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Biochemical Changes in Ascorbate and Peroxidase Activity in the Adrenal Gland during Different Stages of Reproductive Cycle of Rat (Wistar Strain)

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Abstract: **Rapid synthesis of progesterone under the action of ACTH may be controlled by a similar mechanism as reported for LH in the ovary, thus causing increased synthesis and secretion of the progesterone and corticosteroids from the adrenal gland. ACTH is also known to cause depletion of adrenal ascorbate and cholesterol in the hypophysectomized rat which is shown to occur within Minutes of ACTH injection and to exhibit a characteristic time sequence. Peroxidase mediated conversion of pregnenolone to progesterone stimulated in the presence of ascorbate in the rat and rabbit ovarian tissue had also been demonstrated. Since ascorbate is known to be a donor in peroxidase reaction, the possibility of peroxidase system being involved in the rapid depletion of ascorbate during the normal reproductive cycle.**

Keywords: **ACTH, Adrenal, Peroxidase, Ascorbic Acid, & Corticosteroidogenesis.**

1. INTORDUCTION

There adrenal cortex produces a considerable number of different substances which have been classified into three groups i. Glucocorticoids ii. Mineral corticoids iii. Sex hormones. The sex hormones produced by the adrenal cortex of both males and females are progesterone, testosterone and estrogens. The adrenal gland is the source of sex hormones until the testis and ovaries mature at puberty. The secretion of these hormones is controlled by ACTH and not by gonadotrophins which stimulate the testes and the ovaries. The origin of steroidogenic tissue from the same mesothelium that gives rise to gonads is interesting because corticoid tissue and gonads both produce steroid hormones, and they are the only vertebrate Tissue that do so. It appears that at least a part of adrenocortical function is under the control of t he Anterior Pituitary through the agency of a special trophic hormone, adrenocorticotrophin (ACTH).

The various in vivo and in vitro studies have demonstrated that the ovary and adrenal possess the side-chain cleaving system to convert C_{27} cholesterol to pregnenolone which are mainly a C_{22}-C_{20} lyase and hydroxylases (Simmer, 1968). Steroidogenic enzymes such as 3B-OH-steroid dehydrogenase and 20 -hydroxysteroid dehydrogenase have been reported (Beyer et al., 1956; Burstein et al., 1963 and Weist et al., 1963)which are involved in the biosynthesis of progesterone and androgens.

In rat about 83% of cholesterol is esterified and is found in droplets. The adrenal cortex in most species has a high content of cholesterol which is decreased by ACTH (Sayers et al.,1946; Fisher, 1962).At the same time Since adrenals are known to secrete large quantities of progesterone, which is an oxidation product of pregnenolone, it appears probable that conversion of pregnenolone to progesterone may be brought about peroxidatively by the operation of peroxidase as suggested in the ovary (Agrawal and Laloraya, 1977) . The role of peroxidase in the endocrine regulation of hormone action in the adrenals which is closely interlinked in reproductive functioning of different groups of animals remains largely unknown. There appear important gaps in the understanding of the hormone regulation and the enzymic

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mechanism which lead to the rapid formation and secretion of hormones namely progesterone and corticosteroids in the adrenal gland. ACTH is shown to promote cholesterol ester hydrolysis by activating cholesterol esterase (Tyslowitz, 1943; Long 1947); this stimulates the net progesterone secretion by increasing conversion of cholesterol to pregnenolone, the intermediate precursor of progesterone. How this pregnenolone is rapidly converted to progesterone in adrenals remains to be known.

Under the action of ACTH no free pregnenolone is traceable in adrenals indicating a very rapid rate of conversion of pregnenolone to progesterone, far exceeding the formation of pregnenolone from cholesterol esterase activation (Hayano et al. 1956). Progesterone is known to be a precursor of several steroid hormones including androgens, estrogens corticoids (Fig. 1).Samuels and uchikawa (1967) in Vitro studies have shown that it occupies a key position in the biosynthesis of adrenal corticoids. The physiological importance of adrenal progesterone in the rodent or any other species is not yet known.

