Forelimb Deformity of Offspring of Rats Given Dichlorphenamide During Pregnancy. (32352)

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(Introduced by K.-F. Benitz)
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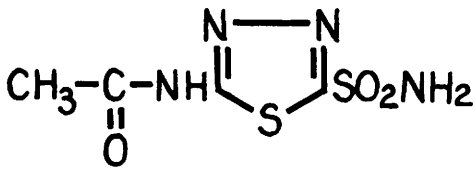
It has recently been shown that diets containing very high doses of the carbonic anhydrase inhibitor, acetazolamide, are teratogenic when given to pregnant rats and mice (1). The deformity is quite reproducible and specific, consisting of a defect of the postaxial portion of the right forelimb. This is the only abnormality found, except for about 10% of the deformed individuals, which have a bilateral forelimb deformity with a more severe defect on the right.

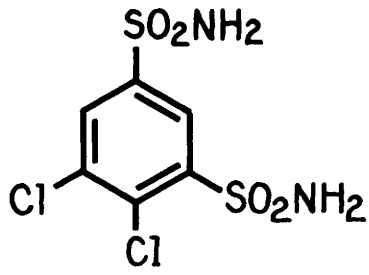
Since the only known pharmacological effect of acetazolamide is carbonic anhydrase inhibition, it seems to us that its teratogenic activity is due to this property. It has been pointed out, however, that the teratogenicity may be due instead, to the action of a metabolite of acetazolamide(2). To investigate this possibility, the experiments described here were done to find if another structurally unrelated carbonic anhydrase inhibitor, dichlorphenamide (Fig. 1) could produce this same specific forelimb deformity.

Methods. Sexually mature male and female Sherman rats from the Lederle colony were placed together in the late afternoon and the female was checked each morning for spermatozoa. On the morning these were found, the female was placed in a separate cage and given a diet consisting of powdered Purina Laboratory Chow to which dichlorphenamide, 0.6% by weight, was added. This was considered to be day 0 of gestation. Control rats were given Purina Laboratory Chow without added drug. Drug intake was calculated from measurements of body weight and food intake which were made every other day.

Two experiments were performed. In Experiment I, rats were treated throughout pregnancy and allowed to deliver spontaneously.



Acetazolamide



Dichlorphenamide

FIG. 1. Chemical structures of acetazolamide and dichlorphenamide.

The pups were examined as soon as possible after birth. Experiment II differed only in that the mother was killed on day 17, 18, or 19 of pregnancy and the fetuses were removed from the uterus for examination. This was done to avoid the problem of maternal cannibalism.

The fetuses and pups were fixed in Bouin's fluid, 10% formalin, or 95% ethanol. The formalin-fixed material was stained with methylene blue and cleared to demonstrate cartilage(3). The ethanol-fixed offspring were stained with alizarin and cleared to demonstrate calcified skeletal structures(4). The pups and fetuses were examined at 10× magnification while still alive, and again after

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TABLE I. Occurrence of Right Forelimb Deformity in Offspring of Rats Given Dichlorphenamide in Their Diet During Pregnancy.

Treatment group	Female No.	Maternal drug intake, mg/kg body wt/day	Offspring Deformed	Offspring Total
		Exp I		
Control diet	68	0	0	6
	79	0	0	7
	84	0	0	13
	98	0	0	9
	112	0	0	11
Total avg		0	0	46
Dichlorphenamide, .6% in diet	90	295	0	0
	93	490	0	8
	104	387	0	0
	106	211	4	11
	110	403	0	0
Total avg		357	4	19
		Exp II		
Dichlorphenamide, .6% in diet	DH 3	317	0	0
	DH 6	325	0	13
	DH 7	362	0	0
	DH12	374	0	10
	DH24	427	0	0
	DH29	322	2	7
	DH41	351	2	8
	DH42	336	0	0
	M16	350	0	0
	P12	335	0	0
Total avg		350	4	38

fixation and clearing.

Results. In both experiments there were deformed offspring from mothers which received dichlorphenamide in their diet (Table I). In Exp. I, 4 newborn rats from the same litter had a similar deformity, an absence of digits 4 and 5 from the right front foot. In Exp. II, 2 fetuses each from 2 treated litters showed a deformity of the right front foot. Two of the fetuses lacked digits 4 and 5 (Fig. 2). One of the remaining animals had digit 5 missing and the other had a defect of the web between digits 4 and 5. Dissection of the newborn rats and examination of cleared fetuses failed to reveal any other defects.

The pregnant rats given dichlorphenamide showed a considerable reduction in food consumption and lost weight. As had been found with acetazolamide, these were the only signs of toxicity seen in the mothers. Control animals showed a weight gain and an increased rate of food consumption during pregnancy. There was a reduction in the number of successfully mated rats found to be pregnant at the time of examination as compared with animals on the control diet. In Exp. II, this was found to be associated with a lack of evidence of implantation sites. All offspring, both newborn and fetuses, of treated and control mothers were alive at the time of initial examination.

