The paper entitled "Ovulation as an Inflammatory Reaction—A Hypothesis" was not part of the symposium to which the rest of this issue is devoted. This paper was reviewed by four colleagues who recommended publication on the grounds that this review will be of interest to a significant number of readers of Biology of Reproduction.

Ovulation as an Inflammatory Reaction—A Hypothesis

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INTRODUCTION

The objective of this paper is to develop a "working model" which integrates the physical and chemical events that are presently known to take place in a mature ovarian follicle during ovulation. The model is based on the hypothesis that an ovulatory surge of gonadotropin induces an inflammatory reaction in mature follicles and that it is this inflammatory condition which brings about the actual rupture of the ovarian surface.

The comparison of ovulation to an inflammatory process is not a farfetched idea. The literature reveals substantial evidence that ovulatory follicles have numerous features similar to those of inflamed tissues.

More importantly, by describing ovulation as an inflammatory reaction, it is possible to formulate a model which incorporates most of the current data on the biochemical changes which take place during ovulation. Of particular interest is the central role which this hypothesis gives to prostaglandins as mediators of the inflammatory process. In addition, the hypo-
Theoretical model establishes a relationship between prostaglandins and ovarian proteolytic activity.

In developing the hypothesis, the first section of this paper will outline the basic features of an inflammatory process. The main portion of the review concentrates on specific similarities between the metabolic events that are known to occur in both inflammation and ovulation. Toward the end, the report considers anti-inflammatory agents as potential regulators of ovulation. The final pages synthesize the data into a "working model" of ovulation as an inflammatory reaction.

CHARACTERISTICS OF INFLAMMATORY PROCESSES

"Inflammation is a process and not a state" (Ebert and Grant, 1974). Inflammation is a process that can be initiated by a wide variety of conditions that cause irritation or injury to tissue. The source of the inflammatory insult can be bacteria, viruses, genetically-based cellular and endocrine abnormalities and physical and chemical stimuli (Van Arman, 1976). Essentially, all tissues in mammalian organisms are capable of undergoing an inflammatory reaction in response to such irritants.

Inflammatory reactions are commonly identified as being either "acute" or "chronic" depending on whether they are of short duration or extend over a long period of time. However, this differentiation is little more than a convenient temporal division, because there is no clear demarcation between acute and chronic inflammation. Furthermore, the features of both conditions often appear in the same lesion.

Still, inflammation is a dynamic process and some changes can be detected as the condition develops. For example, exudation and edema are more prominent features of the acute condition, whereas phagocyte immigration and tissue proliferation are more characteristic of chronic inflammation (Bonta and Parnham, 1978).

An inflammatory process usually ends with permanent destruction of tissue or with complete healing (Van Arman, 1976). Therefore, during the course of an inflammatory process, the tissue may be simultaneously engaged in both damage and repair (Bonta and Parnham, 1978). This dual activity has made it more difficult to decipher continuity within the sequence of events that takes place in the overall process. Nevertheless, a great deal has been learned about inflammation in recent years and the following pages summarize the more established characteristics of the process.

Physical Changes

Vascular dynamics. A century ago, it was recognized that one of the "most important events of inflammation is a change in the vessel wall" (Ebert and Grant, 1974). The principal modifications in the vascular system include local vasodilatation and increased capillary permeability, two distinct phenomena which increase the local blood flow and cause extravasation of serum proteins, respectively. These alterations in the vascular system lead to edematous swelling of the tissue (Bonta and Parnham, 1978; Ebert and Grant, 1974; Owen, 1977).

Immigration of leukocytes and macrophages. If an inflammatory reaction persists for several hours, the inflamed tissue produces chemo-attractive agents which attract a variety of migratory cells into the area (Bonta and Parnham, 1978; Gabbiani, 1977; Perper, 1976). The first cells to reach the site of inflammation are polymorphonuclear leukocytes, which serve as a line of defense against infection (Ebert and Grant, 1974). As these cells enter the inflamed area they release a number of mediators and enzymes which propagate the inflammatory reaction and inadvertently cause tissue damage (Perper, 1976). Macrophages, with their abundance of lysosomes, also appear in the inflammatory exudate as do lymphocytes. Thrombocytes aggregate in the vascular system (Ebert and Grant, 1974). All of these cells, but especially the neutrophils, persist until the wound healing process has set in (Gabbiani, 1977).

Proliferation of fibroblasts. The repair phase of the inflammatory process is characterized by the proliferation of fibroblasts and the multiplication of small blood vessels (Berliner et al., 1967; Arrigoni-Martelli, 1977; Kang, 1978). Fibroblasts are especially important in remodeling the connective tissue elements at the site of inflammation. These cells are the source of collagen and proteoglycans, the macromolecules that are needed in the formation of scar tissue (Kang, 1978).

The mechanisms by which chemotactic substances induce fibroblasts to accumulate at the site of injured tissue and lay down new...
connective tissue are not completely understood. There is evidence that inflammatory exudates contain a "mitogenic factor" which stimulates DNA synthesis and cell division of quiescent fibroblasts and this substance may be involved in the proliferation of cells in the inflamed tissue. Other stimuli which may attract fibroblasts from contiguous connective tissue toward the site of inflammation include 1) lymphokines from activated lymphocytes, 2) C5-derived peptide from the complement system and 3) collaged-derived peptides from tissue that has been degraded by collagenolytic enzymes (Kang, 1978).

**Chemical Changes**

**Cyclic nucleotides.** Cyclic nucleotides have been identified as key intermediates in the reactions of cells to exogenous stimuli. Therefore, it is not surprising that an inflammatory reaction is influenced by cyclic nucleotides (Arrigoni-Martelli, 1977). The cyclic nucleotides appear to exert their effect by controlling the release of the principal mediators of inflammation (Arrigoni-Martelli, 1977; Dunn et al., 1976).

In vitro studies have shown that an increase in intracellular cAMP inhibits the release of inflammatory mediators from basophils, neutrophils, mast cells and thrombocytes, whereas a decrease in this cyclic nucleotide enhances the release. Cyclic GMP has just the opposite effect, at least in neutrophils and mast cells (Arrigoni-Martelli, 1977). Therefore, it has been suggested that cGMP may have a predominantly "proinflammatory" role, while cAMP has an "anti-inflammatory" effect (Dunn et al., 1976). However, such categorical roles may be characteristic only of migrating, phagocytic-type cells. At present, the intracellular concentrations of cyclic nucleotides in the tissue which actually receives the inflammatory insult are not clear.

**Mediators.** An inflammatory response is mediated by several potent substances that are released locally in irritated tissue. The principal mediators of inflammation are histamine, serotonin, bradykinin and prostaglandins (Vane, 1976; Arrigoni-Martelli, 1977). Histamine, with some serotonin, is usually released first, followed by activation of the kinin-forming system and eventually by the production of prostaglandins (Lewis, 1977). The appearance of these mediators at the site of inflammation is transitory: In some instances they are detectable only during the first several hours of an inflammatory reaction (Ferreira et al., 1977).

