Morphine-Induced Fetal Malformations II: Influence of Histamine and Diphenhydramine

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Abstract □ The results of this investigation indicate that teratogenic effects of high subcutaneous doses of morphine sulfate administered on Day 8 or 9 of gestation in CF-1 mice are not augmented by histamine dihydrochloride or decreased by diphenhydramine. On the contrary, histamine caused a decrease in the incidence of exencephaly and cryptorchid testes and diphenhydramine caused an enhancement of the teratogenic response when both of these agents were used in combination with morphine sulfate.

Keyphrases □ Morphine induced—fetal malformation □ Fetal malformation, morphine induced—histamine, diphenhydramine effects □ Teratogenic effects—morphine, high doses □ Histamine, diphenhydramine effects—morphine-induced teratogenesis

A previous investigation (1) demonstrated that high subcutaneous doses of morphine sulfate administered to nontolerant CF-1 mice on the 8th or 9th day of gestation are capable of inducing a variety of fetal malformations. The most prominent anomaly observed with drug challenge on Day 8 was exencephaly, while axial skeletal fusion was the most conspicuous malformation occurring with the drug insult on Day 9.

It can be assumed that an evaluation of the pharmacologic actions of morphine may provide some insight into its teratogenicity. Because a decrease in food consumption is not involved in the teratogenicity of morphine (1), the next most obvious pharmacologic action of the drug which should be investigated is its ability to release histamine.

Many investigations lead one to believe that histamine release may be involved in the production of congenital anomalies by morphine. Among these are those of Ciuchta and Gautieri (2) who, working with perfusion studies on full-term human placentas, showed that histamine consistently caused constriction of placental vessels. This action may result in a decrease in the oxygenation of fetal blood flowing through such vessels and, hence, contribute to fetal asphyxia which, therefore, could conceivably be responsible for the production of congenital malformations. Also, several investigators noted that mice, subjected to anoxia on Day 8 or 9, gave birth to a large number of fetuses with exencephaly and axial skeletal fusions (3, 4). Furthermore, X-radiation has been shown to cause exencephaly and axial skeletal fusions in mice (5), as well as an increase in the histamine content of adult females (6, 7). Consequently, the possibility exists that morphine may produce its teratogenic effects by histamine release and the ensuing placental anoxia.

Therefore, the purposes of the present study were: (*a*) to reconfirm the teratogenic effects of high doses of morphine in CF-1 mice, and (*b*) to ascertain whether histamine is involved in the production of congenital malformations by morphine. The latter may be determined by observing an enhancement of malformations with the prior administration of histamine, or a reduction of malformations with the prior administration of an antihistamine.

EXPERIMENTAL

In all experimental groups, CF-1 albino mice[1] were employed. The females were placed in groups of 25–30 in aggregate cages and were not mated until they weighed at least 25 g. Males were placed in individual metal cages[2] measuring 12.5 × 15 × 10 cm. with a wire mesh front and floor. Excreta pans were elevated to the cage floor to permit coprophagy. The mice were maintained on Purina laboratory chow and tap water *ad libitum*.

Sodium chloride (0.9%), morphine sulfate[3] (4.0%), diphenhydramine HCl[4] (1.0%), and histamine dihydrochloride[5] (1.0%) were prepared weekly in distilled water and kept under refrigeration until the time of administration. At this time, the prepared solutions were allowed to equilibrate to room temperature, and all injections were made using 1-ml. B-D tuberculin syringes equipped with 1.27-cm. (0.5-in.) 26-gauge needles.

To produce timed pregnancies, two female mice were placed in a fertile male's cage at 4:00 p.m. The following morning at 8:00 a.m. the females were removed and inspected for the appearance of a vaginal plug which represents the last part of the ejaculate. Females exhibiting this plug were considered gravid; the morning of its appearance was designated as Day 0 of gestation. These gravid mice were then weighed and placed in individual cages, similar to the males, and remained undisturbed until the morning of Day 7 when they were weighed a second time. Pregnancy was confirmed by a weight gain of 2 g. or more.