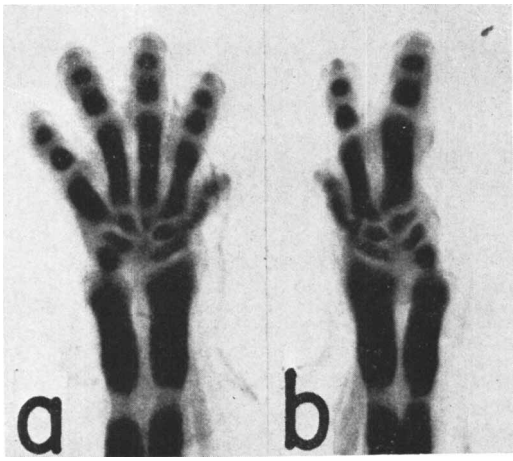

FIG. 2. Left (a) and right (b) forelimbs of 19-day rat fetus from mother given dichlorphenamide. Fourth and fifth digits missing on right. Cleared and stained with methylene blue to show cartilage.

Discussion. The right-sided postaxial defect of the forelimb described here has never been found to occur spontaneously in our rat colony, whether the rats are examined before or after birth. Although treated animals showed reduced food intake and weight loss during pregnancy, these factors alone have not been found to cause this deformity. We are not aware of any way in which this specific isolated deformity has been repeatedly produced except by the use of carbonic anhydrase inhibitors.

Recently, Wilson, Maren, and Takano(5) have described the production of a right-sided defect in the forelimb of the rat using high doses of ethoxyzolamide, another potent carbonic anhydrase inhibitor.

It is not clear whether the teratogenic effect of these carbonic anhydrase inhibitors is associated with enzyme inhibition in the mother, the placenta, or the embryo. It is not known when carbonic anhydrase first appears in embryonic development. Furthermore, the teratological effect may be produced by changes secondary to carbonic anhydrase inhibition,

e.g., acidosis.

This great difference in chemical structure of compounds capable of producing the same deformity is of interest in comparison with the findings of Wilson on the teratogenicity of azo dyes chemically related to trypan blue(6). Here, it was found that a total dose of 135 mg/kg of trypan blue produced 49% deformed fetuses, while the same amount of a closely related structural isomer, Evans blue, resulted in a deformity rate of only 14%.

It should be pointed out that the amount of dichlorphenamide (in mg/kg) used to produce this teratogenic effect is more than 100 times greater than that used in the treatment of human patients.

Summary. Administration of very high doses of dichlorphenamide, a carbonic anhydrase inhibitor, in the diet of rats during pregnancy is associated with the production, in some of the offspring, of a deformity consisting of a postaxial defect of the right forelimb. This is not associated with any other demonstrable anatomical abnormalities. This deformity appears to be the same as that caused by acetazolamide, a compound of quite different chemical structure which is also a potent carbonic anhydrase inhibitor.

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Received January 30, 1967. P.S.E.B.M., 1967, v126.

Effect of Hypervitaminosis D Upon the Phospholipids of Metaphyseal And Diaphyseal Bone.* (32353)

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Previous work has shown that the hypervitaminotic D state affects the quantity of lipids which can be extracted from the matrix of rat bone(1,2). Hypervitaminosis D increased the total lipids, phospholipids, triglycerides, cholesterol, cholesterol esters, and total fatty acids of the diaphyseal and metaphyseal long bones. The greatest change was in the phospholipid fraction. There is no information available in the literature on the nature of the phospholipids of long bones or on the mechanism of action of vitamin D in lipid metabolism. Consequently, the following experiments were carried out to determine which phospholipids were present, whether the effect of vitamin D on phospholipid metabolism is confined to a single phospholipid or concerns all major phospholipid components, and to ascertain whether the changes are the result of increased synthesis or decreased break-down.

Materials and methods. Male hooded rats of the R.V.H. strain averaging 110 g were maintained on a complete synthetic laboratory diet (Purina Labina). The hypervitaminotic D group received 15,000 international units of vitamin D_2‡ dissolved in sesame oil per 100 g of body weight daily. The control animals were dosed with an equal volume of sesame oil. Between days 14 and 18, 11 control

* Supported by Grant MA-1571 from The Medical Research Council of Canada, Grant AM-04071 from USPHS and U. S. Atomic Energy Commission Contract AT (30-1) 2530.

† Holder of USPHS Research Career Development Award 5-K3-AM-15 374-03.

‡ Vitamin D_2 (calciferol) kindly supplied by N. V. Phillips-Roxane Co. The vitamin D was dissolved in a small amount of absolute alcohol and diluted with sesame oil so that the concentration of vitamin D in sesame oil was 15,000 units/ml.