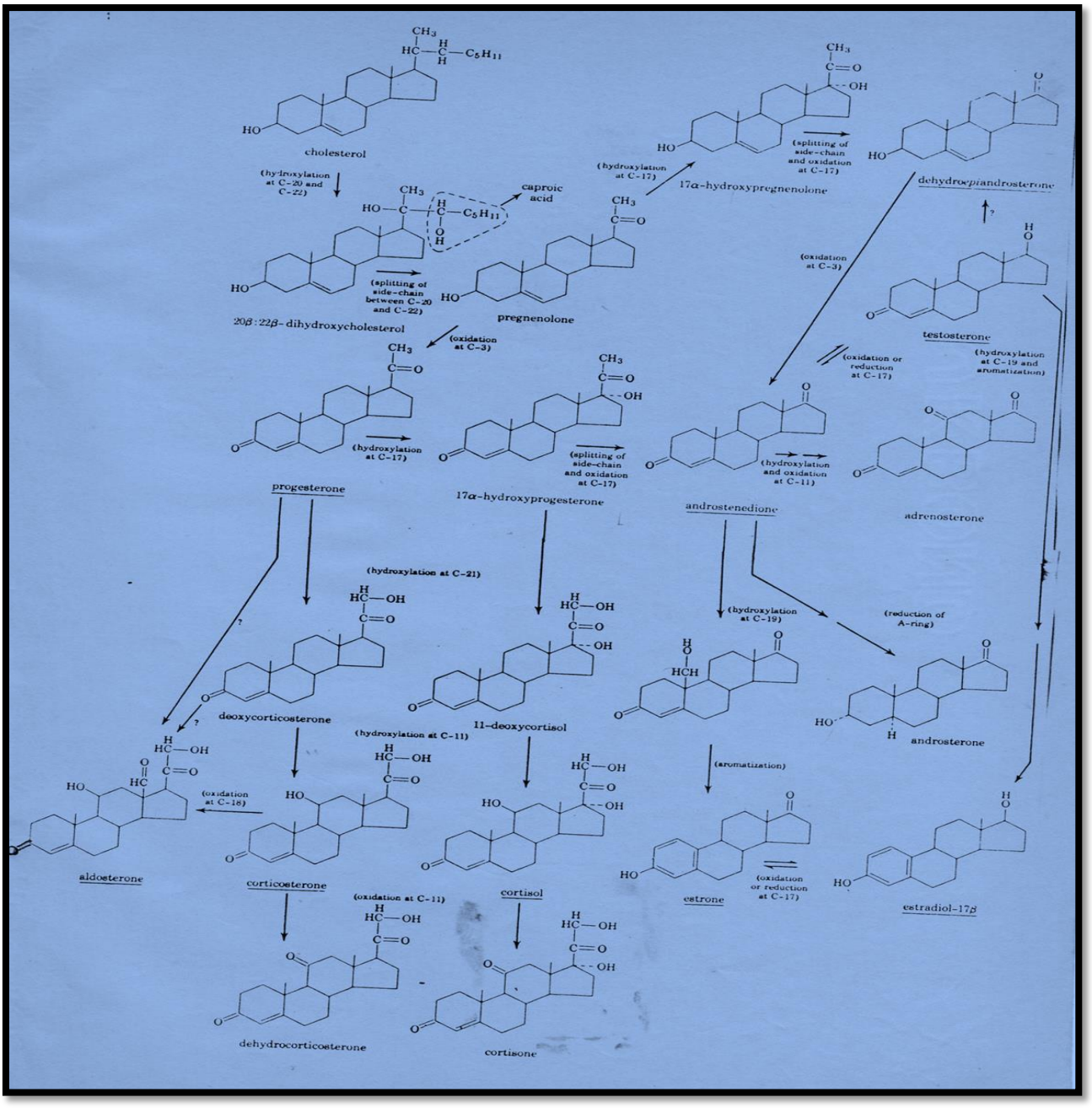

Fig. 1: Major metabolic pathways involved in biogenesis of androgens, estrogens and corticoids from progesterone

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A relationship between adrenal steroidogenesis and reproduction has been demonstrated for several species (Christian,1963; Liptrap,1970; Ramalay1973). Treatment with ACTH blocked ovulation,caused cystic ovaries, and delayed estrous in the pig, whereas hydrocortisone treatment had no effect on ovulation but did delay the onset of estrous(Baldwin & Sawyer,1974; Stoebel& Moberg,1981; and Barb et al. 1982). Progesterone had been found in systemic plasma of rats after ovariectomy. The disappearance of progesterone from systemic plasma eight hours after the removal of ovary and adrenal suggests an adrenal origin for this compound (Feder 1968).

