sulted in paradoxical (inward) displacement of the lower rib cage. The displacement was increased by increasing the amplitude of the stimulus.

Our data clearly indicate that in dogs the diaphragm consists of two muscles that act differently on the rib cage. The costal part of the muscle has a direct inspiratory action on the lower rib cage, even without the aid of increase in abdominal pressure. This is consistent with the arrangement of these fibers, which are inserted into the ribs and directed upward, parallel to the rib cage. On the other hand, the crural part has an expiratory action on the lower rib cage as long as abdominal pressure is not able to increase. These fibers have no insertion on the ribs, and their expiratory effect on the rib cage could be due to the fall in pleural pressure or to a force directed inward and backward. This force would be transmitted to the ribs through the central tendon and costal fibers and would diminish the area of apposition between the costal fibers and the rib cage. In the intact animal the expiratory effect of the crural diaphragm is balanced by the rise in abdominal pressure (Fig. 1).

Figure 2 illustrates a model of a respiratory system behaving in accordance with our data. The diaphragm is represented as two different pressure generators. The costal diaphragm is in series with the intercostal and accessory muscles of inspiration, while the crural diaphragm is in parallel. The summing junction adds the pressure developed across the rib cage by the intercostal and accessory muscles to the change in abdominal pressure and to the pressure directly developed by the contracting part of the diaphragm, producing the total pressure acting across the rib cage. As a result, with the intercostal muscles remaining relaxed, contraction of the crural part would have no net effect on the rib cage if the gain at the summing junction adjusted the change in abdominal pressure so that it was exactly equal and opposite to the change in pleural pressure. On the other hand, contraction of the costal part would expand the rib cage through a direct effect and through the increase in abdominal pressure.

The model of the diaphragm as two separate muscles, one in series, the other in parallel with the rib cage, has a clear anatomical counterpart. It also has an embryological counterpart. The muscular portion of the diaphragm does not arise as a single sheet, but as individual muscle bundles. This is true both in the phylogeny of animal species (*7*) and in man, where the costal part of the diaphragm develops from myoblasts originating in the lateral body walls while the crural part develops in the dorsal mesentery of the esophagus (*8*). These two parts of the diaphragm also differ from each other in terms of fiber composition (*9*) and nerve root innervation (*10*). We have now established that the costal and crural parts have different actions on the chest wall.

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Chloramphenicol Administration During Brain Development: Impairment of Avoidance Learning in Adulthood

Abstract. *Rats treated with chloramphenicol from days 7 to 21 of intrauterine life (50 milligrams per kilogram per day, injected subcutaneously into the mothers) or in the first 3 days of extrauterine life (50 to 100 milligrams per kilogram per day) were trained for avoidance conditioning when 60 days old. The acquisition of the avoidance response was impaired to a highly significant degree in all the treated groups.*

The finding that chloramphenicol, in mammals, inhibits protein synthesis not only in mitochondria but also in brain junctional complexes at concentrations easily attainable with doses in the therapeutic range (*1*), prompted us to study the influence of the administration of this antibiotic during pregnancy or in the neonatal period on the later avoidance learning ability of rats. Chloramphenicol is still widely used in Europe and many Latin countries as a broad-spectrum antibiotic. Our results show that avoidance learning is impaired in rats exposed to chloramphenicol during brain development.

Wistar female rats, 3 months old (Morini, S. Polo d'Enza, Reggio Emilia, Italy), given free access to food, were timemated and thereafter placed in separate cages. The rats were assigned at random to four groups of 15. The date of concep-

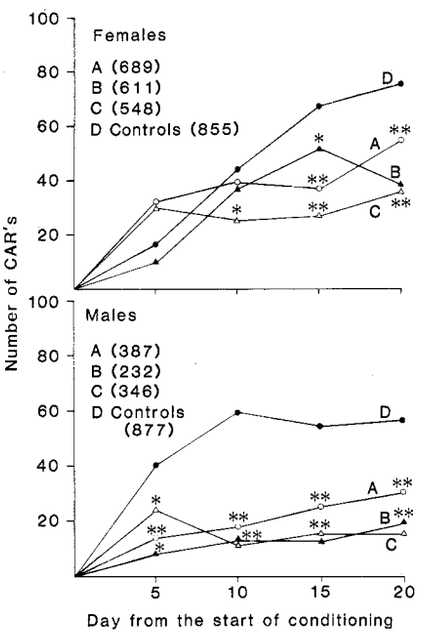

Fig. 1. The effect of early treatment with chloramphenicol on avoidance conditioning in rats (60 days old). The graphs show the number of conditioned avoidance responses (CAR's) on days 5, 10, 15, and 20 after the start of conditioning; each rat was given ten trials per day and there were ten rats in each group. Curve A: the rats had received chloramphenicol (50 mg/kg-day) from days 7 to 21 of intrauterine life. Curves B and C: rats in these groups had received chloramphenicol (50 or 100 mg/kg-day, respectively) for the first 3 days of extrauterine life. Numbers in parentheses show the number of CAR's achieved by each group during the whole period of conditioning (20 consecutive days: 2000 trials per group). Difference from controls: *, $P < .05$; **, $P < .001$ (the number of CAR's achieved by the treated groups on days 5, 10, 15, and 20 of conditioning, as well as the total number of CAR's during the whole period of conditioning were compared with the corresponding values of the control groups by the χ^2 test).