When the mediators are released during the early stages of an inflammatory reaction, they activate positive feedback loops which facilitate the liberation of more and more of their own kind. This feedback results in an "avalanche" of mediators at the site where the inflammatory insult was inflicted. Thus, the mediators are important, not just for bringing about the pathophysiological changes that are characteristic of an inflammatory reaction, but also because they are responsible for the self-propagating nature of the inflammatory response (Chahl and Chahl, 1976).

a) Histamine. Histamine is the primary mediator of inflammatory reactions, producing rapid changes in the microvasculature of irritated tissues (Arrigoni-Martelli, 1977; Lewis, 1977). Although histamine may bring about some degree of vasodilatation (Lewis, 1977), its principal effect appears to be an increase in vascular permeability and exudation of serous components from the vascular compartment (Arrigoni-Martelli, 1977; Owen, 1977; Williams, 1977a). Histamine is preformed and stored in tissue mast cells and in circulating basophils (Arrigoni-Martelli, 1977; Lewis, 1977).

b) Serotonin. The role of serotonin in inflammation is relatively modest (Arrigoni-Martelli, 1977). The appearance of this mediator is limited to the early phases of the inflammatory response, in association with histamine activity. Like histamine, it seems to have a greater effect on vascular permeability than on vasodilatation (Williams, 1977). Serotonin has only a slight effect in some species and it is completely inert in rabbits and guinea pigs (Arrigoni-Martelli, 1977).

c) Bradykinin. The specific function(s) of bradykinin as a "mediator" of inflammation has not been clearly established. Since the kinin-forming system is not activated until after histamine is released, it has been suggested that bradykinin may have a greater responsibility during the intermediate stages of an inflammatory process, whereas the earlier and the more advanced phases are mediated by histamines and prostaglandins, respectively (Arrigoni-Martelli, 1977; Lewis, 1977). Like the other mediators, bradykinin has the ability to increase vascular permeability in most animals tested, including rabbits (Arrigoni-Martelli, 1977).
d) Prostaglandins. Prostaglandins serve as local regulators of cell functions (Arrigoni-Martelli, 1977; Bonta and Parnham, 1978). Even the slightest irritation or distortion of cell membranes results in an increase in the biosynthesis of prostaglandins by activating the hydrolysis of arachidonic acid from phospholipids [i.e., by activating the rate limiting step in prostaglandin synthesis (Arrigoni-Martelli, 1977; Lewis, 1977)]. Therefore, it is not surprising that inflammatory stimuli readily induce prostaglandin synthesis (Vane, 1976; Arrigoni-Martelli, 1977; Bonta and Parnham, 1978).

Although prostaglandins (especially the E-type) are produced relatively early in the overall inflammatory process, they are usually formed subsequent to the release of histamine and bradykinin (Greaves et al., 1976; Lewis, 1977). Unlike the other mediators of inflammation, prostaglandins are not stored within any subcellular compartment, but are produced on physiological demand, a condition which probably helps to restrict their action to the irritated tissue (Arrigoni-Martelli, 1977). The generation of prostaglandins is essential for the continuance of the symptoms of inflammation (Vane, 1976).

Prostaglandins (at least the E-type) cause vasodilatation (Greaves et al., 1976; Ferreira et al., 1977; Lewis, 1977; Owen, 1977; Williams, 1977a; Williams and Peck, 1977; Bonta and Parnham, 1978). In addition, they increase vascular permeability and bring on edema. However, the amount of exogenous prostaglandin needed to bring about this effect is rather high (Vane, 1976); therefore, the contribution of prostaglandins to the development of edema is probably via their capacity to intensify the vascular leakage that is initiated by other mediators such as histamine and bradykinin (Greaves et al., 1976; Messina et al., 1976; Lewis, 1977; Vane, 1976). As an inflammatory condition progresses toward the chronic stages, the local vasculature becomes less responsive to further prostaglandin stimulation (Bonta and Parnham, 1978).

Prostaglandins probably affect more than just the blood vessels in inflamed tissue. They may also facilitate the destruction of connective tissue elements in acute inflammatory reactions (Bonta and Parnham, 1978). In addition, prostaglandins may regulate the formation of granulomas in chronically inflamed tissues by stimulating the proliferation of local fibroblasts and by increasing the deposition of new connective tissue elements (Bonta and Parnham, 1978).

In considering other potential roles of prostaglandins, the E-type prostaglandins appear to have leukotactic properties (Ford-Hutchinson et al., 1977; Till et al., 1979). Upon arriving at a site of inflammation, leukocytes (and macrophages) are capable of releasing additional prostaglandins into the inflamed area (Lewis, 1977). Such a sequence of events might explain, at least in part, how prostaglandin E is able to generate even greater amounts of prostaglandins and propagate the inflammatory response (Chahl and Chahl, 1976).

Prostaglandins do not always provoke a proinflammatory response. Some of the products of arachidonic acid (including the E-type prostaglandins) can modulate the inflammatory response by acting as antiinflammatory agents under conditions that have not been clearly defined (Arrigoni-Martelli, 1977; Bonta and Parnham, 1978). This antiphlogistic action may depend on the phase of the inflammatory process: During the acute stage, prostaglandins obviously induce typical proinflammatory changes, whereas in chronic inflammation a shift in the ratio of E and F prostaglandins may bring about homeostatic restraint of the inflammatory response [i.e., a latent influence which has been interpreted experimentally as an antiinflammatory effect of prostaglandins (Arrigoni-Martelli, 1977; Bonta and Parnham, 1978)]. Thus, in their maintenance of homeostasis in inflamed tissues, prostaglandins (even individual prostaglandins such as E2) may exert, in concert, both pro- and antiinflammatory effects by reinforcing some components of inflammation while suppressing others (Bonta et al., 1977b; Weissman et al., 1976; Smith, 1977; Bonta and Parnham, 1978; Till et al., 1979). In any event, regardless of whether they act as mediators, modulators, or both, prostaglandins certainly have a complex central role in inflammation.

Proteolytic activity. Proteolytic enzymes are also involved in inflammatory processes. During the first hours of inflammation, proteases of the chymotrypsin-type have been identified, while cathepsin-type proteases appear later (Bertelli et al., 1969). The highest concentration of these enzymes is found at the time of greatest fibroblast activity in inflamed tissues.

There is also an increase in pepsin activity in inflamed tissue (Arrigoni-Martelli, 1977).
Some of the plasmin is generated in association with the formation of bradykinin. Evidently, the inflammatory insult initiates the activation of the Hageman Factor (clotting factor XII) and this factor converts plasminogen pro-activator into its active form while simultaneously stimulating the production of bradykinin (Arrigoni-Martelli, 1977).

