forcement schedule from the 2-minute variable interval to the more desirable continuous reinforcement. After a number of such stimulus presentations, a punishment contingency was added so that during the stimulus period the fish could get a worm for every lever response but also had to accept a shock. The shock intensity was 7 volts for 0.3 second. The development of the suppression for one of these fish is shown in Fig. 2*a*. The top record shows that the fish made 21 responses during a light presentation before the punishment contingency was added. The second cumulative record, which represents the second shock punishment trial, shows that the fish was willing to accept only two shocks in order to obtain the worms. The third record shows a complete suppression of lever responding approximately 11 trials later.

Similar data averaged for the three fish are shown in Fig. 2*b*, which shows that under continuous-reinforcement conditions without shock, the mean response rate was 27. On the second punishment conditioning trial, this was reduced to 7. By trial 13, suppression was complete in the three fish during the light period.

The results of this study demonstrate that the conditioned "anxiety" procedure and the punishment technique may be applied to goldfish with the same results that have been observed in other species (*10*).

IRVING GELLER
Wyeth Laboratories, Inc.
Philadelphia 1, Pennsylvania

References and Notes

1. W. K. Estes and B. F. Skinner, *J. Exptl. Psychol.* **29**, 390 (1941).
2. W. K. Estes, *Psychol. Monographs* **263**, 1 (1944).
3. J. B. Appel, *Science* **133**, 36 (1961); L. H. Storms, G. Boroczi, W. E. Broen, Jr., *ibid.* **135**, 1133 (1962); J. V. Brady, *Experimental Psychopathology* (Grune and Stratton, New York, 1957), chap. 3, p. 20.
4. J. V. Brady, *Science* **123**, 1033 (1956); I. Geller, in *Psychosomatic Medicine*, J. H. Nodine and J. H. Moyer, Eds. (Lea & Febiger, Philadelphia, 1962), chap. 33, p. 267.
5. The feeder was built by E. Adams in the instrument shop at Wyeth Laboratories, Radnor, Pa.
6. N. Longo and M. E. Bitterman, *J. Exptl. Analysis of Behavior* **6**, 279 (1963).
7. I am indebted to Dr. Bitterman of Bryn Mawr College for suggestions for painting the electrodes.
8. N. Longo and M. E. Bitterman, *Am. J. Psychol.* **72**, 616 (1959).
9. B. F. Skinner, *Science and Human Behavior* (Macmillan, New York, 1963), pp. 91–97.
10. I am indebted to H. Harner and W. Ridley for assistance with setting up the apparatus needed for programming and delivery of the electric shocks.

15 May 1963

Teratogenic Effects of Meclizine Hydrochloride on the Rat

Abstract. *Congenital malformations were induced in the offspring of pregnant Sprague-Dawley rats by the administration of relatively large doses of meclizine hydrochloride (Bonine). The critical period of gestation for administration was from the 12th to 15th day. Anomalies were produced in the tongue, palatal closure, mouth, lower jaw, vertebrae and limbs.*

Recent reports concerning meclizine and human fetal abnormalities are contradictory. Some authors have reported a direct positive correlation between its administration during gestation and abnormalities in the fetus (*1*), while others have found no correlation (*2*). Reports on the effects of antihistamines in pregnancy and pseudopregnancy in the mouse and rat are also contradictory. Goldstein and Hazel (*3*) found that the antihistamine, "Pyranisamine maleate, administered daily by subcutaneous injection to female mice, in a dosage sufficient to cause marked and prolonged sedative effects, did not interfere with normal ovulation, fertilization or implantation." Shelesnyak and Davies (*4*) found that subcutaneous injection of benadryl in mice and pyrrolazote in rats significantly reduced the number of normal pregnancies and concluded that the antihistamines acted on the developing fetus after implantation had occurred. Finally the report of Tuchmann-Duplessis (*5*) at the University of Paris, on the teratogenic effect of cyclizine on laboratory animals, led the Wellcome Foundation of London, England, to advise the Ministry of Health on the possible teratogenicity of the antihistamine.

