Cobalt-Cortisone Interrelationships in the Induction and Inhibition of Cleft Palate in Mice

GILBERT KASIRSKY, WILLIAM T. SHERMAN, RONALD F. GAUTIERI, and DAVID E. MANN, Jr.

Abstract □ The i.m. administration of cortisone acetate, 2.5 mg., to nulliparous CF-1 mice, on Days 11–14 or 10–13 of gestation, yielded 75 and 70 percent incidences, respectively, of cleft palate in the 18-day fetus. The single i.p. injection of either cobaltous chloride, 25 mg./kg., or sodium cobaltinitrite, 50 mg./kg., on Day 10 or 11, followed by four daily i.m. administrations of cortisone or physiological saline caused a marked reduction in the incidence of fetal clefts in the presence of the steroid, and a significant increase in this anomaly in the presence of saline; the more profound effect in each instance being associated with the earlier day of administration. Replacement of cobaltous chloride with nickel chloride in the treatment regimen neither induced cleft palate with physiological saline nor markedly inhibited clefts caused by cortisone.

Keyphrases □ Cleft palate induction, inhibition—mice □ Cobalt-cortisone effect—cleft palate, mouse fetus □ Fetal age—cleft palate induction, inhibition

Several biological interrelationships have recently been shown to exist between certain inorganic cobalt compounds and the adrenal steroid, cortisone, by this laboratory. Cobaltous chloride and/or sodium cobaltinitrite, when administered individually to mice, have evinced antiinflammatory and antitumorigenic activities, as well as growth retardation and cleft palate formation, in the fetus in the manner of cortisone, while in the presence of the steroid, the production of fetal clefts was markedly inhibited (1–5). The striking similarities of these cobalt-evoked effects with those elicited by cortisone prompted several of the authors to postulate an "ionic hormonal precursor hypothesis" (6) which assumes that some contemporary hormones had their origins from inorganic ions entrapped by primitive aquatic organisms which utilized them to catalyze primeval metabolic processes. According to this concept, the younger the organism (in this instance the mouse fetus) at the time of drug insult with cortisone and cobalt, administered individually or together, the more dramatic the response anticipated because of a reciprocal parallelism between decreasing age and increasing drug sensitivity during the "phylogenic recapitulation of ontogeny." Therefore, the principal objectives of this study were twofold: to substantiate the precepts of the hypothesis by observing the effects of fetal age on the incidence of cleft palate caused by cortisone and cobalt compounds alone and together and to determine the extent of ionic specificity involved through the substitution of nickel chloride in the treatment regimen in place of cobaltous chloride.

EXPERIMENTAL

CF-1 albino mice weighing between 20 and 25 g. were obtained from Carworth Farms, Inc., New City, N. Y. Females were caged in groups of 25 and 30 for at least 2 weeks after arrival and were not mated until they were 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 tapwater *ad libitum*. Handling, environmental control, timing of pregnancies, removal and examination of fetuses, fixing, storage, bone staining, and gross sectioning are described in detail in previous papers (5, 7). Pregnancy was confirmed by a weight gain of 2 or more g. usually noted by Day 8 and/or the appearance of the placental sign on Day 10. Pregnant animals were then assigned to one of 15 experimental groups (number of animals per group and sequence of drug administration are noted in Table I). The following drugs and doses were used: physiological saline, 0.1 ml.; cortisone acetate,[1] 2.5%, 2.5 mg.; cobaltous chloride, 1.0%, 25 mg./kg.; sodium cobaltinitrite, 2.0%, 50 mg./kg.; and nickel chloride, 1.0%, 25 mg./kg.

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

Table I—Influence of Fetal Age on Incidence of Cleft Palate in Mice in Response to Cobaltous Chloride or Sodium Cobaltinitrite Alone and in the Presence of Cortisone

Group	Treatment (day)	No. Litters	Litters with Cleft Palate	No. Fetuses	No. Fetuses with Cleft Palate	% with Cleft Palate
A	Untreated controls[a]	26	0	194	0	0.0
B	Saline (11–14)[a, b]	26	0	201	0	0.0
C	Cortisone (11–14)[a, b]	26	26	185	140	75.6
D	Cortisone (10–13)	11	11	59	41	69.5
E	Cortisone (11–14) saline (10)	23	22	121	85	70.2
F	Cortisone (11–14) $CoCl_2$ (11)[a]	26	6	190	24	12.6
G	Cortisone (11–14) $CoCl_2$ (10)	27	1	212	1	0.5
H	Cortisone (11–14) $Na_3Co(NO_2)_6$ (10)	23	2	153	4	2.7
I	Saline (11–14) $CoCl_2$ (11)[a]	26	13	208	27	12.9
J	Saline (11–14) $Na_3Co(NO_2)_6$ (11)	23	10	162	16	9.8
K	Saline (11–14) $Na_3Co(NO_2)_6$ (10)	15	11	77	47	61.0
L	Saline (11–14) $CoCl_2$ (10)	4	4	29	28	96.5
M	Saline (11–13) cortisone (10)	8	0	42	0	0.0
N	Cortisone (11–14) $NiCl_2$ (10)	7	5	38	20	52.6
O	Saline (11–14) $NiCl_2$ (10)	14	0	111	0	0.0

