DNA, since the mean islet mass is much higher in the obese than in the lean mice[22]. The release of somatostatin from both freshly isolated and cultured *ob/ob* islets was also less than in their lean littermates. The lack of effect of adding theophylline to the incubation medium is in contrast to the report of Schauder et al.[23].

From the present findings, it is concluded that the turnover of somatostatin is very high as indicated by the high ratio between the amount of released somatostatin and the somatostatin content in the incubated islets. Furthermore the results obtained in the cultured specimens indicate also that the synthesis of somatostatin occurs in adult mouse pancreas without neural influence, as has been shown earlier for fetal rat pancreas[24]. Somatostatin is known to inhibit the stimulated release of insulin and glucagon, but it has also inhibitory effects on many gastrointestinal functions[25,26]. A relative deficiency of somatostatin-producing cells might thus explain many of the metabolic disorders found in the obese-hyperglycemic mice, and also explain the marked increase in the number of somatostatin cells in animals with experimental diabetes[26]. These questions, however, need further attention.

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Corpus luteum function in ageing inbred mice[1]

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Summary. Impaired breeding performance of aged female mice was associated with reduced numbers of ovulations and increased mortality of embryos. The amounts of progesterone in the sera, corpora lutea and uterine flushings of these animals were similar to those of young animals when measured by radioimmunoassay.

There is substantial evidence to show that the gametogenic potential of the ovaries of mice and some other animals outlasts the ability of the uterus to maintain conceptuses to term[3]. The results of embryo transplantation experiments in which the age of donors and of recipients were variables showed that the latter was the major factor affecting embryo survival and that the majority of ova from old mice were viable in a young uterine environment[4,5]. The relative contribution of extrinsic and intrinsic factors to the decreased gestational potential of the ageing uterus is not clearly established. Morphological studies[6] and hormone supplementation studies[7] implied that the secretory activity of the corpora lutea (CL) might be sub-optimal for uterine function. Other investigators have doubted this conclusion and implied that unknown intrinsic age changes of the uterus are responsible for most pregnancy wastage[8–10]. We have therefore measured the levels of progesterone in ageing pregnant mice in order to directly assess luteal function.

Materials and methods. Young (2–3 month) and aged (8–12 month) virgin CBA/H female mice were paired either with young albino male mice or proven sterile vasectomized animals. They were examined each day for the presence of a copulatory plug (= day 1 of pregnancy/pseudopregnancy). Mated females were allocated randomly to 3 groups which were either autopsied on day 4 or 8 of pregnancy or used for litter size determination at term. On the day of autopsy the animals were anaesthetized with ether and a blood sample was collected from the orbital sinus. They were killed by cervical dislocation before recovering from the anaesthetic. The number of uterine embryos was determined either by flushing them out with saline and counting them in a watch glass (day 4) or by counting the number of implantation swellings (day 8). The most hyperaemic set of CL was counted under the microscope. 2 CL from 1 ovary of each animal were removed by fine dissection for subsequent hormone determination, closely apposed CL being avoided. Histological preparations showed that CL isolated by this technique were intact and generally free of adhering tissue apart from occasional preantral follicles. The undissected ovaries were prepared by routine histological methods and stained with haemalum and eosin. The dis-

Table 1. Circulating and luteal progesterone levels of young and aged pregnant and pseudopregnant mice

Age (months)	Day of pregnancy	No. of corpora lutea (CL) of pregnancy	Embryos (% of CL)	CL progesterone ng/CL	Serum progesterone ng/ml
2- 3	4	9.5±0.3	8.3±0.3 (86.8)	9.48±0.67	28.02±2.12
	8	9.3±0.5	9.0±0.4 (97.3)	11.28±1.14	29.63±3.05
	8 (pseudopregnant)	9.5±0.3	0	12.14±2.01	27.64±0.84
8-12	4	5.5±1.3	4.3±1.5 (77.3)	11.38±1.50	30.59±6.51
	8	6.3±0.8	2.4±0.8 (38.6)	11.11±1.68	25.27±3.78

Means ± SEM are given. 4–7 animals/group.

