been measured in the plasma of starved or hypophysectomized rats with lowered oxygen requirement after stimulation by hypoxia, cobalt, or anemia. Also, polycythemic rats have been submitted to hypoxia or cobalt. A reduced erythropoietin response was observed in hypophysectomized and starved rats after stimulation by hypoxia. The diminished response was not so striking after stimulation by cobalt, probably because the concentration of the cobalt in the plasma was higher in these groups. Response to cobalt and hypoxia was also reduced in polycythemic rats.

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Multiple Congenital Abnormalities in the Rat Resulting from Acute Maternal Niacin Deficiency During Pregnancy.* (28183)

JACK G. CHAMBERLAIN AND MARJORIE M. NELSON†

Department of Anatomy, University of California, Berkeley and San Francisco

Deficiencies of riboflavin, pantothenic acid, or pteroylglutamic acid, induced by antimetabolites of these vitamins during pregnancy, result in multiple congenital malformations in the offspring(1,2). This study reports the effects of a purified niacin-deficient diet containing the niacinamide antimetabolite, 6-aminonicotinamide (6-AN), on embryonic development. As niacinamide can be synthesized from dietary tryptophan(3,4), deletion of the vitamin from the diet may not affect significantly embryonic development in rats and certain other species(5,6,7).

Methods and materials. Rats (Long-Evans) averaging 100 days of age and 225 g in weight were bred with normal males. All rats were given a stock diet of natural food stuffs‡ and distilled water for drinking; lettuce was furnished twice weekly. Rats were weighed regularly and vaginal smears were examined daily. The morning of finding spermatozoa in the vaginal smear was considered day zero of pregnancy. Rats were transferred to individual cages and given a purified niacin-deficient diet containing 100 mg of 6-AN/kg of diet on days 7-9, 8-10, 9-11 or 10-13 of pregnancy. The animals were then trans-

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‡ Ground whole wheat 67.5%, casein 15%, skim milk powder 7.5%, sodium chloride 0.75%, calcium carbonate 1.5%, hydrogenated vegetable oil 6.75%, 1.0% fish oil (vit. A and D concentrate) and sufficient KI to furnish 0.9 μg iodine/g of diet.

TABLE I. Effects of a Purified Niacin-Deficient Diet Containing 6-Aminonicotinamide (100 mg/kg of Diet) During Pregnancy in the Rat.*

Days of pregnancy fed deficient diet		Rats bred, No.	Rats with living young, %	Living young day 21 of pregnancy: Total No.	Avg wt, g	Abnormal, No. (%)
7- 9	I †	8	100	39	3.8	9 (23)
	II	4	75	17	3.5	5 (29)
8-10	I	11	27	20	5.8	1 (5)
	II	7	30	6	4.0	6 (100)
9-11	I	5	100	37	5.1	2 (5)
	II	7	86	49	5.4	9 (18)
10-13	I	7	86	54	4.4	8 (15)
	II	5	100	42	3.9	7 (17)
Controls‡		12	100%	120	5.7	1 (<1%)

* Casein 24%, sucrose 64%, hydrogenated cottonseed oil 8%, salts 4%, crystalline vitamins/kg of diet: d-biotin 300 mg, 2 methyl-1, 4-naphthoquinone 5 mg, thiamine HCl 5 mg, pyridoxine HCl 5 mg, riboflavin 10 mg, p-aminobenzoic acid 10 mg, pteroylglutamic acid (PGA) 5.5 mg, d-calcium pantothenate 50 mg, inositol 400 mg, choline chloride 1.0 g, and 6-aminonicotinamide 100 mg. All rats received weekly a fat soluble vitamin mixture containing 800 USP units of vitamin A, 115 chick units of vitamin D, 6 mg dl-α-tocopherol and 650 mg corn oil (Mazola).