Collagenolytic activity also increases in inflamed tissue (Bertelli et al., 1969; Ito et al., 1974; Bonta and Pambah, 1978). The precise role(s) of collagenase in inflammation has not been established. This potent enzyme probably facilitates the destruction of foreign bodies which enter inflamed tissues. During the intermediate stages of an inflammatory reaction, collagenase hydrolyzes peptides that may serve as chemotactic stimuli for neighboring connective tissue fibroblasts (Kang, 1978). In the final stages of the inflammatory process, collagenolytic enzymes function in the remodeling of damaged connective tissue.

**COMPARISON BETWEEN OVULATION AND INFLAMMATION**

This section elaborates on those features of ovulation which are similar to an inflammatory process. The discussion of each feature of ovulation will be followed by additional pertinent information on inflammation and related phenomena. Where appropriate, the more significant metabolic reactions in ovulation and inflammation will be summarized in the form of a simple equation. These equations (Eq) will be used later to formulate an overall scheme of the ovulatory process.

In dynamic processes such as ovulation and inflammation, many reactions transpire simultaneously and some follow one another in close sequence. Because of the overlapping nature of this cascade of reactions, it is difficult to single out specific phenomena and deal with them as isolated entities. Nevertheless, in the interest of developing a working model at the end of this review, certain facets of the ovulatory process will be considered as insular phenomena in this section.

Data from rabbits will be emphasized when they are available, because the physical and chemical changes which occur during ovulation in this animal can be accurately related (on a temporal scale) to the actual time of gonadotropin release and the anticipated time of follicular rupture. The ovulatory process normally requires 10 h in the rabbit.

**Vascular Dynamics**

In ovulation. An ovulatory surge of LH causes a conspicuous change in the ovarian vasculature. Within a few minutes after gonadotropin stimulation there is a significant increase in ovarian circulation (Wurtman, 1964; Lee and Noy, 1978). The elevation in ovarian blood flow lasts for at least 9 h, with a distinct peak occurring 4 h after gonadotropin stimulation (Blasco et al., 1975). This increase in circulation is associated with the hyperemic condition that develops in follicles which respond to LH (Burr and Davis, 1951; Zachariae, 1958; Szego and Gitin, 1964; Espey, 1974; Cherney et al., 1975). Along with vasodilatation, there is an increase in vascular permeability in the follicles (Zachariae, 1958; Espey, 1978b).

These vascular changes cause the follicle to become edematous, a condition which persists through the time of follicular rupture (Bjersing and Cajander, 1974; Cherney et al., 1975; Espey, 1967; Parr, 1974; Szego and Gitin, 1964). The changes in the vascular system are of such a magnitude near the time of ovulation that erythrocytes permeate the walls of the vessels and form petechiae in the interstitial spaces of the follicle (Parr, 1975; Espey, 1978b).

Thus, LH induces ovarian hyperemia, vasodilatation, edema and even extravasation of blood in ovulatory follicles:

\[ \text{Eq 1: } \]

\[
\begin{align*}
\text{LH} & \\
\uparrow & \\
\text{normal vessels} & \rightarrow \text{dilated, permeable vessels}
\end{align*}
\]

This effect of LH on the ovarian vasculature can be mimicked by histamine, but not by FSH or serotonin (Wurtman, 1964).

In inflammation. The preovulatory changes in blood vessels of mature Graafian follicles are essentially identical to the vasodilatation and increase in capillary permeability that occurs in the circulation to inflamed tissues. It is well known that such changes are mediated by histamine in the early stages of an inflammatory reaction and by bradykinin and prostaglandins in the later stages (Vane, 1976; Arrigoni-Martelli, 1977; Lewis, 1977). The secre-
otion of these mediators in ovariatory tissue will be discussed in more detail in a later section.

**Leukocytes and Macrophages**

**In ovulation.** Basophils and possibly other types of leukocytes accumulate in Graafian follicles near the time of ovulation (Zachariae et al., 1958; Thonnard-Neumann, 1964; Espey, 1974):

\[ \text{LH} \]
\[ \text{systemic leukocytes} \rightarrow \text{leukocyte migration into follicles} \]

These cells are not conspicuous in the follicles of preovulatory control animals. However, 8 h after rabbits have been mated (\(\sim\)2h before ovulation) histological sections show a moderate number of basophils in the blood vessels around the larger follicles (Zachariae et al., 1958). At 10 h after mating, there is a conspicuous accumulation of basophils in the ovulating follicles and the site of accumulation is characterized by tissue edema. Maximum accumulation is reached a few hours after ovulation, at which time the leukocytes can be seen passing from the vessels into the fresh corpora lutea. The basophils do not begin to dissipate until \(\sim\)24 h after coitus (\(\sim\)14 h after ovulation) (Zachariae et al., 1958).

In addition to basophils, other granulocytes as well as thrombocytes appear in follicles near the time of ovulation (unpublished observations). The accumulation of these cells well ahead of the moment of rupture signifies that chemotactic substances are being produced even before the tissue has been injured by the actual phenomenon of rupture. It also indicates that reactions similar to those found in inflammation are occurring during the ovulatory process. It would be useful to have more quantitative and qualitative information on the distribution of these types of cells in the follicle wall during the ovulatory process.

**Inflammation.** "One of the major characteristics of chronic inflammation is the involvement of several different types of white blood cells, which migrate into the inflamed area" (Bonta and Parnham, 1978). As these cells circulate into a site of inflammation they adhere to the vascular endothelium and eventually enter the tissue by diapedesis (Ebert and Grant, 1974).

The specific roles of these cells in inflammation are not fully documented. It is known that basophils are rich in histamine, which is released at the site of inflammation:

Eq 3:
\[ \text{inflamed tissue} \]
\[ \text{basophils} \rightarrow \text{histamine} \]

It has been suggested that polymorphonuclear leukocytes also contribute prostaglandins beyond the amount released by the irritated tissue itself (Higgs et al., 1975). However, it is not clear whether leukocytes have the capacity to produce significant amounts of prostaglandins (Glatt et al., 1974; Walker et al., 1976; Ford-Hutchinson et al., 1977; Bonta and Parnham, 1978). Either macrophages (Bray and Gordon, 1978) or thrombocytes (Glatt et al., 1974) appear to be more likely sources of supplementary prostaglandins in inflammation:

Eq 4:
\[ \text{inflamed tissue} \]
\[ \text{macrophages} \]
\[ \text{thrombocytes} \rightarrow \text{prostaglandins leukocytes (?)} \]

