Morphine-Induced Fetal Malformations III: Possible Mechanisms of Action

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Abstract □ The teratogenic effects exerted by morphine sulfate in CF-1 mice on Days 8 and 9 appear, from indirect evidence, not to be attributable to cholinesterase inhibition. The anticholinergic drug, atropine, when used in combination with morphine, resulted in the potentiation of morphine-induced defects, exencephaly and axial skeletal fusions. In addition, both atropine and morphine, either alone or in combination, produced constriction of the placental vessels. Morphine administration on Days 8 and 9 resulted in an increase in blood sugar regardless of the dose employed. Previously reported similarities produced by atropine and morphine on the placental vessels, the knowledge that both drugs decrease oxygen tension, the resemblance of hypoxic-induced malformations to the defects observed in this study, the effects of hypoxia on carbohydrate metabolism, and the reported involvement of carbohydrate metabolism in the morphogenesis of the embryonic neural tube all suggest that the malformations observed following morphine administration are related to the reduced oxygen concentration as well as the hyperglycemia produced subsequent to its administration in gravid mice.

Keyphrases □ Morphine-induced fetal malformations—possible mechanisms of action □ Exencephaly, morphine induced—possible mechanisms of malformation □ Teratogenicity, morphine—possible mechanisms of morphine-induced fetal malformations □ Fetal malformations—possible mechanisms of morphine-induced exencephaly

Exencephaly, an anomaly characterized by the extensive exterior protrusion of cerebral tissue due to the absence of skin and bones of the cranial vault, has been shown to be caused by a variety of inducers. The causative agents include genetic mutations (1), irradiation (2), nutrition (3), hypoxia (4), and a number of chemical agents (5) including morphine sulfate (6).

Having established the fact that high subcutaneous doses of morphine sulfate (200, 300, and 400 mg./kg.) are teratogenic (6), the subsequent investigation of a mechanism of action was undertaken in this laboratory. Among the problems encountered in trying to define a mechanism of teratogenicity for morphine are the enigmatic pharmacological reactions produced by it—histamine release (7), food retardation (8), cholinesterase inhibition (9), vasoconstriction of placental vessels (10), and hyperglycemia (11)—plus the knowledge that teratogenic insult may occur at any of three different loci: the mother, the placenta, and the embryo.

The involvement of histamine release and food retardation as the underlying factors causing the malformations observed following morphine administration were negated by Iuliucci and Gautieri (12) and Harpel and Gautieri (6), respectively. Consequently, the objectives of this investigation were to: (*a*) reconfirm the teratogenic potential of high subcutaneously administered doses of morphine in CF-1 mice; (*b*) evaluate the possibility of cholinesterase inhibition by morphine as a causative factor in the production of fetal anomalies, determined by increasing the defects with the prior administration of physostigmine or decreasing them with the prior administration of atropine; (*c*) ascertain whether an effect exerted by morphine, atropine, and physostigmine on the placental vasculature *in vivo*, utilizing ^{22}Na transport, is involved in the production of the abnormalities; and, finally, (*d*) assess the involvement of the hyperglycemic component in the teratogenicity induced by morphine.

EXPERIMENTAL

CF-1 albino mice[1], weighing between 25 and 30 g., were employed in all experimental procedures and subjected to treatment and breeding procedures as reported previously (6, 12).

Injectable solutions of morphine sulfate[2] (40.0 mg./ml.), physostigmine sulfate[3] (0.025 mg./ml.), atropine sulfate[4] (5 mg./ml.), sodium sulfate[5] (10 mg./ml.), and sodium chloride (9 mg./ml.) were prepared fresh weekly by dissolving the drugs in distilled water.

The pregnant female mice were assigned at random to one of 35 experimental categories, with each category being treated on either Day 8 or 9 of gestation as designated in Tables I–IV. Iuliucci and Gautieri (12) described termination of pregnancy, removal of fetuses, gross inspection, and preparation of the excised fetuses for both skeletal (13) and soft tissue examination (14).

A second aspect of this study involved the actions of certain drugs upon the placental vasculature as determined by transport of ^{22}Na according to the procedure described by Robson and Sullivan (15), who employed it to study placental transport *in vivo*.

Five minutes after the subcutaneous administration of the respective drug(s), the isotope, 0.1 ml. delivering 3 μc., was injected intravenously into a tail vein of the mouse. By reporting the activity of the samples as a percentage of the concentration of isotope in maternal blood, each animal served as its own control.

A third aspect of this study involved an evaluation of the effects exerted by morphine (200, 300, and 400 mg./kg.) on the maternal blood sugar of 8- and 9-day pregnant CF-1 mice.

Each animal was initially bled by inserting a fine glass pipet into the medial canthus and removing 0.1 ml. of blood from the veins of the retro-orbital plexus (16). This was followed by the subcutaneous administration of designated test agent and dose. Twenty minutes later, a second 0.1-ml. sample was withdrawn from the mouse. Both samples were then subjected to the Folin–Malmros (17) micromethod for blood glucose determination. By withdrawing two samples, one prior to and one following drug administration, each animal served as its own control.

