Cardiac Malformations in the Rat Induced by Maternal Hypercapnia with Hypoxia

By Olga M. Haring, M.D.

ABSTRACT
Forty-seven pregnant rats were exposed for 24 hours to a gas mixture of 6% carbon dioxide, 10% oxygen and 84% nitrogen on different days during gestation. The earliest exposure was on the fifth day after observed copulation, the latest on the sixteenth. The hearts of 370 newborn test rats were studied in serial sections and compared with those from 111 newborn control rats. The overall incidence of cardiac malformations was 28.1% in the experimental animals and 4.5% in the controls. In the test groups, severe structural anomalies resulted from exposures as early as the fifth day of gestation.

The results of the present and of previous experiments suggest that maternal hypercapnia in the presence of normal and low oxygen tensions is markedly teratogenic for the cardiovascular system in the rat.

ADDITIONAL KEY WORDS teratogenic effects prenatal hypercapnia prenatal hypoxia prenatal asphyxia teratogenic susceptibility

In earlier experiments, the administration of 6% carbon dioxide with 20% oxygen and 74% nitrogen to pregnant rats for 24 hours produced fetal cardiovascular anomalies. A subsequent study showed that 24-hour exposure of pregnant rats to 10% oxygen, 89% nitrogen, and less than 0.5% carbon dioxide only slightly increased the incidence of cardiac defects in the offspring, as compared with control animals. The purpose of the present investigation was to observe the effects of maternal hypercapnia with hypoxia on the fetal cardiovascular system in the rat.

Methods
In the current series of experiments, pregnant Sprague Dawley rats were placed in pairs in closed plastic chambers and exposed to 6% carbon dioxide, 10% oxygen and 84% nitrogen for 24 hours; 2 liters of gas/min flowed through the chamber from compressed gas cylinders. The first two pairs were exposed on day 5 after observed copulation, the second two pairs on day 6, the third two on day 7, and so on. The last two pairs were exposed on day 10 of gestation.

Gas samples from the chamber were analyzed for oxygen and carbon dioxide content at regular intervals with the 0.5 ml Scholander Gas Analyzer or by using a Burell Kwik Chek analyzer. The rate of continuous flow in the chamber was measured by a flowmeter. All rats received a liberal stock diet of Purina Laboratory chow and water. The plastic chamber was transparent and the rats could be observed for unusual behavior during the experiments. After removal from the exposure chamber they were kept in separate cages and breathed room air until delivery. Control animals of the same breed, age and mating time were kept in separate cages and maintained on the same food but breathed fresh air through their entire pregnancy. After delivery the mothers were killed and the placenta sites in the uteri were counted and noted. Seven rats with no evidence of placenta were considered not pregnant and were not considered further in the study. Immediately after birth, the newborn rats were weighed and examined for visible malformations, and their body and tail lengths were measured. Then they were decapitated and preserved in 10% formalin. After fixation, the chest organs were embedded in paraffin, the hearts and lungs were completely sectioned serially, stained with hematoxylin eosin, and studied by the light microscope.

Results
Twenty-four hour exposure of 54 rats to
6% carbon dioxide, 10% oxygen and 84% nitrogen resulted in neither maternal death nor premature termination of pregnancy. At the beginning of the exposure the rats seemed rather restless and breathed rapidly, but after a few hours they seemed to return to normal and consumed food and water in usual quantity. The pregnancies of 47 test rats and 16 control animals are characterized by the data in Table 1. The incidence of stillbirth was higher in the experimental group (7.2%) than in the control group (4.7%), and the difference is statistically significant (P < 0.01). There was no significant difference in litter size, sex predominance or birth weight between the control and the test animals. No malformations were detected on gross examination in the control group. In the experimental group, 1 animal had a shortened tail (the mother was exposed on day 5 of gestation), and 3 were markedly underdeveloped (2 from mothers exposed on day 13 and the other from one exposed on day 15).

CARDIOVASCULAR DEFECTS

From the experimental series 370 hearts, and from the control series 111 hearts, were sectioned serially and studied microscopically; the remaining 17 test hearts and 17 controls were not sectioned for technical reasons.

Control Series

Cardiac anomalies were detected in 5 control offspring (4.5%) (Fig. 1). Three had a small defect in the ventricular septum in the area bordering on the junction of the membranous and muscular interventricular septa; these resulted in a minute and undoubtedly physiologically unimportant communication between the ventricles. A moderate degree of hypertrophy of the myocardium and ventricular septum was seen in 2 specimens, with some narrowing of the pulmonary outflow tract.

The ductus arteriosus was contracted and probably functionally closed in 27 rats (26.2%), only slightly contracted in 39 (37.9%), and wide and patent in 37 (35.9%). In 2 the ductus could not be identified.

Experimental Series

The incidence of cardiovascular defects was 28.1% in the experimental group; 105 of the hearts were abnormal. The varieties of cardiovascular malformations are in Table 2.