Another way in which migratory cells may participate in the inflammatory response and cause tissue damage is by the release of intracellular stores of proteolytic enzymes. Cells such as macrophages, thrombocytes and polymorphonuclear leukocytes are known to secrete collagenase, elastase, plasminogen activator and nonspecific neutral proteases at sites of inflammation (Perper, 1976; Bonta and Parnham, 1978; Vassalli et al., 1976; Tansey and Padykula, 1978; Werb, 1978):

Eq 5:
\[ \text{inflamed tissue} \]
\[ \text{macrophages} \rightarrow \text{plasminogen activator thrombocytes} \rightarrow \text{collagenase leukocytes} \rightarrow \text{nonspecific proteases} \]

Since these same types of cells migrate into the follicle before ovulation, it is possible that they contribute at least a portion of the proteolytic enzymes which degrade connective tissue in preovulatory follicles.
Fibroblasts

In ovulation. Mature ovarian follicles are encapsulated by a rather dense layer of thecal tissue containing many fibroblasts. These cells become mitotically active as the time of ovulation approaches (unpublished observation). The fibroblasts frequently have centrioles in their cytoplasm, and they take on the appearance of proliferating cells, even before the follicle ruptures (Espey, 1971):

\[ \text{Eq 6:} \]

\[
\text{LH} \downarrow \quad \text{quiescent \rightarrow prolaminating} \\
\text{fibroblasts \fibroblasts}
\]

The specific metabolic factors which stimulate this fibroblast activation are unknown.

At the time of ovulation, thecal fibroblasts migrate into the stratum granulosum, laying down collagenous support for the mass of developing lutein tissue (Espey, 1978b). This movement of fibroblasts suggests that the lutein granulosa produces a chemotactic agent which attracts the surrounding fibroblasts from the theca externa. The nature of this chemotactic substance is unknown. In other tissues, lymphokines and the peptides which arise from collagen degradation serve as chemotactic stimuli for fibroblasts (Kang, 1978). However, the granulosa contains neither blood vessels nor collagen; therefore, this layer is an unlikely source of either lymphocytes or collagen fragments.

In inflammation and related processes. Fibroblasts exhibit “two extreme physiological states”: quiescence or active proliferation (DeAsua et al., 1975). Transition to the proliferating state can be regulated by the concentration of essential nutrients, by serum, or by other growth-promoting factors which act on the cell surface (DeAsua et al., 1975). Such factors are obviously present in inflamed tissues, because fibroblasts readily infiltrate sites of inflammation, especially during the repair phase of the process (Arrigoni-Martelli, 1977). It may be quite relevant that prostaglandins, which mediate the later stages of the inflammatory process, promote fibroblast proliferation (DeAsua et al., 1975; Hial et al., 1977):

\[ \text{Eq 7:} \]

prostaglandin (F\(_2\alpha\))

\[ \downarrow \quad \text{quiescent \rightarrow prolaminating} \]

fibroblasts fibroblasts

Fibroblasts, themselves, are a common source of prostaglandins (Newcombe and Ishikawa, 1976; Baenziger et al., 1977; Dayer et al., 1977; Tam et al., 1977; Peters et al., 1977a; Peters et al., 1977b; Chandrabose et al., 1978; Lindgren et al., 1978; Bonta and Parnham, 1978):

\[ \text{Eq 8:} \]

\[
\text{stimuli (?)} \downarrow \\
\text{fibroblasts \fibroblasts \prostaglandins}
\]

Therefore, the proliferation of fibroblasts could be regulated by exogenous prostaglandins, or by other factors which stimulate the synthesis of endogenous prostaglandins within fibroblasts.

Fibroblast proliferation can also be induced by proteases of the type that appear in inflamed tissue (trypsin, plasmin and thrombin) (Burger, 1970; Greenberg et al., 1976; Pohjanpelto, 1977; Zetter et al., 1977):

\[ \text{Eq 9:} \]

\[
\text{serine proteases} \downarrow \\
\text{quiescent \rightarrow prolaminating} \\
\text{fibroblasts \fibroblasts}
\]

In some instances the proteases stimulate as much as a 7-fold increase in the frequency of mitosis in fibroblasts (Pohjanpelto, 1977).

It has recently been established that fibroblasts are a common source of latent collagenase (Bauer et al., 1975; Werb and Reynolds, 1975; Birkedal-Hansen et al., 1976a; Birkedal-Hansen et al., 1976b; Werb et al., 1977; Vae et al., 1977; Dayer et al., 1977; Woolley et al., 1978; Stricklin et al., 1978; Bonta and Parnham, 1978). The secretion of procollagenase can be induced by exposing fibroblasts to any of a number of proteolytic enzymes, including trypsin, chymotrypsin, plasmin and elastase (Werb and Aggeler, 1978):

\[ \text{Eq 10:} \]

\[
\text{serine proteases} \downarrow \\
\text{fibroblasts \procollagenase}
\]
This “secretion” of collagenase is initiated only when the proteases decrease the adhesions between cells, i.e., when the conditions are the same as those which free fibroblasts from contact inhibition and stimulate their proliferation (Werb and Aggeler, 1978).

Fibroblasts, especially transformed ones, also produce large quantities of plasminogen activator (Ossowski et al., 1973; Goldfarb and Quigley, 1978).

Eq 11:
\[
\text{trypsin} \\
\text{fibroblasts} \rightarrow \text{plasminogen activator}
\]

One means of inducing the secretion of plasminogen activator is by treating fibroblasts with trypsin (Werb and Aggeler, 1978).

In summary, fibroblasts are a source of prostaglandins and of proteolytic enzymes such as collagenase and plasminogen activator; in turn, both of these groups of compounds are capable of stimulating fibroblast proliferation. This information may be quite relevant since prostaglandins, proteases and proliferating fibroblasts are collectively involved in both the ovulatory process and the inflammatory process.

Cyclic Nucleotides

In ovulation. It is well known that the ovulatory surge of LH activates the adenylate cyclase system which in turn stimulates the synthesis of cAMP in follicle cells (Hunzicker-Dunn et al., 1979):

Eq 12:
\[
\text{LH} \\
\text{mature follicles} \rightarrow \text{cAMP increase}
\]

An increase in ovarian cAMP is detectable within seconds after the gonadotropin level begins to rise in the blood. The synthesis of cAMP reaches a peak within 30 min and then gradually declines, even though the LH levels remain elevated somewhat longer. At this stage of the ovulatory process, the adenylate cyclase system loses its capacity to be stimulated further by LH (Marsh et al., 1973; Lamprechts et al., 1973; Lamprechts et al., 1979; Hunzicker-Dunn et al., 1979):

Eq 13:
\[
\text{adenylate cyclase} \\
\text{desensitization} \\
\text{follicles} \rightarrow \text{cAMP decrease}
\]

The loss in responsiveness persists through the time of ovulation; this “LH refractoriness” is not caused by prostaglandins (LeMaire et al., 1976).