In the light of these reports it was decided to investigate the effects of meclizine hydrochloride (a compound of very close chemical structure to cyclizine) on fetal development in the rat, as part of an effort to obtain an agent which might specifically induce oral facial malformations.

"Bonine," a preparation of meclizine hydrochloride which is available commercially without prescription, was tested for teratogenic activity on the Sprague-Dawley rat. Tablets were pulverized and force fed by intubation as a suspension in 50 percent by volume of ethanol in water at different stages of gestation. At no time did the volume that was force fed exceed 1 ml. The excipient used in the preparation of the meclizine hydrochloride tablets (Bonine) was used for control purposes.

Three hundred and twenty mature female rats weighing 200 ± 20 g were used for this study. The onset of gestation was established by the demonstration of spermatazoa in vaginal smears. The day following the appearance of a positive smear was recorded as the first day of pregnancy.

Since our purpose was mainly the induction of cleft palate or oral facial malformations, the treatments were limited to the first 16 days of gestation. Emphasis was placed on the 12th to the 15th day, since it has been established by Feild *et al.* (*6*) that the 13th to the 15th gestational days are the critical periods of palatal development and closure in this strain of rats.

One day prior to the expected day of parturition the pregnant rats were killed with sodium nembutal. The young were delivered by cesarean section and examined for gross malformations. They were fixed in 80 percent ethanol, cleared with 1 percent KOH and the skeleton was selectively stained with 0.5 percent Alizarin Red S after which the specimens were examined for skeletal defects.

Fifty-six pregnant rats were used to determine the effects of multiple doses of 50 mg of Bonine per rat after implantation had occurred. Multiple doses before the 11th or after the 13th day produced no malformations. The critical time for two consecutive treatments

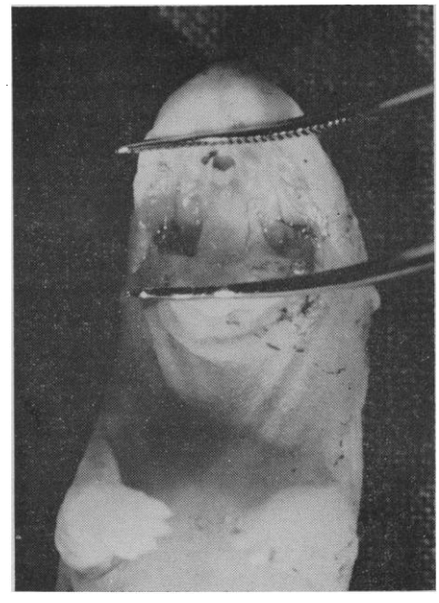

Fig. 1. Offspring with cleft palate and fusion of the tongue to the palatine shelves.

Table 1. Teratogenic effects of meclizine HCl (Bonine) in the rat.

Dose (mg)	No. of pregnant rats	Days administered (inclusive)	No. of viable young	Young resorbed (%)	No. of young malformed	
					Grossly	Shown by clearing
50	4	13–14	37	0	10	0
50	4	13–15	28	15.1	28	0
40	6	11–14	53	3.6	36	26
35	7	9–14	51	32.9	51	51
25	6	7–8	58	0	0	0
25	10	11–14	96	12	0	12
25	3	12–14	32	0	2	0
10	9	7–14	86	5.4	1	17
10	8	10–14	87	1.1	2	0
5	8	7–13	51	30.0	0	0
5	3	1–15	25	0	0	14

was the 13th and 14th days, which resulted in 37 viable young, ten of which had gross malformations (Table 1, line 1). Cleft palate with glossopalatine fusion (Fig. 1), brachygnathia (receded lower jaw), and microstomia (small mouth) were present in varying degrees; they also appeared to have a certain degree of micromelia (short limbs). Clearing of the fetuses made specific skeletal abnormalities evident (Fig. 2). The femur and the humerus were extremely short as compared with those of the control fetuses, while the vertebral bodies had failed to calcify and the lower jaws were square and flat. As the duration of treatment was extended the percentage of resorbed and grossly malformed young was increased, the critical period of gestation being days 13 to 15 inclusive (Table 1, line 2). Treatment for four or more days after the 9th gestational day produced either 100 percent resorptions or malformations, or both, the malformations being very specific and the same as the ones induced with shorter treatment or lower dosage.

