STUDIES ON THE MECHANISM OF NIDATION

XIX. HISTOCHEMICAL CHANGES IN THE OVARIES OF PREGNANT RATS FOLLOWING ERGOCORNINE

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Summary. When pregnant rats are given a single subcutaneous injection of ergocornine methanesulphonate on the 4th day of gestation, physiological indications of changes in the ovary are manifold.

Activities of a number of histochemically demonstrable enzymes were investigated in the ovaries of ergot-treated pregnant rats. The enzymes included Δ^5 -3 β -hydroxysteroid dehydrogenase, succinic dehydrogenase, alkaline and acid phosphatase and adenosine-triphosphatase.

The ovaries were examined at 2-, 4-, 8- and 24-hr intervals after administration of the drug. Macroscopic and microscopic vascular dilatation and engorgement were seen in the ovaries at 2, 4 and 8 hr and had decreased by 24 hr. Activities of Δ^5 -3 β -hydroxysteroid dehydrogenase and succinic dehydrogenase were histochemically demonstrable in the corpora lutea of gestation as well as other sites in the ovary at 2, 4 and 8 hr after ergocornine. At 24 hr, portions of the corpora lutea of gestation showed necrosis and dissolution of cells and many healthy vesicular follicles had developed. These changes in the ovary (luteolysis in the corpora lutea of gestation and development of follicles to the ovulation stage) form the basis for the physiological effects—interruption of gestation and appearance of oestrus—which occur 2 to 3 days after administration of ergocornine to pregnant rats during progestation.

INTRODUCTION

It has been demonstrated that a single injection of the alkaloid ergocornine to rats during progestation interrupts pregnancy and pseudopregnancy (Shelesnyak, 1954, 1955) but when progesterone was given together with the ergocornine this action was prevented, pregnancy or pseudopregnancy being maintained. It was postulated that ergocornine in some way reduced the availability of progesterone to the progestational uterus, brought about by its effect on the ovary, or, more precisely, the corpora lutea which presumably constitute the major site of progesterone production in the rat ovary.

The enzyme Δ^5 -3 β -hydroxysteroid dehydrogenase is involved in the biological synthesis of hormonally active steroids (Samuels, 1960). It is histo-

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chemically demonstrable (Wattenberg, 1958) and its presence has been shown in the parenchymatous cells of steroid producing organs in many species. 3β -Hydroxysteroid dehydrogenase has been shown to occur in the rat ovary in the interstitial tissue, the thecae of follicles and the luteal cells (Levy, Deane & Rubin, 1959). Moreover, the activity of this enzyme has been shown to vary in the ovaries, adrenals and placentae of a number of species during different physiological states and under experimental conditions (Rubin, Deane & Hamilton, 1963; Rubin & Deane, 1965). In addition, the activities of some other histochemically demonstrable enzymes have been shown to reflect the functional state of the ovary (Lobel, Rosenbaum & Deane, 1961; Deane, Lobel & Romney, 1962). The studies reported here were undertaken to find out whether the administration of ergocornine to pregnant rats produced an effect on the ovaries which could be detected with the histochemical techniques employed and thus contribute to an understanding of the effects of the drug on pregnant rats.

MATERIALS AND METHODS

Animals and treatment

The animals used in these experiments were adult, virgin, female rats of the Biodynamics Institute colony, originally Wistar stock. The animals were housed in air-conditioned quarters in which a normal light cycle obtained. Purina laboratory chow and tap water were available *ad libitum*. Vaginal smears were recorded daily, and only animals having regular 4- or 5-day cycles were used for the study.

Pregnancy. Females in pro-oestrus were caged overnight with males and insemination verified on the following morning by the presence of a vaginal plug or spermatozoa in the vaginal smear. The day on which spermatozoa or plug were found in the vaginal smear was designated as Day 0 (L_0) of pregnancy, the days following as L_1 , L_2 , etc.

