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The 1965 recipient of the Meltzer Award was Dr. Edmund H. Sonnenblick of the Cardiology Branch, National Heart Institute, National Institutes of Health. The selection was made by a Committee appointed by the President, consisting of Doctors Dwight J. Ingle, Lewis Thomas, and Maurice B. Visscher.

SECTION MEETINGS

JOINT MEETING DISTRICT OF COLUMBIA—MARYLAND	
University of Maryland	April 3, 1965
ILLINOIS	
Chicago Engineers Club	February 11, 1965
MARYLAND	
Johns Hopkins University	February 11, 1965
MINNESOTA	
University of Minnesota	January 22, 1965

Teratogenic Action in Rats of Reserpine Alone and in Combination with Salicylate and Immobilization.* (29990)

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A study of the teratogenic role of the metabolic consequences of salicylate poisoning has suggested that anomalies are induced by this agent on the tenth day of gestation in rats more through maternal systemic responses to its toxicity than through its direct effect on embryonal cells(1). Salicylate teratogenicity and toxicity are greatly potentiated by maternal immobilization ("immobilization effect") so that anomalies are produced by otherwise non-toxic, non-teratogenic doses of salicylate(2). Immobilization(3) and salicylate poisoning(4,5,6,7) each produce weight loss, stimulation of respiration, hormone release and changes in the size of adrenals, thymus and lymph glands. In salicylate poisoning, these responses are mediated through the central nervous system(6, 7). These observations suggest that the "immobilization effect" is at least partly the result of the activity of the maternal central nervous system. Our observation(8) that the "immobilization effect" is prevented by low doses of the central depressants, sodium pentobarbital and chlorpromazine, supports this hypothesis. Reserpine produces central de-

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pression(9) but also produces the systemic responses(10) common to teratogenic doses of salicylate and to immobilization. Therefore, reserpine might be expected to alter the "immobilization effect" differently than do the other central depressants. In the present report we have undertaken to study whether embryonic development or the "immobilization effect" are modified by reserpine. Further, since both reserpine(11) and salicylate (12) affect tissue catecholamines, we have also tested whether fetal development is affected by epinephrine.

Materials and methods. Virgin Sprague-Dawley females of the Charles River Caesarean-derived albino rat colonies 8 to 9 weeks of age and weighing from 185-215 g were mated by the supplier (Charles River Breeding Laboratories, Brookline, Mass.) according to the procedures described previously(1,2,8). For each experimental section of this report, approximately 60 timed-pregnant rats were received at one time in our laboratory on the 7th day and divided at random into the appropriate experimental and control groups. The animals in the first section were given a single intramuscular injection of varying doses of either reserpine or reserpine solvent on the ninth or tenth day. The animals in the second section were given a single intramuscular injection of either epinephrine base in peanut oil or peanut oil on the tenth day. In the immobilization experiments, the animals were injected intramuscularly with either reserpine, 1 mg/kg, or reserpine solvent 18 hours prior to immobilization on the tenth day and were injected subcutaneously with sodium salicylate immediately after the start of immobilization. The unimmobilized control groups were given the appropriate drugs or solvents by the same routes at the same intervals and were deprived of food and water for the same period that the experimental animals were immobilized. The animals were immobilized for 3¾ hours in a prone position as previously described(2). Throughout the experimental period the animals were kept 3 to a cage measuring 15 × 9 × 6.5 inches. The fetuses were delivered by Caesarean section on the 20th day.

The drugs used were: sodium salicylate, 100 mg/cc; reserpine (Serpasil, Ciba) 2.5 mg/cc and epinephrine in oil (1:500). The preparations were diluted with their solvent so that doses to be given were contained in a volume of 0.5 to 1 cc. Microradiographs were taken on ordinary radiologic film with G. E. low-voltage radiation equipment at 20 ma. and 17 KV.