Large amout of AA is shown to be present in the adrenal cortex and suggestion was made that this may be necessary for corticosteroidogenesis (Lowenstein and Zwemer, 1946). Changes in ascorbic acid content have been used as an index of adrenocortical activity as it is reduced by ACTH at a faster rate than cholesterol (Giroud et al., 1942; Sayers et al., 1946).Ascorbate changes in the rat adrenal during the estrous cycle and pregnancy have also been investigated but the regulatory mechanism remains to be illucidated Dean,1952; Foreman,1963).

The objective of the present investigation therefore was:

In Situ changes of peroxidase and ascorbate during the estrous cycle of rat (albino rat ,Wistar strain) to understand the peroxidative pathways of steroid biogenesis.

2. MATERIAL & METHODS

Colony-bred albino rats (Wistar Strain) of our departmental colony maintained on a regimenof 12hrs.light/12 hrs. dark in a temperature controlled room (25^{0} - + 1^{0} C) were used in this study. They received food & water ad libitum. Vaginal smears were taken daily and at least two complete cycles were observed in each rat prior to its use in an experiment. The mature female rats used for the study showed a regular 4-5 days estrous cycle. The adrenal of sexually mature rat were used. Pregnancy, which lasted 21 days in rats, was induced by mating functional females with proven males on the day of vaginal cornification, which was designated day 0. The day of conception was ascertained by examinintg vaginal smears daily; the observed presence of sperms in a smear being taken to indicate the first day of pregnancy.The pregnant females were sacrificed at various stages of pregnancy by cervical dislocation and dissected. The adrenal were stored at -20^{0}C and were later subjected to various histochemical analyses.

Circadian rhythm in the secretions of the hypothalamo-hypophyseal-adrenal axis hs been reported by a large number of workers (Ganong, 1963; Critchlow, 1963; Critchlow et al.1963). Therefore, it follows that comparable results can be obtained by examination of animals killed at the same time of the day. Hence, keeping this in view, all experimental animals were sacrificed at one fixed time.

3. BIOCHEMICAL ANALYSIS

Total Proteins:

A total protein was estimated by the method of Lowry et al. (1951) after proceeding for calibration of caesin).

Ascorbic Acid:

Ascorbate was determined by the colorimetric method of Mindlin and Butler (1938) by following the decolorization of 2,6 dichlorophenolindophenol in metaphosphoric acid after proceeding for calibration of Ascorbic Acid.

Enzyme Activity:

Peroxidase Activity: Total peroxidase activity was measured using guaiacol as donor by the method of Maehly and Chance (1954).

4. RESULTS

Fig. 2. Shows changes in ascorbate and peroxidase activity in the adrenal gland during different stages of reproductive cycle of albino rats.

Peroxidase activity is seen at estrous and the ascorbate content is high. A sharp rise in peroxidase

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Activity is observed at metaestrous and diestrous which continue to rise up to proestrous, when ascorbate content also begins to deplete sharply. This elevation of peroxidase activity is accompanied by rapid depletion of AA which reaches a steady state at proestrous. Thus it appears that during the period of ACTH action in diestrous and proestrous peroxidase levels are high and this is accompanied by decline in ascorbate levels. A very high peroxidase activity is seen at diestrous and proestrous which correlates very well with our histochemical observations reported earlier.

