without any expensive antisera, radioactive steroids or equipment. (Supported by the Ford Foundation, New York.)

25. Progesterone receptor assays in human endometrium
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Precipitation of progesterone receptor (PR) by polyethylene glycol (PEG) provides a simple assay. Soluble tissue preparations and nuclear extracts, equilibrated with labelled steroid, are treated with PEG (final concentration 15%) after addition of γ-globulin. "Blank" precipitation of free steroid was 10.0 ± 1.6% (mean ± S.D., $n = 40$). The cytoplasmic and nuclear PR from human endometrium, as determined by this method, were saturable, heat labile (40 C, 30 min) and not dissociated from progesterone (P) during PEG precipitation. For a given tissue, the proportion of labelled P bound to a nonsaturable, heat stable ('non-specific') component decreased as the concentration of labelled steroid was increased from 0.29 to 23 nM. Cortisol was found to increase or decrease non-receptor binding of P, the effect varying between tissues and with P and cortisol concentrations. Glycerol (10%), present during equilibration only, did not increase the affinity of the PR for P. This method is capable of high precision, obviates the use of synthetic P analogues and may find application in studying the properties of PR in relation to its natural hormone.

26. A simple method for separation of viable testicular cells
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The role of steroids on spermatogenesis has been a problem for several decades. This is due to the fact that testicular steroid estimation in each cell type could not be performed due to non-availability of a method for isolating the testicular cells suitable for biochemical study. A technique has been described here in which biochemical estimation of steroids could be performed by which the steroid contents and their role in testis in different experimental conditions could be studied. In this method, testis is minced in a buffer medium. After vigorous shaking the supernatant containing spermatogenic cells is removed and treated with hypotonic KCl, first with 0.075 M to isolate spermatocytes and then with 0.06 M to separate and isolate spermatogenia and spermatids. Leydig and peritubular cells from pellet are separated one after another by treatment with collagenase and pancreatin, respectively. The residue is then passed through a syringe needle to break the tubular fragments. The cell suspension is shaken and layered over a 4% sucrose gradient at 4°C to separate Sertoli cells from other cells (spermatogonia and spermatocytes). Approximately 5–10% contamination is found in different fractions. All the cells are found to be 90% viable by their ability to exclude trypan blue.

27. Patterns of labelling in [^{3}H]-steroids by tritium nuclear magnetic resonance spectroscopy
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Tritiated hormonal steroids are widely used in biological research. Tritium nuclear magnetic resonance spectroscopy provides a convenient, reliable and non-destructive method of analysis, giving a direct quantitative measurement of both the positions and stereochemistry of tritium labelling in [^{3}H]-steroids. Conventional methods using chemical and biochemical procedures to obtain such data are usually very time consuming and tedious. Such methods are also often prone to errors due to non-specific hydrogen-tritium isotope exchange and from the lack of specificity of the reactions used. Examples are given which illustrate the importance of knowing the patterns of labelling in tritiated androgens, oestrogens and corticoids for biological research (aromatase, dehydrogenase and protein (receptor) binding studies).

28. Determination of sex hormone binding capacity using a rapid twin-gel micro-column
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A new method has been developed to study steroid–protein interactions in human plasma. In this instance the method is applied to the determination of 5α-dihydrotestosterone binding non-specifically to human serum albumin and specifically to sex hormone binding globulin (SHBG). The method presented uses a twin-gel micro-column incorporating an upper layer of Cibacron Blue F3G-A-Sepharose 4B for the affinity removal of HSA and the steroid bound to it, above Sephadex LH-20 to retard the free steroid. Thus after washing of incubate aliquots through the column the emergent fluid contains the specifically bound steroid. The method is evaluated and compared with equilibrium dialysis, ammonium sulphate and charcoal separation procedures, and SHBG binding capacity data is presented for—(a) ovulatory and non-ovulatory cycles in normal female volunteers, (b) diurnal variations in male and female volunteers, (c) post-menopause women before and during oestrogen therapy regimes, and correlated with testosterone and androstenedione levels determined by radioimmunoassay.

3. BIOSYNTHESIS

29. Androgen production *in vitro* by hamster granulosa cells and theca
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Comparative ability of granulosa cells and theca from hamster follicles to produce androstenedione (A), testosterone (T) and dihydrotestosterone (DHT) from endogenous and exogenous substrate was assessed in 2 h incubations. From endogenous substrate, theca accumulated A(0.41 ng), T(0.11 ng) with DHT non-detectable. LH increased thecal A(1.7 ng), T(0.75 ng) and DHT(0.63 ng). Granulosa cells, ± LH or FSH, did not accumulate androgens. Only theca converted added progesterone to A, T and DHT, with LH increasing conversion. Similarly, only theca converted added 17α-OH progesterone to A, T and DHT, with a slight LH effect. With A as added substrate, granulosa cells accumulated 10× more T than did theca. FSH increased

conversion to T(2×) by granulosa cells. A to T in theca was not increased by LH or FSH. DHT accumulation from added A was detectable in both granulosa (1.1 ng) and theca (1.7 ng), with no further LH or FSH effect. DHT accumulation from exogenous T was approximately the same in both granulosa cell and theca (1.5 ng), with no LH or FSH effect. The results indicate the source of follicular A is the theca and that of T the granulosa cells. Both tissues have a small and comparable amount of 5α-reductase activity. LH appears to increase 17α-hydroxylase activity while FSH increases granulosa cell 17β-dehydrogenase.