RESULTS

Maternal Effects of Test Drugs—The subcutaneous administration of 50 mg./kg. atropine sulfate did not produce readily observable effects, except an apparent increased heart rate and a coldness of the tail. When administered in combination with morphine, no Straub tail was detected at first; when it did appear some 30 min. later, it lasted for only 5 min. as compared with several hours following the injection of morphine alone. Next came a period of depression and labored breathing, followed by a hyperactive period. Atropine sulfate failed to produce any deaths when used alone. However, com-

[1] Carworth Farms, Inc., New York, N. Y.
[2] Morphine sulfate USP, Control No. P0006, Merck and Co., Inc., Rahway, N. J.
[3] Physostigmine sulfate, Control No. 5703, Merck and Co., Inc., Rahway, N. J.
[4] Atropine sulfate, Control No. 1278, Merck and Co., Inc., Rahway, N. J.
[5] Sodium sulfate, Control No. 500813, Fisher Scientific Co., Pittsburgh, Pa.

Table I—Mean Values of Test Groups Receiving Single Injections (Treatment on Day 8)

Treatment	Maternal Weight Ratio, S/T[a]	Fetal Ratio, Right Horn/ Left Horn	Resorption Ratio, Right Horn/ Left Horn	$\bar{X}$ Fetal Weight, g.	Sex Ratio, M/F	Soft Tissue Abnormalities	Skeletal Abnormalities
Control (untreated)	25.5/48.0	6.2/3.7[b]	0.17/0.33	1.07	4.0/5.8	0.0	1.5
Saline, 0.3 ml.	26.8/51.0	5.8/6.2	0.33/0.50	1.13	6.5/5.5	0.17	1.3
Morphine, 200 mg./kg.	27.0/44.3[b]	6.3/5.0	0.50/0.00	0.92[b]	5.8/5.2	0.83[b]	8.8[b]
Morphine, 300 mg./kg.	25.7/48.5	5.5/5.0	0.50/0.33	1.10	5.2/5.3	1.17	2.2
Morphine, 400 mg./kg.	27.3/47.8	5.8/5.5	0.00/1.00	1.01[b]	5.5/5.8	0.67	9.0[b]
Physostigmine, 0.005 mg.	25.7/45.8[b]	4.3/4.0	0.00/0.50	1.00	3.7[b]/4.7	3.67	10.0[b]
Atropine, 50 mg./kg.	25.5/46.0	5.8/4.8	0.07/0.83	1.16	6.0/4.2	0.33	5.5
Sodium sulfate, 60 mg./kg.	26.8/44.7[b]	4.5[b]/4.0	0.00/0.50	1.11	5.3/3.2	0.00	6.0[b]

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

Table II—Mean Values of Test Groups Receiving Single Injections (Treatment on Day 9)

Treatment	Maternal Weight Ratio, S/T[a]	Fetal Ratio, Right Horn/ Left Horn	Resorption Ratio, Right Horn/ Left Horn	$\bar{X}$ Fetal Weight, g.	Sex Ratio, M/F	Soft Tissue Abnormalities	Skeletal Abnormalities
Saline, 0.3 ml.	25.2/49.7	6.2/4.0	0.50/0.33	1.20	5.7/4.5	0.0	0.8
Morphine, 200 mg./kg.	26.2/47.8	4.5/5.8	0.33/0.17	1.02[b]	6.0/4.3	0.17	6.7[b]
Morphine, 300 mg./kg.	25.8/41.3[b]	5.3/3.3	0.00/1.50	1.15	4.3/4.3	0.33	3.3[b]
Morphine, 400 mg./kg.	26.0/43.5	4.7/4.8	0.00/0.17	1.11	5.0/4.7	0.17	4.8[b]
Physostigmine, 0.005 mg.	25.7/44.8	4.7/5.2	0.00/0.33	1.10[b]	5.3/4.5	1.67[b]	8.3[b]
Atropine, 50 mg./kg.	26.8[b]/49.0	5.2/5.8	0.00/0.33	1.20[b]	6.2/4.8	0.00	5.2[b]
Sodium sulfate, 60 mg./kg.	27.0[b]/46.7	4.8/5.7[b]	0.00/0.33	1.10[b]	6.5/4.0	0.00	7.8

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

binations of atropine, followed in 15 min. by varying doses of morphine, resulted in a definite increase in the number of maternal deaths. The higher the morphine dose following the atropine injection, the greater the incidence of maternal death, the greatest incidence (100%) being observed with the 400-mg./kg. dose of morphine.

Treatment with 0.005 mg. physostigmine produced a generalized lethargy in the animals. Salivation and Straub tail were noted, along with hyperpnea that lasted for approximately 1 hr.; at the end of this time, the mice became hyperactive. Unlike atropine pretreatment, which antagonized morphine-induced Straub tail, the prior administration of physostigmine not only did not alter the Straub tail produced following the administration of morphine but actually stimulated a Straub tail itself. Atropine injection before physostigmine resulted in effects quite similar to those noticed following the administration of atropine alone—no Straub tail. The single injection of 0.005 mg. physostigmine sulfate caused no deaths. Conversely, when administered 15 min. prior to morphine, physostigmine produced a substantial increase in the death rate, especially at the higher doses of the latter; 100% fatality, regardless of the day, resulted from the combination of 0.005 mg. physostigmine and 400 mg./kg. morphine.

It was also observed that the injection of saline 15 min. prior to morphine administration did not result in any noteworthy change in the normal maternal symptoms produced by the narcotic.

