in anticholinergic activity between VII and the experimental compound VI-*B* might be due in part to the difference in steric rigidity and bulkiness of the tropane N-function and the acyclic N-function of VI-*B*.

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Keyphrases

Xanthoxime esters, ethers—synthesis
Pyridine-3-aldoxime esters, ethers—synthesis
Anticholinergic activity—screening
Ileum strips—spasmolytic testing

Morphine-Induced Fetal Malformations I

Exencephaly and Axial Skeletal Fusions

By HOWARD S. HARPEL, JR.* and RONALD F. GAUTIERI

High subcutaneous doses of morphine sulfate, 100–500 mg./kg., administered to CF-1 mice on Day 8 or Day 9 of gestation are teratogenic and result in a large number of fetuses with exencephaly and axial skeletal fusions. Retardation in food consumption is not primarily responsible for these effects even though fasting alone affects embryonic development. Based upon the narrow range between the maternal LD_{50} and the teratogenic doses in this species, the teratogenic potential of morphine sulfate is low.

With the current interest in drug-induced embryopathies, it is surprising that the recent literature is devoid of teratogenic studies involving the narcotic analgesics. In 1931, Myers (1) reported that daily morphine administration beginning 3–5 months before mating and continuing throughout gestation had little effect upon the growth and reproduction of the albino rat. Although the litters were of average size and composed of young that appeared normal, the method of examination was not as extensive as the techniques currently employed in teratogenic studies. Moreover, ample time was available for the female rats to become tolerant to morphine before conception, and consequently, all development occurred in a morphine-adapted environment.

Therefore, it was imperative to determine the consequences of morphine sulfate administration during the critical stages of development in a common laboratory mammal by administering high subcutaneous doses to nontolerant CF-1 mice on the eighth or ninth day of gestation. Moreover, during the investigation it became clear that drug administration produced a temporary decrease in food intake, and for this reason the effect of food deprivation on each of these 2 days also was investigated.

EXPERIMENTAL

CF-1 albino mice weighing between 20–25 g. were obtained from Carworth Farms, Inc., New City, N. Y. Females were caged in groups of 25 to 30 for at least 2 weeks after arrival and were not mated until they weighed at least 25 g. Males and gravid females were caged individually in metal cages measuring 12.5 × 15 × 10 cm. with a wire mesh front and floor. The colony was maintained on Purina laboratory chow and tap water *ad libitum*. Sodium chloride (0.9%) and morphine sulfate[1] (4.0%) prepared weekly in distilled water were

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[1] Morphine sulfate, USP, Merck Co., Rahway, N. J.

administered subcutaneously in the upper right abdominal area.

The doses of morphine chosen for use in this study were based on the LD_{50} in nongravid mice. The LD_{50} of 525 mg./kg. (500–551 mg./kg., 95% confidence limits) was established by administration of 4 doses (400–700 mg./kg.) to 10 mice at each dose level (2).

To produce timed pregnancies, two females were placed in the cage of a fertile male at 4:00 p.m. The next day at 8:00 a.m., females exhibiting a vaginal plug of coagulated ejaculate were considered gravid. The day of the appearance of the vaginal plug was designated as Day 0. Gravid females were weighed, caged individually, and left undisturbed until the morning of Day 6 when they were weighed again. Pregnancy was confirmed by a weight gain of two or more grams. At this time the gravid animals were randomly assigned to one of 15 experimental categories: an untreated control group; two saline control groups receiving 0.3 ml. of 0.9% saline solution on either Day 8 or Day 9; 10 morphine-treated groups receiving 100, 200, 300, 400 or 500 mg./kg. of morphine sulfate on either Day 8 or Day 9; and two food-deprivation groups that were deprived of access to food on either Day 8 or Day 9. All solutions were injected on either Day 8 or Day 9 between 8:00 and 9:00 a.m.

Every animal was weighed on the morning of Day 6 through Day 13 to standardize the effects of handling. A laparotomy was performed immediately on mice killed by drug treatment in order to reconfirm pregnancy and count implantation sites. A similar procedure was followed with animals whose body weight changes indicated that complete resorption of the litter was occurring.

On Day 18, after sacrifice by dislocation of the cervical vertebrae, the uterine horns were exposed surgically. The number and position of fetuses and resorption sites were counted and recorded. Fetal viability was determined by reflex movement of the limbs in response to mechanical stimuli applied to the uterine wall with a blunt probe.