Treatment. Twenty-one pregnant rats were used for this study, fifteen experimental and six controls. The experimental animals received a single injection of ergocornine, 1 mg in 0.25 ml 70% alcohol, on the morning of L_4 . The control rats received an injection of the vehicle only. Groups of three experimental animals and one control were killed 2, 4 and 8 hr after injection. One group of six ergocornine treated and three control animals was killed 24 hr after injection.

Preparation of material

The animals were killed by cervical dislocation, the ovaries rapidly removed and representative ones from each group were: (a) frozen in liquid air, mounted on block holders and transferred to the cryostat; (b) placed in chilled neutral formalin and kept overnight at 4° C; and (c) fixed overnight in cold alcohol-formalin-acetic acid.

Preparation of cryostat sections. The frozen ovaries mounted on block holders were cut at 10 μ in a Slee cryostat at -15° C. The sections were picked up on cover glasses and stored in the cryostat or in a deep freezer until used

(maximum period of storage 1 week). The unfixed sections were incubated at 37° C in air for the demonstration of: (a) 3 β -hydroxysteroid dehydrogenase activity (Rubin, Deane, Hamilton & Driks, 1963); and (b) for the activity of succinic dehydrogenase (Barka & Anderson, 1963). Following both types of incubations, sections were rinsed in distilled water, placed in formol alcohol for $\frac{1}{2}$ hr, then rinsed and mounted in glycerol-gel. Control preparations were incubated in media lacking the substrates.

Preparation of frozen sections. The blocks of tissue fixed overnight in cold, neutral formalin were cut at 10 μ on a freezing microtome and sections incubated for: (a) acid phosphatase activity in the metal salt medium (Gomori, 1952), 20 min incubation; (b) alkaline phosphatase activity, 10 min incubation (Pearse, 1961); and (c) adenosine-tri-phosphatase activity; 15 min incubation (Pearse, 1961). Other sections were stained for lipids with oil-red-o dissolved in 60% triethyl phosphate or with Sudan Black (Pearse, 1961), and for histological examination with haematoxylin and eosin, or with dilute methylene blue and eosin at pH 5.1. Control preparations for the enzymic methods were incubated in media lacking the substrates. For lipid methods, control sections were first extracted with acetone for 30 min.

Preparation of paraffin sections. The ovaries fixed in alcohol-formalin-acetic acid were dehydrated, cleared and embedded in paraffin. Sections were cut at 5 μ and representative ones stained as follows: (a) with haematoxylin and eosin; (b) overnight with dilute methylene blue (10^{-3} M) and eosin (2×10^{-4} M); (c) by the PAS method (Pearse, 1961); (d) with Masson's trichrome stain (Lillie, 1954); and (e) with a stain for reticular fibres (Lillie, 1954).

Interpretation of sections incubated for enzymic activity

3 β -Hydroxysteroid dehydrogenase is a diphosphopyridine nucleotide-dependent dehydrogenase capable of oxidizing Δ^5 -3 β -hydroxysteroids to Δ^4 -3-ketosteroids. Histochemically, activity of this enzyme may be visualized by incubation of an unfixed tissue section in a medium containing the substrate dehydroepiandrosterone (DHA), diphosphopyridine nucleotide (DPN), a tetrazolium salt and buffer. Hydrogen ions removed from the substrate by the enzyme are transferred to DPN, then from DPNH to the tetrazole by a portion of the electron transport system related to DPNH-cytochrome-c reductase. Consequently, the method demonstrates the activity of a sequence of enzymes. Thus, when specific substrates and DPN or TPN are used, a part of the diaphorase system (that associated with the specific dehydrogenase) is demonstrated. It is presumed that the specific dehydrogenases have a close structural association with the diaphorases. However, the rate-limiting factor is the activity of the specific dehydrogenase, since the diaphorase in the cell is apparently able immediately to oxidize all the DPNH produced (Barka & Anderson, 1963). A positive result is indicated by the deposition of the formazan (the reduced tetrazole, now insoluble and coloured blue) at sites of enzyme activity. No deposition was seen in sections incubated in media lacking the substrate (DHA).

