Table 2. Progesterone incubations

Amount of incubations	n	Stage	Incubation time (h)	Substrate $^{14}C/^{3}H$ (nmoles)	Recovery in percent of dose	
					Substrate	Metabolites
2	50	3–4	24	5.4	88.7 ± 5.7	0
1	10	5	24	5.4	90.2	0
1	5	5	72	5.4	89.1	0
2	30	3–4	48	0.007	92.9 ± 1.9	0
1	30	4	72	0.007	85.0	Traces
2	10	O+F*	120	0.007	27.7 ± 0.3	60.2 ± 0.2

*O+F, oocytes with follicle cells.

Material and methods. Embryos were F1-hydrids from a cross CBA-HT6 × CB-20. They were obtained from oviducts or from uterus at 1–3.5 days of pregnancy, of mice which were ovulated and copulated normally. The age was determined from the occurrence of the vaginal plug, which was the day 0. The developmental stages[12] were observed under dissecting microscope before and after incubation. Before incubation embryos were rinsed 3 times with medium; 30 incubations were made in disposable organ culture dishes, Auerbach type (Falcon 3010) in 0.5 ml of early medium supplemented with 11.5 mg/10 ml Na-lactate and 10 mg/10 ml bovine serum albumin, at 37 °C and in 5% CO_2-air atmosphere saturated with water vapour. The medium contained 100 units/ml of penicillin and 50 µg/ml of streptomycin. Substrates used were introduced in 10 µl volume dissolved in propylenglycol-ethylalcohol (1:1). Substrates: ^{14}C-pregnenolone 56.7×10^3 cpm, 5.7 nmoles, ^{14}C-progesterone 63.2×10^3 cpm, 5.4 nmoles, ^{14}C-dehydroepiandrosterone 35.2×10^3 cpm, 3.5 nmoles, ^{3}H-progesterone 530×10^3 cpm, 0.007 nmoles. The incubation time varied from 14 to 120 h as did the number of embryos per incubation from 5 to 50. Incubation was stopped by acetone.

Controls were made by incubating flushed uterine content (negative control) and ovarian follicles (positive control) 72–120 h. In 4 pregnenolone incubations, it was checked that the radioactive substrate was taken up by the embryos.

Extraction was done by ether and chromatography by ascending thin-layer systems. These methods have been described elsewhere[13]. Solvent system used in TLC was acetone:chloroform, 15:85. Radioactivity on thin-layer chromatograms was detected and measured by a windowless gasflow scanner (Berthold, Federal Republic of Germany). Recoveries presented in the tables were calculated from the scanning records.

Results and discussion. The results of the pregnenolone and progesterone incubations are shown in the tables indicating no metabolism of these steroids in the embryos. Only some accumulation of radioactivity in 2 polar fractions in 1 progesterone incubation was detected. However, oocytes with follicle cells converted 60.2% of the progesterone dose (0.007 nmoles) to metabolites. Results in dehydroepiandrosterone incubations (48 h, 20 embryos at stages 3–4) remained similarly negative.

We suggest that unlike pig[5] and rabbit[3] preimplantation mouse blastocyst does not possess similar kind of enzyme activities for steroid metabolism. This may be due to differences in blastocyst formation and trophoblast differentiation at the time of implantation. Our results agree with the findings of Chew and Sherman[11], but are contradictory to those histochemical observations made by Dey and Dickman[8]. We find it obscure that, although they used dehydroepiandrosterone as the substrate to demonstrate $\Delta^5 3\beta$-HSD activity histochemically, we were not able to show biochemically this activity by the same substrate. A recent study by Sherman and Atienza[14] shows that progesterone formation and progestin and androgen metabolism in mouse is merely restricted to giant trophoblast cells at postimplantation stages.

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Plasma hormone levels in normal and lead-treated pregnant mice[1]

P. Jacquet, G. B. Gerber, A. Leonard and J. Maes

Centre d'Etude Nucléaire, Department of Radiobiology and Euratom, Boeretang 200, B–2400 Mol (Belgium), 12 April 1977

Summary. Dietary lead (0.5%) was given to mice which, after mating, exhibited a vaginal plug. Estradiol, progesterone and prostaglandins E and F 2α were determined in the plasma by radioimmuno assay at different times thereafter. The increase in estradiol and decrease in prostaglandins prior implantation are not greatly altered by lead treatment, whereas the subsequent increase in progesterone and later in estradiol is abolished. It is concluded that the lower number of pregnancies seen in lead-treated mothers is due to a maternal hormonal imbalance caused by lead.