The initial elevation in cAMP mediates the steroidal and luteal responses of gonadotropin-stimulated follicles (Marsh and LeMaire, 1973; LeMaire and Marsh, 1975; Baumberger and Lindner, 1975; Erickson and Ryan, 1975; Selstam et al., 1976; Baumberger et al., 1978; Hunzicker-Dunn et al., 1979; Armstrong et al., 1979; Mills, 1979a).

Eq 14:
\[
\text{cAMP} \\
\text{mature follicles} \rightarrow \text{steroidogenesis}
\]

It is not clear whether cAMP induces steroidogenesis by accelerating some limiting step between cholesterol and pregnenolone (Marsh and LeMaire, 1973), or by acting at some other biochemical site beyond pregnenolone (Armstrong et al., 1979).

The elevation in steroid production is not continuous. A period of steroidogenic inactivity sets in before the time of follicular rupture. This quiescent period occurs during the time when the cyclase system in the follicle is unresponsive to further gonadotropin stimulation (Hunzicker-Dunn et al., 1979; Hunzicker-Dunn, 1979; Mills, 1979b). This period of steroidogenic quiescence will be discussed in more detail in the next section, which examines qualitative and quantitative changes in follicle steroids.

Cyclic AMP production is distinctly a biphasic phenomenon in rabbits, with a second peak in cAMP appearing 3–4 h after gonadotropin (hCG) injection (Goff and Major, 1975). Recent evidence suggests there is a second phase of cAMP production in gilts also (Tsang et al., 1979). This second peak in cAMP occurs about the same time prostaglandin levels begin to rise in the follicle (LeMaire and Marsh, 1975; Baumberger et al., 1978; Tsang et al., 1979).

There is considerable indirect and direct evidence that cyclic nucleotides stimulate the preovulatory synthesis of prostaglandins in the follicle (Kuehl et al., 1972; Marsh and LeMaire, ...
1973; Marsh et al., 1974; Zor et al., 1977a; Zor et al., 1977b; Bauminger et al., 1978; Clark et al., 1978):

\[ \text{Eq 15:} \]
\[
\text{cAMP} \downarrow \\
\text{mature follicles} \rightarrow \text{prostaglandins}
\]

Following cyclic nucleotide stimulation, \( \sim 3-5 \) h must lapse before an increase in prostaglandin synthesis can be detected (Marsh and LeMaire, 1973; Marsh et al., 1974; LeMaire and Marsh, 1975; Rigler et al., 1976; Zor et al., 1977a; Zor et al., 1977b). Therefore, even though the second peak in cAMP production occurs at approximately the same time that prostaglandins increase in the follicle, it is more likely that the initial peak in cAMP is responsible for the prostaglandin synthesis which appears after a latency of 3-5 h.

It is interesting that the antiinflammatory agent, indomethacin, does not prevent the initial output of cAMP (Rigler et al., 1976), but it does inhibit the second peak that normally occurs 3-4 h after gonadotropin stimulation (Goff and Major, 1975). Since indomethacin is known to inhibit prostaglandin synthetase, it could be that the second peak in cAMP is dependent upon prostaglandin synthesis in the follicle. If this is indeed the case, then it means that the first peak in cAMP may induce changes in the follicle which lead to a prostaglandin mediated second phase of nucleotide synthesis.

The specific site(s) of cAMP synthesis in the follicle is unclear. The increase in production could occur in the granulosa, the theca interna, the theca externa, or in all 3 layers (LeMaire and Marsh, 1975). There is a report that gonadotropins stimulate cAMP production in both the thecal tissue and the granulosa layer (Weiss et al., 1978). Alternatively, it has been suggested that LH stimulates the adenylate cyclase system in the theca interna, while FSH exerts its effect on the granulosa cells (Armstrong et al., 1979).

Ovarian cGMP may also be regulated by LH, but in a reciprocal fashion to cAMP production (Patwardhan and Lanthier, 1978). During the first several hours after LH stimulation in the rabbit, there is no conspicuous change in ovarian cGMP. However, after 4 h there is a significant reduction in the level of cGMP in the ovary and this decline continues through the time of ovulation.

Before closing this section, it is worth noting that analogs of cAMP (but not cGMP) are capable of stimulating granulosa cells to produce plasminogen activator (Strickland and Beers, 1976):

\[ \text{Eq 16:} \]
\[
\text{cAMP} \downarrow \\
\text{mature follicles} \rightarrow \text{plasminogen activator (granulosa)}
\]

It may also be relevant that the production of plasminogen activator by the Chinese hamster ovary is dependent on cAMP synthesis (Mott et al., 1976). The potential role of plasminogen activator in ovulation will be discussed in more detail later.

In inflammation and related processes.

Cyclic nucleotides appear to have a major role in the regulation of mediator release in inflammatory reactions (Dunn et al., 1976; Weissman et al., 1976; Parnham et al., 1977; Lewis, 1977; Arrigoni-Martelli, 1977; Lindgren et al., 1978). However, the precise nature of this role is not clear. According to some reports, cAMP mediates the inflammatory response, whereas in other cases it appears to exert an antiinflammatory effect. The effect seems to vary depending on 1) whether the experiment was conducted in vivo or in vitro, 2) whether the system under analysis was in the early or the late stages of the inflammatory process, 3) whether the cyclic nucleotide was of endogenous origin, or applied exogenously and 4) especially on whether the assay was made on the tissue which actually received the inflammatory insult, or whether it was made on the types of cells that migrate into the area of inflammation, i.e., on macrophages and leukocytes.

The phagocytic cells, basophils, neutrophils, mast cells and thrombocytes in inflamed tissues usually contain low levels of cAMP (Parnham et al., 1977; Lewis, 1977; Arrigoni-Martelli, 1977). In contrast, less motile cells such as synovial fibroblasts and bone cells reportedly have greater amounts of cAMP (Parnham et al., 1977). To complicate the picture further, the cAMP level in lymphocytes can vary, depending on the amount of prostaglandin in the inflamed area (Parnham et al., 1977).

Although it is difficult to decipher whether cyclic nucleotides have predominantly a pro-
or an antiinflammatory effect, it does seem clear that a low level of cAMP is a necessary condition at some stage of the inflammatory process (Dunn et al., 1976; Parnham et al., 1977; Arrigoni-Martelli, 1977; Bonta and Parnham, 1978). The period of reduced cAMP production may be comparable to the interval during which the adenylate cyclase system of ovarian follicles becomes unresponsive.