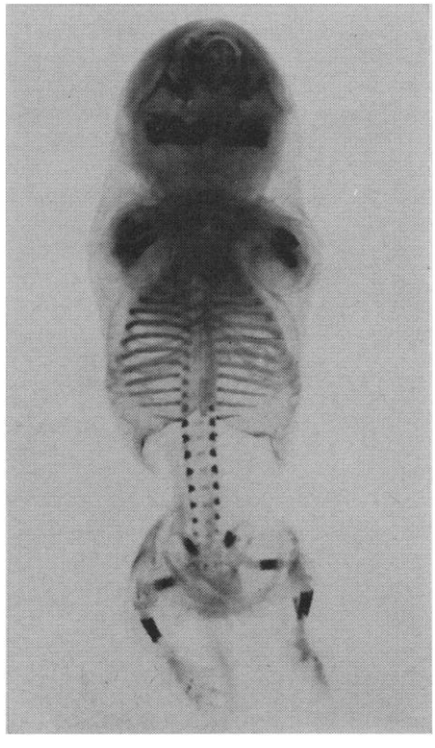

Fig. 2. Skeleton of an alizarin stained specimen demonstrating lack of calcification of the vertebral bodies, short femurs and short humerus.

At 35- and 40-mg levels of Bonine the incidence of cleft palate, brachygnathia, microstomia, and skeletal malformations was still very high. Although the incidence began to decline at 25 mg (Table 1, lines 6 and 7) doses of 10 mg administered from the 10th to 14th day resulted in 2 of 87 fetuses having cleft palate and skeletal malformations. Short femur and humerus, and lack of calcification of the bodies of cervical vertebrae, were still induced when 5 mg of Bonine per rat was administered from days 1 to 15. Multiple doses of Bonine of 1 to 5 mg per rat did not inhibit implantation in this strain of rats.

Single doses of Bonine ranging from 35 to 75 mg per 200-g rat administered from the 9th to the 15th day of gestation to 39 pregnant rats produced three malformed young out of 357 obtained. The malformations were the same as those obtained previously.

To eliminate the possibility that some impurity in the Bonine tablet could be responsible for teratological effects, pure meclizine hydrochloride was administered to 41 pregnant rats in a way similar to that of the previous experiments. The results were indeed similar to those obtained using the commercial preparation. The threshold dose for skeletal malformations was again 5 mg per rat administered in 1 ml of 50 percent ethanol from the 9th to the 14th day. Again, at higher doses of 25 and 50 mg administered from the 11th to the 15th day, the gross malformations of cleft palate with glossopalatine fusion, brachygnathia, microstomia, and micromelia were observed.

An aqueous suspension of 50 mg of Bonine per 200-g rat was force fed to eight pregnant rats during the 12th, 13th and 14th days of gestation. Here again the same type of malformations were observed in all of the 55 viable young obtained, indicating that the alcohol used in the other experiments was not a factor concerned in producing these malformations.

Forty-nine pregnant rats served as controls. They were force fed with 50, 25, 10, 5, 2, or 1 mg of the excipient in the Bonine tablet. This material was suspended in 50 percent by volume of ethanol in water and 1 ml of the suspension was administered daily to each rat from the 9th to the 15th day of gestation. Four hundred and forty-six young were obtained of which 14 (3.13 percent) were resorbed. The remaining 432 showed no evidence of gross or skeletal malformations.

It is obvious that meclizine hydrochloride is a potent teratogen in the rat. The external defects were specific and could be induced at will in 100 percent of the litters and in 100 percent of the fetuses making up each litter. This was also true of skeletal malformations which became evident after clearing. At present it is not possible to state the percentage of internal or soft tissue defects since none of the specimens were sectioned, except to determine the type of fusion which occurred between the tongue and the palatine shelves.

