Toxicity of Progesterone in the New-Born Mouse.* (19471)

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(Introduced by J. H. Burchenal.)

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During a systematic study of the effect of adrenocortical and related steroids on the new-born mouse, progesterone, a relatively innocuous steroid in mature mice, was found to be unusually toxic. Symeonidis(1,2) had previously shown that mice and rats were remarkably susceptible to progesterone during the last trimester of pregnancy; progesterone inducing fetal death with resorption or stillbirths and, in some instances, the mothers died with histological changes suggestive of eclampsia. It was, therefore, of interest to determine the toxicity of progesterone in new-born mice, and to find the age at which the mice developed tolerance to this steroid.

Materials and methods. A variety of pure mouse strains and hybrid mice were used. Pregnant mice were placed in separate cages during the last few days of pregnancy, and examined daily. The mice usually littered during the night, and the new-born found each morning were classified as less than 24 hours old. The mice were divided into age groups of 1) less than 24 hours, 2) 25-48 hours, 3) 49-72 hours, 4) 73-120 hours, 5) 121-168 hours, for injection. Mice in each litter were divided usually into a control and a treated group. Injections of progesterone† were made with a No. 25 needle under the skin of the back, and a special effort was made to prevent leakage. The usual volume was 0.05 cc, but in older mice 0.10 cc was given. The control mice received an equivalent volume of physiological saline. The mice were examined and weighed daily, and usually discarded 10 days after injection. A total of 89 litters consisting of 488 new-born mice was used. In order to confirm the reported toxicity of progesterone during the last third of pregnancy, conspicuously pregnant mice (usually 16-18 days of gestation) were injected with progesterone subcutaneously, and the size of the fetuses and the delivery date were used as evidence for the age of the fetuses when the mother was injected.

Results. Suckling mice. Table I summarizes the toxicity data on the mice injected with progesterone and their litter mate controls. The new-born mice were unusually susceptible to progesterone, with the LD_{50} estimated at 0.1 mg/mouse. Mice obtained immediately after delivery succumbed to 0.02 to 0.05 mg/mouse. It was often difficult to draw definite conclusions from the work with very young mice, since disturbing the litter or intoxicating half the litter sometimes alienated the mother, so that the litter was not cared for and all the mice died, including the untreated ones. Results could be regarded as significant only when the control mice survived. The tolerance of the mice to progesterone increased rapidly, as shown in Fig. 1, so that at 72 hours the LD_{50} was about 20 times the 24-hour figure. Resistance then developed at a relatively slower rate, but at 7 days the LD_{50} dose on a mg/kg basis showed a 40-fold increase over the LD_{50} dose in the new-born mouse.

Following a dose in the range of the LD_{50}, the progesterone-treated mice became lethargic, feeble and failed to nurse. The mice injected soon after birth died within 24 hours or survived, whereas the older mice were sometimes prostrated and continued to lose weight for 3-4 days before death. Occasionally the seriously intoxicated mouse recovered after 2 to 3 days and began to nurse and gain weight.

Progesterone appeared to be somewhat spe-

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† Progesterone was made available through the courtesy of the Schering Corp. and was prepared in aqueous suspension of 200 mg/cc; this was appropriately diluted with saline before use.

TABLE I. Approximate LD_{50} of Progesterone in Suckling Mice at Various Ages.

Age at inj., hr	Dose, mg/mouse	Treated: No. inj. Litters	Treated: No. inj. Mice	Treated: Avg wt at inj., g	Treated: % survival, 5 days	Control: No. mice treated	Control: % survival, 5 days	Estimated LD_{50} of progesterone: mg/mouse	Estimated LD_{50} of progesterone: mg/kg
0–24	.05	2	6	1.4	100	6	100	.1	70
	.10	9	29		52	26	77		
	.20	5	17		12	10	90		
	.5–1	4	12		0	15	100		
25–48	.30	2	10	1.7	90	—	—	.4	235
	.50	6	19		38	15	94		
	1	3	11		18	5	60		
	1.5–2	3	9		0	6	100		
49–72	1	4	10	2.1	90	7	72	2.5	1200
	2	9	25		80	17	88		
	3	4	12		25	6	100		
	5	2	6		0	6	66		
73–120	2	1	3	2.5	100	3	100	4	1600
	5	10	32		22	3	100		
	10	4	12		0	6	100		
121–168	5	3	9	3.3	78	9	100	9	2700
	10	15	104		45				
240	10	3	22	4.8	91	—	—	>10	>2100

cific in inducing acute toxicity in new-born mice, since the injection of 1 mg doses of cortisone acetate, 17 hydroxy-pregnenolone, desoxycorticosterone acetate and Δ^5 pregnenolone did not induce acute deaths regularly during the first 24 hours after birth. The activity of these steroids can not be compared quantitatively with that of progesterone, since they were each suspended differently in saline, and slower absorption from the injection site probably diminishes their acute toxicity.