Classification of results. The effects produced by each procedure were measured according to the following previously described (2) classification: maternal death: No. females dying after treatment; maternal morbidity: magnitude of average weight change day after treatment (normals gain about 7-10 g); fetal mortality: early resorption: No. females with embryos and placentae completely resorbed shortly after treatment showing yellow nodular implantation remnants in parametria at delivery, and late resorption: % conceptions resorbed with only placentae present at delivery and partially resorbed fetuses; fetal morbidity: inhibition of fetal growth in weight and length, and fetal abnormality: % anomalous fetuses of all those alive at delivery. Probabilities for significance of the differences obtained between groups given and not given either reserpine or epinephrine were calculated according to chi square analysis except for maternal weight change when Student's T test was used.

Results. With one notable exception described below, the anomalies produced by reserpine or epinephrine are similar to those produced by salicylate with or without immobilization as previously described(2) and consist of: encephalocele, cranioschisis, craniorachischisis, spina bifida, umbilical hernia, varying degrees of thoraco-abdominal eventrations, microphthalmia, exophthalmia, anophthalmia and various combinations of these defects. The type of anomaly shown in Fig. 1 occurred in the groups treated only with reserpine or epinephrine. These fetuses were shorter than the controls, lacked dorsal and lumbar spines and ribs, and occasionally they had a short, thin tail.

Reserpine at the doses used did produce some sedation. The animals, although not exerting much muscular activity, were responsive to stimuli. At the higher doses they

TABLE I. Maternal and Fetal Effects of Reserpine and Epinephrine.

Substance injected	Day injected	Dose, mg/kg	Maternal effects: Pregnant females, No.	Maternal effects: Death, No.	Maternal effects: Wt change day after inj, g	Fetal effects: Death: Females early resorption, No.	Fetal effects: Death: Ratio late resorptions to total No. conceptions	Fetal effects: Growth: W, g	Fetal effects: Growth: H, mm	Fetal effects: Morphology: Ratio live litters with abnormal fetuses to total No.	Fetal effects: Morphology: Normal No.	Fetal effects: Morphology: Abnormal No. and type§	Fetal effects: Morphology: Abnormal %
Reserpine solvent	9	.2 cc	27	0	+ 7	0	16:259	2.7	32	0:27	243	0	0
	10	.2 "	27	0	+11	1	11:257	2.7	33	0:26	246	0	0
Reserpine	9	.8	12	0	—21‡	5‡	13:79 †	2.3*	31	3:7‡	63	2 A, 1 P‡	5
	9	1.0	22	0	—19‡	11‡	19:104†	2.1*	29	4:9‡	67	16 A, 2 B†	21
	9	1.5	12	2‡	—26‡	6‡	3:46 ‡	2.2*	29	1:4‡	42	1 B†	2
	10	.1	35	0	+10	0	15:372	2.6	30	2:35	350	2 A	.6
	10	.8	23	0	—16‡	9‡	7:147	2.4	29	0:14	140	0	0
	10	1.0	15	0	—25‡	12‡	1:27	2.2‡	28	0:3	26	0	0
	10	1.5	15	10‡	—23‡	3‡	10:24 ‡	1.7‡	25	1:2‡	11	1 AE, 1 C‡ 1 E	22
	10	2.0	12	2†	—22‡	9‡	0:9	1.7‡	26	1:1‡	6	1 B, 1M‡ 1 A	33
Epinephrine solvent	10	.1 cc	12	0	+10	0	9:126	2.7	34	0:12	117	0	0
Epinephrine	10	.5	14	0	+ 8	0	13:155	2.3	29	1:14	142	1 C	.8
	10	1	12	0	— 2	1	14:123	2.4	30	3:12	105	2 C, 2 B†	4
	10	2	12	0	—14‡	3	11:87	2.3	28	0:12	76	0	0
	10	5	12	9‡	—11‡	2‡	—	2.0	28	0:1	13	0	0

* .05 > P > .04.
† .04 > P > .01.
‡ .01 > P > .001.

