18 Klein, R.A., Biochim. biophys. Acta *210* (1970) 486.
19 Kaminskas, E., Fiel, M., and Henshaw, E.C., Biochim. biophys. Acta *444* (1967) 539.
20 Fiske, C.H., and Subarrow, Y., J. biol. Chem. *66* (1925) 375.
21 Kates, M., Techniques of Lipidology. Eds T.S. Work and E. Work. North Holland, American Elsevier, Amsterdam 1975.
22 Brunette, D.M., and Till. J.E., J. Membrane Biol. *5* (1971) 515.
23 Roe, J.H., J. biol. Chem. *107* (1934) 15.
24 Rosier, R.N., Gunter, T.E., Tucker, D.A., and Gunter, K.K., Analyt. Biochem. *96* (1979) 384.
25 Wallach, D.F.N., Soderberg, J., and Bricker, L., Cancer Res. *20* (1960) 397.
26 Schneider, P.B., Lipid Res. *18* (1977) 239.
27 Machida, K., and Ohnishi, S., Biochim. biophys. Acta *596* (1980) 201.
28 Netter, H., Theoretical Biochemistry, p.590. Physico-chemical Principles of Vital Processes, Oliver and Boyd, 1969.

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The effect of 17α-methyltestosterone on the sex of the common carp, *Cyprinus carpio* (L.)

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Summary. Dip treatment of fertilized eggs of *Cyprinus carpio* (L.) in 17α-methyltestosterone, followed by dietary administration of the same to the hatchlings over 50 days resulted in 54% males, 13% females and 33% sterile fish, while in the control group both sexes were in nearly equal numbers. The androgen-treated fish also showed better growth.

The common carp, *Cyprinus carpio*, is an important species used in composite fish culture in India. However, its 'wild spawning' in culture ponds has been found to affect the pond's yield adversely[2]. Studies conducted at the College of Fisheries, Mangalore, have revealed that by administering 17α-methyltestosterone (200 ppm) over 131 days, starting from the 2nd day after hatching, it is possible to produce a brood consisting of only male and sterile fish[3]. Since the duration of treatment in that experiment was too long for field application, an attempt was made to reduce the treatment period to 50 days.

The fish was induced, induced-bred the developing eggs in the gastrula stage were given a dip-treatment in an aqueous solution of 17α-methyltestosterone (17α-MT) at a concentration of 200 ppm for 1 h. The resultant hatchlings were maintained on a feed containing the same hormone at 200 ppm over 50 days, starting from the 2nd day after hatching. They were also given small quantities of plankton during the first week only.

The fry were reared in plastic pools during the treatment period of 50 days and were later transferred to cement cisterns (50 m^2) for further rearing over another 72 days on a hormone-free diet.

On termination of the experiment, the length and weight of all the fish were recorded and their gonads dissected out and sexed, following the methodology described elsewhere[3].

The androgen-treated group had 54% males, 33% sterile fish and 13% females, while the control group had 51% males and 49% females. The growth rate and survival of the treated fish were found to be consistently better than those of the controls. The fish classified as 'sterile' had only filiform gonads, which on histological examination did not show any germ cells. However, a few enlarged cells, resembling the germ cells, were encountered in a few filiform gonads.

The results of this investigation clearly demonstrate the possibility of reducing the period of hormonal treatment. In the earlier work on common carp, treatment with 17α-MT (200 ppm) over a period of 131 days was found to be effective in producing a population completely devoid of females[3]. Even though this could not be achieved in the present work, it has clearly indicated the possibility of reducing the period of hormonal treatment considerably. It appears highly probable that a completely female-free population could be obtained with only 50 days of hormonal treatment or even less by increasing the dosage. Another possible reason for the occurrence of females in the present study could be that the hatchlings initially fed more on the plankton given than on the hormone-containing diet. Therefore, reducing plankton food to only the first couple of days might yield better results.

1 Acknowledgment. The award of Junior Research Fellowship by I.C.A.R., India, to the first author is gratefully acknowledged.
2 Varghese, T.J., Satyanarayana Rao, G.P., and Shetty, H.P.C., Mysore J. agric. Sci., *10* (1976) 681.
3 Sathyanarayana Rao, H.N., and Satyanarayana Rao, G.P., Aquaculture *35* (1983) 83.