Glossopalatine fusion, brachygnathia, microstomia, and micromelia observed in these studies parallel recent findings with hypervitaminosis A reported by Kalter and Warkany (*7*). Whether both of these agents induce these malformations by the same metabolic pathway remains to be determined. One of the interesting facts about meclizine hydrochloride is that it is not strain specific, since our results with the Sprague-Dawley strain have been reproduced in the Osborne Mendel strain (*8*). Furthermore, it is interesting to note that Tuchmann-Duplessis (*5*) was able to induce a variety of malformations in rats, rab-

bits, and mice with cyclizine which is a compound of chemical structure very similar to meclizine hydrochloride.

Until studies on the level of histamine in the liver of the pregnant rat and of the embryo have been carried out it cannot be concluded that meclizine hydrochloride does or does not exert its teratogenic action via its antihistaminic activity (*9*).

C. T. G. KING
National Institute of Dental Research, National Institutes of Health, Bethesda 14, Maryland

References and Notes

1. G. I. Watson, *Brit. Med. J.* No. 5317, 1446 (1962); C. G. Fagg, *ibid.* No. 5320, 1681 (1962); G. W. Mellin and M. Katzenstein, *Lancet* No. 7274, 222 (1963).
2. M. P. Carter and F. W. Wilson, *Brit. Med. J.* No. 5319, 1609 (1962); J. R. James, *ibid.* No. 5322, 59 (1963); A. David and A. H. Goodspeed, *ibid.* No. 5323, 121 (1963); L. Martin-Nahon, *ibid.* No. 5326, 331 (1963); K. D. Salzmann, *ibid.* No. 5328, 471 (1963).
3. A. Goldstein and M. H. Hazel, *Endocrinology* **56**, 215 (1955).
4. M. C. Shelesnyak and A. M. Davies, *Proc. Soc. Exptl. Biol. Med* **89**, 629 (1955).
5. H. Tuchmann-Duplessis, *Brit. Med. J.* No. 5333, 828 (1963).
6. L. E. Feild, S. J. Kreshover, J. E. Lieberman, *J. Dental Res.* **39**, 1240 (1960).
7. H. Kalter and J. Warkany, *Am. J. Pathol.* **38**, 1 (1961).
8. From experiments currently in progress in this laboratory.
9. I thank Chas. Pfizer & Co., Inc., for a supply of meclizine hydrochloride and the excipient used for the manufacture of the Bonine tablet, F. J. Kendrick for his evaluation of histologic material, and Julia Derr and Sallie Weaver for excellent technical assistance.

13 May 1963

Electron Microscope Autoradiography of Bacteria Labeled with Iodine-125

Abstract. *The low-energy extranuclear electrons emitted by iodine-125 can be used for electron microscope autoradiography with results comparable to those obtained with tritium. Autoradiographs of tritium-labeled bacteria showed that 71 percent of all reacted grains localized on the cell. This figure was 86 percent when I^{125}-labeled bacteria were used.*

The low-energy electrons emitted by tritium contribute to accuracy of localization in autoradiography, because electrons that do not travel in paths perpendicular to the plane of the section are apt to be absorbed before they reach the photographic emulsion. Lateral scatter of electrons is reduced further in electron microscope autoradiography because the sections of cells and layers of the photographic emulsion are ultrathin (*1*). Iodine-125 has been used for autoradiography in the light microscope (*2*) and the electron microscope (*3*). The x-rays and gamma rays from I^{125} do not affect the photographic emulsion significantly, and the extranuclear electrons emitted by I^{125} have energies near the range of beta particles of tritium (*2*). In the present experiment, we have used bacteria as small biological objects in order to assess the accuracy of localization in autoradiography with I^{125}.