§ Code for anomalies: A, anophthalmia; B, absence of most of axial skeleton; C, cranioschisis; E, encephalocele; M, microphthalmia; P, cleft lip and palate.

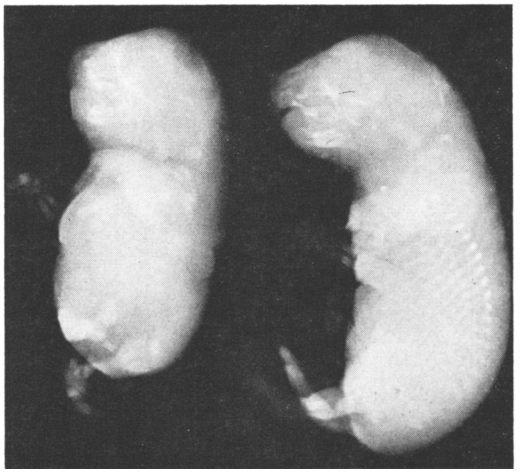

FIG. 1. Stunted fetus, lacking dorsal and lumbar spine and ribs, with a short, thin tail resulting from maternal administration of either reserpine or epinephrine (left). Normal litter mate (right).

showed toxic signs of diarrhea and ptosis which lasted up to one week. The effects of reserpine and epinephrine on the pregnant rats and their fetuses are given in Table I. Reserpine at higher doses produces highly significant maternal weight loss, fetal resorption, inhibition of fetal growth, ratio of live litters with abnormal fetuses, and percent abnormal fetuses. Although the percent of abnormal fetuses resulting at a dose of 0.1 mg/kg is not statistically significant, there were no abnormal fetuses occurring among all the 606 controls of these experiments. Epinephrine at 1 mg/kg (5 μM/kg) also produces highly significant maternal weight loss and a low but significant percentage of fetuses with anomalies similar to those produced by reserpine.

The effect of reserpine at 1 mg/kg on salicylate teratogenicity in immobilized and unimmobilized females is shown in Table II. The Table illustrates the "immobilization effect" by showing that immobilization increases the effect of salicylate at 400 mg/kg upon all parameters studied. Reserpine significantly elevates the maternal morbidity, fetal mortality, and teratogenicity of salicylate. In immobilized salicylate-treated animals pretreatment with reserpine results in a non-significant raising of maternal death rate and weight loss, but a highly significant increase in fetal mortality and anomaly rates. Consequently, it can be seen that reserpine markedly enhances salicylate toxicity and teratogenicity in both immobilized and unimmobilized animals.

Discussion. Previous studies on the effects of administration of reserpine during gestation have shown it to have an abortifacient action but not a teratogenic one(13,14). The present results show that single doses of reserpine produce a significant per cent of abnormalities on the ninth or tenth days in rats in proportion to dose. Reserpine pretreatment greatly enhances salicylate teratogenicity and toxicity while pretreatment with another tranquilizer, chlorpromazine, does not appreciably affect salicylate teratogenicity or toxicity(8). Further, the present report shows that the augmentation of salicylate teratogenicity and toxicity by maternal immobilization, which is prevented by chlorpromazine and sodium pentobarbital(8), is markedly potentiated by prior treatment with reserpine. Thus there is a divergence in effects of reserpine and chlorpromazine on salicylate teratogenicity in immobilized animals. It has also been observed that chlorpromazine, unlike reserpine, does not produce depletion of catecholamines in the brain(15, 16,17). Accordingly it is tempting to seek a connection between the potentiation of salicylate teratogenicity by reserpine and the effects of depletion of brain catecholamines.