Tables I–IV, which contain the cumulative mean values for each experimental group, give the results obtained from the various categories employed in the teratological aspect of this study.

Exencephaly (Herniated Brain)—The observed fetal and litter incidences of offspring manifesting herniated brains are tabulated in Tables V–VIII, and Fig. 1 contains representative specimens exhibiting this anomaly.

By referring to these tables, it can be seen that of all the agents and combinations tested, only 300 mg./kg. morphine and 300 mg./kg. morphine preceded by 50 mg./kg. atropine administered on Day 8 produced a significant incidence of exencephaly when compared with saline controls. The combination of atropine and 300 mg./kg. morphine on Day 8, although insignificant when compared with 300 mg./kg. morphine on Day 8 on a litter basis, was significant when compared on a fetal basis. This combination resulted in the greatest incidence of exencephaly, in which five out of six litters, totaling 13 exencephalic fetuses, exhibited this abnormality.

A significant decrease in the number of litters displaying exencephaly when compared with the respective dose and day of morphine administration was observed only in the group receiving the combination of 0.005 mg. physostigmine and 300 mg./kg. morphine on Day 8. The administration of physostigmine alone on Day 8 produced exencephalies in two litters, which borders on being significant.

The occurrence of this anomaly was not apparently influenced by either uterine horn (right *versus* left) or position, because the defect was evenly divided between the horns and no particular position was favored irrespective of the treatment employed. Furthermore, this defect did not appear to be sex related, since a predominance of the malformation was not observed in either sex. Many of the exencephalic fetuses also had open eyes, a condition not observed even in untreated fetuses at regular parturition.

Other Soft Tissue Defects—In addition to exencephaly and its related defects, retardation of testicular descent (cryptorchid testes) (Fig. 2) was the only other soft tissue anomaly that occurred at a significant degree. The results of the various test agents upon testicular descent are depicted in Tables V–VIII.

Some other soft tissue malformations observed in this study are as follows: At least one fetus exhibiting hydronephrosis was observed in groups administered saline; 200, 300, and 400 mg./kg. morphine; and physostigmine and 200 mg./kg. morphine on Day 8.

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

Treatment	Maternal Weight Ratio, S/T[b]	Fetal Ratio, Right Horn/ Left Horn	Resorption Ratio, Right Horn/ Left Horn	$\bar{X}$ Fetal Weight, g.	Sex Ratio, M/F	Soft Tissue Abnormalities	Skeletal Abnormalities
Saline, 0.3 ml., and saline, 0.3 ml.	26.0/47.2[c]	5.2/6.2	0.30/0.80	1.17	7.0/4.2	0.20	0.7
Saline, 0.3 ml., and morphine, 200 mg./kg.	26.8/44.7	4.2[d]/5.7	0.20/0.20	1.03	4.4/5.0	0.17[d]	3.8
Saline, 0.3 ml., and morphine, 300 mg./kg.	27.8[d]/48.7	4.5/5.0	0.30/0.30	1.05	5.3/5.2	1.50	9.3[d]
Atropine, 50 mg./kg., and morphine, 200 mg./kg.	28.2[d]/42.0[d]	4.0/3.2	0.67/0.67	1.11	3.5/3.7	1.00	10.8
Atropine, 50 mg./kg., and morphine, 300 mg./kg.	27.2/50.7	4.3/6.3	0.67/0.83	1.05	5.0/5.7	3.33	10.3[d]
Physostigmine, 0.005 mg., and morphine, 200 mg./kg.	26.2/48.2	4.3[d]/6.0	0.00[d]/0.33	1.15[d]	5.3/5.0	1.83	8.3
Physostigmine, 0.005 mg., and morphine, 300 mg./kg.	24.8/44.0	6.2/3.3	0.33/0.00	1.03	6.8/2.7[d]	0.83	10.3[d]
Atropine, 50 mg./kg., and physostigmine, 0.005 mg.	28.3/49.5	4.5/6.2	0.00/0.17	1.03	5.8/4.8	1.00	8.5[c]

[a] With 15-min. interval between injections. [b] S = starting weight and 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, $p < 0.05$.

Bloody hemispheres (internal hemorrhage) were a common observance in the physostigmine groups on either Day 8 or 9. The 50 mg./kg. atropine and 300 mg./kg. morphine combination produced the greatest array of soft tissue anomalies (Fig. 3), including sirenomelus, exencephaly, cryptorchid testes, cranioschisis, gastroschisis, facial abnormality involving the snout and jaw, and ophthalomodisgenesis.

Skeletal Abnormalities—Tables I–IV show the high incidence of skeletal malformations that resulted from treatment on either Day 8 or 9 with various agents. Among the most common of these observed defects were delayed ossification in the phalanges, sternebrae, and skull, especially the supraoccipital bone, and rib and vertebral fusions (Tables V–VIII).

Results of Radio-Sodium Experiment—The 12 mice comprising the control group were injected with 0.3 ml. normal saline 5 min. prior to the intravenous administration of the radio-sodium isotope. Three animals then were sacrificed at 5-, 10-, 15-, and 20-min. intervals each.