After transection of the right horn between the ovary and the first fetus, the fetus was removed by blunt dissection, blotted dry with absorbent paper, weighed and measured from the crown to the rump. It was examined for external defects, and the sex determined. Each member of the litter was removed in turn and examined in this manner. Every second fetus was fixed for soft tissue examination, and the remainder were prepared for skeletal examination. To establish the uterine position occupied by the fetus, a straight pin with a colored head was inserted into the base of the tail or axilla before fixation with Bouin's. Specimens were stored in 70% ethanol after 2 weeks in the fixative. Before sectioning, the specimens were reexamined for external defects under a low-power binocular microscope. Free-hand sections were made with a thin, double-edged razor blade according to the method of Wilson (3).

For skeletal examination, specimens were prepared by alkaline maceration, staining with alizarin red S, and clearing according to the method of Staples and Schnell (4).

Food consumption was measured to determine if drug treatment altered food intake in a manner that might correlate with effects upon the conceptus. Feeders were made from 120 g. clear glass ointment jars, 5.5 cm. in height and 7 cm. in diameter, with hard plastic tops. A hole 2 cm. in diameter drilled in the center of the lid allowed free access to food but prevented scattering and loss of uneaten food. These feeders were filled daily with 120 g. of finely ground Purina laboratory chow. They were placed in the cage on the morning the vaginal plug was discovered.

The mice accepted the feeder very readily, but data from Day 0 and Day 1 were often inconsistent with the normal pattern that was established by Day 2. Such inconsistencies for the first 2 days were found with most of the animals tested.

The statistical significance of variations among the experimental groups was estimated by the Student t test for continuous variates and the unadjusted chi-square or Yates' corrected chi-square for binomial proportions (5). Probabilities, p, greater than 0.05 were considered to represent insignificant differences.

RESULTS

Maternal Effects—Regardless of the dose or day of administration, gravid mice given morphine exhibited stimulation followed by depression and a hyperactive period of recovery all of which differed mainly in duration and intensity. After 24 hr., all survivors, irrespective of dosage, appeared normal in spontaneous activity and appearance. Any loss in body weight was regained by the second morning after treatment in conjunction with a return of food consumption to normal levels.

There were no deaths among the gravid mice treated with 100, 200, or 300 mg./kg. of morphine on Day 8 or Day 9. However, deaths did occur with the two higher doses employed; 3 of 14 or 21% were killed by 400 mg./kg. on Day 8, and 2 of 13 or 15% were killed on Day 9. After a dose of 500 mg./kg., 6 of 15 or 40% were killed on either Day 8 or Day 9. These results did not differ significantly from the expected number of deaths in nongravid animals calculated in the LD_{50} determination.

During gestation, 32 control females gained an average of 20.5 g. and the mean maternal weight gain of this and the other experimental groups is tabulated in Table I. It can be seen that the various treatments did not alter significantly the amount of weight gained with the exception of the group which received 500 mg./kg. of morphine on Day 9.

The mean change in body weight 24 hr. after treatment with saline on Day 8 and Day 9 was an increase of 0.2 and 0.7 g., respectively. Among the morphine-treated groups weight loss did not vary significantly with dosage. Irrespective of dosage, mean weight loss in the groups treated with morphine on Day 8 was 1.6 g., and 10 g. in those treated on Day 9. The largest weight losses were seen with the food-deprivation groups; in the Day 8 group the loss was 6.3 g. and in the Day 9 group 4.9 g.

In all mice, with the exception of those which resorbed completely, body weight returned to normal by the second morning after treatment and continued to increase in the normal pattern.

Food Consumption—The absolute amount of food consumed daily varies roughly as a function of body weight, and comparison of absolute consumption was not considered valid. However, net changes

TABLE I—MEAN MATERNAL WEIGHT GAIN, NUMBER OF IMPLANTATION SITES, AND NUMBER OF RESORPTIONS

Treatment[a]	Maternal Weight Gain, g.			Implantation Sites			Fetal Resorptions		
	N[b]	$\bar{x}$[c]	s[d]	N[e]	$\bar{x}$	s	N[e]	$\bar{x}$	s
Control	32	20.5	4.1	43	10.8	1.97	43	1.2	1.22
S-8	8	22.2	4.0	8	11.3	1.06	8	0.6	0.77
S-9	9	18.7	5.0	9	10.3	1.32	9	1.3	1.38
M-1-8	8	23.0	3.8	8	11.4	1.30	8	0.9	0.95
M-2-8	6	20.7	4.2	6	11.7	2.50	6	1.0	1.64
M-3-8	6	19.8	3.1	6	10.2	1.17	6	1.2	1.09
M-4-8	11	20.1	8.1	11	10.8	1.40	11	1.6	1.97
M-5-9	6	23.0	3.0	7	11.6	1.34	7	1.0	1.41
FD-8	6	21.0	6.5	6	11.3	1.21	6	1.7	2.34
M-1-9	5	17.0	4.6	6	11.3	2.14	6	1.3	1.52
M-2-9	9	21.7	4.6	9	10.9	1.27	9	1.3	1.34
M-3-9	10	23.1	4.2	10	11.7	1.70	10	0.7	0.67
M-4-9	9	22.3	5.5	9	11.8	1.64	9	2.6[f]	2.79
M-5-9	5	16.2[f]	4.6	7	11.8	1.68	7	2.6[f]	2.12
FD-9	7	21.3	2.6	7	11.1	2.27	7	1.3	1.79

