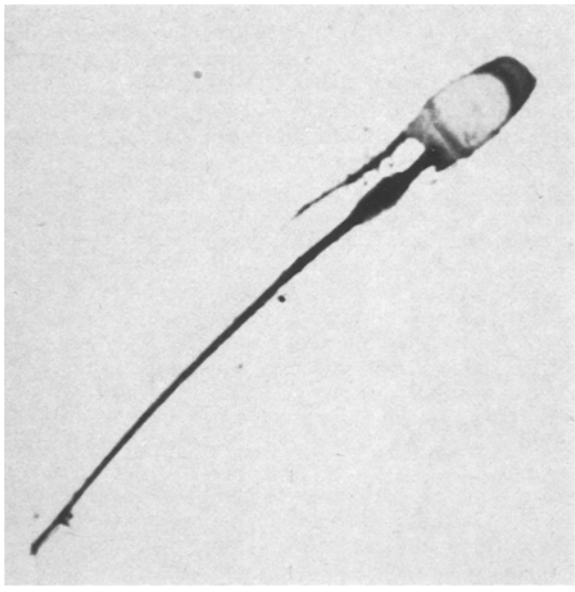

Fig. 2. Sperm of *Ctenomys maulinus* showing a cytoplasmic droplet in the midpiece.

[11] O. A. Reig, personal communication.
[12] E. Bustos and P. Potocnjak, Pan Am. Ass. of Anatomists Meeting, New Orleans (1972).

the sperm appearing paddlelike in shape in the smears. A conspicuous process, caudally oriented, originates from the posterior end of the postacrosomic region and runs parallel to the flagellum, which is seen displaced towards the opposite side of the head.

This postacrosomic process is longer than the anteroposterior diameter of the head (Table). The acrosome forms approximately $^3/_4$ of the head, excluding the postacrosomic process. The width of the head is roughly half its length.

In some spermatozoa, a cytoplasmic droplet is seen at the proximal segment of the tail, i.e. facing the postacrosomic process (Figure 2).

As can be seen in the Table, though head diameters fluctuated from one animal to another, the ratio length of the head/length of the acrosome was quite constant. The length of the postacrosomic process did not change significantly in the animals analyzed. Since it was also observed in unfixed material, it probably corresponds to a distinctive structure of *C. maulinus* spermatozoa.

The postacrosomic process may well be a generic characteristic, since a comparable structure has been found in different species of the same genus[11]. However it has not been observed in testicular sections of *Octodon degus*[12].

Hence it seems advisable to study more species of the genus *Ctenomys* and other Octodontidae, using more refined techniques, in order to gain a better understanding of the morphofunctional and evolutionary meaning of this structure.

Bacterial Endotoxin and Impaired Fetal Development

C. R. Coid[1]

Clinical Research Centre, Watford Road, Harrow (Middlesex HA1 3UJ, England), 21 January 1976.

Summary. Small doses of *E. coli* endotoxin given to pregnant mice on the 13th day of pregnancy caused only a mild maternal illness but induced resorption of approximately half the number of fetuses in each mouse. The remaining live fetuses developed normally and showed no evidence of retarded growth or malformations. The weights of their placentas and maternal spleens increased significantly. Endotoxin given on the 6th day of pregnancy caused a small reduction in fetal weights.

Fetal growth retardation, manifested by low birthweight, is recognized as an important problem in man and one which has also been identified in animals. From clinical studies in women and experiments in animals it is clear that subclinical or mild maternal infections are frequently associated with fetal wastage and retarded fetal development[2–5]. However, the effects of small amounts of endotoxin from Gram-negative organisms, which are frequently associated with bacteriuria in pregnancy[6,7] and premature delivery[8], have received little attention although the abortifacient activity of relatively large non-lethal doses are well established[9–11].

The present investigation, therefore, was undertaken to study the effects on fetal development in the mouse of relatively small amounts of endotoxin which caused only a very mild and transient disturbance of the mother's health. The endotoxin was administered on the 13th day of gestation after the development of placental circulation and on the 6th day of pregnancy before its development.

Materials and methods. An outbred strain of mice, TO/Crc, weighing 20 to 30 g were used throughout the study. The day on which the vaginal plug was found was taken as the 1st day of pregnancy. At the end of the experiment, on the 19th day of gestation, each animal was anaesthetized with ether then killed by dislocation of the cervical vertebrae. The fetuses and placentas were removed, examined macroscopically and then weighed. The fetuses were not examined for cleft palate.

Endotoxin. Each mouse was injected s.c. either on the 13th day of pregnancy, or on the 6th day, with the required amount of endotoxin (*E. coli* 0127:B8; Difco Laboratories) in 0.1 ml sterile phosphate-buffered saline (PBS). Control mice were each injected with 0.1 ml sterile PBS only. The significance of the effects of endotoxin treatment was assessed using *t*-tests.