Large amounts of lead (0.1–0.5%) added to the diet of mice which, after mating, had displayed a vaginal plug, markedly reduce the number of mice which become pregnant[2]. Histological studies indicate that lead interfers with the implantation of the embryo and causes regression of the corpora lutea[3]. Lead also seems to exert a direct action on the embryo, since it delays its early divisions[4]. One possible mechanism by which lead may

1 Supported by contract 038-74-7 ENV B of the E.C. Environmental Research Program. Publication No. 1394 of the Euratom Biology Division.

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Prostaglandin content in the plasma of normal and lead-treated mice after successful mating

Day of pregnancy	Prostaglandin E (ng/ml means ± SE) Control	0.5% lead	Prostaglandin $F_{2\alpha}$ (ng/ml means ± SE) Control	0.5% lead
3	1190 ± 65 [4]	985 ± 181 [4]	402 ± 108	283 ± 21
4	959 ± 16 [7]	989 ± 71 [6]	225 ± 114	330 ± 52
5	1113 ± 102 [7]	1182 ± 125 [6]	305 ± 83	424 ± 43
6	1000 ± 77 [7]	967 ± 127 [7]	320 ± 43	267 ± 45
11	1513 ± 141 [6]	1450 ± 67 [4]	530 ± 54	428 ± 28
16	1665 ± 374 [3]	1637 ± 243 [4]	347 ± 42	233 ± 57

All values except from days 3, 11 and 16 are means from 2 experiments carried out at different times. The number of animals used is indicated in parenthesis.

intervene in implantation could be by changing the hormonal balance during the critical period. In the present investigation, we have, therefore, studied by radioimmunological techniques the plasma levels of estradiol, progesterone, prostaglandin E and prostaglandin $F_{2\alpha}$ in normal and lead-treated mice at different times after they had displayed a vaginal plug following mating.

Methods. Mice of the C57B1 strain were mated and, when displaying a vaginal plug (defined as 'successfully mated'), were given a diet containing 0.5% of lead as lead acetate, or were kept as controls. At different times thereafter, the mice were bled and the hormones were determined using radioimmuno assay kits supplied by the IRE (Fleurus, Belgium) (estradiol and progesterone) or by Calbiochim. (USA) (prostaglandin E and $F_{2\alpha}$). Plasma from 1 mouse (about 0.5 ml) was sufficient to assay either estradiol and progesterone or the 2 prostaglandins. The procedures of extraction had to be modified from those suitable for the larger plasma samples of man, as described previously[5].

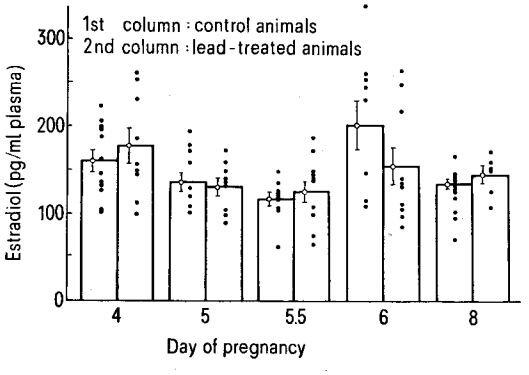

Fig. 1. Estradiol levels in normal and lead-treated mice at different times of pregnancy. The single values as well as the means and SE are shown.

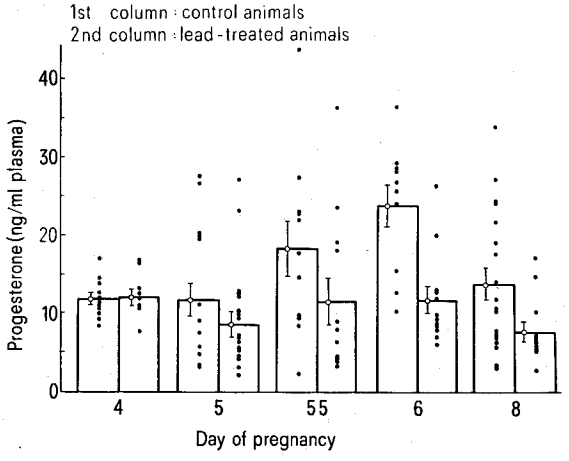

Fig. 2. Progesterone levels in normal and lead-treated mice at different times of pregnancy. The single values as well as the means and SE are shown.