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Alloxan-induced hyperglycemia increases progestin and androgen accumulation by isolated rabbit follicles in vitro[1]

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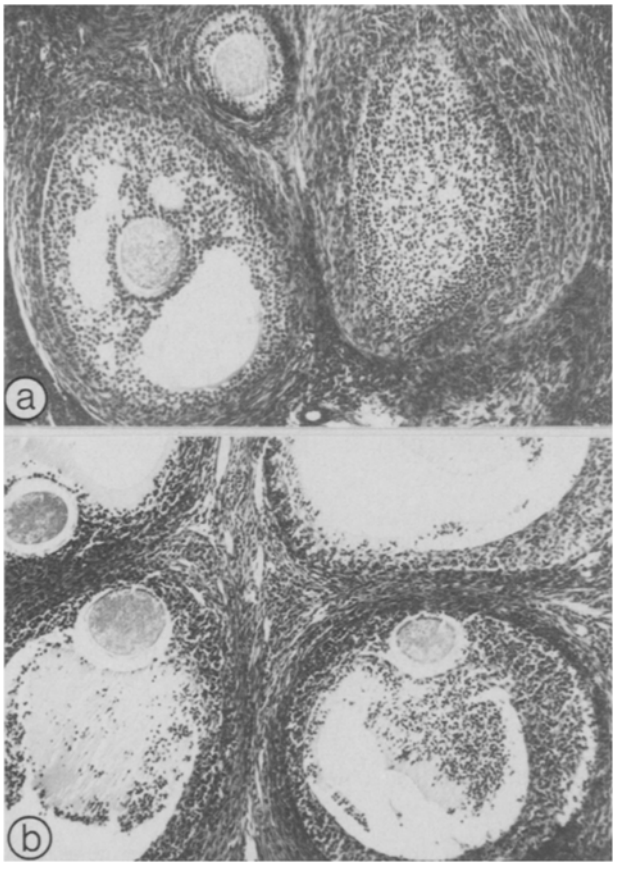
Summary. Follicles isolated from Alloxan-treated rabbits and incubated in vitro, accumulated more progesterone and testosterone than those from saline-treated rabbits. LH augmented the accumulation of these 2 steroids. By contrast, the estradiol response to LH stimulation by follicles from Alloxan-induced hyperglycemic rabbits was diminished when compared to follicles from saline-treated rabbits. Ovaries from hyperglycemic rabbits also appeared to have more cystic follicles.

Diabetes mellitus has long been recognized to be closely related to disturbances in reproductive function (reviewed by Rodriquez-Rigau[2]). In rabbits it was observed that complications of pregnancy usually resulted in abortions, stillbirths and premature deliveries[3]. Decreased ovulations, and loss in weight of ovaries and uteri of rats[4-6] have been attributed to decreased response to gonadotropins[7]. The defect in reproductive function in female rats has been shown to be an abolition of the preovulatory luteinizing hormone (LH) peak and consequent failure to ovulate[8]. Since the rabbit is a reflex ovulator and pregnancy can still occur in diabetic rabbits[3] it was of interest to determine whether Alloxan-induced hyperglycemia could have any effects on ovarian endocrine functions.

Effects of Alloxan-induced hyperglycemia on gonadotropin stimulation of steroid accumulation by isolated rabbit follicles (mean ± SEM)

	Normal (pmoles/mg protein)		Hyperglycemic (pmoles/mg protein)	
	Control	LH	Control	LH
Progesterone	8.9 ± 1.5 (n = 8)	32.7 ± 3.4* (n = 8)	18.6 ± 2.5 (n = 6)	49.4 ± 8.8 (n = 7)
Testosterone	17.7 ± 5.4 (n = 4)	97.6 ± 11.1 (n = 8)	30.4 ± 10.1 (n = 6)	74.3 ± 6.2 (n = 7)
Estradiol	13.4 ± 1.7 (n = 8)	18.5 ± 2.7** (n = 6)	13.8 ± 0.1 (n = 2)	11.9 ± 3.8 (n = 8)

*$p < 0.005$; **$p < 0.01$ between LH-treated follicles from normal and hyperglycemic rabbits. Results represent steroid accumulation in the medium during the 2nd incubation period. Numbers in parenthesis refer to number of incubations performed on pooled follicles.



Photomicrograph of ovary from control (a) and hyperglycemic rabbit (b). Hematoxylin-eosin stain. Note the location of the oocytes in the follicles and signs of atresia in the granulosa of (b). × 145.