Succinic dehydrogenase. This enzyme constitutes one of the main components of the tricarboxylic acid cycle; it is the only histochemically demonstrable dehydrogenase system which requires no co-enzyme. Succinic dehydrogenase

activates the hydrogen atoms of succinic acid and effects their transfer to the cytochrome-b, probably by means of a carrier. Then the tetrazolium salt acts as electron acceptor and the blue formazan deposits occur in the form of dots, presumably on the mitochondria. When the substrate (succinate) was omitted from the media, no formazan was seen in the sections.

Alkaline phosphatase. The phosphate released from the substrate (β -glycero-phosphate) in the incubation medium by activity of the enzyme is deposited as calcium phosphate and this may be rendered visible by treating the sections first with cobalt and then with sulphide. This reaction product of alkaline phosphatase activity occurs in the form of a black precipitate. No precipitate was observed in sections incubated in media lacking the substrate.

Acid phosphatase. The phosphate released by enzymatic hydrolysis from the substrate (β -glycero-phosphate) is precipitated by lead ions present in the incubating medium. The lead phosphate is then transformed into lead sulphide by treatment of the sections with ammonium sulphide, and subsequently appears as a dark brown precipitate. Sections incubated in media lacking the substrate showed no reaction.

Adenosine-triphosphatase. Essentially the same principle as described for the demonstration of acid phosphatase activity using adenosine-triphosphate as substrate and carried out at pH 7.2. Brown-black deposits indicate sites of ATPase activity. Sections incubated in media lacking the substrate showed no precipitates.

RESULTS

L₄ Ovaries of pregnant rats

The ovaries of the control animals, killed 2, 4, 8 and 24 hr after injection of the vehicle only, will be considered together. On macroscopic examination, the ovaries appeared well vascularized and pink, with prominent corpora lutea visible at the surface of the ovaries.

On microscopic examination, in all cases the ovaries contained a variable number of large, well-vascularized corpora lutea, with cords of clearly defined and non-vacuolated luteal cells, typical of the corpora lutea of this stage of gestation. There were also many medium and small follicles and some large ones, most of which appeared atretic. The interstitial tissue was well developed. The ovaries also contained involuting shrunken corpora lutea of previous cycles, marked by the presence of macrophages, connective tissue and the regression or disappearance of the luteal cells.

The enzymic histochemical picture was similar in control ovaries taken at the various time intervals from L₄ to L₅ so that, on the basis of histochemistry, it was not possible to distinguish between them. Consequently, they will be discussed together. The activity of the enzyme 3β -hydroxysteroid dehydrogenase was confined to the cells of the thecae externae of follicles, the cells of the interstitial tissue and those of the corpora lutea, i.e. the sites of steroid synthesis in the rat ovary. In addition, activity of 3β -hydroxysteroid dehydrogenase was seen in the granulosa of atretic follicles.

Succinic dehydrogenase activity was strong in the interstitial tissue, in involuting corpora lutea, the thecae of follicles and in the phagocytes in the

ovary. Moderate activity occurred in the newly formed corpora lutea of gestation and weak activity in the granulosa of follicles. Activity was also observed in walls of the larger blood vessels. Morphologically, the protein which forms the succinic dehydrogenase molecules has been associated with the cristae of mitochondria. Cells and tissues showing high levels of succinic dehydrogenase activity are assumed to be metabolically active (Barka & Anderson, 1963).