Results and discussion. Plasma levels of estradiol and progesterone at different times after successful mating are presented for control and lead-treated mice in figures 1 and 2. On day 4 of pregnancy (3 days after the vaginal plug), estradiol levels increase in both lead-treated and control animals. On day 5 and 5.5 they have returned to normal levels, and increase again on day 6 in controls but not in lead-treated mice.

Plasma progesterone increases significantly on day 5.5 and 6 of pregnancy in control mice but remains at low levels in lead-treated ones. On inspection, one recognizes that at these times the progesterone values appear to be distributed around 2 centers of gravity, one near the progesterone values of non-pregnant mice and the other at a much higher level. The latter group comprises about 50% of the mice, i.e. the same percentage of mice with vaginal plugs which will actually become pregnant, and one may assume that these are the same animals with the high plasma progesterone. In lead-treated animals, only about 10% of the animals have high progesterone levels, and indeed only about 10% of these mice, become pregnant[2].

Prostaglandins E and F2α are slightly reduced on day 4 of pregnancy (table), a finding which may reflect the inhibition of luteolysis prior to the raise of progesterone. Lead-treated rats display, in general higher prostaglandin values on day 4 and 5 and this may be related to the lower progesterone values seen in these animals. It should be pointed out that although the difference between lead-treated and control mice is not statistically significant for any one day, the differences with age of pregnancy, particularly in the lead-treated group are significant.

From the data presented, one can distinguish 2 phases of hormonal reaction during early pregnancy, an increase in estradiol and decrease prostaglandins preparatory to implantation, and an increase in progesterone and later in estradiol during implantation. Apparently the first phase i.e. the preparation of the uterus by estrogen secretion and the inhibition of luteolysis by the depression of prostaglandins are not greatly affected by lead treatment. On the other hand, lead prevents the increase in progesterone and estradiol characterizing implantation. These observations correlate well with our histological studies[3]: the uterus appeared to develop normally until day 5. Then the corpora lutea showed signs of involution, the uterine

5 G. B. Gerber, P. Jacquet, A. Leonard and J. Maes, Proc. Soc. exp. Biol. Med., submitted for publication.

mucosa did not undergo a deciduous reaction and the embryo failed to develop trophoblastic giant cells in lead-treated mice. Nevertheless, as long as our knowledge of the complicated interrelations between mother and embryo remains fragmentary, it is difficult to decide whether the hormonal changes reflect only the non-implantation of a insufficiently developed embryo or are the cause of non-implantation. Indeed, the embryo, although developed to a normal blastocyst at the time of implantation[3], is somewhat smaller after lead treatment and its early divisions had been retarded[4]. More studies on the activation of the embryo and on the possible reversal of the damage to implantation by hormonal treatment will be needed to decide this question. Nevertheless, we presently favour the principal role of the mother in the early effects of lead on pregnancy, because those few lead-treated mothers capable of implanting embryos have a normal litter size, and because the hormonal changes described arise already on day 5 when the activation of the embryos is just beginning.

Effect of actinomycin D on the quail oocyte nucleolus during meiotic prophase I

A. M. Vagner-Capodano, M. H. Pinna-Delgrossi and A. Stahl[1]

Laboratoire d'Histologie et Embryologie II, Faculté de Médecine, 27, boulevard Jean-Moulin, F-13385 Marseille Cédex 4 (France), 18 March 1977

Summary. Actinomycine D alters profoundly the distribution of the nucleolar constituents in the quail oocyte at prophase I of meiosis. As a consequence of nucleolar segregation, the normally existing relations between the nucleolus fibrillar centers and the microchromosomes are ruptured. The relations between the fibrillar center and the dense fibrills which surround it remain intact, suggesting that they constitute together a functional unit.

