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the crude cytosol. These results indicated that progesterone receptor was precipitated by 30% saturated ammonium sulfate, and was changed to 5S protein in the low salt medium, instead of 8S protein in the crude cytosol, and that progesterone receptor consists of 2 components.

144. Effect of hCG and Prostagrandin (PG) $F_{2\alpha}$ on Human Corpus Luteum; in vitro Perfusion of Human Utero-tubo-ovarian Unit

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In vitro perfusion of human utero-tubo-ovarian unit was performed using the extracorporal organ survival device. Experimental design was as follows; (1) control group: no stimulant was added, (2) hCG group: hCG 3000 IU was added, (3) PG-hCG group: PG $F_{2\alpha}$ 500 μg was added by one-shot or 200 μg by drop-infusion and then hCG 3000 IU was added.

Progesterone concentration in the perfusate increased immediately after hCG administration, but did not change after PG $F_{2\alpha}$ administration. No remarkable change was observed in 20α-OH-progesterone concentration by hCG and/or PG $F_{2\alpha}$ administration. Uptake of ^{3}H-uridine and ^{3}H-leucine by luteal cells after hCG administration was greater than that before hCG administration, but there was no difference in uptake before and after PG $F_{2\alpha}$ administration. The enzymatic activities of 3β-HSD, G-6-PDH, and LDH of the luteal cells were well maintained throughout perfusion for 210 min.

The luteotropic action of hCG was demonstrated by either endcrinological or histochemical methods using the human utero-tubo-ovarian unit perfusion technique. However, no apparant effect of PG $F_{2\alpha}$ on human corpus luteum was seen in the doses used throughout this experiment. The results of the present study suggest that human corpus luteum may be refractory to PG $F_{2\alpha}$.

145. Effect of Clomiphene Citrate on Corpora Lutea of Pregnant Rats

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Clomiphene citrate (Clomid) has been suggested to have luteolytic effect both on the rat and human. In this study, its effects on histochemistry, ultrastructure and steroidogenic enzymes of corpora lutea of early pregnant rats as well as plasma steroid levels were investigated. Climid was administered intramuscularly to pregnant Wister-strain rats twice daily 3 to 5 consecutive times in dose of 3-6 mg/Kg/time, beginning on day 7 of pregnancy. At laparotomy 15 or 63 hrs after the last injection, implantation sites were examined and corpora lutea and plasma samples were subjected to analysis. Climid (6 mg/Kg) induced abortions in all rats injected 5 consecutive times 63 hrs after the last injection. Ovaries of this group contained numerous follicles and electromicrographs of lutein cells demonstrated indented nuclei, and abundunt microbody-like membranous structures closely related to mitochondrial membrane, indicating early degeneration. The activities of G6PDH, malic enzyme and ATP citrate lyase in these corpora lutea decreased to around 70% of the control levels, while 20α-HSD activity was maintained at a very low level. Plasma progesterone, 20α-dihydroprogesterone and 17α-hydroxyprogesterone levels decreased significantly in aborted rats with the prior apparent increases of plasma estrone and estradiol levels. It is concluded that Clomid has a mild luteolytic property in pregnant rats, probably mediated by the elevated levels of estrogens secreted from the ovaries.

146. Studies on the Activity and Physicochemical Properties of Macromolecular hCG Extracted from the Normal Chorionic Villus and then Purified

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Human chorionic gonadotropin extracted with very weak alkali from the acetone powder of the chorionic villus in early pregnancy was subjected to

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ammonium sulfate fractionation. As a result, biological activity was found to be present in a 60 per cent saturated fraction, which was then chromatographed on DEAE Sephadex A-50. The elution pattern showed a distinction between biologic and immunologic activities: the former was high only in a fraction eluted with 0.1M NaCl whereas the latter was observed in eluates of different salt concentrations. The immunologic activity of FSH or TSH was absent in all fractions. The fraction eluted with 0.1M NaCl was then purified by means of Ultrogel AcA 44 and DEAE cellulose. Its active component displayed a uniform band in disc electrophoresis, and so was defined as p-hCG. The sample had a molecular weight of approximately 80,000 as estimated by SDS disc electrophoresis. It was split by treatment with 8M urea into subunits of molecular weights of 57,000 and 23,000 respectively. Its biological activity was as low as several hundred I.U. per mg. The amino acid composition of the sample was such as to be richer in lysine, aspartic acid, glutamic acid, glycine and alanine but poorer in arginine, threonine, serine and proline than urinary hCG (u-hCG). Two different N-terminal amino acids were identified: alanine and valine. The sugar content was approximately 20 per cent, being lower than that of u-hCG. Especially low was the sialic acid content. Anti-p-hCG antibody was prepared to establish a new RIA system for p-hCG, which was then compared with the conventional one for u-hCG in respect of the effluent with DEAE Sephadex A-50. As a result, it was found that the anti-p-hCG antibody showed low reactivity with an effluent having 0.2M solvent, and anti-u-hCG antibody with that having 0.1M solvent. It was thus shown that p-hCG is distinct biologically, physicochemically and immunologically from u-hCG.

147. On the Floating Mechanism of the Ovum into the Antrum at an Early Stage of Ovulation

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Of late the hypothalamo-putuitary axis which takes part in ovulation seems to have been reported in detail. But, the mechanism of ovulatation in the ovary appears to have left much to be studied. For the ovum to be released out from the follicle, the cumulus oocyte complex must be set free from granullosa cells (G-cells) of cumulus oophorus and float into the antrum. However, very few reports are there regarding the above phenomena.

Present studies were made about how the ovum surrounding corona radiata were separated from the adjoining G-cells of cumulus oophorus. Female 25-30 day old wister-Imamichi rats were induced to ovulate treatment with FSH and LH (Roulands' method). The ovaries were studied histologically with optical and electron-microscopes periodically on an endocrinological basis.

Result was as follows. (1) G-cells of cumulus oophorus were fundamentally connected with a fragile simple apposition. (2) By administration of FSH, a number of microvilli and large and small annular nexus were found. (3) Four hours after administration of LH following FSH the cell junction began to be loosened. Retention of secretion gradually increased between the cell membranes and at the annular nexus, resulting in the expansion of the intercellular space. Another four hours later the annular nexus got shrunk and slipped out, and cell separation progressed, the cumulus oocyte complex was set free and began to float into the antrum. (4) Following administration of FSH, observations were made for subsequent 72 hours without administration of LH, revealing neither separation of cell membranes nor that of annular nexus.

Thus, it was disclosed that in the follicles of the rat ovary, by the action of LH following FSH, a tight junction of G-cells of the cumulus oophorus began to be loose allowing the ovum covered with corona radiate to float into the antrum.

148. A Gonadotropin-responsive Virilizing Adrenal Tumor and its Endocrinologic Studies

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