all possible combinations of intestinal segments showed statistically significant differences at P 0.05 except two. The jejunum–ileum and A. colon–D. colon contractions per minute comparisons were not significantly different; however, the two other adjacent segments comparisons here were significantly different.

In the transducer recorded series, the contractions per minute again decreased with progression to the lower intestinal tract and the intervals between contractions again increased. The A. colon was the only exception to this trend in these two cases and may be due to more sensitive recording.

In this series the t values indicated statistically significant differences for all comparisons of contractions per minute which would be very important for drug comparison effects upon the intestinal tract.

The comparison of interval between contractions between A. colon and D. colon, however, was not significantly different. The reverse was true in the kymograph series in the comparisons between jejunum–ileum and A. colon–D. colon; the interval between contractions was significant but the contractions per minute were not significantly different. The transducer system would appear to better record the contractions per minute.

Two comparisons of amplitude of contractions in the transducer series also were not significantly different. They were duodenum–A. colon and duodenum–D. colon.

It would appear that this study and evaluation of normal rabbit intestinal activity could serve as a basis for further drug activity studies employing the rabbit intestinal tract and furthermore, in determining the preferential intestinal segment of a drug's activity.

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Inhibition of Cortisone-Induced Cleft Palate in Mice by Cobaltous Chloride

By GILBERT KASIRSKY, RONALD F. GAUTIERI, and DAVID E. MANN, JR.

Young primipara CF-1 mice were mated overnight and those with vaginal plugs (day zero) were considered pregnant. Animals were divided into five groups of 26 each and subjected to the following treatment: group *A* received 2.5 mg. of cortisone acetate i.m. on days 11, 12, 13, and 14; group *B* received cobaltous chloride, 25 mg./Kg. i.p. on day 11 only followed by cortisone acetate, 2.5 mg. i.m. on days 11, 12, 13, and 14; group *C* received physiological saline, 0.1 ml. i.m. on days 11, 12, 13, and 14; group *D* represented untreated controls; group *E* received cobaltous chloride, 25 mg./Kg. i.p. on day 11 only followed by saline, 0.1 ml. i.m. on days 11, 12, 13, and 14. Mice were laparotomized and fetuses were examined on day 18. Group *A* (cortisone), 140 of 185 fetuses developed cleft palate (75.6 per cent incidence); group *B* (cobaltous chloride–cortisone), 24 of the 190 fetuses had clefts (12.6 per cent incidence); group *C* (saline), none of the 201 fetuses showed clefts; group *D* (untreated controls), none of the 194 fetuses had clefts; group *E* (cobaltous chloride–saline), 27 of the 208 fetuses had cleft palate (12.9 per cent incidence). Also, the incidence of cleft palate by litters was group *A*, 100 per cent; group *B*, 23.1 per cent; group *C*, 0 per cent; group *D*, 0 per cent; group *E*, 50 per cent.

DURING A recent evaluation of the relative antitumorigenic properties of cortisone (1), cobaltous chloride (2), and sodium cobaltinitrite (3, 4) in CF-1 mice subjected to the minimal carcinogenic dose$_{50}$ (MCD$_{50}$) of methylcholanthrene, several striking similarities became apparent in the character of the histological responses evoked by each drug, for not only was there a significant reduction of tumor incidence, but also the remarkable absence of cutaneous inflammation. The latter observation may be related to a report of an accentuation of anti-inflammatory activity of δ-1 hydrocortisone by the addition of a cobalt atom to the C-20 carbonyl function (5). Because interference with normal closure of the palatine shelves in certain strains of mice by cortisone is well documented (6), and furthermore, because previous studies in this laboratory have uncovered certain cortisone-like characteristics of two inorganic cobalt compounds, it thus became the primary objective of this investigation to discern the nature and extent of any cobalt–steroid interactions in terms of greater or lesser anomalies in the mouse fetus.