† Rats which promptly consumed diet and gained weight the day following transfer to the complete diet are designated Group I. Group II indicates those animals which did not show a gain in body wt the day after transfer to the complete diet, perhaps prolonging the period of niacin deficiency an additional day or two.

‡ Given complete diet days 7-21 of pregnancy. Composition similar to niacin-deficient diet except that 20 mg of niacinamide was added/kg of diet and 6-AN was omitted.

ferred to a complete diet similar in composition to the niacin-deficient diet except that 20 mg of niacinamide was added/kg of diet and 6-AN was omitted. Control rats were maintained on this complete diet from day 7 to 21 of gestation. Food intake was measured daily during the deficiency period and the amount of 6-AN ingested was calculated for each rat. Living young were removed, counted and weighed on the 21st day of pregnancy, one day before parturition. Implantation sites were counted and measured transversely. Fetuses were fixed in Bouin's fluid or in 95% ethanol and later examined for congenital malformations.

Rats who promptly consumed the diet and gained weight the day following transfer to the purified complete diet are designated Group I in the tables. Group II indicates those animals who did not show a gain in body weight the day after transfer to the complete diet, perhaps prolonging the period of niacin deficiency an additional day or two.

Results. Control rats gained an average of 115 g (range 91-164) during pregnancy, and consumed approximately 15-20 g of diet/day during the second week of gestation. At autopsy all control rats had living young, averaging 5.7 g (5.3-6.2) in weight. Of the 120 living young examined, one had a midline patent thyroglossal duct. On the basis of total implantation sites, less than 1% contained an abnormal or resorbed fetus (Table II). While on the niacin-deficient regimen rats lost an agerage of 19 (2-37) g in weight but gained an average of 84 g (71-93) during

TABLE II. Effects of a Purified Niacin-Deficient Diet Containing 6-Aminonicotinamide (100 mg/kg Of Diet) on Embryonic Development.*

Days of pregnancy fed deficient diet		Implantation sites: Total No.	Abnormal, %	Resorbed, %	Total affected, %
7- 9	I †	83	11	53	64
	II	45	11	62	73
8-10	I	103	1	81	82
	II	87	7	93	100
9-11	I	49	4	25	29
	II	71	13	31	44
10-13	I	73	11	26	37
	II	49	14	14	28
Controls		120	<1%	0%	<1%

* Based on total implantation sites observed at autopsy.

† See Table I for explanation of I and II.

pregnancy. Regardless of the deficiency period each rat consumed approximately 14 g (7-24) of diet, containing 1.4 mg (0.7-2.8) of 6-AN.

Days 7-9. All rats in Group I had living young on the 21st day of pregnancy but they averaged only 3.8 g (3.2-5.0) in weight. Nine fetuses (23%) were subsequently found to be abnormal. On the basis of total implantation sites, abnormal embryonic development occurred in 11% and embryonic death with resorption in 53%. Thus, 64% of the implantation sites were affected by the transitory deficiency during this period. Congenital abnormalities (Table III) included renal aplasia, hydroureter, hydronephrosis, retinal detachment and folding, microphthalmia, missing ossification centers, agenesis of the tail, gastroschisis, exencephaly, and marked kyphosis. Animals in Group II had fewer living young at autopsy. Abnormalities (*e.g.*, bilateral anophthalmia) also were more severe than in the previous group.

Days 8-10. Only 27% of the pregnant rats in Group I had living young at autopsy. Surviving fetuses averaged 5.8 g (3.0-6.0) in weight; one had hydrocephalus. Over 80% of the implantation sites showed embryonic mortality or contained an abnormal fetus at term. Group II. Virtually all implantation sites contained abnormal or dead embryos at autopsy. All 6 surviving fetuses were abnormal. Missing ossification centers, hydronephrosis, and hydrocephalus were found. Anophthalmia, when observed, was confirmed histologically; serial sectioning of the heads showed no evidence of lens or retina on either side.