The mean values generated for the placentas and fetuses at the various time periods following drug pretreatment were then compared with the mean values obtained for saline; significance was determined by Student's t test (Table IX). Only morphine at the 5- and 15-min. intervals exhibited a significant difference when the placental values were compared ($t > 2.77$, $p < 0.05$). Comparison of the fetal values resulted in significant differences being observed for both atropine and morphine at 5 min. and for the atropine-morphine combination at 10 min. ($t > 2.77$, $p < 0.05$).

Results of Blood Sugar Experiment—Two blood samples were withdrawn from each of the six mice constituting one of 10 experimental groups, with the second sample being taken 20 min. after the designated treatment of the respective animal. The mean change per group in either Day 8 or 9 is delineated in Table X.

The increases in blood sugar observed with the 200- and 300-mg./kg. doses of morphine administered on Day 8, when compared with saline controls, resulted in significant differences. All three doses of morphine employed on Day 9 were significantly different. An interdose comparison of morphine, however, resulted in no significant differences being observed on either Day 8 or 9.

DISCUSSION

The most profound effect exerted upon the maternal mouse following the injections of morphine, atropine, physostigmine, and various combinations thereof was death, resulting from acute toxicity. Single injections of the doses employed proved nonlethal

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

Treatment	Maternal Weight Ratio, S/T[b]	Fetal Ratio, Right Horn/ Left Horn	Resorption Ratio, Right Horn/ Left Horn	$\bar{X}$ Fetal Weight, g.	Sex Ratio, M/F	Soft Tissue Abnormalities	Skeletal Abnormalities
Saline, 0.3 ml., and saline, 0.3 ml.	25.3/45.0[c]	5.0/4.2	0.20/0.30	1.10	3.7/4.7	0.20	5.7[c]
Saline, 0.3 ml., and morphine, 200 mg./kg.	25.8/46.4	5.6/4.2	0.40/0.60	1.08	5.8/4.0	0.40	2.6
Saline, 0.3 ml., and morphine, 300 mg./kg.	26.2/42.0	3.7/4.5	0.50/0.50	0.98[d]	3.8/4.3	1.30	9.7[d]
Atropine, 50 mg./kg., and morphine, 200 mg./kg.	26.3/45.3	5.3/5.2	0.33/0.67	0.96	5.3/5.2	0.83	10.8
Atropine, 50 mg./kg., and morphine, 300 mg./kg.	26.0/45.8	5.7/4.5	0.33/0.83	1.05[d]	5.5/4.7	0.33	7.8[d]
Physostigmine, 0.005 mg., and morphine, 200 mg./kg.	26.0/41.8	4.2/3.5	0.17/0.50	1.01	4.7/3.0	1.67[d]	11.7
Physostigmine, 0.005 mg., and morphine, 300 mg./kg.	26.3/45.2	6.3/3.8	0.00/0.67	1.04[d]	6.8/3.3	1.33[d]	12.8[d]
Atropine, 50 mg./kg., and physostigmine, 0.005 mg.	26.5/47.5	5.7/4.5	0.33/0.33	0.95[c]	4.5/6.2	0.67	10.3

[a] With 15-min. interval between injections. [b] S = starting weight and 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, $p < 0.05$.

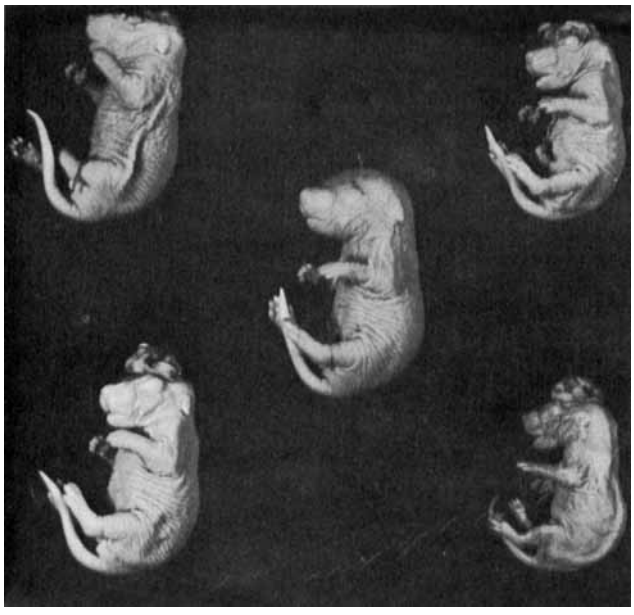

Figure 1—*Exencephalic fetuses and normal littermate after fixation. Upper left and right: exencephalic fetuses with open eye. Middle: normal fetus. Lower left and right: exencephalic fetuses with closed eyes.*

except in mice receiving 400 mg./kg. morphine on either Day 8 or 9. Both 0.005 mg. physostigmine and 50 mg./kg. atropine, administered 15 min. prior to this dose of morphine, resulted in the deaths of all animals so challenged. Consequently, combinations of 400 mg./kg. morphine with other test agents were deleted from the remainder of the study. Atropine or physostigmine administered 15 min. before the 200- and 300-mg./kg. doses of morphine also increased the mortality rate, with the greatest enhancement occurring on Day 8; 81%, 27 out of 33, succumbed following the 50 mg./kg. atropine and 300 mg./kg. morphine combination. The incidence of deaths observed in the various groups should be borne in mind when considering the anomalies produced by different combinations, because defects can be obscured by the production of maternal or fetal death (18). Furthermore, death may actually be secondary to the malformation (18), for the insult may be of such severity that the fetus may not survive.