Acid phosphatase activity was prominent in the luteal cells of involuting corpora lutea, as well as in the phagocytes. Acid phosphatase activity in these structures was localized in large intracellular granules. Activity was not demonstrable in the granulosa of healthy follicles, but was a striking feature in the granulosa of atretic ones and in the phagocytes which often appeared to contribute to the destruction of the ova within these follicles. Only few reactive granules were seen in the luteal cells of the corpora lutea of gestation. The histochemically demonstrable activity of acid phosphatase is frequently used as a marker for the presence of lysosomes (Novikoff, 1961). When enlarged cytoplasmic bodies rich in acid phosphatase occur in cells undergoing regressive changes (i.e. involuting luteal cells or atretic granulosa cells), these bodies have been termed 'cytolysomes', and they are concerned with the degradation of the cells' own organelles (Novikoff, 1963).

Alkaline phosphatase activity was demonstrable in the vessels of the thecae of follicles, in the sinusoids of the corpora lutea and in the smaller ovarian blood vessels. Adenosine-triphosphatase activity occurred in the same locations and in addition in larger ovarian blood vessels. Activity was especially prominent in well-vascularized corpora lutea and in the thecae of small and medium sized follicles. Activities of both enzymes are associated with transport of material across cell membranes (Danielli, 1952, 1958; Novikoff, 1960).

Lipids (i.e. free lipids demonstrable with fat stains) were present in abundance in the form of coarse droplets in the interstitial tissue, in involuting corpora lutea and in the thecae externae of follicles. Lipids also occurred in the phagocytes in the ovary and in the granulosa of atretic follicles. Steroid-producing tissues are generally rich in lipids (Wolman, 1964) but the concentration of lipids within the cells changes with the functional activity of the cells. The lipids in the luteal cells of involuting corpora lutea and the granulosa of atretic follicles are associated with degenerative changes in these structures.

In sections stained with basic dyes for the demonstration of nucleic acids, the small follicles were deeply stained, the corpora lutea of gestation likewise were basophilic, while the old corpora lutea were pale. In sections stained with PAS for the demonstration of glycogen and glycoprotein, PAS positive material was seen in the liquor folliculi of follicles, around the ova, in phagocytes and in muscle fibres in the walls of the blood vessels. Other elements in the ovary were negative with the PAS stain. Collagenous fibres occurred mainly at the periphery of the ovary and at the centre, forming a network around the hilar blood vessels. Cores of connective tissue with coarse collagenous fibres were seen in the corpora lutea, especially those of previous cycles, and fine collagenous fibres around the blood vessels between the cords of luteal cells in the corpora lutea of gestation. However, the reticular network in the ovary is

more prominent than the collagenous. A network of reticular fibres surrounds growing follicles and supports the thecae, but does not penetrate into the granulosa.

Changes following ergocornine

Macroscopically the ovaries of pregnant rats killed 2 hr after ergocornine injection appeared dark red and the ovarian blood vessels were prominent. Microscopically, a pronounced vascular dilatation and engorgement were the most striking gross effects seen in the ovaries of pregnant rats given 1 mg ergocornine 2 hr previously. The hilar and central ovarian vessels were dilated and packed with erythrocytes, the thecal vessels of the corpora lutea, as well as those within them, were likewise widened and engorged; however, the vessels of the follicles did not appear to be affected (Pl. 1, Fig. 1).

The activity of 3β -hydroxysteroid dehydrogenase, 2 hr after ergocornine administration, was histochemically demonstrable in corpora lutea, interstitial tissue and the thecae externae of follicles. Many developing follicles, from primary to large but without antrum formation, showed strong activity of both alkaline phosphatase and adenosine-triphosphatase in the vascular network around them. The pattern of distribution of succinic dehydrogenase activity appeared normal.