Days 9-11. All rats in Group I had living young at term, averaging 5.1 g (4.5-5.6) in weight. Two (5%) abnormal offspring were observed. One-fourth of the implantation sites examined contained resorbed or dead embryos and 4% contained an abnormal fetus. Therefore, 29% of the implantation sites were affected, a significant increase when compared to that of control rats. Persistent developmental pathways of the thyroid (4%) and thymus (4%) were observed. Group II. Incidence of abnormalities and embryonic mortality increased significantly when com-

TABLE III. Types and Incidence of Congenital Malformations Observed in Day 21 Experimental Fetuses.

Days of pregnancy fed niacin-deficient diet containing 6-aminonicotinamide									
7-9		8-10		9-11		10-13			
I* (39) †	II (17)	I (20)	II (6)	I (37)	II (49)	I (54)	II (42)		
Kidney—19%	Eye—12%	Brain—9%	Skeleton—100%	Thyroid—4%	Kidney—11%	Brain—12%	Kidney—8%		
Trunk—12%	Kidney—5%		Eye—100%	Thymus—4%	Thyroid—11%	Cleft palate—9%	Thymus—8%		
Skeleton—5%	Thyroid—8%		Kidney—100%			Thyroid—8%	Limbs—4%		
Eye—5%	Thymus—8%		Brain—33%			Skeleton—7%	Trunk—2%		
Brain—4%						Eye—4%			
						Kidney—4%			

* See Table I for explanation of I and II.

† In parentheses—No. of fetuses examined.

pared to results in Group I (Table II). Of the 49 surviving young, 11% showed hydronephrosis or hydroureter and 11% had a patent thyroglossal duct.

Days 10-13. Only 86% of the rats in Group I had living young, averaging 4.4 g (3.2-4.9) in weight. Of 54 surviving fetuses examined, 8 (15%) were abnormal. On the basis of total implantation sites, abnormal embryonic development occurred in 11% and embryonic death with resorption in 26%. A total of 37% of the implantation sites, therefore, were affected by the transitory deficiency. Abnormalities included hydrocephalus, cleft palate, patent thyroglossal duct, lens vacuolation, hydroureter, and skeletal defects. Group II. Incidence of abnormal development and embryonic mortality was similar to that observed in Group I.

Discussion. A niacin-deficient diet containing 100 mg of 6-AN/kg of diet given to rats during the second week of pregnancy for periods of 48 or 72 hours had deleterious effects on embryonic development. Ingestion of 1-2 mg of the antimetabolite resulted in marked embryonic mortality, averaging 46% (25-81%), and a low incidence of abnormal young by day 21 of gestation. Regardless of when the transitory niacin deficiency was initiated, 29% to 82% of the implantation sites examined showed either embryonic death with resorption or contained an abnormal fetus. Incidence of embryonic mortality and abnormalities in 3 out of 4 experimental groups was increased further when rats did not eat upon being transferred to the complete diet. Days 8-10 appeared to be the period when embryos were most sensitive, as only 27% of the rats had living young at autopsy, and 82% of the implantation sites were resorbed or contained an abnormal fetus. Congenital abnormalities in fetuses removed from mothers on day 21 included defects of the skeleton, central nervous system, eye, urinary system, trunk, thyroid and thymus. Pregnant rats ate less and lost weight while on the diet containing the antimetabolite. However, within 1-2 days after being transferred to the complete diet, all pregnant rats progressively gained weight until autopsy. Fasting animals during the second week of pregnancy to produce a comparable loss of weight did not result in embryonic mortality or congenital malformations (unpublished data). Symptoms of niacin deficiency, induced by oral administration of 6-AN, can be prevented by providing adequate amounts of dietary niacinamide(8).