For the purpose of developing the central hypothesis of this review, it is important to mention that elevated levels of cAMP can stimulate prostaglandin synthesis in a variety of systems, including fat cells (Dalton and Hope, 1974), thyroid cells (Burke et al., 1973), brain cells (Abdulla and McFarlane, 1972), ovarian cells (Marsh et al., 1974) and fibroblasts (Hamprecht et al., 1973; Lindgren et al., 1978; Bonta and Parnham, 1978). In fibroblasts, at least, cAMP increases prostaglandin synthesis probably by stimulating an acyl hydrolase which liberates arachidonic acid, the principal precursor of prostaglandins (Lindgren et al., 1978):

Eq 17:  
\[ \text{cAMP} \rightarrow \text{fibroblasts} \rightarrow \text{arachidonic acid} \]  
(prostaglandins)

Clearly, this stimulation of prostaglandin production is a proinflammatory effect of cAMP.

Cyclic AMP may also function in the regulation of fibroblast growth: increased levels of cAMP reduce the growth rate of this type of connective tissue cell (Johnson and Pastan, 1971; Dubpernell and Gavurin, 1978; Bonta and Parnham, 1978). This inhibitory effect on fibroblasts might further explain why the adenylate cyclase system in the follicle becomes desensitized during ovulation, at a time when theca fibroblasts normally proliferate into the granulosa layer.

**Steroids**

In ovulation. Mature ovarian follicles begin a cAMP-mediated increase in steroid secretion within 1 h following the ovulatory surge in gonadotropins (Hilliard and Eaton, 1971; YoungLai, 1972; Mills and Savard, 1973; Hilliard and Eaton, 1974; LeMaire and Marsh, 1975; Armstrong et al., 1976):

Eq 18:  
\[ \text{LH} \rightarrow \text{cAMP} \rightarrow \text{mature follicles} \rightarrow \text{andro gens, estrogens, progestins} \]

Not only is there a substantial increase in the overall rate of steroid production, but there is also a change in the kinds of steroids produced. There is a significant (10-fold) increase in the proportion of testosterone in comparison to estrogen (Mills and Savard, 1973; Hilliard et al., 1974; Armstrong et al., 1976). There is also an increase in progesterin output, with 17α-OH progesterone being more copious during the preovulatory phase and progesterone being more abundant during the postovulatory stages of luteinization (Hilliard and Eaton, 1971; Mills and Savard, 1973).

As an ovulatory follicle progresses toward luteinization, the production of steroids is not continuous. Within 4–5 h following gonadotropin stimulation, the follicle enters a dormant phase, with steroidogenesis being essentially "turned off" by the time of ovulation (Hilliard and Eaton, 1971; YoungLai, 1972; Mills and Savard, 1973; Hilliard et al., 1974; Armstrong et al., 1976). This intermission in steroid production may be a result of the desensitization of the adenyl cyclase system in preovulatory follicles (Hunzicker-Dunn et al., 1979; Mills, 1979):

Eq 19:  
\[ \text{LH refractoriness} \rightarrow \text{cAMP desensitization} \rightarrow \text{preovulatory follicles} \rightarrow \text{steroid inactivity} \]

Whatever its cause, this ebb in steroidogenic activity is probably a necessary condition for ovulation to take place (LeMaire and Marsh, 1975; Hunzicker-Dunn, 1979).

As regards the site of steroid production, testosterone is secreted predominantly by theca interna cells (Moor, 1977; Armstrong et al., 1979; Fortune and Armstrong, 1979), whereas estrogens (and some progesterone) are produced mainly by the granulosa cells (Erickson and Ryan, 1975; Armstrong et al., 1979; Armstrong, 1979). Since the addition of exogenous androstenedione to cultured granulosa cells results in a substantial increase in estradiol production, it has been suggested that androgens from the theca interna may be
necessary as substrate for the production of estrogen in the granulosa layer (Erickson and Ryan, 1975; Moor, 1977; Armstrong et al., 1979). Androgens may also augment the production of progesterone by granulosa cells (Armstrong, 1979).

Steroids (specifically estrogens) may contribute to the ovulatory process by increasing the synthesis of prostaglandins, particularly PGF$_{2\alpha}$ (Marsh and LeMaire, 1973; LeMaire and Marsh, 1975; Erickson et al., 1977):

\[
\text{Eq 20:} \quad \text{steroids (estrogen)} \rightarrow \text{preovulatory follicles} \rightarrow \text{prostaglandin (F$_{2\alpha}$)}
\]

Conversely, prostaglandin synthesis apparently is not essential for steroid production and luteinization of the follicle (LeMaire and Marsh, 1975; Goff and Major, 1975; Armstrong et al., 1976; Phi et al., 1977; Maia et al., 1978; Lee and Noy, 1978).

There are conflicting suggestions that ovarian steroids may activate (Rondell, 1974), or may inhibit (Hunzicker-Dunn, 1979) ovarian collagenolytic enzymes. However, there is no concrete evidence to date to show that sex steroids have a direct effect on either collagenase activity (Espey and Coons, 1976), or plasmin activity (Strickland and Beers, 1976) in follicles in vitro. Still, it is possible that steroids could have some indirect influence on proteolytic activity in follicles in vivo; therefore, it would be premature to rule out any relationship between ovarian steroidogenesis and ovarian proteolytic enzymes.

In inflammation and related processes. There is sparse information about the effect of sex steroids on inflammatory reactions. There is a single report that exogenous estradiol can provoke the formation of edematous fluid which resembles the fluid that is present in experimentally-induced inflammation (Langgard and Hvidberg, 1969). In addition, it has been shown that low doses of estradiol liberate intrinsic histamine from uterine tissue (Szego, 1965):

\[
\text{Eq 21:} \quad \text{estrogen} \rightarrow \text{uterus} \rightarrow \text{histamine}
\]

Estrogens also stimulate an increase in prostaglandin F in the uterus (Kuehl et al., 1976):

\[
\text{Eq 22:} \quad \text{estrogen} \rightarrow \text{uterus} \rightarrow \text{prostaglandin} \ F
\]

Thus, as in ovulation, reactions similar to those in inflammation take place in the uterus at a time when uterine steroid output is undergoing significant transition (Tansey and Padykula, 1978).

It would be helpful to know whether ovarian steroids influence the proliferation of theca fibroblasts during ovulation. Such an influence seems quite possible in light of the evidence that some types of fibroblasts possess receptors specific for androgens (but not estrogens) (Hsie et al., 1971; Bauknecht, 1977), while other fibroblasts can be stimulated by estradiol (Schonhofer et al., 1974).

The literature is confusing with regard to the effect of steroids on proteolytic activity. Progesterone reportedly inhibits collagenase in the uterus (Jeffrey et al., 1971; Jeffrey et al., 1974; Woessner, 1976) and in the pubic symphysis (Wahl et al., 1977), but not in the skin (Jeffrey et al., 1974). Estrogen inhibits collagenolytic activity in uterine tissue in vivo to some extent (Ryan and Woessner, 1974; Woessner, 1976), but not in vitro (Jeffrey et al., 1971). Estrogen also reduces collagenase in the pubic symphysis (Wahl et al., 1977a), yet it stimulates collagenolytic activity in breast cancers (Heuson et al., 1975). Testosterone presumably has no effect on collagenase activity (Jeffrey et al., 1974).