In sections stained with methylene-blue-eosin (used for demonstration of nucleic acids) the granulosa of growing follicles were deeply stained. In sections of ovaries stained with the fat stains ORO or Sudan Black, the thecae of the follicles and the interstitial tissue showed much free lipid (Pl. 2, Fig. 3). Less was present in the corpora lutea, and it appeared that when the ovaries of ergocornine-treated rats were compared to those of control animals (killed at the same time, but not given the drug) there was a decrease in the amount of free fats in the corpora lutea of the ergocornine-treated animals. However, this is a subjective impression, no quantitative estimations were carried out. There was only moderate acid phosphatase activity in the luteal cells of the large corpora lutea of gestation and strong acid phosphatase activity in the phagocytes of earlier corpora lutea. Many follicles in various stages of development up to antrum formation appeared to have no demonstrable acid phosphatase activity within the granulosa and therefore were adjudged to be healthy. The distribution of PAS positive material, collagenous and reticular fibres, was similar to that of control ovaries.

The ovaries of animals killed 4 and 8 hr after ergocornine administration were also dark red in appearance and showed engorgement of the hilar blood vessels, and of the vessels of the corpora lutea. There was no apparent decrease in the demonstrable activity of 3β -hydroxysteroid dehydrogenase in the luteal cells, in the follicular thecal cells, or in the interstitial tissue (Pl. 3, Figs. 4 and 5). Likewise, activity of succinic dehydrogenase remained strong (Pl. 4, Fig. 6). However, acid phosphatase activity in older corpora lutea was increased (Pl. 7, Fig. 12). There was little intracellular free lipid demonstrable in the corpora lutea of gestation (Pl. 5, Figs. 7 and 8). At 8 hr, a slight increase in the activity of acid phosphatase within the luteal cells was observed (Pl. 6, Figs. 9 and 10). The activities of alkaline phosphatase and adenosine-triphos-

FIG. 1. Cryostat section of rat ovary 2 hr after ergocornine administration; stained with haematoxylin and eosin, $\times$ circa 90. Note dilated and engorged vessels in central part of ovary; bv, blood vessels; cl, corpus luteum.

FIG. 2. Paraffin section of rat ovary 24 hr after ergocornine administration; trichrome, $\times$ circa 22; note decrease of vascularity around corpora lutea. The corpus luteum of gestation, marked cl, is necrotic in its lower part. Other small corpora, to the left of the two follicles, show no areas of necrosis.

FIG. 3. Cryostat section of rat ovary 2 hr after ergocornine administration; stained with Sudan Black for demonstration of free lipids, $\times$ circa 30; cl, corpus luteum; f, follicle; i, interstitial tissue.

FIGS. 4 and 5. Cryostat sections of rat ovary 4 hr after ergocornine administration; incubated for demonstration of 3β -hydroxysteroid dehydrogenase activity; cl, corpus luteum; f, follicle; i, interstitial tissue; th e, theca externa; both $\times$ circa 75. Note strong activity in corpora lutea as well as in interstitial tissue and thecae of follicles.

FIG. 6. Cryostat section of rat ovary 4 hr after administration of ergocornine; incubated for demonstration of succinic dehydrogenase activity; $\times$ circa 75. Note strongly reactive old corpora lutea and interstitial tissue, moderately reactive corpora lutea of gestation at top and lower right (cl), little activity in follicle (f); and strong activity in interstitial tissue (i).

Figs. 7 and 8. Sections of rat ovary 4 hr after administration of ergocornine; stained with Sudan Black for demonstration of lipids. Fig. 7. Cryostat section, $\times$ circa 30. Note that lipids are not demonstrable in granulosa of both follicles, but marked accumulation is seen in old corpus luteum at left. Fig. 8. Little free lipid occurs in young corpus luteum of gestation (ycl), and much free lipid is seen in old corpus luteum (ocl).

FIGS. 9 and 10. Sections of rat ovary 4 hr after ergocornine administration; incubated for demonstration of acid phosphatase activity; $\times$ circa 75. Fig. 9 shows part of a corpus luteum of gestation; Fig. 10 part of an old corpus luteum (ocl) and a follicle, upper right.