1. High Ventricular Septal Defect (VSD). Thirteen newborn (3.5%) had an isolated defect in the membranous part of the ventricular septum. Although such defects were similar in nature and location to those found in the controls, they were usually larger (Fig. 2). They occurred in rats whose mothers were exposed on days 5, 6, 7, 9, 10, 11 or 14 of gestation.

2. Low Ventricular Septal Defect. A large septal defect in the midportion of the muscular interventricular septum was found in 2 newborn (0.5%) whose mothers were exposed on days 5 and 9.

3. Overriding Aorta. In 3 newborn (0.8%), the interventricular deficiency was large and involved not only the membranous but also adjacent portions of the muscular septum, and the defect was straddled by the dextroposed
OF EXPOSURE

370 TEST RATS
III CONTROL RATS

<table>
<thead>
<tr>
<th>Agent</th>
<th>IVSD+</th>
<th>Over-riding aorta</th>
<th>Common trunk†</th>
<th>Partial transposition</th>
<th>Aortic, pulmonic stenosis</th>
<th>Others</th>
<th>Total</th>
</tr>
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<tr>
<td>Control‡</td>
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<td>.0</td>
<td>.0</td>
<td>0.5%</td>
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</table>

*Isolated ventricular septal defect.
†In Figure 1, this column is included with partial transposition.
‡Data pooled from the 3 control series.

Total incidence of cardiac malformations in test animals whose mothers were exposed to 10% oxygen with 6% carbon dioxide during gestation and incidence in control animals.

Incidence of Cardiac Malformations

aorta (Fig. 3). This defect occurred only in those exposed on day 5 (Fig. 1).

4. Common Trunk. In 2 newborn (0.5%), a single large vessel resembling the aorta emerged from a ventrally placed right ventricle. The pulmonary arteries originated from the ascending portion of this vessel (Figs. 4 and 5); the common carotid and subclavian arteries branched independently from the arch. There was no trace of either the pulmonary trunk or ductus arteriosus. The ventricular septum was defective, but the truncus did not override the defect. The valve of the trunk consisted of four cusps. The mothers were exposed on days 7 and 8.

5. Partial Transposition. A double outlet right ventricle was found in 4 newborn (1.1%). Both the aorta and the pulmonary artery arose from the right ventricle. All 4 had a high interventricular septal defect also.
FIGURE 2
Frontal section through the heart of a test rat with high ventricular septal defect. RA = right atrium; LA = left atrium; RV = right ventricle; LV = left ventricle; Ao = aorta; PA = pulmonary artery; VSD = ventricular septal defect.

FIGURE 3
Frontal section through the heart of test rat with ventricular septal defect and overriding aorta.

This was usually larger than that accompanying overriding aorta and was at some distance below the aortic outlet (Figs. 6 and 7). All 4 had a normal pulmonary artery. The mothers of these rats were exposed on days 5 (2), 6 (1), and 10 (1).

FIGURE 4
Frontal section through the heart of test rat with common trunk. T = truncus communis.

FIGURE 5
Same heart as in Figure 4, showing connection of the trunk with the aortic arch.
6. Muscular Pulmonic or Aortic Stenosis. This condition was found in 70 rats (18.8%). In 40, pulmonary stenosis was predominant and in 4, aortic stenosis; 26 had both. The narrowing of the aortic outflow tract was due to marked thickening of the ventricular septum (Fig. 8). Thickening and displacement of the septal band of the crista supraventricularis produced stenosis of the pulmonary outflow chamber. The number and structure of the valvular cusps were normal. In many instances the ventricles had markedly hypertrophied walls and a small lumen. There was also a thickening of the atrial musculature and increase of the subendocardial tissues.

7. Other Defects. A much reduced left ventricle with markedly hypertrophied walls was found in 5 newborn (1.3%); maternal exposures were on days 6 (2), 8 (1) and 9 (2). This chamber had no communication with the left atrium through the mitral valve (Fig. 9). The atria communicated with each other through a large foramen ovale, and there was also a high ventricular septal defect. An abnormal tricuspid valve was found in 1 (0.3%) (day 10) and multiple defects were seen in the septum primum in 5 (1.3%). The latter anomalies occurred only in off-
INDUCED CARDIAC MALFORMATIONS

FIGURE 9

Frontal section through the heart of test rat with mitral atresia. MV = mitral valve.

spring of rats exposed on day 5 of pregnancy. The ductus arteriosus was closed in 75 (20.8%), open in 148 (40.5%), large and widely open in 139 (38.6%). In 2, the right, rather than the left, ductus was retained, and this was accompanied by a right-sided arch; in 2 others (with common trunk) the ductus was absent and in 6 it could not be identified for technical reasons.