Salmonella typhosa was grown in broth, killed with formaldehyde at *p*H 7.0, and iodinated with 2 mc of NaI^{125} (*4*). Uncombined I^{125} was removed by washing four times in balanced salt solution. The bacteria were fixed in buffered osmium tetroxide, dehydrated through an alcohol series, and embedded in methacrylate by the usual methods. Thin sections were prepared with a Porter-Blum microtome, mounted on stainless-steel grids, coated with jelled Ilford L4 emulsion (*5*), and exposed for 5 to 21 days at 4°C. Coated grids were developed with Kodak D-19, Microdol-X, or a solution of *p*-phenylenediamine and sodium bisulfite (*5*). Most sections were treated with 0.05*N* sodium hydroxide to remove excess emulsion (*6*) and stained with 0.07*M* uranyl acetate prior to examination in an electron microscope (RCA EMU-3).

A thymine-requiring strain of *Escherichia coli* was grown in medium that contained tritiated thymidine. They were then fixed in buffered osmium tetroxide without prior treatment in formaldehyde and prepared for electron microscopy as described above.

In sections of formaldehyde-treated bacteria, large clear spaces occupied the central or nuclear areas, and the bacteria had a shell-like appearance. This aided in assessing localization, for silver grains were located on or near the cell walls and were rarely present in the clear zone (Figs. 1 and 2). Background was negligible as determined by observation of portions of the section in which there were no bacteria. Silver grains located near the bacteria but not over them were attributed to lateral scatter of electrons.

With Microdol-X or D-19 as a developer, of a total of 887 grains counted in a series of fields of I^{125}-labeled bacteria (Fig. 1), 768, or 86 percent, lay partially or completely over bacteria.

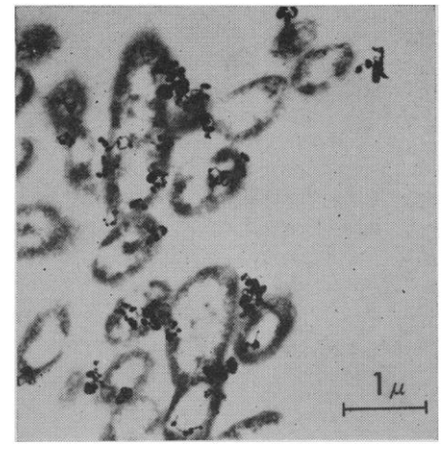

Fig. 1. Autoradiograph of formaldehyde-treated bacteria labeled with I^{125} and developed in Microdol-X.

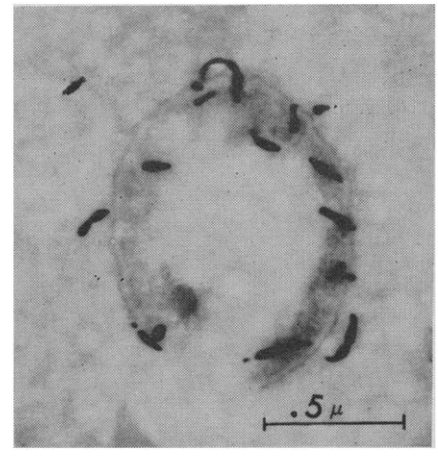

Fig. 2. Autoradiograph of formaldehyde-treated bacteria labeled with I^{125} but not treated with sodium hydroxide. *P*-phenylenediamine was used as the developer.

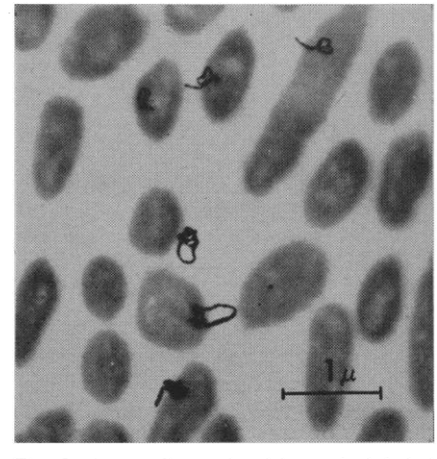

Fig. 3. Autoradiograph of bacteria labeled with tritiated thymidine and developed in Microdol-X.