Table 2. Progesterone and protein content of uterine flushings of young and aged mice on day 4 of pseudopregnancy

Age (months)	Number	No. of CL of pseudopregnancy	Dry uterine weight (mg)	Protein/uterus (μg)	Progesterone/uterus (ng)
3	9	9.0±0.4	15.2±0.3	<25*	0.065±0.016
9-10	10	7.2±0.7	26.0±1.3	<25*	0.053±0.016

Means ± SEM are given. * Below the sensitivity of the protein measurement technique (25 μg).

sected CL were homogenized individually in ether (0 °C) with trace amounts of ^{3}H-progesterone added for the determination of hormone recovery. The homogenates were dried in glass vials and stored with the sera at −20 °C. A further group of animals was killed by decapitation and exsanguinated on day 4 of pseudopregnancy. The uterine horns were dissected and blotted gently with damp filter paper to remove surface blood. Individual horns were then flushed with 0.25 ml of cold sterile phosphate-buffered saline (pH 7.0). The flushings from each animal were pooled and immediately centrifuged at 3000×g for 20 min to remove cellular debris. The supernatants were stored at −70 °C until the protein[11] and progesterone concentrations were measured.

The progesterone content of the sera, CL homogenates and uterine flushings was measured by radioimmunoassay procedures[12]. After extraction of the samples with ether, steroids were separated on celite microcolumns using a stationary phase of ethylene glycol[13]. The chromatographic step was omitted for plasma and CL samples because preliminary results showed there were very large amounts of progesterone in the samples relative to any other steroids that might cross-react with the anti-serum used (S-257 No. 2, kindly supplied by Dr G.E. Abraham). The separation of bound from free steroid was effected by dextran-coated charcoal. A standard curve was constructed from concentrations of the progesterone standard measured in triplicate. The samples were measured in duplicate and the concentrations of progesterone were determined from the standard curve using a non-linear asymptotic model which had been fitted to the curve by iterative methods[14]. The smallest amount of progesterone that could be measured per sample was 0.04 ng. Each of the variables recorded in table 1 was analyzed as a 2-way classification with maternal age and day of pregnancy as the factors. An additional class, 'pseudopregnant', was included in the analysis of some variables. The mean values in table 2 were compared by Student's t-test.

Results and discussion. The number of live offspring born to 14 young mice ranged from 8 to 11 whereas only 2 out of 13 old mice gave birth, and their litter sizes were 1 and 3. The small litter sizes in old females cannot be fully accounted for by the observed reduction in the number of CL (table 1, $p < 0.001$), assuming that each CL represents an ovulation, because the number of embryos as a percentage of CL is also reduced ($p < 0.01$). The latter effect appears as an interaction of age and day of pregnancy ($p < 0.05$) and is due to a reduction in the percentage of embryos from day 4 to day 8 in old mice compared to the high percentage of embryos maintained in young animals. There was no evidence of any significant effects of maternal age upon the serum or CL progesterone levels during pregnancy or pseudopregnancy. Furthermore, the CL of young and old animals were morphologically indistinguishable in histological sections. Examination of the raw data revealed that the absence of implants on day 8 did not affect the progesterone levels in either the young pseudopregnant animals or the 2 old animals that lacked signs of implantation. Serum progesterone levels were not correlated with the number of CL and in 1 old animal 2 CL supported implantation. This result may indicate that some compensatory secretion of progesterone occurs in old mice that have reduced numbers of CL though, contrary to expectation, there was no corresponding change in the progesterone content of individual CL. These results taken together with those obtained by measurement of Δ^5-3β-hydroxysteroid dehydrogenase activity in C57BL mice[10] strongly suggest that the high incidence of prenatal mortality in aged animals is not a result of defective luteal function. A similar conclusion has been drawn in studies of reproductively senescent rabbits[15] and hamsters[16]. The results support the view that intrinsic age changes of the uterus are primarily responsible for decreased breeding performance prior to the onset of anovulation.

Changes in target tissue response to progesterone and other hormones might occur during ageing which could explain the beneficial effects of supplementary progesterone on implantation[7]. A decreased uterine response to progesterone could be due to changes in the uterine vascular supply or the uterine collagen content[8] with increasing age. In addition, there may be changes in factors affecting the concentration of progesterone binding sites in the uterus, e.g. oestrogen levels[17]. Each of these factors can be investigated experimentally but oestrogen deserves special consideration because the numbers of Graafian follicles are reported to be diminished in aged ovaries[18] and the reduced numbers of CL suggests that this may be so in our animals (table 1). We have, however, been unable so far to detect differences in the serum levels of oestrogen in individual young and aged mice because the absolute amounts of oestrogen present in each case are very small (< 30 pg/ml)[19].