The pregnant animals were assigned at random to one of 31 experimental categories. Each category was treated on either Day 8 or 9 of gestation. The treatments included: two saline control groups receiving 0.3 ml. of normal saline solution; six morphine-treated groups receiving single injections of 200, 300, or 400 mg./kg. of morphine sulfate; four groups receiving 50 or 100 mg./kg. of the antihistamine diphenhydramine HCl; two groups receiving 50 mg./kg. of histamine dihydrochloride; six groups injected with 50 mg./kg. of diphenhydramine HCl followed in 15 min. by an injection of 200, 300, or 400 mg./kg. of morphine sulfate; four groups injected with histamine followed in 15 min. by an injection of either 200 or 300 mg./kg. of morphine sulfate; four groups injected with 0.3 ml. of saline followed in 15 min. by the injection of either 200 or 300 mg./kg. of morphine sulfate; and two groups injected with saline followed in 15 min. by a second injection of saline. One group represented the untreated controls to establish the norms of the mice.

The morphine injections, single saline injections, and the second injection of the saline–saline treatment were administered subcutaneously in the upper right abdomen. All other injections were given subcutaneously in the upper left abdomen.

Following the respective treatments, the mice were allowed to continue their gestation uninterrupted until Day 18 which is 1 day prior to the expected delivery. On this day, each pregnant mouse was sacrificed by cervical dislocation and its weight was recorded. The abdominal cavity was opened, exposing the uterine horns. The numbers of fetuses and resorption sites (metrial glands) were determined and recorded. The exposed fetuses were removed by blunt dissection, blotted dry, and weighed to the nearest hundredth of a

[1] Obtained from Carworth Farms, Inc., New City, N. Y.
[2] Norwich Wire Works.
[3] Morphine sulfate USP, Merck and Co., Rahway, N. J.
[4] Marketed as Benadryl by Parke-Davis and Co., Detroit, Mich.
[5] Histamine dihydrochloride, Nutritional Biochemicals Corp., Cleveland, Ohio.

Table I—Mean Values of Test Groups Receiving Single-Injection Treatments on Day 8

Treatment	Number of Litters	Maternal Wt.[a] S	Maternal Wt.[a] T	Fetuses Right Horn	Fetuses Left Horn	Resorptions Right Horn	Resorptions Left Horn	$\bar{X}$ Fetal Weight, g.	Number of Males	Number of Females
Control (untreated)	6	25.5	48.0	6.2	3.7[b]	0.17	0.33	1.07	4.0	5.6
Saline, 0.3 ml.	6	26.8	51.0	5.8	6.2	0.33	0.50	1.13	6.5	5.5
Morphine, 200 mg./kg.	6	27.0	44.3[b]	6.3	5.0	0.50	0	0.92[b]	5.8	5.2
Morphine, 300 mg./kg.	6	25.7	48.5	5.5	5.0	0.50	0.33	1.10	5.2	5.3
Morphine, 400 mg./kg.	6	28.0	47.8	5.7	4.8	0.50	0.67	1.03	7.5	3.0
Diphenhydramine, 50 mg./kg.	6	27.8	40.2[b]	5.5	5.3	0	0.17	0.85[b]	5.6	4.6
Diphenhydramine, 100 mg./kg.	5	25.8	43.6[b]	6.2	5.2	0	0.60	0.96	4.6	6.8
Histamine, 50 mg./kg.	6	27.5	48.3	5.2	4.8	1.0	1.20	1.15	5.7	4.3

[a] S = starting weight; T = terminal weight[b]. Statistically significant in comparison with saline control, $p < 0.05$.

Table II—Mean Values of Test Groups Receiving Single-Injection Treatments on Day 9

Treatment	Number of Litters	Maternal Wt.[a] S	Maternal Wt.[a] T	Fetuses Right Horn	Fetuses Left Horn	Resorptions Right Horn	Resorptions Left Horn	$\bar{X}$ Fetal Weight, g.	Number of Males	Number of Females
Saline, 0.3 ml.	6	25.2	49.7	6.2	4.0	0.50	0.33	1.20	5.7	4.5
Morphine, 200 mg./kg.	6	26.2	47.8	4.5	5.8	0.33	0.17	1.02[b]	6.0	4.3
Morphine, 300 mg./kg.	6	25.8	41.3[b]	5.3	3.3	0	1.50	1.15	4.3	4.3
Morphine, 400 mg./kg.	6	27.5[b]	44.5[b]	6.0	4.7	0.17	0.17	1.05	6.3	4.3
Diphenhydramine, 50 mg./kg.	6	26.0	39.8[b]	6.5	3.8	0.33	0.50	1.0	6.0	4.3
Diphenhydramine, 100 mg./kg.	4	26.0	46.3	6.0	5.8	0	0.50	0.95[b]	5.0	6.8
Histamine, 50 mg./kg.	5	22.2	44.2	4.0[b]	6.0	0.20	0.80	1.06	5.6	4.8

