Osteolathyrogenic Effects on the Developing Rat Foetus

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WITH ONE PLATE

Skeletal and aortic changes in foetal rats treated with osteolathyrogenic agents via the maternal system throughout pregnancy or very late in pregnancy have been described by Stamler (1955). Developmental changes in Xenopus larvae (Chih, Witschi, & Ponseti, 1954) and in salamander and toad larvae (Levy, 1955) have also been described. The abnormalities produced in these forms by these agents have apparently been related to a direct effect of the agent on the mucopolysaccharide ground substance. No attempt has been made in a mammal to determine if there are specific time-susceptible periods in embryogenesis when this type of stimulus may induce true teratogenic effects. The present study was devised to determine the effect of a known osteolathyrogenic agent during specific stages of gestation and especially to observe the effect of this agent during the early critical stages of embryogenesis.

MATERIALS AND METHODS

Cystamine dihydrochloride was used as the lathyrogenic agent. It has proven activity (Dasler & Milliser, 1958), is obtainable commercially in a pure form (from Nutritional Biochemicals Corp., Cleveland, Ohio), and is a stable compound. The animals were fed by gavage to regulate the amount and time of feeding. Rats fed 100 mgm. of cystamine daily fared very poorly and usually succumbed within 3 or 4 days, but when the dosage was reduced to 50 mgm. of cystamine daily over a 5-day period the animals remained in good health. Five groups of pregnant rats of the Sherman strain were fed 50 mgm. of cystamine in 1 ml. of saline by gavage for 5 consecutive days of gestation beginning on days 7, 9, 11, 13, and 15. Control animals were fed 1 ml. of normal saline by gavage beginning on day 14 for 5 consecutive days. All animals were allowed the regular Purina rat chow and water ad lib. The rats were killed on the day of term and the

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foetuses fixed in 95 per cent. alcohol, eviscerated, cleared in 2 per cent. KOH, and stained with Alizarin red. Two specimens from each litter were fixed in Bouin's fluid and kept for further examination. Resorption sites were sought and recorded.

RESULTS

The feeding of cystamine increased foetal resorption beyond that found in control rats as shown in Table 1. No gross abnormalities in the fresh or bone-stained foetuses of the control group were found. The only structural abnormalities noted in the experimental series were defects in rib structure and a rather marked kyphosis in some animals (Plate, figs. 1, 2, 3). The rib defects were first manifest in the series of rats treated on days 9–13 as a rather subtle twisting of the medullary region of the rib (Plate, fig. 3). In the series of rats treated later in gestation this basic rib defect increased in severity so that even more pronounced deformities appeared (Plate, fig. 2). The defect appeared in all members of an affected litter in about the same degree, even though there was some variance in the extent of the malformation among litters treated on the same days of gestation. The more posterior ribs were affected most and the lesions were almost always bilaterally symmetrical in each foetus. Although the curvatures of the ribs tended to be uniform in distance from the mid-saggital plane within a given litter, there was enough variation of this distance among the various litters to indicate that the site of deformity was not dependent upon the site of a single muscle attachment.

The most severe deformities appeared in those animals treated on days 15–19 of gestation. These foetuses showed a marked kyphosis, foreshortening of the thoracic cage, and severely altered rib morphology involving all of the ribs bilaterally (Plate, fig. 2). It is of special interest to note that none of the animals treated on days 7–11 of pregnancy produced foetuses with skeletal or other gross abnormalities.

Microscopic examination of the affected ribs revealed rather marked disorganization of the osseo-cartilaginous model as compared with controls (Plate, figs. 4, 5, 6). The core appears to be pushed toward the periphery of the