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EXPERIMENTAL

Groups of 30 female mice of the CF-1 strain, weighing approximately 25 Gm., were individually

TABLE I—INHIBITION OF CORTISONE-INDUCED CLEFT PALATE IN MICE BY COBALTOUS CHLORIDE

Group	Treatment	No. Litters	Litters with CP	No. Fetuses	No. Fetuses with CP	% with CP
A	Cortisone, 2.5 mg. i.m., days 11, 12, 13, 14	26	26	185	140	75.6
B	Cortisone, 2.5 mg. i.m., days 11, 12, 13, 14 Cobaltous chloride 25 mg./Kg. i.p., day 11	26	6	190	24	12.6
C	Physiological saline, 0.1 ml. i.m., days 11, 12, 13, 14	26	0	201	0	0
D	Untreated controls	26	0	194	0	0
E	Physiological saline, 0.1 ml. i.m., days 11, 12, 13, 14 Cobaltous chloride, 25 mg./Kg. i.p., day 11	26	13	208	27	12.9

placed in a breeding cage with a male of the same strain at 4 p.m. every Wednesday and Thursday. The following morning (9:00–9:30 a.m.), females were returned to their own cages and those with vaginal plugs were considered pregnant and put aside in individual cages. The morning of the appearance of the vaginal plug was designated day 0 (gestation period 0–19 days). Pregnancy was confirmed by the weight gain which becomes apparent on or after day 8, or the placental sign which appears on day 10. The diet consisted of Purina laboratory chow and tap water, both given *ad libitum*. Food was placed in ample amounts on the floor of each cage and water was obtained through stainless steel drinking tubes which were attached to 4-oz. bottles. Excreta pans were arranged to permit coprophagy. Care was taken to minimize drafts and noise, and environmental temperature was maintained at 25° throughout the experiment.

Pregnant animals were divided into 5 groups comprising 26 mice each and subjected to the following treatment.

Group A—Each mouse received an intramuscular injection of 2.5 mg. of cortisone acetate[1] on days 11, 12, 13, and 14.

Group B—Each animal received an intraperitoneal injection of cobaltous chloride, 25 mg./Kg. (10% aqueous solution), on day 11 only followed by cortisone acetate, 2.5 mg. i.m., on days 11, 12, 13, and 14.

Group C—Each animal received an intramuscular injection of physiological saline solution, 0.1 ml., on days 11, 12, 13, and 14.

Group D—Untreated controls.

Group E—Each animal received an intraperitoneal injection of cobaltous chloride, 25 mg./Kg., on day 11 only followed by saline, 0.1 ml. i.m., on days 11, 12, 13, and 14.

All compounds were injected with a 26-gauge, 0.5-in. needle attached to a 1-ml. syringe which had been previously rinsed with sterile water for injection.

A single injection of the cobalt compound was administered intraperitoneally at 9:30 a.m. on the eleventh day of gestation. Injections of cortisone acetate were administered 5 min. after the cobalt administration on day 11. Subsequent injections of the steroid on days 12, 13, and 14 were also given intramuscularly, alternating the sites of injections from one hind leg to the other (biceps femoris muscle).

[1] Merck Sharp and Dohme, West Point, Pa.

EXAMINATION OF FETUSES

On day 18, which is 12 to 24 hr. prior to expected delivery, the mice were sacrificed by cervical dislocation and laparotomized immediately.

The abdominal wall of the mother was incised from the vaginal opening to an area just below the xiphihumeralis along the linea alba. Incisions were also made diagonally through the rectus abdominis, obliquus externus, and transverse abdominis to form a flap.

Before opening the uterine horns, fetal swellings and resorption sites (metrial glands) were recorded and the uterus was excised for removal of the fetuses. Before weighing and sexing, the fetuses were placed on blotting paper to remove excessive amniotic fluid, whereupon they were observed under an Ednalite magnifier for the presence of gross anomalies (2 × magnification).

Following the preliminary examination, the fetuses were prepared for further study. In order to evaluate skeletal structure, every third fetus was immersed in a modified clearing and staining solution according to the Staples and Schnell modification of the Spaltheholtz method (7). The remainder of the fetuses was fixed in Bouin's solution for 2 weeks in preparation for free hand razor blade sectioning as described by Wilson (8). This method allows for the evaluation of malformations in a large number of animals with a minimum expenditure and delay.

RESULTS

The incidence of cleft palate obtained for the various groups of mice at the termination of this study is recorded in Table I as follows.

Group A—Twenty-six pregnant mice which received cortisone acetate, 2.5 mg. i.m., on days 11, 12, 13, and 14 of gestation responded with a

75.6% incidence of fetal cleft palate. All of these litters had some clefts, the total number of fetuses having been examined was 269, with 3 born dead (84 of these were placed in alizarin red S solution for bone staining). Average weights of pregnant mice and fetuses were 46.2 and 0.86 Gm., respectively; number of resorptions was 49.

