those of other strains(8). Similar lesions have been reported to occur in response to irradiation as well as to a variety of other agents (see 6). Hence, the specificity of the disease is highly uncertain, at least without more refined study. The possibility that it may denote an autoimmune lesion deserves consideration (see 9). The role of virus in the etiology of the lesion is being explored in view of its induction by leukemia virus preparations of diverse types and origins.

Summary. Intercapillary glomerulosclerosis was found in mice of the RF strain bearing passaged myeloid leukemia whether these mice developed the leukemia from inoculation with leukemic cells or subcellular materials. The glomerular lesions occurred as early as 2 weeks after inoculation in mice with rapidly developing leukemia. The glomerulosclerosis was indistinguishable histologically from that induced by irradiation or that developing spontaneously in aging control animals.

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Prevention of Salicylate Teratogenicity in Immobilized Rats by Certain Central Nervous System Depressants.* (29009)

ALLEN S. GOLDMAN† AND WILLIAM C. YAKOVAC (Introduced by T. N. Harris)
Department of Pathologic Anatomy, Children's Hospital of Philadelphia and Departments of Pediatrics and Pathology, School of Medicine, University of Pennsylvania

Congenital malformations have been produced in the offspring of pregnant rats by single injections of large doses of sodium salicylate on the 10th day of gestation(1). An investigation of the teratogenic role of the known metabolic consequences of salicylate poisoning has suggested that this agent is dysembryogenic more through the systemic responses of maternal central nervous system poisoning than through its direct embryonic cytotoxicity(2). The teratogenicity of salicylate has been shown to be enhanced quantitatively by maternal immobilization so that anomalies are caused by otherwise non-toxic, non-teratogenic doses of salicylate ("immobilization effect")(3). Either immobilization (4) or salicylate poisoning(5,6,7,8) produces systemic responses characteristically seen following environmental stress, *i.e.,* weight loss, eosinopenia, stimulation of respiration, hormone release, and changes in size of adrenals, thymus and lymph glands. In salicylate poisoning, these responses have been shown to be mediated through the central nervous system(7,8). Therefore, immobilization may enhance salicylate teratogenicity by augmentative effects on the maternal central nervous system. In the present report, this hypothesis was tested by determining whether the "immobilization effect" can be prevented by the central nervous system depressants, sodium pentobarbital and chlorpromazine.

Materials and methods. Virgin Sprague-Dawley females of the Charles River Caesarian derived albino rat colonies 8 to 9

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† Fellow of USPHS.

weeks of age and weighing from 185-215 g were mated by the supplier (Charles River Breeding Laboratories, Brookline, Mass.) according to procedures described previously (2,3). In each of the 4 experiments described here, approximately 60 timed-pregnant rats were received in our laboratory on the 7th day and divided at random into 2 experimental (immobilized) and 3 control (unimmobilized) groups. The experimental animals were injected intramuscularly with either a central depressant, sodium pentobarbital or chlorpromazine in solvent, or solvent alone 1 hour prior to immobilization and were injected subcutaneously with sodium salicylate 1 hour 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 experimental animals were immobilized in a prone position on a modification(3) of a device described by Renaud(9). The animals given 400 mg/kg sodium salicylate were immobilized for 3¼ hours. The length of immobilization of the animals receiving 300 mg/kg salicylate was increased to 4½ hours in an attempt to produce a significant frequency of anomalies at this lower dose. Throughout the experimental period the animals were kept 3 to a cage measuring 15 × 9 × 6.5 inches. The fetuses were delivered by Caesarian section on the 20th day and examined grossly.

The drugs used were: sodium salicylate, dissolved in distilled water, 100 mg/cc; sodium pentobarbital, dissolved in a mixture of 10% alcohol and 20% propylene glycol in distilled water; and chlorpromazine, dissolved in saline, 25 mg/cc. The central depressant preparations were diluted with saline so that the dose to be given, 40 mg/kg sodium pentobarbital or 2 mg/kg chlorpromazine, was contained in a volume of 0.5 to 1 cc.

Classification of results. The effects produced by salicylate with and without immobilization were measured according to the following previously described(3) classification: ***maternal death:*** No. females dying after treatment; *maternal morbidity:* magnitude of weight change day after treatment (normal gain about 7 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 experimental groups given and not given a central depressant were calculated according to chi square analysis except for maternal weight change when student's T test was used.

Results. The anomalies produced by salicylate with or without immobilization as previously described(3) consist of: encephalocele, cranioschisis, craniorachischisis, spina bifida, umbilical hernia, varying degrees of thoraco-abdominal eventrations, microphthalmia, exophthalmia, anophthalmia and various combinations of these defects. Pretreatment with either central depressant did not show significant changes in the types of anomaly produced in any given situation.

The dose of pentobarbital used produced sedation with relaxed muscle tone for about 6 hours in both immobilized and unimmobilized animals. These animals could be aroused with strong stimuli. Chlorpromazine at the dose used did not produce sedation. These animals, although exerting somewhat less muscular activity, were as alert and responsive as animals not receiving chlorpromazine.

The results of these experiments are presented in Table I. The results obtained in 2 separate experiments upon the unimmobilized control groups were not significantly different and are presented as averages.