Embryonic abnormalities here reported to follow ingestion of 6-AN during the second week of pregnancy, the so-called "critical period" of development in the rat, were also observed after a single maternal injection of the niacin antimetabolite on any day during the third week of pregnancy(9). No typical pattern of abnormalities was seen as practically all systems were affected to some extent. The close resemblance between many of the anomalies resulting from a transitory niacin deficiency and those following other transitory vitamin deficiencies(1) lends support to the hypothesis that the timing of a teratogenic procedure is of more critical importance than the nature of a metabolic insult.

Antimetabolite 6-AN was teratogenic when injected into 24- or 96-hour chick embryos (10). It also produced embryonic abnormalities in the rat(11) and mouse(12,13) when given by single injection to mothers between the 10th and 12th day of pregnancy. The congenital abnormalities previously reported following injection of 6-AN include various skeletal defects, cleft palate and cleft lip, exomphalos and open eye. In all cases the maternal signs of niacin deficiency and fetal anomalies produced by 6-AN were prevented by simultaneous or prior administration of niacinamide or its precursors.

Summary. Pregnant rats were given a purified niacin-deficient diet containing the niacinamide antimetabolite, 6-aminonicotinamide (100 mg/kg of diet), on days 7-9, 8-10, 9-11 or 10-13 of pregnancy. Days 8-10 appeared to be the period of greatest sensitivity as 80% embryonic mortality or abnormality was observed at autopsy on day 21 of pregnancy. A high incidence of embryonic mortality with resorption and a low incidence of abnormal young were observed, regardless of when the diet was first instituted. Malformations in 21-day fetuses from mothers consuming 1-2 mg of 6-AN included defects of the skeleton, central nervous system and eye,

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Erythropoietic Effect of Red Blood Cell Components and Heme-Related Compounds. (28184)

JAMES R. BROWN, NOE ALTSCHULER* AND JOHN A. D. COOPER
(Introduced by Allen Lein)
Radioisotope Service, Veterans Administration Research Hospital and Department of Biochemistry, Northwestern University Medical School, Chicago, Ill.

Several investigators(1-3) have suggested that products of hemolyzed blood are a stimulus to red blood cell production. Other investigators, Verzar(4), Zih(5), and Bomford (6), established the erythropoietic action of bilirubin using increased red blood cell counts as a quantitative indicator. Further studies have not been done with the more quantitative techniques now available. The present work was undertaken to determine the possible involvement of red blood cell moieties in a feedback mechanism. This report presents data on the erythropoietic activity of lysed red blood cells from various species and on compounds that are either metabolically or structurally related to the heme moiety(7).

Materials and methods. Albino female rats of the Sprague-Dawley (Holtzman) strain weighing 150-250 g were used. For bioassay of erythropoietic activity, fasting "dehydrated" rats were generally used. Rats on a complete nutritional diet were used for comparison. Subcutaneous injections of the test material or control solution were given daily, for 3 days, to rats which had been deprived of food and water for 48 hours prior to the first injection. Water was provided *ad libitum* for 24 hours between the second and third subcutaneous injections. Food was withheld during the bioassay. There was no apparent local inflammatory response to injection of lysed red blood cells, hemin, or related compounds. Immediately after the animals had received the third subcutaneous injection, they were anesthetized lightly with ether and injected intravenously (*via* the tail vein) with 0.5 μc of Fe^{59}-citrate in 0.25 ml of physiological saline solution. The specific activity of the Fe^{59} iron ranged from 23.6 to 30.8 μc/μg. The maximum amount of iron administered to an animal was 1.6×10^{-2} μg.

Twenty-four hours after Fe^{59} injection, the tip of the tail was cut and blood was taken by capillary tube for micro-hematocrit and reticulocyte determinations. There was a distinct advantage in obtaining blood from the tail for these procedures since large numbers of animals were sacrificed. The animals were next anesthetized, the abdomen opened, and blood drawn from the abdominal aorta with an oxalate-rinsed syringe. Two ml were then measured into a plastic counting tube for counting in a well scintillation counter to per-

* Research Fellow of the Nat. Research Council of Argentina.