The combination of 50 mg./kg. atropine followed in 15 min. by 0.005 mg. physostigmine proved to be nontoxic on Days 8 and 9, attesting to the relative innocuousness of this combination in the doses employed when compared with their lethal actions when used in combination with morphine. Another interesting observation was the increase in acute toxicity following the saline and 300 mg./kg. morphine injections. Evidently, the mere trauma of an injection preceding morphine administration is substantial enough to cause death.

The present study demonstrated that more exencephalic fetuses were observed when the gravid animal was treated with 300 mg./kg. morphine on Day 8, while treatment on Day 9 with morphine (all three doses) resulted in a greater number of fetuses displaying axial skeletal fusions. This combination of defects, *i.e.*, exencephaly and axial skeletal fusions, is not unusual. On the contrary, Book and Rayner (19) contend that deficiencies in skeletal and muscle development are secondary to a primary anomaly of the central nervous system (CNS).

Pretreatment with saline 15 min. prior to the morphine injection produced no significant defects compared with morphine alone, thereby negating the possibility that trauma of injection is involved in the subsequent induction of anomalies when a drug is administered before morphine. Therefore, the observance of a significant difference in the incidence of anomalies, *i.e.*, exencephaly and axial skeletal fusions, following pretreatment of morphine administration with a drug must be interpreted as an interaction between the agent and morphine.

Another soft tissue anomaly found in significant numbers was cryptorchid testes, which was generally observed to be associated with a decreased mean fetal weight. Although the importance of

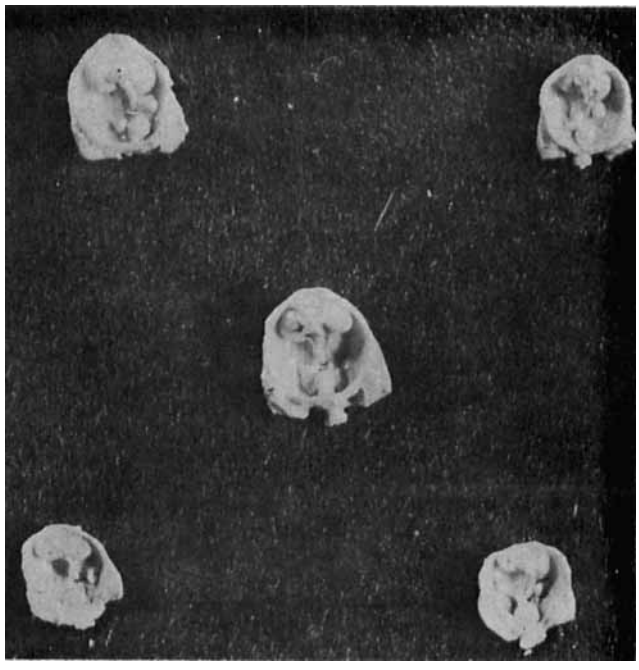

Figure 2—*Specimens exhibiting cryptorchidism. Orientation of section: spinal cord and kidneys at top; bladder and testes on each side, in center at bottom. Upper left and right and lower left: right unilateral cryptorchidism. Middle: normal section. Lower right: bilateral cryptorchidism.*

retarded testicular descent is not clear at this time, conceivably as growth continues the normal descent of the testes may occur. Zondek and Zondek (20) also reported that failure of testicular descent and anencephaly occur concomitantly.

The reversible anticholinesterase agent, physostigmine, when used alone, produced a total of two exencephalic fetuses (one in each of two different litters), significant retardation of testicular descent, and no axial skeletal fusions. The cryptorchid testes and the ob-

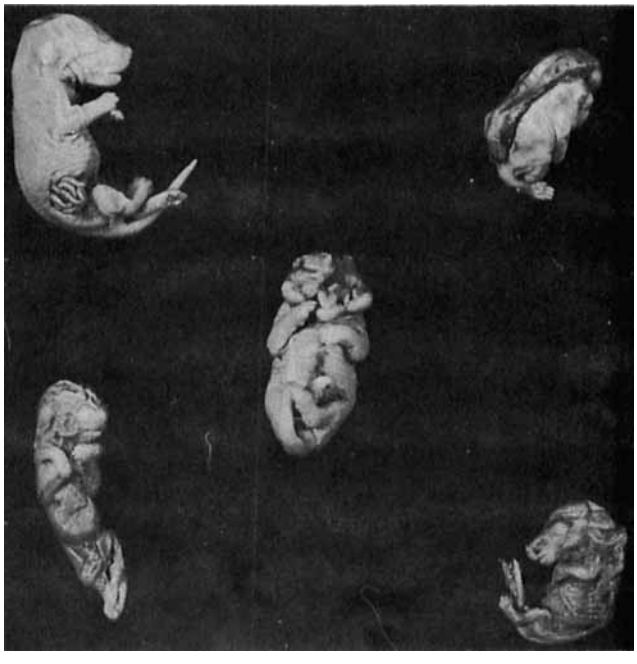

Figure 3—*Examples of observed gross defects following the 50-mg./kg. atropine–300-mg./kg. morphine combination administered on Day 8. Upper left and right: fetuses demonstrating gastroschisis and cranioschisis, respectively. Middle: fetus exhibiting facial anomaly. Lower left and right: fetuses displaying sirenomelus and runt formation with open eye, respectively.*