[a] S = starting weight; T = terminal weight. [b] Statistically significant in comparison with saline control, $p < 0.5$.

gram on a torsion balance. Each fetus was examined for external defects, and the sex was determined on the basis of their external genitalia. Fetal viability was determined by reflex movement of the limbs in response to mechanical stimulation of the fetus with a blunt probe after removal from the uterus. Every second fetus was processed for skeletal examination according to the method of Staples and Schnell (8). The remaining fetuses were fixed and decalcified in Bouin's fixative. After 2 weeks, the fixative was replaced by 70% ethyl alcohol in which the specimens were stored until freehand sections were made with a thin double-edged stainless razor blade according to the method of Wilson (9).

Statistical Methods and Analysis—The significance of observed variations among the experimental groups was determined by using standard statistical routines. These included the Student t test for continuous variables and the uncorrected χ-square test for binomial proportions (10). The t values were calculated on the IBM 360-60 computer. The probability, p, was determined for the t and χ^2 values by utilization of standard probability tables.

RESULTS

Maternal Effects of the Drugs—Maternal stimulation, followed by depression and a recurrent hyperactive period of recovery, was experienced as a result of all morphine treatments. With the higher doses of morphine, convulsive seizures accompanied the excitement stage and continued to interrupt the depression. Acute toxicity to morphine was found to be very pronounced when the mice were treated with 400 mg./kg. This dosage caused deaths to occur in 13 of 25 animals (52%) when given on Day 8 and in 11 of 23 (43%) when injected on Day 9.

Excitatory responses were also evoked upon the administration of diphenhydramine. When given alone, this antihistamine produced no convulsive activity at the dosage levels employed. However, when it was administered in combination with morphine, convulsive seizures appeared more frequent and severe than those observed with morphine alone. In no case did single injections of 50 mg./kg. diphenhydramine cause any maternal deaths. However, when it was administered 15 min. prior to the 400-mg./kg. dose of morphine, a definite increase in death rate was observed. When this combination treatment was performed on Day 8, four of five of the animals were killed; with this same combination on Day 9, all eight pregnant mice succumbed. As a result of this extremely high death rate, the decision was made to delete the 400-mg./kg. dose of morphine in combination with other test drugs. By employing the two lower doses of 200 and 300 mg./kg. in these combinations, enhancement or reduction in fetal effects as compared to those produced by morphine alone could still be observed and would satisfy the purpose of this investigation. In all cases, local tissue irritation occurred at the site of the diphen-

Table III—Mean Values of Test Groups Receiving Combination Treatments[a] on Day 8

Treatment	Number of Litters	Maternal Wt.[b] S	Maternal Wt.[b] T	Fetuses Right Horn	Fetuses Left Horn	Resorptions Right Horn	Resorptions Left Horn	$\bar{X}$ Fetal Weight, g.	Number of Males	Number of Females
Saline, 0.3 ml. Saline, 0.3 ml.	5	26.0	47.2[c]	5.8	6.2	0.40	0.80	1.14	7.0	4.2
Diphenhydramine, 50 mg./kg. Morphine, 200 mg./kg.	6	26.8	46.3	5.8	5.0	0	0.30	1.03	5.8	5.4
Diphenhydramine, 50 mg./kg. Morphine, 300 mg./kg.	6	26.0	42.8[d]	5.0	4.7	0.70	0.50	1.02	4.0	5.3
Histamine, 50 mg./kg. Morphine, 200 mg./kg.	5	25.8	46.4	5.2	4.4	0.20	0.20	1.08	5.4	4.6
Histamine, 50 mg./kg. Morphine, 300 mg./kg.	5	25.2	41.2[d]	4.6	4.0	0.40	0	1.04	4.0	4.2
Saline, 0.3 ml. Morphine, 200 mg./kg.	6	26.8	44.7	4.2[d]	5.7	0.20	0.20	1.03	4.4	5.0
Saline, 0.3 ml. Morphine, 300 mg./kg.	6	27.8[d]	48.7	4.5	5.0	0.30	0.30	1.05	5.3	5.2

[a] 15-min. interval between injections. [b] S = starting weight; T = terminal weight. [c] Statistically significant in comparison with saline control, $p < 0.05$. [d] Statistically significant in comparison with single injections of the respective dose of morphine, $p < 0.05$.

