during 2 weeks showed no significant effects on the histamine LD_{50} values of test *versus* control groups despite findings of increased adrenocortical activity (6). This may be related to the time interval factor between mescaline and histamine challenge. Histamine in this last instance was administered 24 hr. after the last mescaline injection. No significant effects were noted on body weights and growth patterns of mice in the 2-week investigation as demonstrated by the present and previous findings (6).

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Teratogenic Effects of Audiogenic Stress in Albino Mice

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Abstract □ The teratogenic effects of audiogenic stress were studied in 60-day-old pregnant Swiss-Webster mice by exposing the pregnant dams to the effects of a noise generator for varying periods during pregnancy. The most severe fetal effects were noted when the pregnant dams were stressed on Days 8–17 and Days 10–15 of pregnancy. In an attempt to discern the days of greatest susceptibility to stress, a second group of mice was studied, and it was determined that Days 7–8 was the period of maximal susceptibility. The teratogenic effects produced by audiogenic stress included a reduction in fetal weight, complete or partial resorption of fetuses, cranial hematoma, dwarfed hind limbs, and tail defects.

Keyphrases □ Teratogenicity—audiogenic stress effects, albino mice □ Audiogenic stress, effects—teratogenicity, albino mice

The maternal organism has been exposed to a wide variety of noxious chemicals and environmental changes in an effort to determine the effects of such exposure on fetal development (1–4). Procedures that only affect the maternal organism have, however, received little attention until recently. Environmental stresses to which virtually all maternal organisms are subjected during pregnancy have been proven to exert real and deleterious effects on fetal development (5–8). Geber (5) recently demonstrated that decreased fetal weight as well as resorption frequency can be produced in the offspring of rats subjected to audiovisual stress for varying periods during development. The stress of handling pregnant dams (6) and severe audiogenic stress (7) have been shown either to block pregnancy completely or to reduce the chance for successful fetal development. The purposes of this investigation were to study the effects of audiogenic stress on pregnant albino mice in an attempt to determine the effects of such stress on fetal development and also to determine the period of pregnancy in which the fetus is most susceptible to the deleterious effects of stress.

METHODS

Sexually mature Swiss-Webster mice, received at 60 days of age, were used throughout this study. They were kept in a separate room in the animal quarters for 1 week prior to breeding to allow for adjustment to their surroundings. When proestrus was determined by the technique of vaginal smearing (9), two females were placed with each male for an 11-hr. period. When the sexes were separated, the presence of a vaginal plug was taken as proof of pregnancy. This was then Day 0. Between 20–35% of the mated females became pregnant using this technique. Pregnant females were then isolated and kept in separate cages for the duration of pregnancy.

The stress chamber was rectangular [25.40 × 20.32 × 60.96 cm. (10 × 8 × 24 in.)] and constructed of stainless steel. Pregnant mice as well as controls were placed in this chamber in a separate room, and the stressed dams were subjected to the effects of a noise generator which consisted of a motorcycle horn connected to a microswitch timer and set to deliver an output of 82–85 decibels[1] at a

[1] Sound Level Meter, Type 1551-A, General Radio Co., Cambridge, Mass.

Table I—Teratogenic Effects of Audiogenic Stress in Albino Mice

No. of Pregnant Dams	Period of Stress, Days	Hours Stressed per Day	Total No. Normal *vs.* Abnormal Fetuses	Mean Litter Size	Mean Fetal Weight, g.	Mean CR/TU[a] Distance, mm.	No. Litters Delivered *vs.* No. Resorbed	Teratogenic Effects
5	Control	0	52/0	10.5	1.45	20/9	5/0	None
5	8–17	8	18/2	10.0	0.44	15/6	2/3	Hematoma kinked tail resorption
6	10–15	5	43/14	9.5	0.87	18/7	6/0	Hematoma dwarfed hind limbs straight tail resorption
4	7–8	5	All resorbed				0/5	Resorption
3	9–10	5	24/0	8.0	1.80	25/10	3/0	None
4	11–12	5	52/0	13.0	1.70	23/9	4/0	None
4	13–14	5	38/1	9.5	1.60	20/8	4/0	Hematoma
3	15–16	5	29/0	9.0	1.40	21/9	3/0	None
2	17–18	5	18/0	9.0	1.59	24/10	2/0	None

[a] CR = crown-rump distance in millimeters; TU = transumbilical distance in millimeters.

frequency of 320–580 c.p.s. The timer delivered these noise levels for 60–75% of each hour in an intermittent fashion, *i.e.*, 3 min. on, 2 min. off. Background noise was measured as 35–50 decibels at a frequency of 20–32 c.p.s.