With regard to the effect of steroids on a protease which activates latent collagenase, estrogen stimulates the production of plasminogen activator in the lung (Ambrus et al., 1971) and in the uterus (Katz et al., 1976).

Adding to the above confusion, sex steroids may (Wahl, 1977), or may not (Werb, 1978), inhibit macrophage collagenase. Thus, collectively, the existing data are not very helpful in clarifying the question of whether sex steroids have a positive or a negative effect on ovarian proteolytic activity.

Histamine

In ovulation. The release of histamine may also be a physiologically significant event
during ovulation (Szego and Gitin, 1964; Schwartz and McCormack, 1972; Wallach et al., 1978; Knox et al., 1979). Ovarian histamine depletion is achieved within 2 h after the i.v. administration of LH, during the period when the follicle first becomes hyperemic (Szego and Gitin, 1964):

\[
\text{Eq 23:}
\]

\[
\text{LH (cAMP?)}
\]

\[
\text{mature follicles} \rightarrow \text{histamine release}
\]

Further support for a role for histamine in ovulation comes from experiments which show that antihistamines, such as chlortrimeton and chlorpheniramine, inhibit ovulation in vivo in rabbits (Knox et al., 1979) and in vitro in the perfused rabbit ovary (Wallach et al., 1978). The addition of prostaglandin \( F_{2\alpha} \) to the perfusion fluid reverses the antiovulatory effect of chlorpheniramine. This intriguing information suggests that histamine may serve as a mediator of prostaglandin \( F_{2\alpha} \) production in the preovulatory follicle:

\[
\text{Eq 24:}
\]

\[
\text{histamine}
\]

\[
\text{preovulatory follicles} \rightarrow \text{prostaglandin (F}_{2\alpha}\text{)}
\]

Collectively, the above results indicate that histamine probably has a significant function in ovulation.

**In inflammation and related processes.** The role of histamine in inflammation appears to be related primarily to its effect on the circulatory system:

\[
\text{Eq 25:}
\]

\[
\text{histamine}
\]

\[
\text{inflamed tissue} \rightarrow \text{increased vascular permeability}
\]

Histamine interacts in target tissues with specific receptors located on the plasma membranes of endothelial cells, especially along postcapillary venules with diameters ranging from 20–30 \( \mu \text{m} \) (Arrigoni-Martelli, 1977). Histamine causes the endothelial cells to contract, thereby producing gaps between adjacent cells and allowing plasma to filter into the surrounding tissue.

Histamine may have other significant effects on inflamed tissues. There is a report that this agent can stimulate mitotic activity (Szego, 1965):

\[
\text{Eq 26:}
\]

\[
\text{histamine}
\]

\[
\downarrow \text{luminal epithelium} \rightarrow \text{mitotic activity}
\]

\[
\downarrow \text{endometrial glands}
\]

Mitogenic activity is also initiated in quiescent fibroblasts that have been exposed to inflammatory exudates, but the specific mitogenic factor has not been identified (Adolphe et al., 1977).

It may also be relevant that histamine, with other mediators of inflammation, can stimulate cAMP formation and induce prostaglandin \( F_{2\alpha} \) synthesis in various tissues (Arrigoni-Martelli, 1977; Platshon and Kaliner, 1978):

\[
\text{Eq 27:}
\]

\[
\text{histamine}
\]

\[
\downarrow \text{(cAMP)}
\]

\[
\downarrow \text{various tissues} \rightarrow \text{prostaglandin (F}_{2\alpha}\text{)}
\]

(human lung)

These results are of special interest since there is parallel evidence that histamine may also influence prostaglandin \( F_{2\alpha} \) production in ovarian follicles (Wallach et al., 1978).

**Serotonin**

**In ovulation.** There is negligible information on the level of serotonin in the follicle during ovulation. One brief report suggests that serotonin increases in the rat ovary near the time of ovulation (Clausell and Soliman, 1978):

\[
\text{Eq 28:}
\]

\[
\text{gonadotropin}
\]

\[
\downarrow \text{ovary (rat)} \rightarrow \text{serotonin}
\]

**In inflammation.** It is difficult to find data on serotonin in inflammatory processes. It apparently has the same basic effect as histamine on the vascular system in inflamed tissues, but to a lesser extent (Arrigoni-Martelli, 1977):

\[
\text{Eq 29:}
\]

\[
\text{serotonin}
\]

\[
\downarrow \text{inflamed tissue} \rightarrow \text{increased vascular permeability}
\]
One should be aware, however, that serotonin may be "inert" in rabbits.

**Bradykinin**

*In ovulation.* No information could be located on the concentration of bradykinin in ovarian follicles. It would be useful to know if this mediator of inflammation is released along with histamine, serotonin and prostaglandin during the ovulatory process.

*In inflammation and related processes.* It still serves the purpose of this review to describe several properties of bradykinin, even though this substance has not yet been identified in ovarian follicles. Like the other mediators of inflammation, bradykinin acts on the vascular system:

\[
\text{Eq 30:} \quad \text{bradykinin} \downarrow \text{inflamed tissue} \rightarrow \text{increased vascular permeability}
\]

However, bradykinin has a more potent effect on vascular permeability in rabbits and rats, than does histamine (Arrigoni-Martelli, 1977).

Beyond its effects on the circulatory system, bradykinin stimulates an increase in cAMP in fibroblasts (Schonhofer et al., 1974; Fahey et al., 1977; Arrigoni-Martelli, 1977). This bradykinin-induced elevation in cAMP initiates prostaglandin synthesis in fibroblasts (Hong and Levine, 1976; Chandrabose et al., 1978; Muroto et al., 1978):

\[
\text{Eq 31:} \quad \text{bradykinin (cAMP)} \downarrow \text{fibroblasts} \rightarrow \text{prostaglandin (F2α)}
\]

An interesting footnote here is that the adenylate cyclase system of fibroblasts which have been stimulated by bradykinin becomes refractory to rechallenge with additional bradykinin; also, similar to the desensitized cyclase system of gonadotropin stimulated follicles, additional bradykinin cannot induce a second increment in cAMP production in fibroblasts (Fahey et al., 1977).

Bradykinin also activates the Hageman factor (clotting factor XII) in inflamed tissue (Arrigoni-Martelli, 1977). Once activated, the Hageman factor can stimulate the formation, not only of more bradykinin, but also of plasminogen proactivator, leading to plasmin formation:

\[
\text{Eq 32:} \quad \text{bradykinin (Hageman factor)} \downarrow \text{inflamed tissue} \rightarrow \text{plasminogen activator}
\]

The potential role of plasmin in ovulation will be discussed in more detail in a later section on proteolytic enzymes.