<table>
<thead>
<tr>
<th>Days of treatment</th>
<th>Number of mothers</th>
<th>Living foetuses</th>
<th>Resorption sites</th>
<th>Percentage resorption</th>
</tr>
</thead>
<tbody>
<tr>
<td>Controls 14–18</td>
<td>5</td>
<td>51</td>
<td>2</td>
<td>3.9</td>
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<tr>
<td>7–11</td>
<td>5</td>
<td>40</td>
<td>9</td>
<td>18.6</td>
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<td>9–13</td>
<td>7</td>
<td>47</td>
<td>13</td>
<td>21.6</td>
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<tr>
<td>11–15</td>
<td>5</td>
<td>42</td>
<td>9</td>
<td>17.6</td>
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<tr>
<td>13–17</td>
<td>5</td>
<td>39</td>
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<td>27.7</td>
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<td>5</td>
<td>37</td>
<td>13</td>
<td>26.0</td>
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developing rib especially near the point of greatest distortion. In addition, there is a considerable proliferation of the connective tissue immediately surrounding the model. This again is most evident in the areas of greatest distortion.

**DISCUSSION**

It is apparent that this osteolathyrogenic agent should not be considered to have a true teratogenic effect on the rat foetus. In spite of the unusual rib patterns mentioned above, there has been no basic developmental alteration in rib morphology or in the skeleton as one might expect to find with a true teratogen. The critical stages of teratogenic susceptibility in the rat are generally considered to be during the 8th to 13th days of gestation. Nevertheless, the gross deformities apparent at birth and after skeletal staining in those animals treated in the later stages of gestation necessitate some classification. The stimulus used here is effective at some time between the period of true teratogenic susceptibility and the immediate prenatal period. For lack of any present terminology with which to label these types of agents it is suggested that the term 'foetal pathogen' be used.

Cartilaginous models of all ribs in the rat are present on day 15, and ossification begins in ribs 3–9 on day 16 of development and has begun in all ribs by day 17½ of development (Wright *et al*., 1958). This would explain the failure to produce any effects during the 7–11-day period, and the slight changes in those rats treated from day 9 to 13. Metabolic interference with the mucopolysaccharide ground substance during these stages of ossification could account for much of the torsion appearing in the osseo-cartilaginous models at term. Further histochemical studies are needed to analyse this point.

**SUMMARY**

1. Foetal rats exposed to an osteolathyrogenic agent, cystamine, in the later stages of gestation revealed marked deformities of the thoracic ribs.
2. Foetuses exposed during the so-called critical stages of embryogenesis in early gestation showed no abnormalities at birth.
3. It is suggested that the term 'foetal pathogen' be used to describe those stimuli which affect foetal growth patterns after the period of teratogenic susceptibility and which produce marked variations from the normal at birth, to distinguish them from the 'foetal teratogen' which produces basic alterations in organogenesis.

**RÉSUMÉ**

*Actions ostéolathyrégéniques sur le fœtus de rat en voie de développement*

1. Des fœtus de rat exposés à l'action d'un agent ostéolathyrogénique, la cystamine, aux stades avancés de la gestation, montrent des déformations nettes des côtes thoraciques.
2. Des fœtus exposés à la même action pendant les stades prétendus critiques de l’embryogenèse, c’est-à-dire au début de la gestation, ne montrent aucune malformation à la naissance.

3. Il est suggéré que le terme ‘action fœtale-pathogène’ soit utilisé pour qualifier les stimuli qui affectent les rythmes de croissance fœtaux après la période de sensibilité tératogène et qui produisent des modifications marquées après la naissance, par opposition aux actions ‘fœtales-tératogènes’ qui provoquent des altérations fondamentales de l’organogenèse.

REFERENCES


EXPLANATION OF PLATE

Fig. 1. 18-day foetus showing distortion of ribs. Treated from days 11-15. × 4.
Fig. 2. Term foetus showing kyphosis and rib distortion. Treated from days 15-19. × 3.
Fig. 3. Term foetus showing minimal rib distortion resulting from treatment on days 9-11. × 8.
Fig. 4. Cross-section 10th thoracic rib from control foetus. Gomori’s trichrome stain. × 80.
Fig. 5. Cross-section 10th thoracic rib of foetus treated on days 15-19. Gomori’s trichrome stain. × 80.
Fig. 6. Cross-section of same rib as shown in fig. 5, through area of maximum distortion. Gomori’s trichrome stain. × 80.

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