[a] Treatment: Control, untreated; S, saline-treated; M, morphine-treated; FD, food deprivation; 1,2,3,4, or 5 indicates dose of morphine in hundreds of mg./kg.; 8 or 9 indicates the day of treatment. [b] Number of observations. [c] Arithmetic mean. [d] Standard deviation. [e] Does not include pregnancies in which complete resorption of litter occurred. [f] Statistically significant in comparison with control, $p < 0.05$.

in consumption for each animal are comparable and have been used to calculate the mean net change before and after treatment for each group. The mean net changes in food consumption on the critical days of the test are tabulated in Table II. The effect of treatment on Day 8 is seen by comparison of consumption on Day 7 and Day 8, while the effect of treatment on Day 9 is seen by comparison of Day 8 and Day 9.

In all morphine-treated groups food consumption was decreased significantly; saline injection produced minimal effect; and in the food-deprivation group consumption was nil, because access to food was denied.

For animals treated on Day 8, comparison of consumption on the day following treatment, Day 9, with that on the day before treatment, Day 7, indicated that in all groups food intake returned to normal levels. For animals treated on Day 9, comparison of consumption on the day following treatment, Day 10, with that on the day before treatment, Day 8, indicated that food consumption was still slightly reduced in the groups administered 100, 200, 300, and 500 mg./kg. of morphine; however, consumption returned to normal levels by the next day.

Implantations and Resorptions—The mean number of implantation sites observed at term in each treatment category are tabulated in Table I.

Any area on the uterine wall showing evidence that nidation had occurred was counted as an implantation site. Therefore, the number of implantation sites represented the total number of viable fetuses and resorption sites observed at term.

In 43 control litters the mean number of implantation sites was 10.8. The mean number of implantation sites in mice that did not survive treatment with morphine on either Day 8 or Day 9 was 12.3 and 11.7, respectively. Although the mean value in many of the treatment groups was slightly higher, none of these values differed significantly from the control group.

Complete resorption occurred in only one of the 44 control pregnancies. After administration of 500 mg./kg. of morphine on Day 8, one of nine mice resorbed completely; and with the same dosage on

TABLE II—FOOD CONSUMPTION

Treatment[a]	N	Mean Changes in Food Consumption, g.					
		Between 2 Days Before Treatment[b]		Between Day Before and Day of Treatment[c]		Between Day Before and Day After Treatment[d]	
Nongravid	7	+0.4	−0.2	−0.2	+0.2	+1.0	+0.7
Control	7	+0.8	+0.8	+0.8	+1.1	+0.6	+0.8
S-8	6	−0.9		+0.3		+0.7	
M-1-8	6	+0.5		−3.1		−0.4	
M-2-8	6	+0.5		−2.5		0.0	
M-3-8	5	+0.8		−2.2		0.0	
M-4-8	5	+0.7		−4.4		+0.5	
M-5-8	5	+1.7		−6.1		−0.7	
FD-8	5	−0.2		—		+0.5	
S-9	6		−0.6		−0.1		−0.4
M-1-9	4		+1.6		−4.0		−0.9
M-2-9	5		+0.1		−3.4		−0.4
M-3-9	6		+0.5		−4.1		−0.8
M-4-9	5		+0.6		−4.5		+0.1
M-5-9	5		+1.3		−6.1		−1.4
FD-9	7		+0.6		—		+0.9

[a] See Table I. [b] For mice treated on Day 8, represents Day 6–7. For mice treated on Day 9, represents Day 7–8. [c] For mice treated on Day 8, represents Day 7–8. For mice treated on Day 9, represents Day 8–9. [d] For mice treated on Day 8, represents Day 7–9. For mice treated on Day 9, represents Day 8–10.

Day 9, one of eight mice resorbed completely. After food deprivation on Day 8, three of nine pregnancies terminated in complete resorption. This rate of resorption, 33 %, was significantly higher than the 2% seen in the control group. There were no complete resorptions in any of the other groups.