Comments

The present series of experiments represents the third stage of a larger study designed to establish an experimental method by which different types of cardiac malformations can be induced in a high percentage of laboratory animals. We thought that by using a 'growth stimulator' in one series of experiments, a 'growth inhibitor' in the other and the combination of both agents in the third, different types of malformations might result. The growth-stimulating effect of increased carbon dioxide tensions in the atmosphere was observed empirically\(^4\) and also experimentally in birds.\(^5\) It was not used as a teratogenic agent in mammals before our studies. The growth-inhibiting effect of hypoxia has been recognized since the classical experiments of Geoffroy-St.-Hilaire.\(^8\) This effect was confirmed in birds,\(^6,\,8,\,9,\,10,\,11\) fish,\(^12\) rabbits,\(^13\) and rats,\(^15\) but only few authors have reported malformations of the cardiovascular system in mammals.\(^16\) The effects of hypoxia combined with hypercapnia have not been reported in mammals, to our knowledge. Riddle,\(^17\) Romanoff\(^8\) and Gallera,\(^7\) however, have reported a teratogenic effect of this gas mixture in birds without mention of any findings in the cardiovascular system.

Results of our experiments of prenatal exposure to hypercapnia alone and of prenatal exposure to mild hypoxia alone have been published in detail.\(^1,\,2\) Briefly, we found that pregnant rats subjected to an increased carbon dioxide tension only, for 24 hours, produced offspring with a high incidence of cardiac malformations (Table 2). There was also evidence of localized tissue overgrowth in the lungs, thymus and heart of the test animals. The average body weight was 18.9% higher in the test group than in the control group. It is also conceivable that anomalies such as transposition complexes may be the consequence of early overgrowth of the ventricular sinusoids followed by abnormal looping of the cardiac tube and a defect in septation.\(^16,\,18-20\) Overgrowth of some portions of the interventricular septum that disturbs coordinated development may produce a low ventricular septal defect. Carbon dioxide given to the mother during the period of fetal cardiogenesis caused a decreased incidence of high ventricular septal defect. This defect in the membranous portion of the septum presents a persistence of the interventricular foramen, closure of which occurs usually around the seventeenth day of gestation,\(^21\) and it was found in 6.8% of the control animals. Perhaps through its stimulating effect on growth, the increased carbon dioxide actually caused a more ready closure of the interventricular foramen.

Exposure of pregnant rats to 10% oxygen for 24 hours resulted in a slight increase of the incidence of cardiac malformations as compared with the control group.\(^2\) There was one example of overriding aorta (0.3%) and four of moderate myocardial hypertrophy with some outflow tract obstruction (1.0%); the
only other abnormality was a single small ventricular septal defect in the membranous portion of the septum (4.0% of cases) similar to those found in the control group. Although the over-all incidence of cardiac malformations was only slightly higher in the test group (5.3%) than in the control group (3.2%), the difference was significant on days 7 and 10 of exposure. The incidence of cardiac anomalies on these days was 13.6%, and this figure compared with the control group is statistically significant (0.05 > P > 0.01).

It is interesting that most significant structural defects occurred in offspring of rats exposed before day 12 of gestation. A high incidence of cardiac anomalies was found as early as days 5, 6, and 7 of development. It has long been claimed that the 'teratogenic susceptibility' (the stage or age at which a teratogenic response to a suitable stimulus can first be induced in a rat embryo) occurs approximately on the eighth day of gestation.22 Because this is also the time at which the embryonic germ layers are forming, it was natural to associate the beginning of teratogenesis with the beginning of tissue differentiation. Although several well-studied agents and conditions such as X-Rays,23 hypervitaminosis A,24 trypan blue,25 and a variety of vitamin deficiencies26 caused malformations only if applied on or after the eighth day, there are others such as Actinomycin D,27 hyperthermia,28 hypoxia,29 hypercapnia3 and (as in our current experience) hypercapnia with hypoxia, that interfere with normal development at an earlier stage. Apparently congenital malformations may originate in at least three different periods in development: (1) before fertilization, probably as a result of germ-cell mutations, (2) before the period of organogenesis, and (3) during the period of organogenesis, owing to environmental disturbances. The mechanism by which hypercapnia with hypoxia exerts its teratogenic action at or before the period of organogenesis is not known. Disturbed maternal physiology, interference with placental exchange, and direct fetal toxicity are possibilities, but there is no direct evidence to support any of them. The mechanisms by which malformations are produced are a matter of conjecture. The ultimate effect of a teratogen is either cell death or alteration in the rate of cell growth, which may cause secondary effects on neighboring organs or tissues due to changed physical relationships. The regulative powers of the embryo may compensate for these changes, but temporary slowing or acceleration may put a tissue or organ out of phase with the growth pattern of adjacent structures. Although the ultimate defect may be the result of different mechanisms, useful information could be expected concerning this problem by applying a teratogen on a given developmental day and studying the embryos through progressive phases of development until birth.

References


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