Materials and methods. Female New Zealand white rabbits were injected with a dose of 60 mg/kg Alloxan in normal saline after overnight fasting. Blood glucose as determined with dextrostix and quantitated with Ames Eyetone (Ames Co., Elkhart, Illinois 46514, USA), averaged 6.71 mmol/l. Within 2 days of injection blood glucose rose to more than 22.2 mmol/l and remained at these levels until the time of sacrifice 7–10 days later. One animal died before it could be used. All hyperglycemic animals suffered from polydipsia. Control animals received saline.

Unless otherwise stated the methods of isolating follicles, incubation and analyses for protein and steroids were identical to those previously described[9]. In the first series of experiments, 5 of 6 animals became hyperglycemic. After a piece of ovary was fixed for histology, follicles were isolated from each animal and incubated in Krebs-Ringer bicarbonate buffer containing 200 mg% glucose and 0.1% bovine serum albumin (BSA) for 2 h at 37 °C. The media were then replaced with new medium with or without 1 μg/ml of LH (NIH-oLH-S17). Incubation was carried out for a further 3 h. Duplicate incubations were done for each animal and the results averaged. Media were removed and stored until analyzed for steroids by radioimmunoassay. Follicles were washed with buffer containing no BSA and homogenized in the same buffer before protein determination.

In the 2nd study 7 rabbits were injected with Alloxan, 4 of which became hyperglycemic within 2 days and remained so until they were killed 7 days later. Follicles were isolated and pooled. 4 follicles were used per incubation which was carried out in minimum essential medium containing 5% normal rabbit serum and L-glutamine[9] for 1.5 h at which time medium was replaced with and without LH. Incubation was carried out for a further 2.5 h. Analysis for steroid and protein were carried out as before. In addition, a piece of ovary was frozen for later analysis of steroids.

Results. Although the weights of hyperglycemic rabbits were depressed ovarian weights did not differ. The ovaries of the hyperglycemic rabbits, however, contained follicles which showed definite signs of atresia (fig. b) when compared to saline-treated rabbits (fig. a). A distinctive feature of some but not all of these ovaries was the location of the oocyte which appeared to be embedded in the granulosa layer without acquiring a cumulus oophorus.

Estradiol was not detectable in extracts of ovaries from both normal and hyperglycemic rabbits. There was no significant difference in ovarian content of progesterone (16.7±4.8 pmoles/mg protein, saline and 10.9±3.1 pmoles/mg protein, Alloxan) but the concentration of testosterone was higher in the normal ovaries (11.4±3.0 pmoles/mg protein) compared to those of hyperglycemic rabbits (4.4±0.9 pmoles/mg protein).

Follicles from hyperglycemic rabbits accumulated more progesterone (7.9 pmoles/mg protein) and testosterone (13.9 pmoles/mg protein) in the medium than those from normal rabbits (4.4 and 9.8 pmoles/mg protein respectively, mean of 2 experiments).

LH stimulated the accumulation of all 3 steroids in the medium in experiment 2 (table). LH stimulation of progesterone production was significantly enhanced in follicles from hyperglycemic rabbits. However, the LH-induced increase above control levels was 265% in normal follicles and 165% in hyperglycemic rabbits, reflecting the higher endogenous ability of the latter follicles to produce progesterone. Although there were differences in the composition of the incubation media the direction of changes in steroid accumulation was similar in both experiments. In experiment 1 LH caused a 40-fold increase in progesterone accumulation by normal follicles but a 26-fold increase by hyperglycemic follicles.

Discussion. The results of this study suggest that hyperglycemia may alter the pattern of steroid accumulation by isolated follicles in vitro. The most significant change was the increased endogenous production of progesterone by follicles from hyperglycemic rabbits. In view of the apparent increase in atretic follicles present in these rabbits (fig.) it could be postulated that the shift to progesterone production may be a result of this change in morphology. Similar findings have been reported in the hamster[10] and rabbit[11,12]. The induction of atresia by hyperglycemia is difficult to assess. It is possible that the deprivation of insulin may decrease gonadotropin support as seen in the rat[8] leading to degenerative changes in the ovarian follicle. Such a mechanism can also account for decreased ovulations and loss in weight of reproductive organs previously described[4-6]. Whether Alloxan has a direct toxic effect on the ovary could not be determined but it is expected that the time interval between drug administration and sacrifice would minimize acute drug effects.