The uterine luminal environment is undoubtedly critical for normal implantation and development. Uterine flushings were examined because some embryos were apparently being lost during the peri-implantation period. In the

rabbit there are correlated changes in the levels of progesterone and protein in the uterine flushings during early pregnancy and pseudopregnancy, though the biological significance of these changes has not yet been demonstrated[20]. The total amounts of uterine luminal progesterone on day 4 of pseudopregnancy were low but similar in young and aged mice (table 2). These progesterone levels were not standardized for uterine weight, even though old uteri were heavier than young ones ($p < 0.001$), because there was no evidence of an association between increased uterine weight and glandular surface area. The levels of protein in uterine flushings of individual mice were also low and below the sensitivity of the technique (table 2), although Aitken[21] has demonstrated a small transient increase in the protein content of pooled samples of uterine flushings on day 5, which is the day on which implantation occurs in pregnant animals. If the relationship between levels of progesterone and of protein holds in the mouse as it does in the rabbit, the results of table 2 imply that the amounts of uterine protein are unaffected by ageing of the uterus.

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The effect of gonadotrophins on the steroidogenesis, in the ovary and testis of gonadotrophin-deprived fresh water teleost, *Cyprinus carpio*

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Summary. Studies have been made on the effect of LH, FSH, and LH+FSH on the gonadal steroidogenesis in gonadotrophin-deprived common carp. LH alone and in combination with FSH was more effective than FSH in stimulation of steroidogenesis.

The surgical hypophysectomy followed by replacement therapy has been the most convincing experimental method for the demonstration of pituitary control of gonadal functions. Since the hyophysectomy disrupts the endocrine balance, several chemical compounds, most of which are steroids, have been used for specific gonadotrophic suppression. These steroids invariably interfere with the feedback pathways[2]. In recent times, several workers have advocated the use of a nonsteroidal antigonadotrophic compound (methallibure, ICI 33,828) in place of surgical hyophysectomy[3-15]. The gonadotrophic inhibitory properties of this compound have been substantiated by studies showing arrest of gametogenesis[3,4,8,11,13], inhibition of development of secondary sexual characters[3,8], histological and cytological changes in the pituitary gonadotrophs[5,9,15,16] plasma gonadotrophin level[10], ^{32}P uptake by ovary[14], and histochemical[4,13] and biochemical[17] activity of steroid dehydrogenases.

The level of Δ5-3β-hydroxysteroid dehydrogenase (3β-HSD), along with that of various other steroid dehydrogenases, is indicative of steroidogenesis in the gonadal tissue[18]. During the present studies, investigations have been made on the effect of LH, FSH and LH+FSH, on the steroidogenesis (with regard to the activity of 3β-HSD) in the ovary and testis of gonadotrophin-deprived teleost fish, *Cyprinus carpio.*

Materials and methods. 30 mature specimens (sex ratio 1:1), of *Cyprinus carpio* measuring 25-30 cm and weighing 100-120 g, were divided into 5 separate groups (each group consisting of 3 female and 3 male specimens), and housed in 300-l aquaria, which were kept aerated periodically. No feeding was done during the experimental period. Groups 2-5 were treated with methallibure as described earlier[17]. The gonadotrophin treatment consisted of 5 i.p. injections (on alternate days), of 7.5 µg/g each of bovine FSH and LH to groups 3 and 5, respectively, and FSH+LH (7.5 µg/g each), to group 4. Group 1 served as untreated control and group 2, as a control for the gonadotrophin treated groups. Both of these groups were given 5 injections of 0.9% saline (0.2 ml in each injection), at the same time when the gonadotrophin treatment (dissolved in 0.2 ml of 0.9% saline for each injection), was being given to other groups.

The procedure regarding the determination of enzyme activity has been reported earlier[17,19]. The relative differences in the enzyme activity between the untreated control (group 1) and various experimental groups (2-5), and among the various experimental groups (2-5), were worked out and Student's t-test was applied to the data for making comparison of the effect of gonadotrophin-deprivation and various gonadotrophin-treatments with respect to enzyme activity.

Results. Table 1 summarizes the results of the experiments.