Table IV—Mean Values of Test Groups Receiving Combination Treatments[a] on Day 9

Treatment	Number of Litters	Maternal Wt.[b] S	Maternal Wt.[b] T	Fetuses Right Horn	Fetuses Left Horn	Resorptions Right Horn	Resorptions Left Horn	$\bar{X}$ Fetal Weight, g.	Number of Males	Number of Females
Saline, 0.3 ml. Saline, 0.3 ml.	5	25.4	45.0[c]	5.0	4.2	0.20	0.40	1.10	3.6	5.6
Diphenhydramine, 50 mg./kg. Morphine, 200 mg./kg.	6	25.0	41.0	3.8	3.8	1.70	2.20	1.12	3.3[d]	4.3
Diphenhydramine, 50 mg./kg. Morphine, 300 mg./kg.	6	25.8	46.3	4.8	4.8	1.20	0.80	1.03	5.2	4.5
Histamine, 50 mg./kg. Morphine, 200 mg./kg.	6	25.5	42.5	5.2	4.7	1.0	0.50	1.07	5.3	4.5
Histamine, 50 mg./kg. Morphine, 300 mg./kg.	5	25.4	46.0	5.2	6.2	0.40	0.80	1.08	5.6	4.8
Saline, 0.3 ml. Morphine, 200 mg./kg.	5	25.8	46.4	5.6	4.2	0.40	0.60	1.08	5.8	4.0
Saline, 0.3 ml. Morphine, 300 mg./kg.	6	26.2	42.0	3.7	4.5	1.0	1.0	0.98[d]	3.8	4.3

[a] 15-min. interval between injections. [b] S = starting weight; T = terminal weight. [c] Statistically significant in comparison with saline control, $p < 0.05$. [d] Statistically significant in comparison with single injections of the respective dose of morphine, $p < 0.05$.

hydramine injection; ulceration and necrosis at the injection site became evident a few days after the administration.

Histamine when given alone caused an overall depressive symptom in the animals. The administration of either histamine or saline 15 min. prior to morphine had no effect on the normal maternal symptoms caused by the narcotic.

Tables I–IV represent the cumulative mean values for each experimental group employed in this study. It appears from the results shown in Tables I and II that subcutaneous injections of morphine tend to cause a reduction in the mean terminal weights of the pregnant mice when compared to saline controls. This result was usually more apparent when the drug was administered on Day 9 of gestation. It can be seen from Tables II and III that in no instance did the administration of normal saline 15 min. before the injection of morphine cause any significant change in the effect of morphine alone on this parameter. It can also be seen from these tables that in almost every case the administration of diphenhydramine on either Day 8 or 9 resulted in a decrease in the mean terminal weight compared to saline, but the greater effect usually occurred at the lower dose. Diphenhydramine or histamine, when employed in the combination treatments with 300 mg./kg. morphine, both caused a reduction in the maternal mean terminal weight compared to single injections of morphine at this same dose.

Fetal Resorptions and Sex Ratios—In practically all cases, there were no differences in the fetal ratio (number of fetuses in the right horn *versus* number of fetuses in the left horn). In the few cases where there were significant differences, they only occurred on one side of this ratio which, therefore, would tend to lessen their significance. No significant differences were observed among the resorption ratios in any of the test groups. In every case, no change was observed in the male *versus* female ratio except when 50 mg./kg. diphenhydramine followed by 200 mg./kg. morphine was administered on Day 9. Here the number of males was less compared to 200 mg./kg. morphine given on the same day.

Mean Fetal Weight—A significant decrease in the mean fetal weight was observed with the administration of the lowest dose of morphine on both Days 8 and 9 of gestation. Diphenhydramine on Day 8 at the 50-mg./kg. dosage and on Day 9 at the 100-mg./kg. dosage caused a decrease in the fetal weight as compared to the saline controls. Although 300 mg./kg. morphine on Day 9 had no effect on the mean fetal weight, in combination with saline a significant decrease in this weight was observed.

