-9^* )</td>
<td>0-312</td>
<td>66</td>
</tr>
<tr>
<td>Series X</td>
<td>12</td>
<td>29-5</td>
<td>58</td>
<td>58-0</td>
<td>1,248 ( \pm 13-7^* )</td>
<td>442 ( \pm 12-1^* )</td>
<td>0-354</td>
<td>29</td>
</tr>
<tr>
<td>Series XV</td>
<td>12</td>
<td>25-2</td>
<td>39</td>
<td>73-7</td>
<td>1,202 ( \pm 15-4^* )</td>
<td>390 ( \pm 11-5^* )</td>
<td>0-324</td>
<td>18</td>
</tr>
</tbody>
</table>

* Standard error.

hamsters as evidenced by the finding of anomalies in 13 foetuses from 8 litters of the total of 24 treated litters of the experimental groups. Only one of the 14 control litters contained anomalies. The teratogenic effects were apparently limited to the cephalic axial region, though careful internal dissections or serial microscopic sections were not done. Anomalies noted were hydrocephalus,
encephalocoeles, and exencephaly. There was one litter of nine hydrocephalic foetuses in the control series. No reference to this type of anomaly in hamsters could be found in the literature, and it has not been noted in the numerous stock animals used in other experimental work in this laboratory.

There appears to be no effect of the dye on the number of ova ovulated or fertilized, or on the number of blastocysts that begin implantation, since the mean litter size, including both the living foetuses and the observable resorption sites, is nearly equal in the control (11.3) and experimental (12.0) groups. This, however, does not preclude a possible sublethal toxic effect of the dye directly on the ovum in the follicle or in the early cleavage stages. There was a marked effect on the intrauterine resorption of implanted blastocysts. This probably occurred well after implantation began since resorption was recognized by macroscopic means. Well-developed placental tissue (chorio-allantoic) was found in most of these resorption sites and, in one case, a well-preserved limb-bud embryo was found at the time of sacrifice, which was one day before term. The placenta of this animal was as large as placentae of the control series of the same developmental age.

There is a decided effect of trypan blue on foetal weight. The foetuses of the experimental series weighed less than did the controls, and this decrease in weight appears to be in proportion to the amount of dye given the mother. The effect of the dye treatment on placental weights and on the placental-foetal weight ratios was less striking. The observed sex ratios of the living foetuses showed that there was no significant sex susceptibility to the dye.

The vitelline membrane of all the experimental foetuses was stained a deep blue colour and those from animals receiving larger doses of the dye had a more intense coloration. By visual examination, all placentae of a single litter appeared to stain with the same degree of intensity. The finding of litters in which only one or two of the foetuses were malformed and in which all of the vitelline membranes appeared to be stained with the same intensity, tends to diminish the possibility that the teratogenic action of the dye occurred through an alteration in the permeability of the yolk-sac placenta. The failure of the dye to affect maternal weight gain during pregnancy reduces the possibility that a maternal metabolic disturbance is responsible for the teratogenicity of trypan blue. The remaining possibility is a direct toxic action on the developing embryo.

**SUMMARY**

1. In a series of 24 treated litters of hamsters, trypan blue was a teratogenic agent, causing malformations mainly in the cephalic axial region. The dye had no effect on the number of ova ovulated, the number of blastocysts that began implantation, or the maternal weight gain during pregnancy.

2. Embryos of the experimental series weighed less than those of the control series injected with saline only, but trypan blue had little effect on placental weights or placental–foetal weight ratios.
3. A naturally occurring hydrocephalic anomaly was found in the control stock.

4. It is suggested from these data that trypan blue has a direct teratogenic effect upon the embryo.

REFERENCES


EXPLANATION OF PLATE

Fig. 1. Foetus at left is a normal one from a control litter. Hydrocephalic foetus at right is from a mother of Series XV. Hydrocephalic head is enlarged and rounded. × 3. See fig. 4.

Fig. 2. Hamster litter-mates from a Series X mother. Both show a small dorsal midline evagination which arose from the mid-brain region. × 3. See fig. 4.

Fig. 3. Foetus from a mother of Series XV, showing a well-developed encephalocele. This foetus was living at the time of sacrifice and the amniotic fluid was grossly bloody. × 2. See fig. 4.

Fig. 4. Brains and upper spinal cords of selected typical anomalies. From left to right: (1) normal control animal, (2) small dorsal encephalocele, (3) brain removed from an exencephalic foetus, (4) brain removed from a typical hydrocephalic foetus showing marked dilatation of the cerebrum with thinning of its walls. × 3.

(Manuscript received 13: ix: 57)