The number of intrauterine deaths was increased significantly only in the groups administered 400 or 500 mg./kg. of morphine on Day 9.

Intrauterine deaths and subsequent resorption sites were further classified according to their gross appearance. The three classifications were: first, total resorption in which the site was marked by a small green knob with no evidence of any other placental or fetal tissue; second, partial resorption in which the placenta still possessed a discrete disk shape and fetal tissue, if detectable, was an amorphous mass; and third, recent death in which necrosis had begun but the external features of the fetus were still recognizable. The tabulation of fetal resorption by type is given in Table III.

It can be seen in the groups treated with 400 or 500 mg./kg. on Day 9 that the number of total resorptions was not extraordinary, but the number of partial resorptions was very high. This type of resorption was responsible for the significant difference in the resorption rate when compared to the controls.

TABLE III—CLASSIFICATION OF FETAL RESORPTIONS

Treatment[a]	Type of Resorption		
	Total	Partial	Recent Death
Control	47	3	1
S-8	4	1	
S-9	10	1	1
M-1-8	6	1	
M-2-8	5	1	
M-3-8	7		
M-4-8	11	3	
M-5-8	5	1	1
FD-8	8	2	
M-1-9	8		
M-2-9	11	1	1
M-3-9	5	2	
M-4-9	10	16	
M-5-9	6	10	1
FD-9	8	1	

[a] See Table I.

The mean weight and crown-rump length of the fetuses in each treatment group are tabulated in Table IV.

Soft Tissue Malformations—The soft tissue defect with the highest incidence was exencephaly which is characterized by the extensive exteriorization of cerebral tissue due to the absence of the skin and bones of the cranial fault (Fig. 1). Diagnosis of exencephaly could be made upon inspection of the litter before the uterine horn was opened because each uterine swelling associated with this defect was dark red and edematous. This hydramnios was caused by bloody cerebrospinal fluid entering the amnionic sac from the open cerebral ventricles.

All of the exencephalic fetuses were viable, and their respiratory movements were initiated spontaneously as quickly as normal littermates. However, in contrast to the pinkish skin color of the normal fetus, all exencephalic fetuses were blanched and anemic in appearance.

Tissue sections from the malformed fetuses show the neural elements to be markedly disorganized, though in some instances rudimentary organization of the hemispheres had occurred.

The number of exencephalic fetuses and the number of litters containing defective young are tabulated in Table V.

Fetuses with exencephaly were evenly divided between the two uterine horns; irrespective of maternal treatment, 13 were found in the right horn and 14 in the left. Furthermore, within each horn the defective fetuses appeared to be randomly distributed with respect to uterine position. The number of male and female exencephalic fetuses were 12 and 15, respectively.

A number of other defects was found in association with exencephaly induced by morphine. The eyes which usually are closed until 14 days after birth were often open. In two fetuses one or both eyes were closed but misshapen. In one fetus both eyes were absent, and in another the right eye was missing. Two fetuses had cleft palate. In seven of the fetuses the snout was shortened so that the lower jaw and tongue appeared to protrude beyond the nose. In four fetuses the internal ear appeared to be larger than in littermates.

TABLE IV—WEIGHTS AND CROWN-RUMP LENGTHS OF FETUSES

Treatment[a]	Weight, g.			Length, cm.		
	N	$\bar{x}$	s	N	$\bar{x}$	s
Control	320	1.2	0.224	298	2.3	0.100
S-8	80	1.2	0.100	74	2.3	0.141
S-9	84	1.2	0.161	82	2.3	0.173
M-1-8	84	1.2	0.100	84	2.3	0.141
M-2-8	64	1.1[b]	0.100	64	2.3	0.141
M-3-8	54	1.2	0.0	54	2.3	0.173
M-4-8	101	1.1[b]	0.100	101	2.2[b]	0.100
M-5-8	86	1.1[b]	0.141	86	2.2[b]	0.141
FD-8	58	1.1[b]	0.141	57	2.2[b]	0.141
M-1-9	54	1.1[b]	0.469	53	2.1[b]	0.141
M-2-9	85	1.1[b]	0.118	85	2.3	0.173
M-3-9	110	1.1[b]	0.141	109	2.3	0.173
M-4-9	111	1.1[b]	0.141	111	2.2[b]	0.173
M-5-9	66	1.0[b]	0.155	65	2.1[b]	0.100
FD-9	69	1.1[b]	0.141	69	2.2[b]	0.100

[a] See Table I. [b] Statistically significant in comparison with control, $p < 0.05$.