FIGS. 11 and 12. Sections of rat ovary 8 hr after administration of ergocornine. Fig. 11. Cryostat section stained with methylene blue eosin; $\times$ circa 75. Note well-defined and non-vacuolated cells of the corpus luteum (cl) and deeply stained granulosa cells of the follicle (f). Fig. 12. Frozen section incubated for acid phosphatase activity; $\times$ circa 75. Note large atretic vesicular follicle (vf), with enzymatic activity in granulosa cells, and part of strongly reactive old corpus luteum to the left.

FIGS. 13 and 14. Cryostat sections of rat ovaries 24 hr after ergocornine administration. Fig. 13, $\times$ circa 75, section incubated for 3β -hydroxysteroid dehydrogenase activity; cl, corpus luteum; f, follicle. Note activity in the theca externa (th e) of follicle in the interstitial tissue and in part of the corpus luteum of gestation at left. Fig. 14, $\times$ circa 75. Section incubated for succinic dehydrogenase activity; cl, corpus luteum; f, follicle; i, interstitial tissue.

FIGS. 15 and 16. Sections of rat ovaries 24 hr after ergocornine administration. Fig. 15, $\times$ circa 75. Cryostat section stained for free lipids. Note absence of lipids in the healthy follicles (f), and accumulations of free lipids in the interstitial tissue (i). Fig. 16, $\times$ circa 75. Frozen sections incubated for acid phosphatase activity. Note activity in corpus luteum (cl) strong in the inner portion of the corpus at top and at right and weaker in the area of necrosis, at bottom and left.

FIGS. 17 and 18. Sections of rat ovary 24 hr after ergocornine administration; $\times$ circa 30. Fig. 17. Frozen section incubated for adenosine-triphosphatase activity. Note stronger activity in old corpora than in corpora lutea of gestation, and lack of activity in granulosa of large follicles (f). Fig. 18. Parallel section incubated for acid phosphatase activity. Note stronger activity in old corpora lutea (at bottom), than in large corpora lutea of gestation (at top) and lack of activity in granulosa of large follicles.

FIGS. 19 and 20. Sections of rat ovary 24 hr after ergocornine administration; $\times$ circa 75; incubated for demonstration of alkaline phosphatase activity. Note highly reactive vascular network around growing follicles of various sizes.

FIGS. 21, 22 and 23. Sections of rat ovaries 24 hr after administration of ergocornine; stained with trichrome. Fig. 21, $\times$ circa 30. Note large corpus luteum of gestation showing necrosis in its outer portion. Fig. 22, $\times$ circa 75. Necrotic area marked by arrows in Fig. 21. Fig. 23, $\times$ circa 75. Corpus luteum of gestation showing necrosis and cords of apparently healthy cells.

phatase were strongest in walls of the follicles and in the vessels of the older corpora lutea. The large corpora lutea of gestation, whose peripheral and internal blood vessels were widely dilated and engorged, showed considerably less alkaline phosphatase and adenosine-triphosphatase activities in the vascular walls. Structurally, however, those corpora lutea showed cords of well-defined and apparently healthy luteal cells (Pl. 7, Fig. 11).

By 24 hr after the administration of ergocornine, some (but not all) of the ovaries on macroscopic examination appeared considerably paler than at 2, 4 and 8 hr after ergot. Microscopically there was decreased vascular dilatation and engorgement (cf. Pl. 1, Figs. 1 and 2). A patchy necrosis was observed within the corpora lutea of gestation. The areas of necrosis were located peripherally, i.e. that part of the corpus luteum which is situated towards the external surface of the ovary (Pl. 1, Fig. 2). These corpora lutea showed an increase in acid phosphatase activity (Pl. 9, Fig. 16). Other portions of the same corpora lutea showed cords of apparently healthy cells with distinct cell outlines and unimpaired 3β -hydroxysteroid dehydrogenase activity and succinic dehydrogenase activity (Pl. 8, Figs. 13 and 14). These areas of dissolution and necrosis of parts of the corpora lutea were seen only in the large corpora of gestation and not in those of previous cycles (Pl. 12, Figs. 21, 22 and 23).

