Protective Effect of Chlorpromazine Against Endotoxin-Induced Abortion. (29404)

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Injection of sublethal quantities of bacterial endotoxins can interrupt pregnancy(1, 2,3). This effect has been reported to be unaltered by cortisone and by chlorpromazine(1,4) even though these agents can protect animals very efficiently against the lethal action of lypopolysaccharides (LPS)(5,6). Little is known about the manner in which LPS causes stillbirth. This phenomenon, which does not seem to be mediated by an endocrine mechanism(4), may be triggered by the injury of the ovum (fetus or placenta), or by direct action on the uterus (3,4,7).

We have studied this problem by measuring the toxicity and the distribution of a chromium labeled endotoxin injected directly into the fetus and by investigating the influence of cortisone and chlorpromazine on endotoxin-elicited abortion.

Materials and methods. The endotoxin was extracted by Boivin's method from *Salmonella enteritidis*, strain *Danysz*. This product was labeled with Cr^{51} (sodium chromate) and sedimented by ultracentrifugation at 40,000 rpm for 8 hours according to a modification of Braude's technique(8). Swiss mice between the 16th and 18th day of pregnancy were used in all experiments. These females were selected by palpation, 2 weeks after having been isolated from males with which they had been previously kept during 3 days.

In experiments involving laparotomy (Table I), endotoxin was injected by the transuterine route in a volume of 0.05 ml into the peritoneal cavity of fetuses approximately 18 days old. To counteract the uterine irritability resulting from such manipulation, 1 mg of progesterone was injected subcutaneously into the mother twice, 1 and 24 hours before endotoxin administration. Preliminary experiments showed that this progesterone treatment does not alter the response to the abortive effect of 1 μg of endotoxin injected intravenously to the mother. Therefore these progesterone treated mice were distributed in 4 groups. In 3 groups, 2 fetuses of each litter were injected either with saline, with 1 μg of LPS or with 10 μg of LPS per fetus; mothers of the fourth group received 20 μg of endotoxin intravenously. Six hours later, the fetuses were checked for survival, the criteria being their motility and their heart beat. These fetuses were then separated from their placentas and sectioned at the level of the diaphragm so as to control the presence and the distribution of the labeled material. Radioactivity of the blood and of various organs was measured in well-type counter according to previously published techniques(8).

In other experiments (Table II), the pregnant mice were injected intravenously in a volume of 0.25 ml with LPS, serotonin (5 HT) or histamine at different doses. The abortive effect of these compounds was checked by sacrificing the mother and ascertaining the survival of the fetuses 24 hours later, except in one experiment in which the litters were checked 3 hours after injection of 10 μg of LPS (Table II). Cortisone acetate was injected subcutaneously in amounts of: a) 50 mg/kg one hour before, or b) 125 mg/kg seven hours before LPS or 5-HT. Chlorpromazine, when used, was injected by the same route 30 minutes before 5-HT and 2 hours before LPS. It was less effective when injected only 30 minutes before the lypopolysaccharide.

Results. 1. Administration of radioactive endotoxin by transuterine route into fetus as compared with intravenous injection of mother. Radioactivity measurements showed that the endotoxin, when administered by transuterine route, was well distributed and had not crossed the placental barrier. Seven

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TABLE I. Toxicity of Endotoxin Injected into Fetus of Progesterone Treated Mice.

Type of injection	No. of pregnant mice	Injected fetuses	Non-injected fetuses	Total
Saline to the fetus	18	4/36*	1/89	5/125
LPS 1 μg "	18	8/36	0/82	8/118
LPS 10 μg "	9	16/18	7/50	23/68
LPS 20 μg to the mother	18	—	72/89	72/89

* No. dead fetuses/total after 6 hr.

TABLE II. Effect of Chlorpromazine on Endotoxin-Induced Abortions in Mice.