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2 Rodriquez-Rigau, L.J., J. Androl. *1* (1980) 105.
3 Miller, H.C., Endocrinology *40* (1947) 251.
4 Foglia, V.G., Borghelli, R.F., Chieri, R.A., Fernandez-Collazo, E.L., Spindler, I., and Wesely, O., Diabetes *12* (1963) 231.
5 Chieri, R.A., Pivetta, O.H., and Foglia, V.G., Fert. Steril. *20* (1969) 661.
6 Lawrence, A.M., and Contopoulos, A.N., Acta endocr. *33* (1960) 175.
7 Farina, J.M.S., Chieri, R.A., Basabe, J.C., and Foglia, V.G., Fert. Steril. *22* (1971) 794.
8 Kirchick, H.J., Keyes, P.L., and Frye, B.E., Endocrinology *102* (1978) 1867.
9 Losier, A.J., and YoungLai, E.V., Biochim. biophys. Acta *562* (1979) 331.
10 Terranova, P.F., Endocrinology *108* (1981) 1885.
11 Nicosia, S.V., Evangelista, I., and Batta, S.K., Biol. Reprod. *13* (1975) 423.
12 Wielgosz, G.J., Low, M.J., and YoungLai, E.V., Acta endocr. *94* (1980) 235.

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Lack of long term effect of p-chlorophenylalanine on brain 5-hydroxytryptamine and electrocortical activity in conscious fetal sheep[1]

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Summary. Daily infusion of p-chlorophenylalanine (p-CPA) into unanesthetized fetal sheep for 3-6 days did not reduce brain 5-hydroxytryptamine (5-HT) concentrations or produce long-term changes in the pattern of electrocortical activity.

In fetal sheep the electrocorticogram (ECOG) differentiates into episodes of high and low voltage activity between approximately 115 and 125 days gestation; after this breathing movements only occur during the low voltage phase and occupy about 50% of the time[4]. 5-HT has been implicated in the genesis and control of high voltage (quiet) sleep in the adult[5], and infusion of the 5-HT precursor 5-hydroxytryptophan has been shown to prolong high voltage ECOG activity and increase breathing movements in fetal sheep[6]. To test the hypothesis that the appearance and maintenance of high voltage ECOG activity in the fetal lamb is due to the functional maturation of central 5-HT pathways we infused p-CPA, a substituted amino acid which depletes 5-HT stores by inhibition of the enzyme tryptophan hydroxylase, into unanesthetized fetal lamb in utero.

Materials and methods. Catheters were implanted into a carotid artery, jugular vein, trachea and amniotic sac of 6 fetal lambs (116-122 days gestation; term is 147 days) at a sterile operation under maternal halothane/O_2 anesthesia[7]. Pairs of stainless steel multistrand wire electrodes were implanted over the parietal dura to measure the ECOG, across 1 eye to record electro-ocular (EOG) activity, and into the nuchal, diaphragm and intercostal muscles to record the electromyograms (EMG's). ECOG and EOG activities, blood pressure, heart rate and breathing movements (from intratracheal pressure, diaphragm and intercostal EMG's) were recorded on a polygraph continuously for 9-15 days from the first day after surgery. Beginning on the 4th-6th post-operative days 600-700 mg p-CPA (as the methyl ester) dissolved 35-40 ml of warm 0.9% (w/v) NaCl was infused into the carotid artery at approximately 6 ml/h. One fetus received one infusion, but since this had no prolonged effect on ECOG or breathing the others were treated once daily for 3-6 days. 24 h after the final infusion (at 129-133 days gestation) the ewe was killed and the fetus removed from the uterus and weighed. Samples of brain tissue from 5 of the 6 fetuses were taken from the cortex (parietal) hippocampus, caudate nucleus, cerebellum, hypothalamus, pons, medulla and cervical spinal cord, placed in chilled tubes and immediately frozen. The samples were assayed for 5-HT and the metabolite 5-hydroxyindoleacetic acid (5-HIAA) within 7 days[8]. One fetus died in utero overnight and samples from that brain were not collected.

In addition to the brains of treated fetuses, samples were collected from a further 28 fetuses of gestational ages between 95 and 138 days and assayed for the indoleamines. This range of ages precedes and follows that during which the ECOG differentiates into high and low voltage activity[4].

Results are presented as mean ± SEM. The unpaired t-test was used to assess the effects of p-CPA treatment on brain concentrations of 5-HT and 5-HIAA.

Results. The concentration of 5-HT and 5-HIAA in the brains of control fetuses at 95 and 116 days, 122-127 days