In sections stained with basic dyes the corpora lutea of gestation were pale and the follicles deeply stained. Free fats were demonstrable in the corpora lutea of gestation as well as in the thecae of follicles and the interstitial tissue (Pl. 9, Fig. 15). Only moderate activity of alkaline phosphatase and adenosine-triphosphatase was demonstrable within the corpora lutea of gestation, and then only in those portions that were not necrotic.

A striking aspect of the ovaries of pregnant animals 24 hr after ergocornine administration, was the presence of large vesicular follicles as well as the numerous smaller follicles in various stages of development (Pl. 1, Fig. 2; Pl. 10, Figs. 17 and 18; Pl. 11, Figs. 19 and 20). The follicles possessed external thecae with strong 3β -hydroxysteroid dehydrogenase activity, succinic dehydrogenase activity and a dense vascular network with strong alkaline and adenosine-triphosphatase activities. Most of the large follicles showed no demonstrable acid phosphatase activity, while some of the smaller ones had intense activity of this enzyme in the granulosa cells, indicating atresia. This was also, but rarely, seen in a large, vesicular follicle. Where the large follicles projected from the surface of the ovary the walls of the follicles were stretched and thinned out; at these places the thecae appeared less vascularized and activities of alkaline phosphatase and adenosine-triphosphatase decreased.

DISCUSSION

There is some suggestive evidence that ergot alkaloids act through the pituitary, though the primary site of action is not yet established. Sommer (1953) demonstrated that ergotamine and ergotoxine given to pregnant rats in the second half of gestation resulted in inhibition of mammary gland development and failure of lactation.

Zeilmaker & Carlsen (1962) put forward the hypothesis that ergocornine

causes a temporary inhibition of the secretion of luteotrophic hormone by the pituitary, that this brings about irreversible changes in the corpora lutea, and that the subsequent progesterone deficiency is responsible for the onset of oestrus 3 days after administration of the drug in the progestational period. However, little histological and histochemical evidence of degenerative change was given in support of this hypothesis.

The histochemical observations presented here indicate that marked changes occur in the ovaries within a short period of time following the administration of ergocornine to pregnant rats on L₄. However, these changes are similar to those which accompany the structural alterations taking place in the ovary during the phases of its cyclic activity. Thus, activity of Δ^5 -3 β -hydroxysteroid dehydrogenase is not seen in the granulosa of healthy follicles but does occur after ovulation when they develop into corpora lutea or when atresia occurs (Levy, Deane & Rubin, 1959; Deane, Lobel & Romney, 1962). Likewise, an increase in acid phosphatase activity and accumulation of free lipids are associated with atresia of follicles and involution of corpora lutea (Deane, 1952; Guraya & Greenwald, 1964). On the other hand, growth and development of follicles are characterized by activities of alkaline phosphatase, adenosine-triphosphatase and succinic dehydrogenase (Arvy, 1963; Velardo & Rosa, 1963; Bulmer, 1964). It is evident that the enzymic alterations seen in the ovaries following ergocornine are typical, but not causative of the structural changes which occurred in the ovaries as a result of the drug. These changes—involution of the corpora lutea of gestation and development of healthy follicles to the ovulatory stage—are such as are known to occur in the ovary on modifications of the hormonal support, i.e. a drop in the levels of LTH and a rise in FSH and LH.

Within 2 hr after the administration of ergocornine to pregnant rats a vascular response is seen in the ovarian vessels, the thecal luteal vessels and subsequently also in the sinusoids within the corpora lutea. Apparently a dilatation of the venous channels is the principal change involved. It is possible that this dilatation leads to stasis of the blood and this in turn to a reduced oxygen tension which may be one of the causes of the necrosis seen in the corpora lutea of gestation 24 hr after ergocornine administration. Short, McDonald & Rowson (1963) have shown that, in the ewe, interference with the blood supply of the corpus luteum inhibits the secretion of progesterone.