Production of Anomalies—*Exencephaly*—The number of exencephalic fetuses and the number of litters containing defective young are tabulated in Tables V–VIII. From Table V it can be seen that in the groups treated with morphine alone, exencephaly was found in three of six litters after treatment with 300 mg./kg. on Day 8. Only one fetus with exencephaly was found in the group treated with 200 mg./kg. on Day 8, which has been shown to be insignificant. The administration of diphenhydramine alone produced no exencephalic fetuses. However, with the combination of 50 mg./kg. diphenhydramine and 200 mg./kg. morphine given on Day 8 (Table VII), five more exencephalic fetuses were found than with morphine given alone.

Although exencephaly was not observed in the groups receiving the combination of 50 mg./kg. histamine and 300 mg./kg. morphine on Day 8, this was a significant decrease in this effect compared to the three litters with exencephaly occurring with 300 mg./kg. morphine on the same day.

In all cases, the injection of saline 15 min. before the morphine administration compared to the injection of morphine alone produced no significant changes in the occurrence of the anomaly.

One case of exencephaly appeared in the following groups having morphine administered on Day 9: 200 mg./kg. morphine; 50 mg./kg. diphenhydramine in combination with 300 mg./kg. morphine; and saline in combination with 300 mg./kg. morphine. Two exencephalic fetuses were found in the group treated with 50 mg./kg. histamine in combination with 300 mg./kg. morphine. In no case was the appearance of exencephaly found to be significant as a result of any treatment given on Day 9.

In contrast to normal fetuses where the eyes are usually closed until 14 days after birth, all but one of the exencephalic fetuses observed had both eyes open. Sections made through these eyes disclosed the vitreous chamber and the lens as being stellate in shape

Table V—Occurrence of Exencephaly, Axial Skeletal Fusions, and Cryptorchid Testes with Treatment on Day 8

Treatment	Exencephaly Litters Absent	Exencephaly Litters Present	Exencephaly Normal Fetuses	Exencephaly Abnormal Fetuses	Cryptorchid Testes Litters Absent	Cryptorchid Testes Litters Present	Cryptorchid Testes Normal Fetuses	Cryptorchid Testes Abnormal Fetuses	Axial Skeletal Fusions Litters Absent	Axial Skeletal Fusions Litters Present	Axial Skeletal Fusions Normal Fetuses	Axial Skeletal Fusions Abnormal Fetuses
Control (untreated)	6	0	59	0	6	0	31	0	6	0	28	0
Saline, 0.3 ml.	6	0	72	0	6	0	38	0	6	0	34	0
Morphine, 200 mg./kg.	5	1	68	1	3	3[a]	37	3	6	0	29	0
Morphine, 300 mg./kg.	3	3[a]	60	3	4	2	29	3	6	0	31	0
Morphine, 400 mg./kg.	6	0	63	0	3	3[a]	29	3	5	1	29	2
Diphenhydramine, 50 mg./kg.	6	0	65	0	4	2	32	2	6	0	31	0
Diphenhydramine, 100 mg./kg.	5	0	57	0	2	3[a]	25	4[a]	5	0	28	0
Histamine, 50 mg./kg.	6	0	61	0	5	1	31	1	6	0	29	0
Saline, 0.3 ml. Saline, 0.3 ml.	5	0	56	0	4	1	23	1	5	0	32	0

[a] Significant $(\chi)^2 > 3.84$ = significantly different from saline control.

Table VI—Occurrence of Exencephaly, Axial Skeletal Fusions, and Cryptorchid Testes with Treatment on Day 9

Treatment	Exencephaly				Cryptorchid Testes				Axial Skeletal Fusions			
	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses
Saline, 0.3 ml.	6	0	61	0	6	0	31	0	6	0	30	0
Morphine, 200 mg./kg.	5	1	61	1	6	0	32	0	5	1	27	3
Morphine, 300 mg./kg.	6	0	52	0	4	2	25	2	3	3[a]	20	5[a]
Morphine, 400 mg./kg.	6	0	64	0	6	0	34	0	4	2	26	4[a]
Diphenhydramine, 50 mg./kg.	6	0	62	0	4	2	29	2	6	0	31	0
Diphenhydramine, 100 mg./kg.	4	0	47	0	3	1	22	1	3	1	22	2
Histamine, 50 mg./kg. Saline, 0.3 ml.	5	0	52	0	5	0	25	0	5	0	27	0
Saline, 0.3 ml.	5	0	46	0	4	1	20	1	5	0	25	0

[a] Significant $(\chi)^2 > 3.84$ = significantly different from saline control.

rather than the respective crescent and circular shapes seen in the normal state.