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The effects on fetal growth, maternal food intake, spleen weights and carcase weights, of small doses of *Escherichia coli* endotoxin injected s.c. into pregnant mice examined on the 19th day of gestation

Dose of endotoxin (days injected)	No. pregnant mice	No. implantations	No. resorptions	No. live fetuses	Mean tissue weights and food intake (g)					
					Fetuses	Placentas	Maternal food intake		Maternal spleens	Maternal carcases	Maternal spleen wt. / carcase wt. $\times 100$
							Days 1–2	Days 3–6			
Endotoxin											
(0.25 mg, day 13)	11	101	56	45	1.24	0.151	2.08	21.68	0.342	28.56	1.11
Saline controls	13	113	9	104	1.29	0.127	7.48	21.72	0.158	29.11	0.53
Difference (± SE)					-0.05 ± 0.05	$0.024^b \pm 0.007$	$-5.40^b \pm 0.37$	-0.04 ± 0.72	$0.184^b \pm 0.045$	-0.55 ± 0.91	$0.58^b \pm 0.12$
							Days 1–3	Days 4–6			
Endotoxin											
(0.147 mg, days 13, 14, 15, 16)	15	163	113	50	1.13	0.126	7.10	18.32	0.443	30.09	1.503
Saline controls	11	120	12	108	1.22	0.108	14.50	18.94	0.154	30.25	0.503
Difference (± SE)					-0.09 ± 0.05	$0.018^a \pm 0.007$	$-7.40^b \pm 1.00$	-0.62 ± 1.07	$0.289^b \pm 0.047$	-0.16 ± 1.15	$1.000^b \pm 0.125$
							Days 1–2	Days 3–6			
Endotoxin											
(0.25 mg, day 6)	6	68	9	59	1.16	0.125	2.81	17.05	0.180	31.71	0.568
Saline controls	4	40	3	37	1.25	0.111	8.38	18.86	0.158	30.68	0.515
Difference (± SE)					$-0.09^a \pm 0.04$	0.014 ± 0.009	$-5.57^b \pm 1.26$	-1.81 ± 1.38	0.022 ± 0.029	1.03 ± 1.23	0.053 ± 0.092

$^a p < 0.05$; $^b p < 0.01$.

Results. A single dose of 0.25 mg endotoxin was injected into mice on the 13th day of pregnancy. This amount did not appear to cause any serious illness though after the first 24 h, their coats were ruffled and food intake dropped significantly during the first 2 days (see Table). In addition, histological examinations were made of the liver, kidney, spleens and pancreas from 3 animals, 6, 18, 24, 48 and 72 h, and 6 days after injection of endotoxin. No significant changes attributable to the treatment with endotoxin were observed. On the 19th day of pregnancy when this experiment was terminated, approximately half the number of fetuses in each mouse had died and were undergoing resorption. However, the weights of the remaining live fetuses did not differ significantly from controls and on macroscopic examination appeared to be the same in every other respect. The placentas from the endotoxin-treated animals weighed more than those of controls ($p < 0.01$). After recovery from the initial anorexia the mothers remained healthy for the rest of the pregnancy and there was no apparent reduction of maternal weight, as indicated by their weights after removal of the uterus and contents (Table). The weights of the spleens of the treated animals, however, were more than double those of the controls.

When the dose of endotoxin was reduced to 0.147 mg, and given daily for 4 days from the 13th day of pregnancy, in an attempt to simulate a more chronic form of endotoxaemia, approximately similar results were obtained (Table).

On the 6th day of gestation, before development of the placental circulation, 6 mice were injected also with 0.25 mg of the endotoxin. As in the previous experiments, the endotoxin did not cause any serious illness and the mice showed the same signs of ruffled coat and a reduced food intake during the first 2 days after injection. It can be seen from the Table that the endotoxin caused only a slight but not a significant increase in the number of resorptions, and the fetuses weighed slightly less than the controls.

Discussion. A healthy placental blood supply is an important factor necessary for normal fetal growth and development[12–14]. However, since endotoxins, products of Gram-negative organisms appear to have a toxic predilection for vascular tissue, particularly the placenta[9] it was expected that small amounts, insufficient to cause a severe effect on maternal health, might be given to produce impaired placental blood supply, thus causing retarded fetal growth. In fact placental weights were increased in all three of the treated groups but only slight growth impairment was observed in surviving fetuses even when approximately half of their littermates died in utero.

Whilst it cannot be claimed that experimental endotoxaemia in animals simulates exactly the consequences of infection with viable Gram-negative bacteria, it is apparent in the mouse, and perhaps it occurs in other species, that endotoxin is not responsible for severe fetal growth retardation. Nevertheless, it is clear that after implantation, when the embryo becomes dependant on its placental blood supply, endotoxaemia may severely interfere with the course of a normal pregnancy by inducing fetal resorption without causing anything more than a mild maternal illness.

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