During the first few hours after ergocornine administration there is no apparent change in the ability of the luteal cells to react when incubated for activities of succinic dehydrogenase and 3 β -hydroxysteroid dehydrogenase. The latter observation was not surprising, since it has been shown in this laboratory that the ability of ovarian homogenates to convert Δ^5 -pregnenolone to progesterone is unimpaired during the first 8 hr after ergocornine treatment (Lamprecht, Shelesnyak, Zmigrod & Lindner, unpublished). This would indicate that the luteal cells are not yet irreversibly damaged, but, on the other hand, the presence of 3 β -hydroxysteroid dehydrogenase does not imply that the luteal cells were actually synthesizing progesterone and/or releasing it into the circulation. However, vapour-phase chromatographic analysis has shown that endogenous progesterone is actually present in near-normal amounts

in ovaries of pseudopregnant rats during the first 12 hr following ergocornine treatment (Lindner, Shelesnyak & Zmigrod, unpublished).

Noteworthy also was the lack of increased acid phosphatase activity in the luteal cells and the fact that they did not show an abundance of free lipid droplets initially. Subsequently, at 24 hr, acid phosphatase activity and free lipids were demonstrable only in the cords of luteal cells that remained in the corpora lutea of gestation, and not in the necrotic portions of these corpora.

It is evident that there are differences between the regression of the corpora lutea of gestation following ergocornine and their regression as it normally occurs in cyclic ovarian activity. Corpora lutea, both of the cycle and of gestation, involute by a process of cellular shrinkage and resorption brought about by the activity of the cells' own hydrolytic enzymes and that of the phagocytes, and accompanied by a relative increase in the connective tissue. This process does not take place rapidly; therefore corpora lutea of two or three generations are generally present in the rat ovary at any one time. Quite a different picture was seen in the corpora lutea of gestation following administration of ergocornine. Large portions of the corpora lutea of gestation showed a dissolution and necrosis of luteal cells 24 hr after administration of the drug. Undoubtedly, this would lead to a drop in the levels of progesterone. Recently, Rubin & Deane (1965) have shown that once a maximum degree of activity of 3β -hydroxysteroid dehydrogenase is attained within existing steroid-producing cells, further increases are related to increases in ovarian weight, i.e. to the amount of tissue producing the hormone. Consequently, if a sudden reduction in the number of luteal cells occurs, the amount of hormone produced would also drop.

According to Zeilmaker & Carlsen (1962) the progesterone deficiency is responsible for the ovulation 3 days after administration of ergocornine. However, it would appear that the follicular growth is stimulated early after administration of the drug, since 24 hr later large healthy vesicular follicles were observed. These may also be seen in the ovaries of pregnant animals not treated with ergocornine but then they are usually atretic, as shown by acid phosphatase activity within the granulosa cells.

Thus, 24 hr after administration of ergocornine to pregnant rats on L_4 , two main effects on the ovary are clearly visible: initiation of luteolysis of the corpora lutea of gestation and incipient ovulation. One cannot judge, however, from the histochemical picture alone which came first, or whether one is a consequence of the other. However, it should be remembered that in the rat, in the absence of a growing crop of new follicles, corpora lutea do not degenerate (i.e. in hypophysectomized animals), and that they retain their functional capacity during long periods. It may well be that ergocornine causes a temporary lack of luteotrophic secretion and thus of luteal support, but it remains an open question whether this alone initiates luteolysis. In the rat, both follicles and corpora lutea are transiently developed and their growth and regression are dependent on a delicate hormonal balance. It is possible that a temporary hormonal imbalance brought about by the ergocornine is sufficient to initiate a spurt of growth of new follicles and this in turn contributes to the luteolysis seen 24 hr after the ergocornine administration.

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