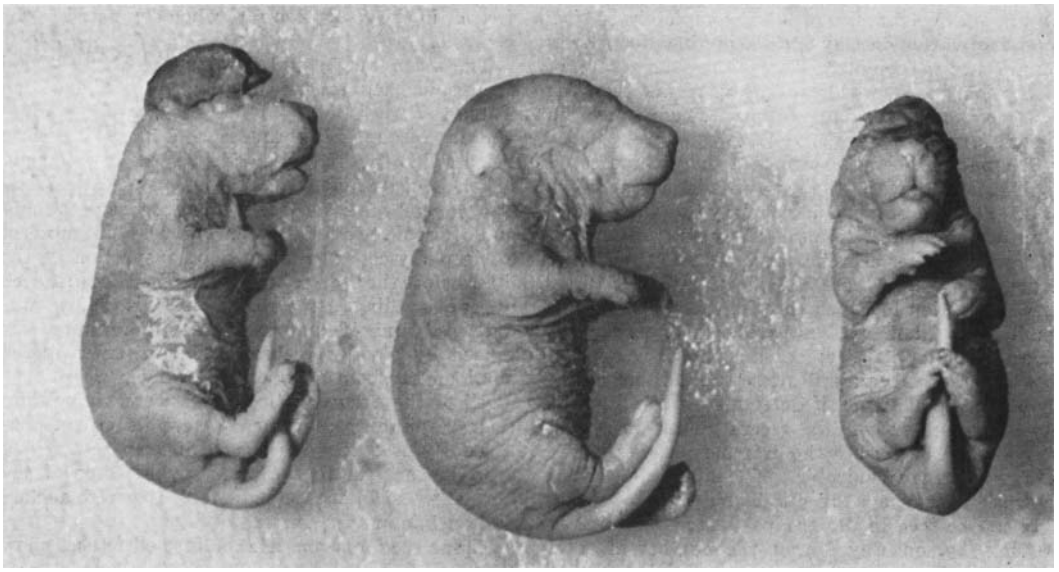

Fig. 1—Near-term mouse fetuses from a female treated with 500 mg./kg. of morphine sulfate on Day 8. In the exencephalic fetuses note the open eye and shortened snout (left) and protrusion of the tongue (right). The middle fetus is a normal littermate.

The weight of the exencephalic fetuses was often but not always less than the mean weight of the normal fetuses. The mean fetal weight of the defective fetuses from the 300 mg./kg. group on Day 8 was equal to the control weight; after 400 mg./kg. it was 0.9 g., $s = 0.08$; and after 500 mg./kg. it was 1 g., $s = 0.07$. The latter two values are significantly lower than the mean fetal weights of all fetuses in their respective groups.

The crown-rump length of exencephalic fetuses was not reduced in comparison to the control fetuses. In fact, the mean crown-rump length was greater in the groups treated with 300 or 400 mg./kg. of morphine on Day 8. Some difficulty was encountered in measuring the total length of fetuses with exencephaly due to the protrusion of neural tissue. This may account for the apparently greater length of these fetuses. Therefore, the fetal weight appears to be a better indication of actual fetal size.

Missing from the skull of the exencephalic fetal skeletons were the frontal, parietal, interparietal, and occipital ossification centers which at this time normally show extensive ossification. In many exencephalic skulls the premaxilla and nasal bones were shortened, but the mandible was of normal size and shape.

Other than exencephaly and defects associated with exencephaly, only a few other soft tissue malformations were found in all of the fetuses examined. In six fetuses testicular descent was retarded; bilaterally in one fetus in the 100 mg./kg. morphine Day 9 group; on the left in a single fetus from the 500 mg./kg. morphine Day 8 group, the 200 mg./kg. morphine Day 9 group and food-deprivation Day 9 group; and on the right in a single fetus from the saline Day 8 group and the food-deprivation Day 9 group. A single fetus with the left pinna misshapen and one fetus with a cleft palate were found in the group given 300 mg./kg. of mor-