Other Soft-Tissue Defects—It can be seen from Tables V and VI that morphine caused a retardation of testicular descent (cryptorchid testes) and that this anomaly usually occurred more frequently when the drug was administered on Day 8 of gestation. This defect was found to be significant in the groups treated with 200 mg./kg. morphine on Day 8 and 400 mg./kg. morphine on Day 8 when compared to the saline controls. In no case did any drug combination significantly alter the effect of morphine on either day with respect to this defect. The group treated with 100 mg./kg. diphenhydramine on Day 8 showed a statistical significance in the appearance of this anomaly.

Other than exencephaly and cryptorchid testes, only a few soft-tissue malformations were found. One fetus demonstrating hydrocephalus was observed in the saline control group injected on Day 8. One fetus with hydronephrous was seen in each of the three groups treated with single injections of morphine on Day 8 and also in the group treated with 400 mg./kg. morphine on Day 9. This same anomaly was found in one fetus in each of the following groups: histamine, 50 mg./kg., on Day 8; diphenhydramine, 50 mg./kg., in combination with morphine, 200 mg./kg., on Day 8; and saline in combination with morphine, 200 mg./kg., on Day 9. An ectopic kidney was found in one fetus belonging to the group treated with 50 mg./kg. diphenhydramine on Day 8.

Rib and Vertebral Fusions—Table VI shows that 300 and 400 mg./kg. morphine, when given on Day 9, produce a significant occurrence of rib and vertebral fusions. With 200 mg./kg. morphine administered on Day 9, three fetuses occurred with either rib or vertebral fusions, the results being of borderline significance. However, in the combination of 50 mg./kg. diphenhydramine with 200 mg./kg. morphine given on this same day (Table VIII), the incidence of these fusions was found to be significant not only when compared with the saline controls but also when compared with results seen with 200 mg./kg. morphine given alone on Day 9.

In no case did the administration of saline 15 min. before the treatment with morphine significantly alter the effect of the single injections of morphine with respect to skeletal fusions.

DISCUSSION

The observations in this study concerning the CNS stimulation and convulsive seizures with higher doses of morphine have been routinely shown in experimental animals (1, 11, 12). Also, Gruhzit and Fiskin (13) reported that stimulation was observed with diphenhydramine in mice and rats irrespective of the mode of administration. Therefore, one would anticipate that a combination of diphenhydramine with morphine would produce more severe convulsive reactions than morphine alone, which was shown to be the case, due possibly to additive effects.

The local irritation observed at the site of diphenhydramine injection must be related to its cytotoxic action due to its local anesthetic properties (14), and this further reconfirms the observations reported by Gruhzit and Fiskin (13).

It can be concluded from the results that single injections of saline do not affect the maternal mean terminal weight when compared to untreated controls. This would indicate that when single injections of test drugs were found to cause a decrease in the maternal terminal weight, it was due to the drug injected and not the trauma of the injection itself.

In contrast to single injections, it was found that double injections of saline did significantly alter the maternal mean terminal weights on both days when compared to the single injection of saline, although this decrease did not differ greatly from that occurring with morphine alone. It was also shown that when the combination of saline and morphine was compared to morphine alone, no significant difference occurred. These results suggest that the trauma experienced by the double injection has some influence on the maternal mean terminal weight. However, a significant change in this parameter found with the combinations employed must be

Table VII—Occurrence of Exencephaly, Axial Skeletal Fusions, and Cryptorchid Testes with Treatment on Day 8 (Compared with Morphine, 200 or 300 mg./kg.)

Treatment	Exencephaly				Cryptorchid Testes				Axial Skeletal Fusions			
	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses
Saline, 0.3 ml. Morphine, 200 mg./kg.	6	0	59	0	5	1	30	1	6	0	28	0
Saline, 0.3 ml. Morphine, 300 mg./kg.	5	1	53	4	2	4	25	5	5	1	24	3
Diphenhydramine, 50 mg./kg. Morphine, 200 mg./kg.	3	3	59	6[a]	4	2	31	3	6	0	31	0
Diphenhydramine, 50 mg./kg. Morphine, 300 mg./kg.	4	2	55	3	3	3	27	3	5	1	27	1
Histamine, 50 mg./kg. Morphine, 200 mg./kg.		1	49	1	4	1	24	2	5	0	24	0
Histamine, 50 mg./kg. Morphine, 300 mg./kg.	5	0[a]	43	0	5	0	23	0	5	0	20	0

[a] Significant $(\chi)^2 > 3.84$ = significantly different from single morphine injection.

