cells[13], but also in cells just going through cytokinesis. These additional basal bodies are physically attached to 2 small roots which form an X-configuration with 2 larger roots (figure, c). In Chlamydomonas, extra basal bodies are present as a rule only in predivision (preprophase) cells[9–11].

Investigation of P. papillatum division stages with 2, 4 and 8 separate daughter cells, either completely enclosed within the mother wall or just leaving the opened mother wall, revealed that the flagella-bound basal bodies of the mother cell, without exception, detach from their axonemes at the proximal end of the plate-like transitional region (triplet-to-doublet transition) (figure, d, e). This event of detachment corresponds to that of Chlamydomonas[9–11]. The mother flagella are constricted at the point where they join the posterior end of the daughter cell (figure, f–h). Corresponding indentations in Chlamydomonas[9,10] were interpreted as the places at which flagellar abscission or breakage occurs. Withdrawal[8] or regression[11] of the flagella are also suggested; these are the results of a gradual shortening or disassembly which probably starts at the flagellar tip and proceeds sequentially to the base until it reaches the point just below the transitional region. Resorption of flagellar protein prior to cytokinesis and re-utilization of protein during the formation of daughter cell flagella have been taken into consideration[14].

Light microscopic observation confirmed the motility of Polytoma papillatum division stages containing 2 and 4 separate daughter cells. From the present experiments, it was determined that: a) In contrast to theories of Lenhossék[15] and Henneguy[16], basal bodies are not kinetic centers. b) In contrast to the supposition of Johnson and Porter[9], basal bodies are not essential for the maintenance of flagella. Thus, flagellar motility in Polytoma clearly does not depend on the presence of basal bodies. Reactivation experiments with ATP also showed that in cilia and flagella lacking basal bodies the mechanism of flagellar motility takes place in the axonemes[17].

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Steroid metabolism by mouse preimplantation embryos in vitro

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Summary. Mouse preimplantation embryos were incubated with radioactive pregnenolone, progesterone or dehydroepiandrosterone for various periods of time. These substrates were not converted to metabolites even after incubation of 120 h. We suggest that preimplantation mouse embryo does not possess enzyme activities for steroid metabolism.

The idea that the blastocyst could contribute to the implantation by its own steroid hormone production has been proposed by Dickman et al.[2]. The pioneer work of early steroid metabolism was done by Huff and Eik-Nes[3] in 1966. They demonstrated that 6-day-old rabbit blastocysts possessed enzyme systems for synthesizing cholesterol and pregnenolone in vitro from acetate. Progesterone was metabolized further to 5β-pregnandione, 3α-hydroxy-5β-pregnan-20-one and 20α-hydroxy-4-pregnen-3-one.

Competitive protein-binding techniques have revealed endogenous levels of progestins (progesterone [0.003 to 0.165 nmoles/ml], 20α-hydroxy-4-pregnen-3-one and 17α-hydroxy-4-pregnene-3, 20-dione) in rabbit blastocysts[4].

Perry et al.[5] using radioimmunoassay found progesterone, estrone and 17β-estradiol in pig blastocysts. Incubation studies still indicated weak $\Delta^5$3β-hydroxysteroid dehydrogenase, 17–20-desmolase, aromatase, 17β-hydroxysteroid dehydrogenase and 3-sulphatase activities.

The presence of $\Delta^5$3β-hydroxysteroid dehydrogenase has been shown histochemically in rat[2,6,7], mouse[8] and hamster[9,10] preimplantation blastocysts, and it was concluded that these embryos could synthesize progesterone. The biochemical studies of Chew and Sherman[11] with preimplantation mouse blastocysts did not support these results. The authors suggested that the apparent discrepancy may be explained by the histochemical method. Dickman and coworkers[8] used dehydroepiandrosterone instead of pregnenolone as substrate.

However, the mammalian embryos bath in a steroidal environment which offers them the possibility to bind and metabolize steroids. Our aim was to show biochemically what kind of steroid metabolism may occur in preimplantation mouse blastocysts.

Table 1. Pregnenolone incubations

Amount of incubations	n	Stage	Incubation time (h)	Recovery in percent of dose Substrate	Metabolites
3	10	2–3	14	93.2 ± 3.0	0
3	50	2–3	24	84.6 ± 3.6	0
1	5	4–5	48	87.5	0
6	10	3–5	48	89.1 ± 1.8	0
3	10	3–4	120	83.0 ± 3.4	0

n, Amount of embryos in incubation.

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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]

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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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