TABLE V—THE OCCURRENCE OF EXENCEPHALY AND AXIAL SKELETAL FUSIONS

Treatment[a]	Exencephaly				Axial Skeletal Fusions			
	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses	Litters Absent	Litters Present	Normal Fetuses	Abnormal Fetuses
Control	42	1	386	1	30	0	134	0
S-8	7	0	74	0	7	0	37	0
S-9	10	0	88	0	10	0	45	0
M-1-8	8	0	83	0	8	0	40	0
M-2-8	6	0	64	0	6	0	30	0
M-3-8	3	3[b]	49	5[b]	6	0	26	0
M-4-8	8	3[b]	86	6[b]	6	3[b]	42	4[b]
M-5-8	4	4[b]	75	11[b]	5	3[b]	40	5[b]
FD-8	5	1	57	1	2	4[b]	24	8[b]
M-1-9	4	2[b]	51	2[b]	4	1	17	2[b]
M-2-9	9	0	79	0	3	5[b]	31	10[b]
M-3-9	10	0	108	0	6	4[b]	46	10[b]
M-4-9	10	1	110	1	4	7[b]	40	18[b]
M-5-9	8	0	66	0	3	4[b]	23	9[b]
FD-9	7	0	69	0	7	0	35	0

[a] See Table I. [b] Statistically significant in comparison with control, $p < 0.05$.

phine on Day 9. A fetus from the group treated with 400 mg./kg. of morphine on Day 9 had cleft palate, spina bifida, misshapen external ears, anophthalmia on the right, and microophthalmia on the left.

Skeletal Malformations—The number of fetuses with rib and vertebral fusions as well as the number of litters with these defects are tabulated in Table V. Fetuses with rib and vertebral fusions after treatment with 500 mg./kg. of morphine on Day 9 are illustrated in Fig. 2.

The extent of the rib fusion varied from fusion only at the base where the ribs join the vertebral column to fusion throughout their entire length. The number of ribs involved on any one side varied from a minimum of two to a maximum of eight. Also, fusions involving the same ribs occurred both bilaterally and unilaterally in about equal numbers. After treatment with morphine on Day 8, none of the ribs below the eighth pair were involved in fusions, while the number of fusions involving each of the ribs one through eight was about equal. After food deprivation on Day 8, only ribs four through twelve were involved; a few fusions were seen in ribs four through eight and in the twelfth, but the highest number were in ribs nine through eleven. After treatment with morphine on Day 9, fusions were found involving all ribs, but 90% involved ribs posterior to the sixth pair.

DISCUSSION

The results of this investigation clearly demonstrate that morphine sulfate administered in high doses on Day 8 or Day 9 is teratogenic in CF-1 mice.

The administration of 100 mg./kg. of morphine on Day 8 had no apparent effect upon development. Doses of 200 mg./kg. lowered the mean fetal weight, but no morphological abnormality was detected. With doses of 300 mg./kg. exencephaly was found in one-half of the litters examined even though the mean fetal weight was not decreased significantly. After doses of 400 or 500 mg./kg. exencephaly and axial skeletal fusions were found in significant numbers along with a decrease in the mean fetal weight and crown-rump length.

All doses of morphine employed on Day 9 produced fusions in the axial skeleton and a decrease in the mean fetal weight. Doses of 400 and 500 mg./kg. produced a significant increase in the number of partial fetal resorptions as well as a decrease in the mean crown-rump length. The mean maternal weight gain was significantly decreased after 500 mg./kg. In contrast to Day 8, the number of exencephalic fetuses produced was significant only in the 100 mg./kg. group. However, it does appear that on Day 9 mouse embryos are more susceptible to morphine-induced abnormalities.

Food deprivation on Day 8 was also teratogenic. Axial skeletal fusions were produced in significant numbers as well as one exencephalic fetus. Furthermore, the mean fetal weight and crown-rump length were decreased significantly. However, food deprivation on Day 9 did not result in teratogenesis, although the mean fetal weight and crown-rump length were decreased significantly.