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

Treatment	Exencephaly				Cryptorchid Testes				Axial Skeletal Fusions			
	Litters		Fetuses		Litters		Fetuses		Litters		Fetuses	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Control (untreated)	6	0	59	0	6	0	12	0	6	0	28	0
Saline, 0.3 ml.	6	0	72	0	6	0	24	0	6	0	34	0
Morphine, 200 mg./kg.	5	1	68	1	3	3[a]	11	3[a]	6	0	34	0
Morphine, 300 mg./kg.	3	3[a]	60	3	4	2	12	3[a]	6	0	31	0
Morphine, 400 mg./kg.	5	1	67	1	5	1	15	1	5	1	33	1
Atropine, 50 mg./kg.	5	1	60	1	6	0	18	0	6	0	31	0
Physostigmine, 0.005 mg.	4	2	48	2	3	3[a]	7	7[a]	6	0	25	0
Saline, 0.3 ml., and saline, 0.3 ml.	6	0	68	0	5	1	15	0	6	0	34	0
Sodium sulfate, 60 mg./kg.	6	0	51	0	6	0	13	0	6	0	25	0

[a] Significant $(X)^2 > 3.84$ = 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		Fetuses		Litters		Fetuses		Litters		Fetuses	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Saline, 0.3 ml.	6	0	61	0	6	0	16	0	6	0	30	0
Morphine, 200 mg./kg.	5	1	61	1	6	0	17	0	5	1	27	3
Morphine, 300 mg./kg.	6	0	52	0	4	2	12	2	3	3[a]	20	5[a]
Morphine, 400 mg./kg.	6	0	57	0	5	1	17	1	5	1	25	3[a]
Atropine, 50 mg./kg.	6	0	66	0	6	0	12	0	5	1	32	1
Physostigmine, 0.005 mg.	6	0	59	0	4	2	10	3[a]	6	0	30	0
Saline, 0.3 ml., and saline, 0.3 ml.	6	0	45	0	5	1	21	0	6	0	23	0
Sodium sulfate, 60 mg./kg.	6	0	63	0	6	0	15	0	6	0	32	0

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

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		Fetuses		Litters		Fetuses		Litters		Fetuses	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Saline, 0.3 ml., and morphine, 200 mg./kg.	6	0	59	0	5	1	12	1	6	0	28	0
Saline, 0.3 ml., and morphine, 300 mg./kg.	5	1	53	4	2	4	10	5	5	1	24	3
Atropine, 50 mg./kg., and morphine, 200 mg./kg.	6	0	43	0	5	1	6	4	4	2	20	2
Atropine, 50 mg./kg., and morphine, 300 mg./kg.	5	1	51	13[a]	4	2	8	3	3	3[a]	26	6[a]
Physostigmine, 0.005 mg., and morphine, 200 mg./kg.	6	0	62	0	1	5	8	8	6	0	31	0
Physostigmine, 0.005 mg., and morphine, 300 mg./kg.	6	0	57	0	2	4	18	1	6	0	28	0

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

servable skeletal defects were, in all cases, associated with the inhibition of fetal growth. The administration of physostigmine 15 min. prior to morphine did not increase the incidence of exencephaly or axial skeletal fusions. However, on the contrary, a significant decrease in the number of exencephalic fetuses resulted from the combination of physostigmine and 300 mg./kg. morphine administered on Day 8. Also, the combination of physostigmine and 200 mg./kg. morphine administered on Day 8 resulted in a significant reduction in the resorption ratio as compared with morphine, while the same combination on Day 9 resulted in a significant increase in the incidence of cryptorchid testes. Therefore, although apparently not involved in the production of the two major anomalies observed following morphine administration, the effect of growth retardation exerted by physostigmine possibly is responsible for the augmented number of fetuses exhibiting cryptorchid testes.

Atropine injection alone resulted in the production of no significant anomalies. Only one exencephalic fetus and one fetus exhibiting axial skeletal fusions were induced following 50 mg./kg. atropine on Days 8 and 9, respectively.

The results obtained from the combination studies with atropine and morphine clearly indicate a potentiation of the teratogenic capability of morphine to induce both exencephaly and axial skeletal fusions. This is evident from the increased incidence of exencephalic fetuses: five out of six litters, totaling 13 fetuses, following the 50-mg./kg. atropine and 300-mg./kg. morphine combination on Day 8. Most combinations of atropine and morphine on both Days 8 and 9 resulted in greater numbers of axial skeletal fusions than with morphine alone. Although atropine has been used as an antidote in morphine poisoning (21) and has been shown to antagonize the Straub tail reactions produced by morphine (22), recent investigations conducted elsewhere indicate an "additive synergism" between atropine and morphine in producing death in mice (23). Hence, the enhancement of acute toxicity by atropine pretreatment of morphine-injected mice observed in the present study is in agreement with the latter study.

The fact that morphine has been shown to exert an effect upon the placental vasculature (10), plus the awareness that atropine has been reported to abolish certain actions produced by acetylcholine upon this structure (24), provided the impetus for this segment of the investigation. The importance of the placenta in the exchange occurring between the maternal and fetal organisms with respect to agents such as oxygen, nutrients, and glucose cannot be overemphasized.