Group B—Twenty-six pregnant mice which received cobaltous chloride ($CoCl_2.6H_2O$), 25 mg./Kg. i.p., on day 11 only and cortisone acetate, 2.5 mg. i.m., on days 11, 12, 13, and 14, had a 12.6% incidence of cleft palate. Only six litters in this group had clefts. The total number of fetuses examined was 259, with 3 born dead (69 of these were placed in the alizarin red S solution for bone staining). The average weights of pregnant mice and fetuses were 44.3 and 1.10 Gm., respectively; number of resorptions was 38.

Group C—Twenty-six pregnant mice received physiological saline solution, 0.1 ml. i.m., on days 11, 12, 13, and 14 of gestation. None of these litters had cleft palate; however, one fetus was exencephalic. The total number of fetuses examined was 288, with 3 born dead (87 of these were placed in alizarin red S solution for bone staining). The mean weights of pregnant mice and fetuses were 51.7 and 1.21 Gm., respectively; the number of resorptions was 13.

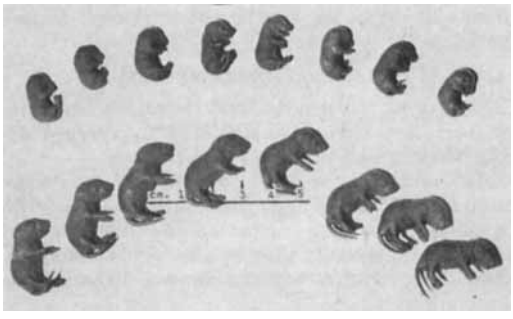

Fig. 1—Key: top, growth retardation produced by cortisone acetate, 2.5 mg. i. m.; bottom, inhibition by cobaltous chloride pretreatment, 25 mg./Kg. i. p.

Group D—This group of 26 pregnant mice was untreated and showed no incidence of cleft palate. The mean weights of pregnant mice and fetuses were 52.8 and 1.47 Gm., respectively; the number of resorptions was 13.

Group E—The final group of 26 pregnant mice received cobaltous chloride, 25 mg./Kg i.p., on day 11 only and saline solution, 0.1 ml. i.m., on days 11, 12, 13, and 14. Thirteen of these litters had some fetuses with clefts. The total number of fetuses examined was 293 (85 of these were placed in alizarin red S solution for bone staining). The incidence of cleft palate, average weights of pregnant mice and fetuses, and number of resorption sites were 12.9%, 49.6 and 0.99 Gm., and 31, respectively. A high incidence of complete fetal resorptions was observed in this group, 13 of the 26 animals.

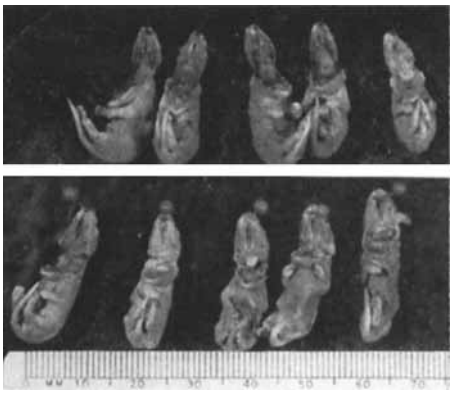

Fig. 2—Key: top, cleft palate induced by cobaltous chloride, 25 mg./Kg. i. p.; bottom, cleft palate induced by cortisone acetate, 2.5 mg. i. m.

DISCUSSION

The original premise which prompted this study concerning cobalt–steroid interrelationships in modifying fetal development was based upon recent findings during experiments with methylcholanthrene carcinogenesis in mice that two inorganic cobalt compounds, cobaltous chloride (2) and sodium cobaltinitrite (3, 4), manifested cortisone-like characteristics (*i.e.*, anti-inflammatory and antitumorigenic actions). The results (Table I) reveal additional similarities such as growth retardation, as denoted by group *A* (cortisone) and group *E* (cobaltous chloride–saline) having the lowest mean fetal weights, and cleft palate induction (Fig. 2 and Table I) by cobaltous chloride alone, and the prevention of both of these events by the cobalt compound in the presence of cortisone (Fig. 1 and Table I). It appears that the cobalt–steroid interaction may occur at the same site through competitive inhibition, for each alone induces clefts while together marked inhibition occurs. It should be noted, however (Fig. 2), that cobaltous chloride alone does not produce a macroscopically identical fissure to that caused by cortisone. On the contrary, cortisone-induced cleft palate is measurably more pronounced than that produced by cobaltous chloride. Whether this reflects a dosage relationship or difference in mechanism of action from that of cortisone remains to be clarified.

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