		Cortisone acetate		Chlorpromazine	
	Controls	50 mg/kg	125 mg/kg	2.5 mg/kg	5 mg/kg
Endotoxin 10 μg	15/18* (48/71)	—	—	—	1/10* (1/67)
" 1 "	26/39 (96/183)	12/17 (48/81)	5/7 (20/34)	3/11 (15/100)	6/22 (13/111)
" 2 "	35/40 (203/252)	—	—	7/30 (25/91)	5/10 (21/65)
" 10 "	18/18 (106/109)	10/10 (75/79)	8/8 (53/59)	12/12 (35/52)	11/17 (62/108)
Serotonin 1 mg†	17/18 (152/163)	14/14 (95/109)	9/9 (59/59)	—	4/10 (6/49)
" 1 "	8/9 (47/72)	—	—	—	3/14 (5/103)
" 2 "	8/10 (54/71)	—	—	—	1/7 (9/59)
Histamine 2 mg	1/15 (2/124)	—	—	—	—
" 3 "	0/7 (0/58)	—	—	—	—
" 5 "	0/10 (0/92)	—	—	—	—
Saline		—	7/7 (68/68)	—	10/10 (49/49)

* No. abortions/total checked after 3 hr; in all other cases, No. abortions/total checked after 24 hr. The values in parentheses = No. dead fetuses/total.

† Injected subcutaneously. In all other cases, serotonin as well as endotoxin, histamine and saline were injected by i.v. route.

per cent of the labeled material was found in the placenta. The remaining radioactivity was equally divided between the cephalic and caudal portions, indicating that the injected material had entered into the fetal circulation and had remained there, since no radioactivity was found in the maternal blood or liver even when as much as 10 μg of LPS had been administered.

Whereas 20 μg of LPS injected into the mother induced a high percentage of stillbirths, 1 μg administered directly into the fetus was almost as well tolerated as saline (Table I). Ten μg of LPS administered to the fetuses resulted in the death of 90%, while only 14% of the non-injected fetuses of the same litter succumbed. It must be kept in mind, however, that the latter dosage represents a very large amount of endotoxin.

2. Influence of chlorpromazine and cortisone on endotoxin-induced stillbirths. In our laboratory, the LD_{100} values for non-pregnant mice injected intravenously are as follows: 300 μg for endotoxin, 6 mg for serotonin and 8 mg for histamine. Although susceptibility to endotoxin is increased during gravidity, in the following experiments none of the pregnant mice died.

One μg of LPS administered to the mother causes 67% abortions 24 hours later. Ten μg of LPS causes 100% of stillbirths within the same period, and 83% within 3 hours (Table II).

One mg of 5-HT causes abortion whereas 5 mg of histamine do not interrupt pregnancy even though animals are seriously shocked by the latter treatment.

Cortisone acetate does not protect pregnant mice against abortions induced by 5-HT or even small (1 μg) amounts of LPS. This hormone has no harmful effects by itself: all the litters were alive 24 hours after injection of 125 mg/kg to the mothers which had received only saline.

Chlorpromazine protects mice against the abortive effects of serotonin and endotoxin. This protection is pronounced following injection of 1 or 2 μg of LPS but weaker against 10 μg. Nevertheless, even in the latter case, only 1 fetus out of 67 died

during the first 3 hours, while 48 out of the 71 controls died during this period. Since 10 μg of LPS can persist in the blood for at least 24 hours(10), an attempt was made to determine whether repeated administration of chlorpromazine could produce better protection against large doses of endotoxin. The results of such tests were negative. Our data are not in disagreement with Rieder and Thomas' observation that chlorpromazine fails to protect pregnant mice(1) since these authors attempted to prevent the death of the fetuses 24 hours after injection of large doses of endotoxin. Finally, 5 mg/kg of chlorpromazine injected alone into pregnant mice which had received saline had no harmful effect as judged by the survival of all the litters 24 hours later.

Discussion. According to Kass(2), endotoxin administration produces uterine contractions by sensitizing the myometrium to oxytocin. Although the influence of pituitary seems excluded in the mouse(4), the results reported here favor the concept that endotoxin induces abortion by its action on the mother.

One could assume, on the basis of body weight and blood volume, that, when 20 μg of LPS are injected into the mother, each fetus receives 0.4 μg. However, only 1/10,000th of the initial radioactivity actually crosses the placental barrier(4). Since 1 μg of LPS inoculated directly into the fetus has no toxic effect, it would appear most unlikely that fetal death is primarily responsible for abortion even when large doses of endotoxin are administered to the mother.