Food and water were continuously supplied to both control and experimental groups; control mice were placed in the exposure chamber for 5-hr. periods on each day of pregnancy.

Both experimental and control females were sacrificed on Day 18 of pregnancy; a laparotomy was performed, the uterus was exposed and incised, and the fetuses were removed. Care was taken to note uterine distribution of fetuses as well as any vacant uterine sites which were indicative of fetal resorption. The fetuses were then weighed on a Class A torsion balance to the nearest 0.01 g.; transumbilical and crown-rump measurements were taken to the nearest millimeter, and the fetuses were then divided into two groups. Every third fetus was placed in 90.0 ml. of a 1.0% potassium hydroxide solution to which 6.0 mg. of Alizarin red S was added to stain the bones selectively. The remaining fetuses were placed in Bouin's solution for several days and then examined microscopically after gross dissection, according to established techniques (9). The following parameters were recorded: total number of normal and abnormal fetuses, litter size, fetal weight, crown-rump and transumbilical distances, number of litters resorbed or delivered, and any demonstrable fetal malformations.

RESULTS AND DISCUSSION

The results of this investigation, summarized in Table I, clearly demonstrate the deleterious effects of audiogenic stress on the developing fetus. The most severe fetal effects were observed when the pregnant dams were stressed 8 hr./day on Days 8–17 of pregnancy, with 40% of the litters resorbing and a mean fetal weight of 0.44 g. compared to a control value of 1.45 g. The next period of greatest susceptibility was Days 10–15 of pregnancy when, although no litters resorbed, the mean fetal weight was 0.87 g. In all cases, the crown-rump and transumbilical distances were decreased when the mean fetal weight was decreased, indicating that this parameter is also a good indication of fetal development. In none of the stressed groups was the litter size or the uterine distribution altered over control values. These observations are in agreement with Geber (5) and Sontag *et al.* (7), who reported decreased fetal weight as a result of audiovisual and audiogenic stress. But the observations contradict the findings of Geber (5) as well as those of Thompson and Sontag (10) with regard to litter size, because these investigators reported decreased litter size and no effect on either litter size or fetal weight, respectively. The latter, however, stressed their experimental groups only during the last trimester of pregnancy.

It is evident from Table I that, when murine pregnancy was studied in 2-day intervals, the period of greatest susceptibility to the effects of audiogenic stress was Days 7–8 of pregnancy, with 100% of the litters resorbing before Day 18.

The teratogenic effects observed (cranial hematoma, dwarfed hind limbs, and tail defects) compare favorably with those reported by other investigators (5). These effects have been attributed to the endocrinologic effects of stress on either the maternal or fetal organism, including the discharge of catecholamines and steroid hormones from the adrenals and decreased maternal and fetal blood flow, especially of the uterine and placental vasculature, thus causing fetal hypoxia and even possibly delaying or preventing the implantation of the fertilized ova (5). This observation correlates also with the findings of Weir and DeFries (6) who reported a decreased rate of pregnancy when fertilized dams were subjected to the stress of handling prior to implantation. The adaptation to maternal stress, suggested by Geber (5), did not appear to be a factor in this investigation, because the data demonstrate that, in general, the more severe the stress, the greater the effect on fetal development. Maternal adaptation would also not be a factor in the portion of this investigation in which the murine pregnancy cycle was studied in 2-day segments. While maternal influences in fetal development as the result of stress are the most plausible explanation for the spectrum of teratogenic effects, the direct effects of sound on the fetus itself cannot be disregarded (7).

The significance of this study is that teratogenic effects have been produced with no manipulations of either the fetus or the maternal organism other than environmental alterations of maternal and/or fetal physiological mechanisms. Also, the period of greatest susceptibility to environmental audiogenic stress appears to be during the period of implantation (Table I), which compares favorably to the period of susceptibility to chemical stress factors, *i.e.*, the administration of known teratogenic drugs (11).

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