Table VIII—Occurrence of Exencephaly, Axial Skeletal Fusions, and Cryptorchid Testes with Treatment on Day 9 (Compared with Morphine, 200 or 300 mg./kg.)

Treatment	Exencephaly				Cryptorchid Testes				Axial Skeletal Fusions			
	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses
Saline, 0.3 ml. Morphine, 200 mg./kg.	5	0	49	0	4	1	25	1	5	0	23	0
Saline, 0.3 ml. Morphine, 300 mg./kg.	5	1	48	1	1	5	21	6	3	3	16	6
Diphenhydramine, 50 mg./kg. Morphine, 200 mg./kg.	6	0	46	0	4	2	22	2	1	5[a]	11	11[a]
Diphenhydramine, 50 mg./kg. Morphine, 300 mg./kg.	5	1	57	1	5	1	30	1	4	2	20	7
Histamine, 50 mg./kg. Morphine, 200 mg./kg.	6	0	59	0	6	0	30	0	4	2	26	3
Histamine, 50 mg./kg. Morphine, 300 mg./kg.	4	1	55	2	4	1	28	1	4	1	20	8

[a] Significant $(\chi)^2 > 3.84$ = significantly different from single morphine injection.

caused by the drug treatment because no change was observed with the combination of saline and morphine when compared to morphine alone.

A direct correlation between the degree of maternal mean terminal weight gain and the mean fetal weights was only noted in the two groups treated on Day 8 with 200 mg./kg. morphine and 50 mg./kg. diphenhydramine. It was also observed that in the groups treated on Day 9 with 200 mg./kg. morphine, 100 mg./kg. diphenhydramine, and saline with 300 mg./kg. morphine, the decreased mean fetal weights were not reflected in the maternal mean terminal weights. Furthermore, many groups showed significant decreases in the maternal mean terminal weights with no reflection on the mean fetal weights. Therefore, it must be concluded that the maternal weight gain during gestation cannot serve as an indicator of fetal size or development.

The fetal defects observed consequent to single injections of morphine sulfate reconfirm the results of those in a previous investigation (1), in which it was demonstrated that the drug administered in high doses on either Day 8 or 9 caused the pronounced fetal abnormalities, exencephaly and axial skeletal fusions. As in the previous investigation, it was also noted in the present study that the occurrence of exencephaly was more prominent when the drug was administered on Day 8, while the skeletal fusions were more pronounced with the treatment on Day 9.

Injection of normal saline 15 min. before the morphine did not modify the occurrence of exencephaly or axial skeletal fusions caused by the drug. This implies that if any drug, administered 15 min. before morphine, does generate a significant variation in the incidence of these two anomalies, this variation must be due to some action or interaction of both drugs and not to any trauma that might occur from the first injection.

The importance of cryptorchid testes found in the groups treated with morphine is not very apparent at this time. The retardation in testicular descent may be a result of the overall retardation of fetal growth, with the testes descending to their normal position at a later period of development. This possibility is supported by the observations that cryptorchid testes were more numerous in smaller fetuses and that they frequently occurred in the groups where other treatments produced smaller and lighter fetuses.

In the combination studies with morphine and diphenhydramine, only the 50-mg./kg. dose of the antihistamine was employed. The results of this investigation suggest that when such combinations are utilized, there is an enhancement in the ability of morphine to induce both exencephaly and axial skeletal fusions. This was demonstrated by the observation that when morphine was given alone at the 200-mg./kg. dose, there was no significant occurrence of either of the two anomalies. However, when this same dose was given in combination with diphenhydramine, the occurrence of both defects on Days 8 and 9, respectively, becomes significant not only when compared to saline but also when compared to the single injection of morphine.

Histamine, when administered alone, produced no significant soft-tissue anomalies and only skeletal defects that could be attributed to the retardation of fetal growth. Combinations of histamine and morphine did not enhance the incidence of exencephaly or axial skeletal fusions. On the contrary, the combination with morphine, 300 mg./kg., on Day 8 resulted in a significant decrease in the occurrence of exencephaly as well as a lower incidence of cryptorchid testes. Therefore, the results seem to indicate that morphine is not eliciting its teratogenic effects *via* histamine release. The fact that diphenhydramine is ineffective in preventing the anomalies further supports the conclusion that histamine release is not an etiologic factor in the production of morphine-induced congenital defects.