Neither chlorpromazine nor pentobarbital alone had an effect on mother or fetus. The maternal and fetal morbidity and teratogenicity of salicylate in unimmobilized animals are not significantly altered by either central depressant. The Table shows that immobili-

TABLE I. Effects of Central Depressants on Toxicity and Teratogenicity of Salicylate.

Procedure				Maternal effects			Fetal effects: Death		Fetal effects: Growth		Fetal effects: Morphology			
Group	Substance inj	Dose (mg/kg)	Length immobilization (hr)	Gravid females No.	Death No.	Wt change day 11 (g)	Females early resorption No.	Ratio late resorptions to total No. conceptions	W (g)	H (mm)	Ratio litters with/without abnormal fetuses	Normal No.	Abnormal No.	Abnormal %
Immobilized	P S	40 400	3¼	10	0	− 7	1	7:88 *	2.3	30	2:9 *	73	8‡	10
	PS S	.5-1 cc 400	3¼	10	1	−11	3	11:56	1.9	23	2:6	32	13	29
	P S	40 300	4½	13	0	− 4‡	0	13:130	2.3	30	0:13‡	123	0‡	0
	PS S	.5-1 cc 300	4½	12	0	−13	2	15:96	2.2	25	6:10	62	19	24
	C S	2 400	3¼	12	1	+ 7‡	1*	10:104	2.1	29	1:10‡	89	5‡	5
	Na S	.5-1 cc 400	3¼	12	4	−12	4	8:44	1.9	23	4:4	26	10	28
	C S	2 300	4½	11	0	− 6‡	1	13:102	2.4	29	0:10*	89	0†	0
	Na S	.5-1 cc 300	4½	11	0	−14	0	23:118	2.5	29	4:11	87	8	9
Unimmobilized	PS S	.5-1 cc 500	—	24	0	− 9	1	29:245	2.2	26	15:23	155	51	24
	Na S	.5-1 cc 400	—	22	0	− 6	1	17:225	2.1	28	4:21	197	11	6
	PS S	.5-1 cc 300	—	11	0	+ 2	0	12:103	2.4	29	0:11	91	0	0
	Na S	1 cc 300	—	10	0	− 4	0	7:106	2.5	30	0:10	99	0	0
	P H_2O	40 .5-1 cc	—	5	0	+ 3	0	4:60	2.2	30	0:5	56	0	0
	C H_2O	2 .5-1 cc	—	11	0	+ 9	0	3:118	2.2	30	0:11	115	0	0
	P S	40 500	—	24	0	− 5	4	45:219	1.8	25	18:20	134	40	23
	C S	2 400	—	22	0	− 2	0	32:213	2.2	29	3:22	181	5	3

* .04>P>.02 † .02>P>.01 ‡ .01>P>.001

P: Pentobarbital S: Salicylate C: Chlorpromazine PS: Pentobarbital solvent Na: Saline

zation increases the effect of salicylate upon maternal and fetal death and morbidity, the proportion of live litters with anomalous young, and the total anomaly rate.

Pretreatment with either pentobarbital or chlorpromazine results in a significant lowering of the maternal weight loss produced by salicylate with immobilization. Although not significant, the numbers of maternal deaths and degree of fetal growth inhibition are lower. Either central depressant also produces a very significant lowering of fetal anomaly rates and of the proportion of live litters with anomalous young. As can be seen in the Table, the dose of 300 mg/kg salicylate produces no anomalies in unimmobilized animals. Immobilized animals receiving this dose of salicylate and pretreatment with only central depressant solvent produce 19% anomalous offspring (27:149) in 10 out of 21 litters; while no anomalies result in the immobilized group given this dose of salicylate and pretreated with either central depressant. Consequently, these central nervous system depressants provide striking protection from the "immobilization effect."

Discussion. An enhancement has been reported in the anomaly production of teratogenic doses of Vit. A by immobilization(10) and of teratogenic doses of trypan blue by noise stimulation(11). Although it has been pointed out that immobilization may achieve its effects through excessive neuromuscular exertion(12), Hartel and Hartel(10) have concluded that immobilization acts as an emotional stress in its enhancement of hypervitaminosis-A teratogenicity. This interpretation is supported by our observation that equal protection is achieved both with the complete muscular relaxation obtained in pentobarbital anesthesia and with the alert state accompanied by only slight muscular relaxation produced by chlorpromazine.

The predominant action of pentobarbital is generally accepted to be depression of the transmission of sensory impulses through the ascending reticular activating system(13). Chlorpromazine is believed to affect similarly the lower centers of the brain, including the reticular activating system, basal ganglia, and sympathetic centers of the hypothalamus (14). The present study has demonstrated that prior treatment with either of these drugs prevents the augmentation of salicylate effects by maternal immobilization. These observations are cogent evidence that in immobilized animals given otherwise nonteratogenic doses of salicylate the production of anomalies is mediated through the maternal neural centers.

Summary. Prior treatment of pregnant rats with the central nervous system depressants, sodium pentobarbital or chlorpromazine, prevents the augmentation by maternal immobilization of the maternal morbidity and teratogenic action induced by sodium salicylate on the tenth day of gestation. The prevention by either central depressant of the production of congenital malformations in immobilized animals given otherwise nonteratogenic doses of salicylate appears to be mediated through the maternal central nervous system.

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