Epinephrine injected directly into rat fetuses on the 17th day has produced necrosis of extremities but no teratogenic effect has been demonstrated by parenteral administration at earlier gestational ages(18,19). Epinephrine placed on the chorioallantoic membrane of chicks produces cephalhematomas and hemorrhage of the distal extremities, which effects are prevented by prior treatment with chlorpromazine(20). The present findings show that large doses of epinephrine have a slight teratogenic action similar to that of salicylate and reserpine. The mechanism of this teratogenicity is unclear but the fact that the hitherto unreported anomaly of fetuses lacking most of their axial skeleton is produced by both epinephrine and reserpine suggests that there is a teratogenic pathway common to these two agents. Such a pathway might involve the effects of deple-

TABLE II. Effects of Reserpine on Toxicity and Teratogenicity of Salicylate.

Group	Procedure: Substance injected	Day	Dose, mg/kg	Maternal effects: Gravid females, No.	Death, No.	Wt change day 11, g	Fetal effects: Death: Females early resorption, No.	Death: Ratio late resorptions to total No. conceptions	Growth: W	Growth: H	Morphology: Ratio litters with/without abnormal fetuses	Morphology: Normal No.	Morphology: Abnormal No. and type§	Morphology: Abnormal %
Immobilized 3¾ hr day 10	R	9	1.0	15	1	—27	10	27:33*	1.8	23	2:2	0	6 A‡ 1 RNT	100
	S	10	400											
	RS	9	.2 cc	13	0	—21	7	28:58	1.7	23	4:6	19	3 C, 3 RN, 1 RH, 2 R, 2 A	37
	S	10	400											
Unimmobilized	R	9	1.0	12	0	—35†	8	38:46‡	1.6	21	1:1‡	2	2 A, 4 S†	75
	S	10	500											
	RS	9	.2 cc	12	0	—24	5	19:77	1.8	26	5:7	40	5 C, 1 CS, 3 R, 1 ARN, 1 RN, 1 S, 4 A 1 AS, 1 AT	31
	S		500											
	R	9	1.0	10	0	—25	5	15:57	2.0	28	1:5	34	6 A, 2 B	19
	Na	10	1 cc											
	RS	9	.2 cc	12	0	0	1	2:102	2.1	28	3:11	90	7 A, 1 C, 2 AE	10
	S	10	400											

* .04>P>.02
† .02>P>.01
‡ .01>P>.001

R = Reserpine
S = Salicylate
RS = Reserpine solvent
Na = Saline

§ Code for anomalies: A, anophthalmia; B, absence of most of axial skeleton; C, cranioschisis; E, encephalocele; H, intestinal hernia; N, absent abdominal musculature; R, craniorachischisis; S, spina bifida; T, absent thoracic musculature.

tion or of storage blockade of tissue catecholamines.

Summary. Large doses of reserpine administered to pregnant rats on the ninth or tenth days of gestation are highly teratogenic. Pretreatment with reserpine significantly increases salicylate teratogenicity and augments the effect of immobilization on salicylate teratogenicity. A low, but significant teratogenicity also results from epinephrine administration on the tenth day. The types of anomaly produced by all these agents are similar except that reserpine or epinephrine also produce the anomaly of stunted fetuses lacking most of the axial skeleton. These findings suggest that disturbed catecholamine metabolism may be an important teratogenic factor.

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Some Biological Properties of a Urinary Norethynodrel Metabolite.* (29991)

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Some biological properties of a urinary metabolite of norethynodrel. The biological properties of norethynodrel and norethindrone have been extensively investigated(1, 2). This laboratory has been interested in the chemical nature and biological properties of metabolites of norethynodrel and norethindrone. The estrogenicity of norethynodrel has been reported to be 3-7% that of estrone (2). The extent to which this estrogenicity is due to aromatization to phenolic estrogens has been a subject of discussion.

Paulsen *et al*(3) bioassayed urines of patients receiving both norethindrone and norethynodrel and observed high estrogenic potency which they claimed to be due to the metabolites of the parent substances and not to the presence of ethynyl estradiol-3-methyl ether in the administered compounds. The present communication presents some biologi-

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