The present data confirm that endotoxin mimics the action of serotonin as far as abortion is concerned. In this connection, it is of interest that endotoxin has been shown to release 5-HT from blood platelets(11,12) and that this substance has an elective action *in vitro* on blood vessels and on the gravid uterus(9,14) and can interrupt pregnancy *in vivo* by producing placental hemorrhage(9, 13). Furthermore, tumor damage as well as placental hemorrhages can be induced both by endotoxin and by serotonin(12,13,14). Since 5-HT can cross the placenta(13), one would assume that endotoxin injected into the fetus releases little or none of this substance. Histamine, which can also be released by endotoxin(15), however, cannot interrupt pregnancy in the mouse, even when administered in high doses. Chlorpromazine which has been shown to counteract the effect of serotonin on uterine contraction *in vitro*(14) and *in vivo*(9), can also protect pregnant mice against endotoxin-induced stillbirths. Although the time and dose requirements would seem to limit its practical use, related substances, administered with antibiotics, may be useful in protecting against the effects of Gram negative infections during pregnancy.

Summary. 1. The mechanism of endotoxic abortion does not seem to be triggered by fetal death because endotoxin injected directly into the fetus is innocuous and no radioactivity crosses the placental barrier when labeled endotoxin is administered to the pregnant mouse. 2. Whereas cortisone is not effective, chlorpromazine protects pregnant mice against endotoxin-induced stillbirth. These results suggest that endotoxin may interrupt pregnancy by releasing serotonin or some related substance.

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The Effect of Crystalline Dihydrotachysterol on Milk Secretion in Thyroparathyroidectomized Lactating Rats.* (29405)

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When lactating rats were thyroparathyroidectomized on day 4 of lactation and given thyroxine replacement therapy without parathyroid hormone, some mothers died of tetany, and the young either died or grew very poorly to day 14. Milk yield averaged 1.6 g on day 14 after a 10 hour separation period(1).

Replacement therapy was successfully achieved in thyroparathyroidectomized lactating rats using 60 to 100 μg Hytakerol (AT 10)/100 g BW/day given orally from day 7 to 13 post-partum(2). The effect of AT 10 on milk secretion in normal rats was also investigated and it was observed that subcutaneous injection of 125 μg of the material to normal rats from day 7 to 20 of the lactation period increased milk yield 57% and 15% on day 14 and 20 of lactation respectively(3). It was claimed that this material contained dihydrotachysterol. Biochemical study carried out to investigate the nature of commercial Hytakerol, however, indicated that this material did not contain dihydrotachysterol but was dihydrovitamin D_2II(4). Consequently the effect of dihydrotachysterol on milk secretion has not been explored. Preliminary study in this laboratory indicated that administration of crystalline dihydrotachysterol to normal rats increased milk secretion 8% on day 14 and 34% on day 20 of lactation. The hypercalcemic and hyperphosphatemic effects of crystalline dihydrotachysterol in rats has been reported(5).

Evidence is presented here which suggests that crystalline dihydrotachysterol is able to take the place of the parathyroid hormone in milk synthesis in lactating thyroparathyroidectomized rats.

Materials and methods. Pregnant rats of the Sprague-Dawley-Rolfsmeyer strain were housed in individual cages and on day 4 post-partum the litters were reduced to 6 young. The animal room was maintained at a temperature of 78° ± 1°F and the rats were fed Purina Lab Chow and water *ad lib.*

The dams were thyroparathyroidectomized (TPEX) on day 4 post-partum under sodium Nembutal anaesthesia with a dose of 30 mg/kg BW. One hour before the operation a blood sample was collected for serum calcium determination by the chloranilate method(6). In seeking evidence for the completeness of removal of the glands, a second blood sample was collected 8 hours after the operation for serum calcium determination. Animals which showed a fall of serum calcium of less than 3 mg% after thyroparathyroidectomy were excluded from the experiments and were considered as "failures." A third and fourth blood sample was collected on day 14 and 20 of lactation for determination of serum calcium. Controls were treated in the same way. The thyroid and parathyroid glands were exposed but not removed. Immediately after collection of the second blood sample, 23 animals were injected with 100 μg crystalline dihydrotachy-

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