Pregnant mice. Five mg of progesterone injected subcutaneously during the 16th to 20th day of gestation caused fetal death in 6 of 8 mice, and 10 mg given to 2 mice caused fetal death in both cases. This observation confirms the detailed report by Symeonidis(1).

Discussion. Symeonidis(2) has presented the following possible explanations for the lethal effect of progesterone on the fetus and occasionally the mother during the last third of pregnancy in the mouse and rat: 1) progesterone induces a hormonal imbalance, 2) it directly damages the embryo and placenta, resulting in substances toxic to the mother, 3) it has a direct toxic action on the maternal tissues. The marked susceptibility shown by the new-born mouse to progesterone may be interpreted as supporting the second hypothesis, that progesterone is acting directly on the fetus in causing fetal death, and maternal toxicity is secondary to fetal injury. A dose of 5 mg of progesterone in a 35 g pregnant mouse, if evenly distributed, would give a concentration of 0.15 mg/g of tissue, which is well above the dose that would be lethal if injected into the new-born mouse. Five mg of progesterone, when given during the first and second thirds of pregnancy is not injurious to the fetus or mother, indicating that the susceptibility to progesterone appears during an advanced stage of embryonic development.

The mechanism whereby the new-born mouse develops resistance to progesterone shortly after birth is not known. Selye(3) has found that certain steroids have anesthetic properties in the rat, and this activity is enhanced by partial hepatectomy. Progesterone exhibited a high degree of anesthetic activity by this test. It seems reasonable to suggest that the toxic action of progesterone in the new-born mouse is due to its anesthetic action, as indicated by the behavior of the mice. As specific systems develop in the mouse, possibly in the liver, the ability of the mouse to cope with progesterone rises rapidly. The

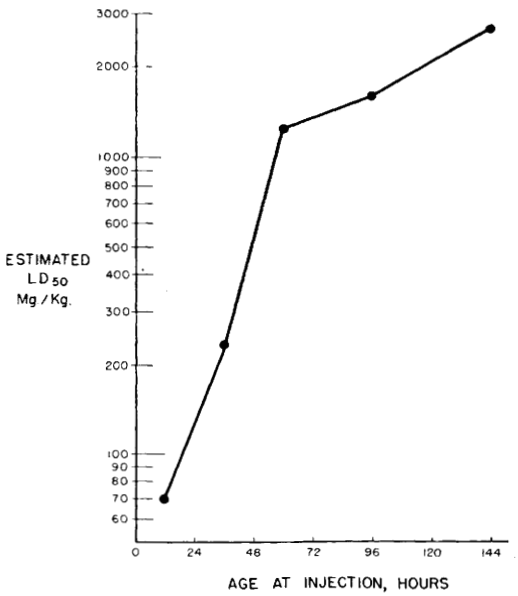

FIG. 1. Rise in tolerance of the baby mouse to progesterone with increasing age.

method of progesterone detoxification and catabolism is poorly understood(4). The data obtained on the development of resistance to progesterone within 2 to 3 days after birth may lead to an understanding of the mechanism whereby progesterone is inactivated or excreted.

Summary and conclusions. The new-born mouse is highly susceptible to the toxic and lethal action of progesterone but, within 2 to 3 days, resistance develops rapidly, and by the 7th day the mouse can tolerate about 100 times as much progesterone as when one day old. Closely related steroids, at equivalent and even higher doses, do not cause the acute lethal action of progesterone in the new-born mouse. The report that progesterone given during the last third of gestation will cause fetal death has been confirmed, and it is suggested that this is due to a direct toxic effect of progesterone on the fetus. The rapid development of resistance to progesterone within a few days after birth suggests that a system or mechanism has appeared which is capable of detoxifying, catabolizing, counterbalancing or accelerating the excretion of progesterone.

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3. Selye, H., *Endocrinology,* 1942, v30, 437.

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An Evaluation of the Role of Ferritin (VDM) in Traumatic Shock.* (19472)

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Introduction. In studies of shock, a correlation has been noted between irreversibility of the syndrome and a phase of hyporeactivity of the finer vessels of the exteriorized omentum and mesentery(1,2). The latter has been attributed to a blood-borne vasodepressor material (VDM) from ischemic liver, etc., which has been identified as a form of ferritin(3-5). The inference has been made that a release of ferritin occurring during shock contributes to the syndrome by rendering vessels unreactive (3). Few other vascular sites, however, have been demonstrated to react to VDM the way the mesoappendix does. The present experiments were designed to test whether 1) increasing the concentration of ferritin in tissues or 2) removing it from the circulation affects the ability of rats to survive after shock-producing trauma.

Materials and methods. Ferritin was crys-

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