[a] Reported previously, *J. Pharm. Sci.*, **56**, 1330(1967). [b] Saline and cortisone administered i.m. except saline i.p. in E. $CoCl_2$, $Na_3Co(NO_2)_6$, and $NiCl_2$ administered i.p.

RESULTS AND DISCUSSION

The administration of cobaltous chloride or sodium cobaltinitrite to pregnant mice on Day 10 or 11 of gestation with physiological saline caused cleft palates in the fetus, the higher incidence being associated with the earlier cobalt challenge. Because molar cobalt equivalents elicited qualitatively comparable palate defects, the role of the metallic ion in causing this malformation in mice is established beyond doubt. The administration of either cobaltous chloride or sodium cobaltinitrite on Day 10 or 11 (Groups F, G, and H) with cortisone (Days 11–14) inhibited cleft palates significantly ($p = <0.005$) when compared to the incidence attained with cortisone alone (Groups C, D), with the greater protection afforded on the earlier day of challenge. Group O, nickel chloride (Day 10) and saline (Days 11–14), was devoid of clefts. Although a significant inhibition ($p = <0.005$) was noted when nickel chloride (Day 10) was administered with cortisone (Days 11–14, Group N), it was not as marked as that observed with either cobalt compound and cortisone (Groups F, G, H). No alteration in the incidence of cortisone-induced cleft palate ($p = <0.5$) was noted when the steroid was administered on Days 11–14 (Group C) or 10–13 (Group D).

Several aspects of the ionic hormonal precursor hypothesis have been verified by this study:

1. The ability of cobalt ion to induce cleft palates in mice in the manner of cortisone and to prevent this malformation when caused by the steroid has been confirmed with cobaltous chloride and sodium cobaltinitrite.
2. The younger the fetus at the time of cobalt challenge, the more dramatic are the responses of cleft palate induction and inhibition in the absence and presence of the steroid, respectively. Age, however, did not influence the incidence of cleft palate caused by cortisone. Thus, from the evolutionary standpoint, one would anticipate a greater biological response to the "hormone precursor"—cobalt—than to that of its more recent counterpart—cortisone—which was indeed the case.
3. Cobalt displays greater ionic specificity than nickel because the former both induces and inhibits cleft palate alone and in the presence of the steroid, while the latter only possesses an inhibitory capability which implies a different mechanism of action at the palatine tissue level.

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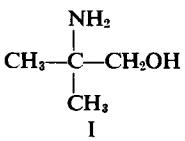
N-Substituted-2-amino-2-methyl-1-propanols as Potential Antitumor Agents

JOHN H. BILLMAN, FRED KOEHLER, and RALPH MAY

Abstract □ Twenty-seven Schiff-base derivatives of 2-amino-2-methyl-1-propanol have been prepared and submitted for antitumor testing. Thirteen *N*-substituted-2-amino-2-methyl-1-propanols were prepared by the reduction of the above Schiff bases. These also were submitted for antitumor testing.

Keyphrases □ 2-Amino-2-methyl-1-propanols, *N*-substituted—synthesis □ Schiff-base derivatives—2-amino-2-methyl-1-propanols □ Antitumor activity—Schiff bases □ IR spectrophotometry—structure □ NMR spectroscopy—structure

2-Amino-2-methyl-1-propanol (I), is an effective antitumor agent (1).



I

Few *N*-substituted derivatives of this compound have been made and apparently none have been tested for antitumor activity. The authors' interest is centered in modifying the structure of 2-amino-2-methyl-1-propanol (I)[1] in the hopes of improving the effectiveness of this drug.

It may be assumed that due to the reactivity of the amino group in 2-amino-2-methyl-1-propanol (I) a reasonable percentage of the applied dose may never reach the tumor site. One means of averting this problem is to protect the amino group in a manner that will allow the blocking group to be removed selectively at the tumor site. Ideally, this might be expected to dramatically improve the therapeutic indexes. The approach that was selected in this project was to form Schiff-base derivatives of the type shown in Scheme I.

[1] 2-Amino-2-methyl-1-propanol is sometimes referred to as AMP.