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		Fetuses		Litters		Fetuses		Litters		Fetuses	
	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal	Normal	Abnormal
Saline, 0.3 ml., and morphine, 200 mg./kg.	6	0	59	0	5	1	13	1	6	0	28	0
Saline, 0.3 ml., and morphine, 300 mg./kg.	5	1	48	1	1	5	9	6	3	3	16	6
Atropine, 50 mg./kg., and morphine, 200 mg./kg.	5	1	62	1	4	2	12	2	0	6[a]	18	14[a]
Atropine, 50 mg./kg., and morphine, 300 mg./kg.	5	1	60	1	5	1	11	1	2	4	23	7
Physostigmine, 0.005 mg., and morphine, 200 mg./kg.	4	2	44	2	2	4[a]	7	5[a]	3	3	17	6
Physostigmine, 0.005 mg., and morphine, 300 mg./kg.	6	0	61	0	1	5	17	8	1	5	23	7

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

Consequently, the possibility exists that interference in the transport of any or all of the above by the action of an agent on the placental vessels, on either the maternal or fetal side, could have detrimental effects on the conceptus.

The results of the radioisotope study (Table IX) demonstrated that, following morphine administration, constriction of the maternal placental vessels occurred at the 5-min. interval, followed by vasodilation at the 15-min. interval. Comparison of the effects of the drugs upon the fetal side showed that both atropine and morphine produced constriction at the 5-min. interval, while the combination of atropine and morphine still caused constriction after 10 min.

The initial vasoconstriction of the placental vasculature by morphine *in vivo* is similar to that observed in the perfused human placenta (10). This action conceivably could precipitate a reduction in the amount of oxygen accessible to the fetus, with the subsequent production of congenital malformations or even death. Therefore, the fact that atropine prolonged morphine vasoconstriction of the placental vessels, plus the increased incidence of anomalies following the administration of this combination, leads to a good cause-and-effect relationship. Further substantiation of this possibility has been provided by others who clamped the uterine vessels in the rodent, which resulted in both severe malformations and death of the fetus (25). In addition, atropine has been reported to cause a fall in mean arterial oxygen tension (26), a condition not unlike that produced by morphine (27), which also reduces the extraction of oxygen from the blood circulating through the brain. Also, morphine has been reported to decrease fetal brain oxygen for at least 50 min. (28). Furthermore, an overdose of morphine early in gestation has resulted in the birth of a malformed child (29), attributed to the acute anoxia caused by morphine.

Ingalls *et al.* (4) induced anencephaly, cryptorchid testes, and rib fusions by exposing mice to decreased oxygen concentrations. These anomalies were the most prominent defects observed in the present study. Moreover, hydramnios, also observed in this study in association with every case of exencephaly, was present where anencephalic fetuses were found in the above study (4). Also, Ingalls and Curley (30), in another investigation, found cranioschisis, rib and vertebral malformations, and open eye in mice rendered hypoxic. Again, these abnormalities were also observed in the present study. In addition, Grabowski (31), utilizing chicks, noted "tremendous edema," blisters, hematomas, and the ultimate defects of "exocephaly," defective eyelids, and short upper beaks following exposure to reduced oxygen concentrations.

Table IX—Mean Percent of ^{22}Na Activity in Placentas and Fetuses of Selected Test Groups Compared with Saline

Group	Time Elapsed after Isotope Administration, min.	Mean Percent of ^{22}Na in Placenta	*SD*	Mean Percent of ^{22}Na in Fetus	*SD*
Saline	5	36.79	±3.40	9.81	±1.61
	10	36.61	±4.49	14.47	±1.42
	15	38.29	±1.66	17.29	±1.00
	20	39.40	±8.18	20.69	±2.62
Atropine sulfate, 50 mg./kg.	5	32.82	±3.04	7.63[a]	±0.29
	10	41.06	±7.22	13.98	±3.20
	15	39.95	±5.76	16.03	±2.29
	20	38.30	±6.30	20.26	±1.54
Physostigmine sulfate, 0.005 mg.	5	34.18	±3.73	7.93	±0.73
	10	40.09	±2.30	13.24	±0.61
	15	38.68	±8.20	17.43	±1.30
	20	48.78	±5.32	21.98	±2.38
Morphine sulfate, 300 mg./kg.	5	21.57[a]	±11.40	5.39[a]	±3.10
	10	37.93	±5.05	11.42	±2.38
	15	43.25[a]	±2.36	16.06	±1.92
	20	47.68	±7.70	20.97	±6.17
Atropine, 50 mg./kg., and morphine, 300 mg./kg.	5	32.69	±1.92	8.17	±0.52
	10	30.69	±5.74	8.60[a]	±2.69
	15	42.65	±5.12	18.15	±3.69
	20	43.12	±2.41	22.31	±1.21

[a] Significant $t > 2.776 = p < 0.05$ calculated from Student's t test = significantly different from saline control.

