Effects of Acute Vitamin Replacement Therapy on 6-aminonicotinamide Induced Cleft Palate Late in Rat Pregnancy.* (31878)

JACK G. CHAMBERLAIN (Introduced by I. W. Monie)
Department of Anatomy, University of California, San Francisco

Numerous studies have been reported employing specific vitamin antimetabolites as teratogenic agents(1,2). Few investigations have been done in the area of inhibitor reversal or metabolic replacement therapy under such conditions(3). Recent studies have shown that a single injection of the niacin antimetabolite 6-aminonicotinamide (6-AN), given 12-24 hours prior to commencement of normal palate closure in the rat, results in 100% abnormalities at term(4,5). The present study was undertaken to test the ability of nicotinamide (NAM) to modify or further delimit the action of 6-AN when the vitamin is given at specific times before or after 6-AN administration. The high incidence of 6-AN-induced cleft palate late in rat gestation was selected as a model to study such niacin countertherapy. NAM was used as it has greater ability than nicotinic acid to rapidly increase nicotinamide adenine dinucleotide (NAD) formation in mammalian tissues(6,7).

Methods and materials. Long-Evans rats averaging 100 days of age and 225 g in weight were bred with normal males and fed a stock diet of natural food stuffs.† The morning of finding spermatozoa in the vaginal smear was considered day zero of pregnancy. Following determination of the dosage response late in gestation, groups of 6-12 rats were each given single injections of 8 mg 6-AN/kg body wt i.p. on the 15th day of gestation. Single injections of 8, 16, 24, 50 or 100 mg of NAM/kg body wt were similarly given at ¼, 1, 2, 12, and 24 hours after administration of the antimetabolite.‡ Additional rats received vitamin therapy 2, 12 or 24 hours prior to 6-AN injection or 48-72 hours after 6-AN treatment. Control rats were given 6-AN and NAM concurrently or alone. Living young and placentae were removed, counted and weighed on the 21st day of pregnancy, one day before parturition. Evidences of fetal death or resorption also were noted. Fetuses were fixed in Bouin's fluid and later examined (x 7-30) for palatal abnormalities.

Results. Results observed following NAM treatment 2 or 24 hours after 6-AN administration are presented in Table I. Averages of the combined data on fetal survival and palatal development are shown in Fig. 1. Young from control rats injected only with 6-AN had 100% palatal abnormalities; simultaneous injection of an equal dosage of nicotinamide prevented the effects of 6-AN. Injection of NAM alone resulted in normal young. When vitamin administration was started 2 or 3 days after injection of 6-AN, no reduction in the incidence of cleft palate was observed at term; fetal and placental weights were similar to those observed in the 6-AN treated controls. If NAM was given 12 or 24 hours after antimetabolite administration, 80-100% of the surviving young had palatal defects (Fig. 1). When vitamin treatment was initiated 2 hours after 6-AN injection, even at high dosages (50-100 mg/kg), 40-80% of the fetuses still had palatal abnormalities (Table I). In general, mothers continued to lose weight 24-48 hours after vitamin replacement therapy. In certain cases rats who responded rapidly following a 2 or 12 hour activity of 6-AN had normal young. However, as shown in Table I, maternal weight gain the day after NAM therapy was not necessarily a reliable guarantee of subsequent normal fetal development. Thus, NAM counter-injections may ameliorate the maternal effects of 6-AN while the antimetabolite continues to act in the embryos.

Injection of NAM 2 hours prior or within an hour after 6-AN treatment prevented virtually all effects of the antimetabolite, suggesting that biochemical abnormalities

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† Composition of the stock diet was reported previously(8).

‡ 6-AN (California Corp. for Biochemical Research) and NAM (Nutritional Biochemicals Co.) were dissolved in sterile distilled water.

TABLE I. Nicotinamide (NAM) Replacement Therapy 2 or 24 Hours After 6-Aminonicotinamide (6-AN) Injection (8 mg 6-AN/kg b wt, i.p. Day 15 of gestation).

NAM (mg/kg)	Rats bred	Avg wt change		Fetal resorption*	Living young day 21 of pregnancy			
		Total	+24 hr after NAM		Wt	Placental wt	Secondary palate: Complete cleft	Secondary palate: Total affected†
+ 2 hr	(No.)	(g)	(g)	(%)	(g ± S.E.)	(g)	(%)	(%)
8	10	+ 83	— 8	18	4.5	.36	20	80
16	10	+ 93	— 9	17	4.4	.35	18	54
24	10	+ 99	— 3	6	4.3	.36	2	60
50	7	+ 94	—10	12	4.3 ± .38	.37 ± .38	8	67
100	7	+ 94	— 9	9	4.8 ± .20	.44 ± .04	5	41
+24 hr								
8	11	+ 62	+ 2	11	3.6 ± .20	.42 ± .02	67	100
16	12	+ 68	— 3	9	3.7	.37	28	90
24	10	+ 65	+ 3	13	3.9 ± .13	.41 ± .02	58	100
50	6	+ 71	+ 3	9	3.5	.39	45	100
100	6	+ 66	— 1	10	3.7 ± .25	.33 ± .03	45	95
Controls:								
6-AN‡	11	+ 57	—17	14	3.3 ± .26	.32 ± .02	58	100
6-AN‡ + NAM‡	10	+116	+ 6	8	5.6 ± .12	.50 ± .03	0	0
NAM§	7	+100	+ 7	15	5.7	.50	0	0

* Based on total implantation sites observed.
† Includes incidence of partial defects (rostral and caudal).
‡ 8 mg/kg body wt.
§ Combined data from 50, 100 mg/kg body wt.

were formed within 1-2 hours after 6-AN injections. Administration of the vitamin 12 hours before 6-AN resulted in 35% cleft palate; pretreatment by as little as 24 hours resulted in 100% palatal abnormalities. There appeared to be no direct relationship between incidence of cleft palate and percentage of fetal death following vitamin treatment (Fig. 1).