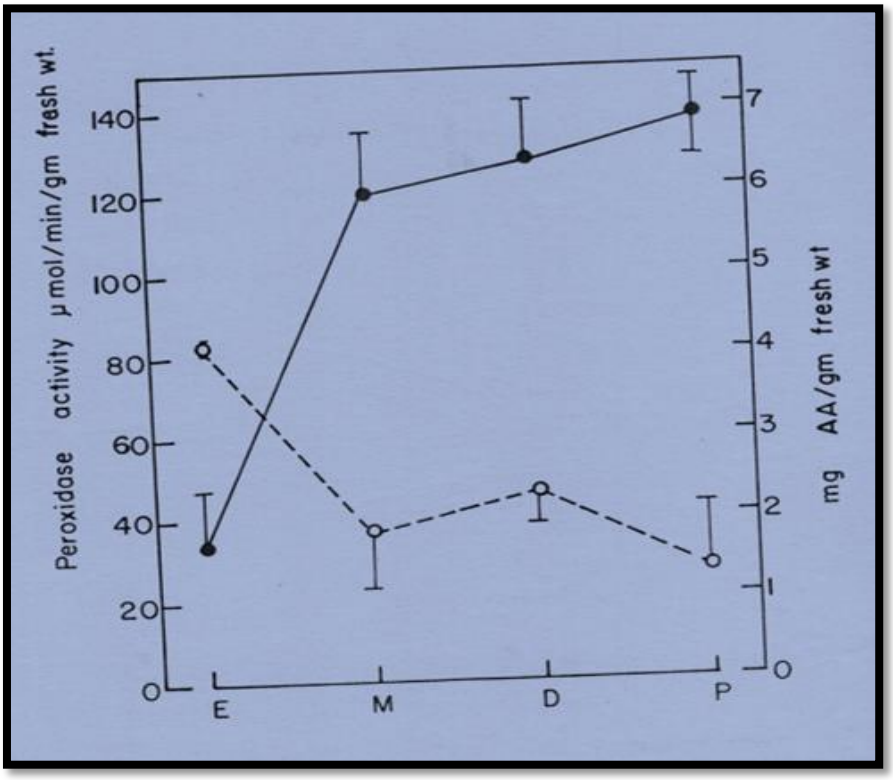

Fig. 2: Changes in ascorbate and peroxidase activity in the adrenal gland during different stages of reproductive cycle of albino rat.

5. DISCUSSION

Peroxidase is present in the inner layer of adrenocortical Cells but not in chromaffin cells of ovulatory animals of different groups of vertebrates which are associated with the functioning of ACTH hormone and progesterone & corticosteroid secretion. Progesterone is known to be a precursor of several steroid hormones including androgens estrogens and corticoids. (Fig. 1), Samuels and Uchikawa (1967) in vitro studies have shown that it occupies a key position in the biosynthesis of adrenal corticoids. Recently, progesterone had been found in systemic plasma of rats after ovariectomy. The disappearance of progesterone from systemic plasma eight hours after removal of the ovary and the adrenal suggests an adrenal origin for this compound (Feder,1968).

Δ^5 - 3β –Hydroxysteroid dehydrogenase and Cytochrome oxidase are present in the adrenocortical cells during the entire period of sexual cycl The enzyme activities like Δ^5-3β-hydroxysteroid dehydrogenase, 21- hydroxylase(21-OHase) and 11β-hydroxylase (11β-OHase) in the adrenals of mammals have been correlated with the synthesis of progesterone and corticosteroids in the cells of zona fasciculata and zona reticularis (Rubin et al.,1957; Chester Jones, 1957 and Rubin et al., 1963).e. Thus the characteristic function of adrenocortical cells regulating large secretion of progesterone during increased sexual activity appears to be related to the presence of peroxidase in these compartments.The adrenal cortex of many non-mammalian species are recognized as the chief site of conversion of ^{14}C acetate to progesterone (Vinson and Whitehouse, 1973a). Also the biochemical studies have shown that adrenal cortex is the chief site for the synthesis of steroid hormones namely Progesterone, cortisol and corticosterone (Hayano et al., 1956; Resko, 1969; Holzbauer, 1969). Marston & coworkers (1985[39]) employing in vitro biochemical techniques, have demonstrated that the bovine adrenocortical cells are able to biosynthesize a variety of steroids, including progesterone, 17- hydroxy progesterone and

androgens under the action of ACTH and it has been suggested that ACTH increases the synthesis of 17 – hydroxylase while reducing the 21-hydroxylase, thereby causing an increased formation of cortisol in the bovine adrenocortical cells (Kramer et al., 1983).