An interesting outcome of this investigation was that diphenhydramine potentiated the teratogenic action of morphine. Since it can be concluded from the findings that it was not the antihistaminic activity of diphenhydramine that enhanced the teratogenicity of morphine, it must be assumed that some other activity of diphenhydramine was responsible for the increased effect. Diphenhydramine, aside from being an antihistaminic, is a potent anticholinergic (15) and mild local anesthetic (14); therefore, it seems imperative that these and other properties of diphenhydramine should be investigated to determine whether they are involved in the enhancement of the teratogenic ability of morphine.

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Interaction of 3-Methylcholanthrene with Lecithin–Cholesterol Mixed Films

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Abstract □ The interaction of a carcinogenic hydrocarbon, 3-methylcholanthrene, with mixed films of various mole fractions of cholesterol and lecithin was investigated. The extent of interaction between 3-methylcholanthrene and cholesterol in the mixed films is greatly influenced by the competitive interaction between cholesterol and the phospholipid. At 50:50 cholesterol–lecithin molar ratios, where these lipids interact to the greatest extent, the interaction with 3-methylcholanthrene is weakest. These competitive interactions may account for the experimental observations that phospholipids retard while cholesterol accelerates the formation of tumors induced by polycyclic aromatic hydrocarbons.

Keyphrases □ 3-Methylcholanthrene interaction—lecithin–cholesterol mixed films □ Lecithin–cholesterol mixed films—competitive interaction □ Cholesterol–lecithin film competitive interaction effect—3-methylcholanthrene–film interaction □ Surface pressure—lecithin–cholesterol mixed film

Monomolecular films represent a relatively simple type of membrane model having a well-defined organized structure. They provide one of the most convenient and promising methods of studying molecules in a fixed orientation, as well as in a single layer where orientation can be changed by compression of the monomolecular film. As such, they constitute an important model system for the study of many natural phenomena that involve surfaces of an oriented array of molecules.

The production of cancer by pure hydrocarbons was discovered by researchers in England (1, 2). Since that time numerous substances have been demonstrated to have carcinogenic activity. These include polycyclic aromatic hydrocarbons, azo dyes, chemical agents of known and unknown composition, carcinogenic viruses, and physical agents (3).

Dickens and Weil-Malherbe (4, 5) observed that phospholipids retard, whereas cholesterol promotes, the formation and growth of tumors when injected simultaneously with the carcinogen, 3,4-benzpyrene.

Altman (6) also demonstrated that phospholipids retard the formation of tumors induced by a polycyclic hydrocarbon. He postulated that the protective activity of phospholipids must be due to their ability to form molecular associations with the polycyclic hydrocarbons, just as they do with cholesterol. Such associations promote the transportability of the carcinogen and thus prevent them from acting on the cell membrane. He felt that the question of why phospholipids only temporarily retard, and not permanently prevent, the formation of tumors must be seen in the scope of quantitative proportions in which phospholipids, on the one hand, and carcinogen plus cholesterol, on the other, occur in animal tissue.

Altman (7) suggested that carcinogenic substances induce cancer because they act as lipophilic sensitizers. They produce this effect either by occupying the available open spaces or by displacing the lipophilic sensitizers naturally occurring in the cell membrane. "Every change (even the slightest) in the structure of the membrane, imperatively leads to a change in its permeability and selectivity and consequently in the composition and properties of the whole cell." Therefore, a modification in the structure of the cell membrane can lead to the transformation of the original normal cell into another cell which accidently might be a cancer cell (6, 7).

Altman (6) felt that since phospholipids increase cell permeability while cholesterol decreases permeability, an excess of sterol (which condenses the cell membrane) promotes the fixation of carcinogenic molecules, while an excess of phospholipids (which expands the cell membrane) prevents such fixation. Absolute values of the ratio of phospholipids to cholesterol would then have a decisive influence on the incidence of cancer. Since values of phospholipids–cholesterol ratios are specific for each tissue, this situation offers an explanation of why one tissue is more accessible to tumor formation than another.

Finean (8) also explained the interactions of the polycyclic aromatic hydrocarbons on the basis of molecular association. Phospholipids and cholesterol both react with the carcinogenic compound; the association with the former promotes the motility of the carcinogen which is then prevented from sticking to the cell membrane.