Discussion. Congenital cleft palate was prevented in rats injected with 8-100 mg of nicotinamide/kg body wt 2 hours before, simultaneously, or up to 2 hours after a teratogenic dose of 8 mg 6-aminonicotinamide/kg body wt. Preliminary results also suggest that nicotinamide adenine dinucleotide (NAD) has a similar protective ability when administered concurrently with 6-AN. Vitamin countertherapy instituted 12 or 24 hours before or 2-72 hours after 6-AN injection was unable to restrict the activity of 6-AN to specific time periods based on (1) embryonic death, (2) cleft palate formation, (3) fetal and placental weights, and (4) maternal weight change during gestation. These results are in contrast to recent reports by Pinsky and Fraser(9) and Goldstein *et al*(10) of 2-hour inactivity of nicotinamide induced by 6-aminonicotinamide and cleft palate formation in mice.

The present study suggests that 6-AN acts very rapidly in producing its effects on embryonic development. Transitory neurological abnormalities have been reported in adult rats within 3-6 hours after administration of 6-AN(11). Once biochemical abnormalities form, presumably non-physiological pyridine nucleotides(12,13,4), they cannot be reversed quickly by NAM. Inability to protect

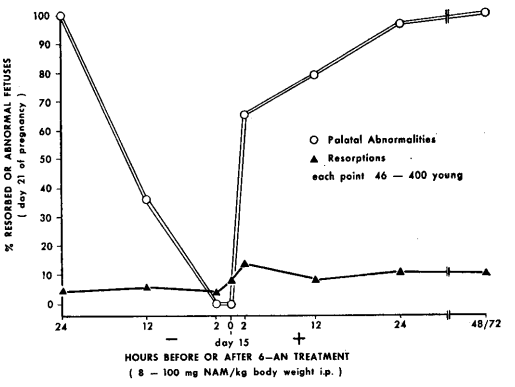

Fig. 1. Effects of nicotinamide (NAM) countertherapy during 6-aminonicotinamide (6-AN) induced cleft palate late in rat pregnancy (8 mg 6-AN/kg body wt i.p. day 15)

against 6-AN when the vitamin is given 12-24 hours prior to antimetabolite treatment may reflect the rapid metabolism of NAM and its decrease to normal levels with 8-24 hours after injection(6).

Summary. Pregnant rats were given single injections of nicotinamide (NAM) (8-100 mg/kg body wt) before or after a teratogenic dose of the vitamin antimetabolite 6-aminonicotinamide (6-AN) late in gestation. NAM effectively competed with 6-AN in preventing cleft palate when given 2 hours before, simultaneously, or up to 2 hours after antimetabolite treatment. However, vitamin replacement therapy was unable to prevent palatal abnormalities or restrict the action of 6-AN to specific time periods when given 12-24 hours before or 2-72 hours after 6-AN administration.

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Response of the Isolated Dogfish Gastric Mucosa to Histamine. (31879)

C. ADRIAN M. HOGBEN (Introduced by B. A. Schottelius)
Mount Desert Island Biological Laboratory, Salisbury Cove, Maine and Department of Physiology and Biophysics, University of Iowa, Iowa City

To simplify an aspect of gastric physiology, many have embraced a proposal that histamine is the final common mediator of acid secretion(1,2,3). At least one scientist has expressed his reservations(4). To date most work has been conducted on the whole animal. Such studies cannot be interpreted stoichiometrically because of our uncertain knowledge of the kinetics of disposition of the several agents that excite secretion of hydrochloric acid.

Davidson *et al* found that the concentration of gastrin required to increase acid secretion by the isolated bullfrog gastric mucosa is less by several orders of magnitude that that of histamine (5). Many years ago Babkin and coworkers failed to evoke acid secretion with histamine in the intact skate (6). I am reporting that though the isolated gastric mucosa of another elasmobranch, the spiny dogfish, does respond to histamine, the interstitial concentration of histamine eliciting maximal secretion of acid is so much that it is unlikely that an extracellular release of histamine mediates cholinergic excitation of acid secretion in this species.

Materials and methods. Dogfish *(Squalus acanthias)* were caught on a trawl line in Frenchman's Bay and held in live cars. In less than 10 minutes after removal of a fish from the sea its mucosa was mounted in a plastic chamber. The mucosa was separated as a flat sheet by dissection on a cold block. When placed in the chamber, the sheet of mucosa became two portions, each having an area of 2.9 or 3.5 cm^2 and whose surfaces were bathed by 20 ml of saline. Serosal surfaces were bathed by a solution having Na^+ 257, K^+ 10, Ca^{++} 10, Mg^{++} 4, Cl^- 240, HCO_3^- 30, $PO_4^{\equiv}$ 3, SO_4^+ 4 mEq/1, and 28 mM/1