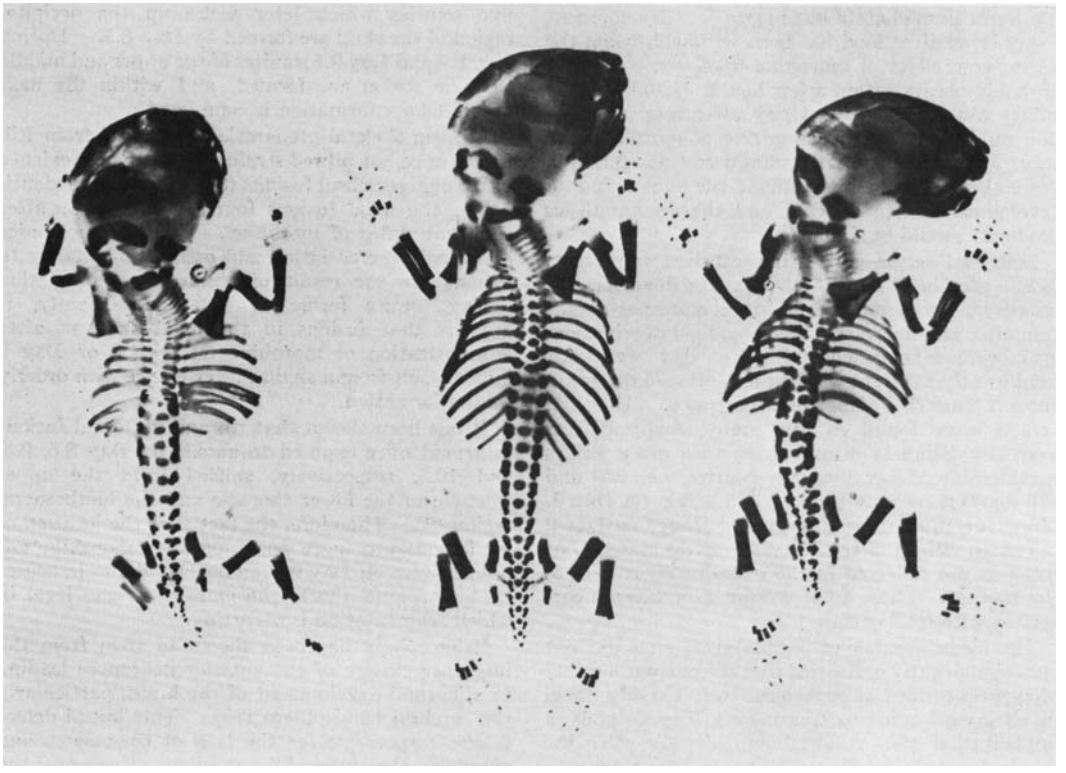

Fig. 2—Alizarin skeletal preparations of near-term mouse fetuses from a female treated with 400 mg./kg. of morphine sulfate on Day 9 showing fusions of the lower ribs and vertebral column. The middle fetus is a normal littermate.

In three of nine mice subjected to food deprivation on Day 8, complete resorption occurred. Death of entire litters may have resulted from an embryocidal effect upon all embryos or maternal effects incompatible with the maintenance of pregnancy. Among the six litters carried to term, the number of resorptions was not increased significantly. After food deprivation on Day 9 all litters were carried to term without an increase in the number of fetal resorptions.

Retardation of food consumption by morphine does not seem to be responsible for the teratogenic effects of the drug even though food deprivation does have definite effects upon embryonic development; for, if the teratogenic effects of morphine were caused by a decrease in food consumption, abnormalities in both the morphine-treated groups and the food-deprivation groups would be similar after treatment on the same day.

Although morphine treatment on Day 8 produced a large number of exencephalic fetuses, only one was found after food deprivation on this day. Moreover, unlike axial skeletal fusions after morphine on Day 8, a large number of fusions were found posterior to the eighth pair of ribs after food deprivation on this day. In fact, the low incidence of exencephaly and the location of axial skeletal fusions after food-deprivation on Day 8 compared quite favorably to the results obtained with morphine treatment 1 day later. Although a large number of axial skeletal fusions were found after morphine treatment on Day 9, the axial skeleton was not affected by food deprivation on this day.

It appears the teratogenic effects of starvation do not occur immediately but may affect development a day or so after food has been withheld, while the teratogenic effect of morphine upon the conceptus probably occurs within a few hours. If teratogenic effects were delayed for 1 day after food deprivation on Day 8 and if the effects of morphine did occur almost at once after administration on Day 9, the embryos would be in about the same stage of development when affected and the abnormalities produced should be similar.

Maternal weight gain during gestation would seem to be a good indication of fetal size and development. However, the differences in mean maternal weight gain after administration of saline, food deprivation, or morphine treatment on either day were not significantly different with the exception of the group given 500 mg./kg. of morphine on Day 9. Although defects were found in this group, many defects were also found in other groups with mean values considerably higher than the control, *i.e.*, 300 and 400 mg./kg. on Day 9 and 500 mg./kg. on Day 8. Moreover, in all morphine-treated groups on Day 9 the mean weight of the fetuses was decreased, but this was not reflected in the overall weight gain of the mother. Thus, total weight gain was not correlated with fetal effects.

The mean number of implantation sites did not vary significantly indicating that the average number of zygotes formed at conception was roughly equal in all groups prior to treatment. The number of implantation sites counted immediately after the death of mice killed by morphine on either day was not significantly different from the control indicating that gestation was proceeding normally up to the time of treatment.

The time of intrauterine death can be estimated by the degree of resorption that has occurred because autolysis, maceration, and disappearance of the products of conception may take several days.