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tion was considered day 0 of gestation.

Chloramphenicol (CAF hemysuccinate, solution containing 50 mg of chloramphenicol per milliliter) was administered to the pregnant mothers or to the newborn pups. For the rats in group A, 50 mg of chloramphenicol per kilogram of body weight per day was injected subcutaneously into the mothers from days 7 to 21 of gestation. Since chloramphenicol passes freely through the placental barrier, one can assume that the fetuses received roughly the same dose as the mothers. For the rats in groups B and C, the chloramphenicol, 50 or 100 mg/kg-day, respectively, was injected subcutaneously into the newborn pups for the first 3 days after birth. The rats in group D served as controls; both the pregnant rats and their young received subcutaneous injections of saline.

Weight gain during pregnancy, litter size, fetal weight, gross malformations of the fetuses, and weight gain of the offspring were recorded. After birth the litter size was adjusted to eight to ten pups in order to maintain a standard nutritive status, equal numbers of each sex being left when possible.

At the doses used, chloramphenicol did not affect the course of pregnancies, litter size, fetal weight, or postnatal weight gain; furthermore, no gross malformations were noticed in the offspring of rats treated with chloramphenicol during pregnancy, and there was no mortality in the groups treated both before or after birth.

At weaning, rats from each group were divided into two subgroups of males and females and, when they were 60 days old, ten rats were selected at random from each of the eight subgroups for the avoidance learning study.

Shuttle boxes divided into two equal and communicating compartments were used. The conditioned stimulus was the sound of a buzzer: if the rat did not cross the passage between the compartments within 5 seconds, the unconditioned stimulus (an electrical shock of 25 V, 1.8 mA) was delivered through the grid floor of the box. Ten consecutive trials at 40-second intervals were performed daily (in the morning, from 0800 to 1200) for 20 consecutive days.

As shown in Fig. 1, the administration of chloramphenicol both during fetal and neonatal life impaired the acquisition of a conditioned avoidance response. The difference between treated and control animals was highly significant in all cases, although it was more marked in males than in females. The pain threshold was evaluated (hot plate test, temperature of the plate 55.5°C) before we conducted these behavioral tests, and no significant differences were observed among the eight groups (mean values ranged from 4.90 ± 0.43 to 6.15 ± 0.79 seconds).

Since chloramphenicol selectively inhibits the protein synthesis in the brain junctional complexes of mammals (*1*), reduced synaptogenesis might play a role in the learning deficit that we report here. One cannot exclude the possibility that the antibiotic interferes with neuron or glial production or migration, or with other aspects of brain differentiation. Metabolic or endocrine changes are other possible mechanisms for the observed effects.

Like other agents described as "pure behavioral teratogens" (*2*), chloramphenicol induces abnormalities in the behavioral capacities of the offspring, unaccompanied by weight loss, mortality, or gross malformations.

Although we cannot extrapolate the present results to humans, the finding that chloramphenicol impairs the acquisition of a conditioned avoidance response in rats should, in our opinion, induce clinicians to be even more cautious in the use of this antibiotic during pregnancy and in infancy. The doses we used are not far from those given to patients (25 to 50 mg per kilogram per day).

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Fetal Female Rats Are Masculinized by Male Littermates Located Caudally in the Uterus

Abstract. *Female rats are masculinized in utero by male littermates sharing the same uterine horn. Increased anogenital distances in neonatal females and mounting behavior in adult females are related to the presence of males on the caudal side of the females in the uterine horn. Contrary to current beliefs, interamniotic diffusion may not be responsible for the exchange of masculinizing agents among fetuses. Since uterine blood flow in the rat is from the direction of the cervix toward the ovary, masculinizing hormones secreted by fetal males may be carried via the uterine vasculature to female littermates located further downstream.*

Normal female rats occasionally display male-like mounting patterns (*1*). Indeed, under various treatment conditions some female rats show the motor pattern of ejaculating males (*2*). The capacity of normal females to exhibit male sexual behavior apparently depends on prenatal exposure to androgen, since such behavior potentials are reduced markedly by prenatal treatment with antiandrogenic drugs (*3, 4*).

A major source of androgen in fetal females is believed to be the male littermates. Female rats developing between two males in utero have longer anogenital distances (an androgen-sensitive morphological measure) at birth and higher frequencies of mounting in adulthood than females located three positions from the nearest male (*4, 5*). Clemens (*5*) proposed that interamniotic diffusion of androgen from contiguous male fetuses masculinized neighboring females. Simple interamniotic diffusion should yield gradations of masculinization in direct proportion to distance from males in utero, but equivalent masculinization occurred in females separated from the closest male by another female and those contiguous to one male (*4, 5*). This discrepancy suggested that diffusion across adjacent amniotic membranes might not be the mechanism of intrauterine exchange of androgen among fetuses. An alternative mechanism is offered by the uterine vasculature.

The rat has a separate vascular system for each uterine horn, with both the arterial and venous flow proceeding from the cervical end toward the ovary (*6*). The uterine vein and artery in the rat parallel one another and are in close apposition (Fig. 1). This organization of the uterine vessels prompted the suggestion that the luteolytic effect exerted by the uterus on the ovary in the rat may be mediated by substances that pass directly from the venous drainage into the arterial supply (*6*). Using a similar line of reasoning, we proposed that the venous drainage from male fetuses introduces substantial amounts of androgen into the

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