Campbell et al. (1977) have shown that steroid secretion from the adrenal cortex of the rat fluctuates during the various stages of estrous cycle. In ovariectomised animals the levels of estradiol remains unchanged at proestrous or metaestrous for 24hrs. Suggesting that this steroid does not exert negative feedback control on FSH. However, in ovariectomised and adrenalectomised animals estradiol levels fall to baseline, suggesting that adrenal can maintain the normal levels of estradiol in the serum.

Oocyte maturation and ovulation in several amphibians can be induced by adrenocortical hormones such as cortisone, hydrocortisone, corticosterone,deoxycorticosterone,progesterone &testosterone among the gonadal steroids (Bergers and Li, 1960;Wright,1961; Ramaswami,1962; Edgren and Carter,1963; Masui 1967; Schuetz, 1967)., whereas maturation in the catfish is stimulated mainly by adrenocortical hormones,principally hydrocortisone and deoxycorticosterone (Goswami and Sundararaj,1971a; Sundararaj Goswami, 1971). Mammalian hypophyseal hormones (FSH, TSH, GH & and ACTH) elicit a marginal ovulatory response, while ovine (LH) and corticosteroids are effective in inducing ovulation and spawning in catfish (Sundararaj and Goswami, 1966). Suggestion has been made that LH may not act directly on the ovary but via the interregnal and corticosteroids. (Goswami & Sundararaj, 1971).

The presence of high peroxidase activity in the adrenocortical cells of the non-mammalian vertebrates during the ovulatory phase may be correlated with the synthesis of progesterone which act synergistically with corticosteroids to cause ovulation in these species as suggested in the fowl (Soliman et al., 1974).

Furthermore, since peroxidase mediated reactions are many fold faster than dehydrogenase reactions, the association of high peroxidase activity in these regions, and lack of activity in adrenocortical cells at follicular phase, in growing follicle of the ovary and IGT of the ovary would suggest that the high rate of progesterone formation may be associated with the functioning of this enzyme at specific sites. Peroxidase thus appears to be involved in the biosynthetic machinery controlling corticosteroidogenesis.

LH first stimulates the interrenal to produce corticosteroids which then directly act on the oocyte to induce maturation and ovulation. Potencies of different steroids in inducing oocyte maturation and ovulation differ in different species. However, it is not clear whether the two systems operate independently or in complement with each other.

Correlated morphological and biochemical studies have shown that the membrane of the agranular ER plays significant role as sites for enzymes involved in corticosteroidogenesis Sander et al.,1976). It is the site of at least three major steroidogenic enzyme systems Inano et al., 1969b), allowing the conversion from exogenous or endogenous cholesterol to the definitive hormones: Δ^5-3β-hydroxysteroid dehydrogenase which catalyses the transformation of pregnenolone to progesterone (Beyer and Samuels, 1956), 21-hydroxylase (Ryan 1973) and 17- hydroxylase (Plager and Samuels, 1954) which transforms progesterone to corticosterone or cortisol.

The high activity of peroxidase seen in the hypertrophied cortical cells of the interrenal gland during the ovulatory phase and the zona fasciculata and zona reticularis of the pregnant rat and rabbit suggest that this enzyme may indeed be involved in the biosynthesis of progesterone, known to be the precursor of several other steroid hormones including androgens, cortisol, corticosterone that control oocyte maturation and ovulation.Δ^5-3β-hydroxysteroid dehydrogenase being present during the entire sexual cycle viz., follicular, prespawning and spawning period, the specific function of the adrenocortical cells appears to be characterized by the presence of peroxidase, which is observed in the cortical cells alone during the ovulatory phase and pregnancy, the well known site for the secretion of progesterone and corticosteroids. The zona fasciculata and zona reticularis which had been suggested to be actively involved in sex hormone secretion also are characterized by the presence of peroxidase lends further support to the view.

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