30. Steroidogenesis in the accessory genital organs of adult male rats

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Synthesis and metabolism of testosterone in the caput and cauda regions of the epididymis and ductus deferens of adult male rats was investigated. In *in vitro* incubation studies with ^{14}C-labelled pregnenolone and acetate, caput epididymis synthesized more of testosterone than other tissues. Addition of LH to these tissues had no effect on steroidogenesis. Labelled testosterone was mainly converted to 5α-dihydrotestosterone(DHT) in caput epididymis and 4-androstene-3,17-dione in ductus deferens. The caput also had higher levels of progesterone, testosterone, DHT, 4-androstene-3,17-dione and dehydroepiandrosterone than other regions of the epididymis and ductus deferens. Unilateral ligation for 24 h decreased the levels of DHT in cauda epididymis and ductus deferens. Thus, both the epididymis and ductus deferens have all the enzymes necessary for the synthesis of testosterone. The biological actions of testosterone, however, may be manifested through different metabolites in different segments of the epididymis and ductus deferens and the caput may need testicular secretions for converting testosterone to DHT.

31. Serum testosterone, Leydig cell population and activities of marker enzymes during sexual maturation in the rat

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To define hormonal and morphological changes related to testicular function during sexual maturation, serum testosterone and LH were estimated by specific radioimmunoassays in male rats aged between 5 and 90 days. Simultaneously, the Leydig cell population in H & E stained testis sections and 5ene-3β hydroxy steroid dehydrogenase (5ene-3β-HSD) and alcohol dehydrogenase (ADH) were quantitated histochemically. Serum testosterone was 0.95 ± 0.04 (SE) ng/ml at day 5, decreased to a minimum (0.21 ± 0.05) by day 25 and thereafter increased progressively to the maximum level (2.94 ± 0.19) by day 90. No significant differences in serum LH were noted at any age. The relative proportion of Leydig cells per testis increased progressively throughout maturation. Concurrent to decrease in serum testosterone in early postnatal life, both 5ene-3β-HSD and ADH activities decreased. By day 30, the 5ene-3β-HSD activity was comparable to that in adults. The ADH activity progressively increased till day 50. Thus, in the absence of a parallel increase in serum LH the increasing testosterone levels during pubertal development could be due to increase in Leydig cell sensitivity.

32. Origin of oestrogen in pre-implantation rabbit blastocysts

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It has been hypothesized that pre-implantation embryos of rabbits, certain other rodents and pigs can synthesize oestrogen which plays an important role in implantation. The present study, carried out to investigate the oestrogen synthesizing potential of day-6 rabbit blastocysts, consisted of incubation of blastocysts in medium 199 containing ^{3}H-labelled steroid substrates (pregnenolone, progesterone, dehydroepiandrosterone, androstenedione or testosterone) followed by separation of radiometabolites by thin layer and paper chromatography and their identification by coincidence of peaks of radioactivity with authentic steroids and recrystallization. Under these conditions blastocysts were unable to convert any of these substrates into oestrogen. There was a significant metabolism of all the substrates into other less potent neutral steroids suggesting detoxifying action of blastocysts. Using radioimmunoassay (RIA) we have also demonstrated the presence of oestrone and oestradiol in blastocysts and uterine fluid. [^{3}H]-Oestradiol administered (i.v.) to rabbits was localized in the blastocysts and uterine fluid. These studies reveal that rabbit blastocysts do not contain the enzymes necessary for oestrogen synthesis: the steroid present in them is of maternal origin.

33. On inhibition of testosterone synthesis by the rat Leydig cells following incubation with gonadotropin inhibiting material from human urine

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Gonadotropin inhibiting material (GIM) has been subjected to extensive studies in our laboratory to describe its presence in various patho-physiological states, and define its physico-chemical characterization. The present communication elucidates the mechanism of action of this anti-LH material at the cellular level. For this, Leydig cells were incubated with HCG both in the absence and presence of GIM and a graded decrease depending on the dose of GIM was observed in the HCG induced testosterone production. Our results also reveal that GIM inhibits, *in vitro*, the HCG induced conversion of [^{3}H]-ATP to cAMP. Further, when Leydig cells were preincubated with GIM, the binding of [^{125}I]-HCG to membrane receptors was prevented. This was further substantiated, using [^{125}I]-GIM as well as FITC-tagged GIM. GIM did not prevent the binding of prolactin to its Leydig cell receptors.

34. Estradiol receptors and steroid aromatization in the etiology of gynecomastia

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Pathogenic mechanisms for the development of gynecomastia in a large majority of young subjects remain obscure. Estradiol receptors (ER) were demonstrated in all the 17 gynecomastic tissues from males ranging from 17–48 years. Further, incubation of 12 breast tissues with [^{3}H]-androstenedione revealed formation of significant quantities of estrone (E_1) and estradiol (E_2) in addition to small amounts of testosterone (T) and 5α-dihydrotestosterone (DHT) in all the subjects irrespective of their genotypes. Furthermore, preoperative high urinary estrogen levels in these cases, dropped significantly following mas-