Resorptions classified as recent deaths must have occurred only a few days before sacrifice, because the fetal and placental tissue is necrotic but intact. The number found in the control and treated groups would not suggest any increase as a result of treatment.

The number of total resorptions in each group compared favorably to the number found in the control group indicating no effect referable to treatment. In fact, many of the deaths marked by total resorption sites could have occurred even before treatment on Day 8 or Day 9.

It seems logical to assume that deaths marked by partial resorption occurred after the treatment days. Compared with the control group, the incidence of partial resorptions in the treated groups was not increased markedly with the exception of those administered 400 or 500 mg./kg. of morphine on Day 9. The increase in intrauterine death during this time must have been caused by the embryocidal action of morphine.

The axial skeleton, derived mainly from mesoderm, originates from the primitive streak at the posterior end of the embryo. As the embryo grows, the primitive streak moves in a cranio-caudal direction leaving behind a dense band of undifferentiated mesenchyme. This mesenchyme is divided into segments or somites by intersegmental fissures and intersegmental vessels from the dorsal aorta (6). According to Otis and Brent (7), the first five somites which later make up the occipital region of the skull are formed by Day 8.5. During Day 8.5 and Day 9.5 somites of the upper and middle thoracic region are formed, and within the next 48 hr. somite formation is completed.

Alizarin skeletal preparations prepared from Rib fusion mice, an inbred strain with a high incidence of rib and vertebral fusions (8), appear to be identical to the axial fusions found in CF-1 mice after sufficient doses of morphine. In Rib fusion mice abnormal differentiation and ossification leading to fusions are the result of abnormal segmentation during somite formation (8). Consequently, it appears that fusions in the axial skeleton after administration of morphine on Day 8 or Day 9 might result from a similar interference with orderly somite formation.

It has been shown that rib and vertebral fusions in gravid mice exposed to anoxia on Day 8.5, 9.5, and 10.5, respectively, shifted from the upper thoracic to the lower thoracic and the lumbosacral region (9). Therefore, the fact that the location of rib fusions was more posterior after morphine administration on Day 9 than on Day 8 also indicates that morphine affects the embryo at the level in which segmentation is occurring.

Exencephaly has been shown to stem from the improper closure of the anterior neuropore leading to abnormal development of the brain, particularly the cerebral hemispheres (10). This initial defect is also responsible for the lack of the osseous and cutaneous structures of the cranium. Consequently, the brain is subject to degenerative and hemorrhagic changes as it lies unprotected on the floor of the skull. In man and other mammals with gestation

periods longer than the rodent most of the cerebral tissue may be completely destroyed leading to anencephaly (10).

In mice exencephaly and axial skeletal fusions have been reported after many types of treatment including the administration of insulin (11), galactoflavin (12), vitamins (9) and urethan (13) as well as riboflavin deficiency (12), X-ray (12), and anoxia (14). Although the mechanisms are not completely understood, all of these must interfere with one or more processes necessary for proper closure of the neural tube and segmentation.

The description of exencephaly produced in mice by the administration of urethan on Day 7 (13) is quite similar to the appearance of this defect after morphine administration on Day 8. According to Sinclair (13), the antimitotic effects of urethan did not seem to be the cause of the deformity because the usual number of mitotic figures was seen in the germinal layers of treated embryos. The fact that both agents are central nervous system depressants might indicate a common mechanism of action.

Gautieri and Ciuchta (15) have shown that morphine produces marked and sustained constriction of the fetal vasculature of the perfused human placenta that is characterized by a slow progressive decrease in the volume flow rate of the perfusate ranging from 35 to 92% after infusion of 0.2–0.4 mg. of the sulfate salt; this effect is reversed by the subsequent administration of nalorphine hydrochloride. Moreover, several investigators have shown that mice subjected to anoxia on Day 8 or Day 9 gave birth to a large number of young with exencephaly and axial skeletal fusions (14, 16). Therefore, evaluation of the extent and consequences of maternal and fetal anoxia after morphine administration should provide some insight into the mechanism of the teratogenic action of morphine.

Although congenital abnormalities were produced by morphine, the doses required were extremely high. Even the lowest dose utilized far exceeded the usual effective analgesic dose in this species by at least 10 times. Moreover, doses of 400 and 500 mg./kg. were lethal to 18 and 40% of the gravid animals. These higher doses are quite close to the LD_{50} of 525 mg./kg. in nongravid mice. Therefore, if the criterion for judgment of safety is the ratio of the LD_{50} dose to the teratogenic dose as has been suggested (17), it must be concluded that the teratogenic potential of morphine sulfate is low.

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Keyphrases

Morphine—teratology, mice
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