Table X—Observed Change in Blood Sugar on Either Day 8 or 9

Treatment	$\bar{X}$ Blood Sugar Change, mg./100	*SE*	p^a
Untreated control—Day 8	0.9	±0.95	0.20
Saline control—Day 8	8.0	±4.13	—
Morphine, 200 mg./kg.—Day 8	57.9[a]	±10.04	0.001
Morphine, 300 mg./kg.—Day 8	42.2[a]	±10.30	0.02
Morphine, 400 mg./kg.—Day 8	33.7	±12.05	0.10
Untreated control—Day 9	4.5	±0.89	0.80
Saline control—Day 9	5.2	±1.38	—
Morphine, 200 mg./kg.—Day 9	50.7[a]	±15.35	0.02
Morphine, 300 mg./kg.—Day 9	49.1[a]	±6.85	0.001
Morphine, 400 mg./kg.—Day 9	34.6[a]	±3.07	0.001

[a] Significant $t > 2.776 = p < 0.05$ calculated from Student's t test = significant increase in glycemic response compared with saline control.

The fact that hypoxia produces various biochemical changes, *e.g.*, an increased utilization of glycogen stores with a subsequent increase in blood glucose and lactic acid (32), plus the knowledge that hyperglycemia in mice precipitates a decrease in brain glycogen concentration (33) stimulated the third aspect of this particular study. All three doses of morphine employed on both Days 8 and 9 produced increases in blood glucose when compared with controls (Table X), with the greatest increase occurring at the lowest dose and the smallest increase occurring at the highest dose, respectively. This phenomenon can possibly be explained by the term "autointeraction" (34), in which a drug noncompetitively antagonizes its initial effect, as the concentration is increased, by becoming attached to a second receptor. Besides, it has been reported that high maternal blood sugars are accompanied by high fetal blood sugars (35). The acceptance of these facts enables one to assume that the aforementioned decrease in brain glycogen (33) precipitated by glucose administration also occurred in this study in response to the morphine-induced hyperglycemia. Since Runner (3) hypothesized that carbohydrate metabolism may be selectively critical for the morphogenesis of the embryonic neural tube, the above-mentioned hyperglycemia and resultant effects produced by anoxia upon carbohydrate metabolism cannot be completely excluded as possible factors contributing to morphine-induced congenital malformations.

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Residual Ethylene Oxide in Gas-Sterilized Medical-Grade Silicones

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Abstract □ Residual gas was extracted from ethylene oxide-treated silicone sheets by high vacuum distillation, and the residue subsequently was analyzed by GLC. To indicate the necessary aeration time of the materials before they can be considered safe to use, the desorption characteristics of ethylene oxide in three types of the silicone polymer were studied. Very rapid desorption of the gas to low residual levels was observed in the two nonreinforced silicone rubbers examined: medical-grade silicone sheeting and a medical-grade elastomer. The third material, Dacron-reinforced silicone sheeting, lost the gas at a considerably slower rate, after an initial rapid drop, due to retention by the Dacron reinforcement.

Keyphrases □ Silicone sheets, medical grade—absorption and diffusion of ethylene oxide after gas sterilization □ Ethylene oxide gas sterilization—absorption and diffusion from medical-grade silicone sheets □ Sterilization with ethylene oxide—absorption and diffusion from medical-grade silicone sheets

During the last couple of decades, there has been an ever increasing trend to utilize synthetic rubber and plastics in medical instruments and equipment as well as in permanent or semipermanent implants and prostheses. Because many synthetic materials are heat labile, conventional dry heat or autoclaving processes have been substituted by low temperature sterilization methods.

One such method is ethylene oxide gaseous sterilization, and its relative merits have already been well documented. However, one serious disadvantage of sterilization with this gas is that ethylene oxide remains in the material, and its presence in a polymeric device can present potential toxicological problems if the gas is released to tissue. Only in recent years, with the increasing use of GLC, has it been discovered that various types of polymeric materials may contain reaction products of ethylene oxide other than ethylene glycol, such as the highly toxic ethylene chlorohydrin (2-chloroethanol) which is formed in the presence of chloride ions (1, 2).

Medical-grade silicones are widely used in tissue implants and can be successfully sterilized both by conventional methods and by ethylene oxide. With the latter method, there is uncertainty concerning the retention time of the gas, together with the possibility of cytotoxic reaction products. These considerations prompted this study.

THEORY

If there is a uniform distribution of a dissolved gas in a thin slab of material and the surfaces are suddenly exposed to zero partial pressure of the gas and maintained at that, the gas begins to clear from the material. It can be shown (3) that, after an initial period during which the mean gas concentration in the medium decreases by 10–20%, the concentration at all points decreases exponentially with the same half-time:

$$T_{1/2} = \frac{2.77\left(\frac{L}{2}\right)^2}{\pi^2 D} \qquad \text{(Eq. 1)}$$

where:

$T_{1/2}$ = half-time (sec.)
L = thickness of material (cm.)
D = diffusion coefficient (cm.^{2} sec.$^{-1}$) —constant for a given gas, temperature, and material

Thus, if the total amount of dissolved gas can be calculated as a function of time, the diffusion coefficient can be deduced from the slope of the exponential (which indicates the time taken for the concentration of dissolved gas to decrease by half) and the thickness of the medium; repeating the experiment with a different thickness of the material provides a check on the value.

The $T_{1/2}$ of Eq. 1 is also a measure of the rate of diffusion *into* the material.

Table I shows the diffusion coefficient of ethylene oxide in several plastics at 23°. Knowledge of the diffusion coefficient of ethylene oxide in a particular material at room temperature should permit