The quail oocyte during prophase I of meiosis is a very suitable material for studying the nucleolar organizers and the stages of nucleolar synthesis. Active ribosomal RNA synthesis takes place during late pachytene and the onset of diplotene[2]. Ultrastructural study of the oocyte nucleus at these stages demonstrates that the euchromatic portion of the microchromosomes containing the ribosomal cistrons penetrates into the fibrillar center of the nucleolus[3]. This constant relation suggests that the fibrillar center may be the site of the nucleolar organizer, as Goessens[4,5] has postulated.

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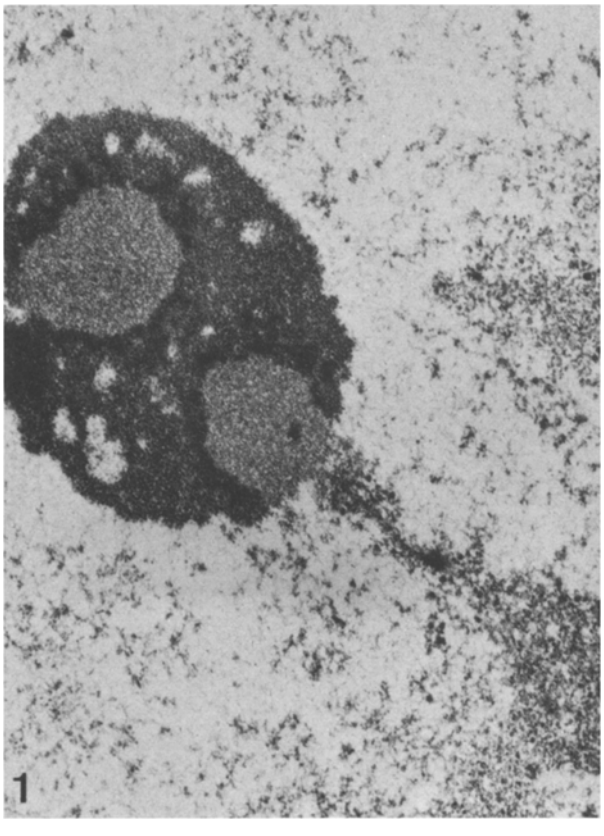

Fig. 1. Quail oocyte nucleolus at diplotene following 1 h treatment with actinomycin D (25 µg/ml). Connections between microchromosome and fibrillar center remain intact.

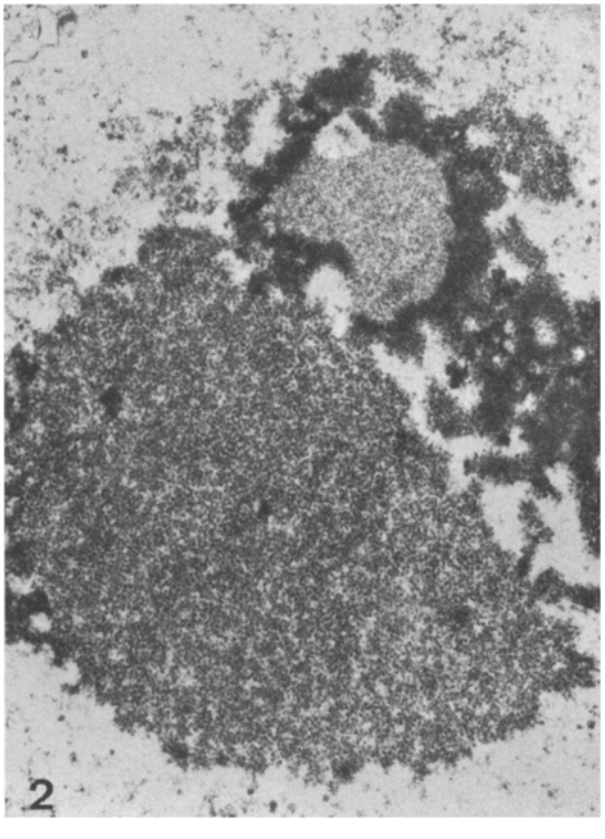

Fig. 2. After treatment of the quail oocyte with actinomycin D (25 µg/ml) for 2 h, segregation of fibrillar and granular constituents of the nucleolus is observed. Connection between the fibrillar center and microchromosomes is no longer visible.