**Prostaglandins**

*In ovulation.* In recent years it has become clear that an ovulatory surge of gonadotropin induces an elevation in prostaglandin synthesis in mature ovarian follicles (LeMaire et al., 1973; Marsh and LeMaire, 1973; LeMaire and Marsh, 1975; LeMaire et al., 1975; Bowring et al., 1975; Armstrong and Zamecnik, 1975; Iesaka et al., 1975; Wallach et al., 1975; Tsang et al., 1979):

\[
\text{Eq 33:} \quad \text{LH} \downarrow \text{mature follicles} \rightarrow \text{prostaglandin synthesis}
\]

In rabbits, there is a substantial increase in the production of prostaglandins E and F within 4–5 h after gonadotropin stimulation (LeMaire et al., 1973). Prostaglandin F reaches a maximum level in the follicle by the time of ovulation and then rapidly declines, whereas prostaglandin E continues to be produced for several hours after ovulation (Yang et al., 1974). There are no data on the follicular concentrations of other prostaglandins, such as thromboxane and prostacyclin, which may also be involved in the ovulatory process. These less stable prostaglandins are difficult to detect in tissue since they are metabolized within seconds after they are synthesized.

The synthesis of prostaglandins in the follicle is apparently not limited to one principal site. Granulosa cells produce significant amounts of prostaglandin (Plunkett et al., 1975; Erickson et al., 1977; Clark et al., 1978), but so do theca cells which generate prostaglandin F (Erickson et al., 1977).

The preovulatory increase in prostaglandin is essential for ovulation. This vital role is demonstrated by the massive evidence that
inhibition of prostaglandin synthesis prevents ovulation, but not luteinization (Orczyk and Behman, 1972; Armstrong and Grinwich, 1972; Grinwich et al., 1972; Behman et al., 1972; O’Grady et al., 1972; Tsafriri et al., 1972a; Tsafriri et al., 1972b; Tsafriri et al., 1973; Yang et al., 1973; Lau et al., 1974; Armstrong and Zamecnik, 1975; Wallach et al., 1975). Exogenous prostaglandin F2α is especially effective in reestablishing the ovulatory process in animals in which prostaglandin synthesis has been inhibited (Armstrong et al., 1973; Diaz-Infante et al., 1974; Wallach et al., 1975; Hamada et al., 1977; Hamada et al., 1978; Wallach et al., 1978):

Eq 34:

\[
\text{PGF}_{2\alpha} \downarrow \quad \text{preovulatory follicles} \downarrow \quad \text{ovulation}
\]

In contrast, prostaglandin E2 seems to suppress ovulation (Richman et al., 1974; Diaz-Infante et al., 1974; Hamada et al., 1977); there are, however, exceptions to this effect (Tsafriri et al., 1973).

It has been repeatedly suggested that prostaglandins (particularly PGF2α) cause rupture of the follicle by increasing ovarian contractility (Espey, 1978a). However, there is no convincing evidence that ovarian contractions are an essential part of the ovulatory process (Espey, 1978a). Therefore, other potential roles of prostaglandins deserve greater consideration in future studies on mammalian ovulation.

Prostaglandin E may function as a mediator of ovarian hyperemia during the later stages of the ovulatory process (Lee and Novy, 1978):

Eq 35:

\[
\text{PGE} \downarrow \quad \text{preovulatory follicles} \downarrow \quad \text{follicular hyperemia}
\]

In contrast, PGF2α appears to diminish blood flow to the ovary, at least during the luteal phase of the cycle (Batta and Martini, 1975; Janson et al., 1975; Ford et al., 1977). It would be useful to have more information on the specific effects of different prostaglandins on blood flow to preovulatory follicles.

Prostaglandins, especially the E-type, stimulate the formation of cAMP in the follicle (Kuehl et al., 1970; Lamprech et al., 1973; Zor et al., 1973; Nilsson et al., 1974):

Eq 36:

\[
\text{PGF}_{2\alpha} \downarrow \quad \text{preovulatory follicles} \downarrow \quad \text{cAMP production (theal fibroblasts?)}
\]

However, prostaglandins stimulate cAMP by a mechanism different from LH action on cAMP because the effects of each are separable and additive (Armstrong et al., 1976; Rigler et al., 1976; Lee and Novy, 1978). Furthermore, follicles which have become refractory to LH remain fully responsive to prostaglandins (Lamprech et al., 1973). These findings suggest that LH and prostaglandins act at different sites in the ovary. For instance, LH could be acting on the secretory cells in the granulosa and theca interna, while prostaglandins might stimulate cAMP formation in the fibroblasts in the theca externa and adjacent thecal tunic.

In light of the above information it seems quite possible that prostaglandins are responsible for the second phase of cAMP formation during the ovulatory process in rabbits. If this deduction is correct, then an inhibitor of prostaglandin synthesis, such as indomethacin, should prevent the second peak in cAMP, and it does, without impairing the initial (LH-induced) increase in cyclic nucleotide (Goff and Major, 1975).

Systemic prostaglandins can induce the release of hypophysial LH (Tsafriri et al., 1973; Labhsutvar, 1971; Batta et al., 1978), which leads to subsequent depletion of ovarian ascorbic acid (Sato et al., 1974). However, there is no evidence that prostaglandins either effect LH secretion during the normal ovulatory process or mediate the acute steroidogenic and luteinizing actions of LH (Grinwich et al., 1972; O’Grady et al., 1972; Armstrong et al., 1976; Phi et al., 1977; Bauminger et al., 1978, Lee and Novy, 1978; Maia et al., 1978; Young Lai, 1978). Instead, prostaglandins appear to function specifically in the physical process of ovulation (O’Grady et al., 1972; Grinwich et al., 1972; LeMaire and Marsh, 1975; Maia et al., 1978).

It has been suggested that prostaglandins might stimulate the synthesis, release and/or activation of a "collagenase-like ovulatory enzyme" (Marsh and LeMaire, 1973; LeMaire and Marsh, 1975; F. Fuchs in the discussion at the end of Wallach et al., 1978):
Eq 37:
\[ \text{prostaglandin} \downarrow \]
\[ \text{preovulatory} \rightarrow \text{collagenolytic} \]
\[ \text{follicles} \rightarrow \text{enzyme (?)} \]

Indirect support of this idea comes from evidence that prostaglandin F\(_{2\alpha}\) causes the release of lysosomal enzymes in luteal tissue (Kaley and Weiner, 1975). However, if prostaglandins do mediate proteolytic activity in the preovulatory follicle, they probably do so by some mechanism other than by direct activation of a latent collagenase (Espey and Coons, 1976).

Prostaglandins of the E-type can stimulate follicular cells to produce plasminogen activator (Strickland and Beers, 1976):

Eq 38:
\[ \text{PGE}_2 \downarrow \]
\[ \text{preovulatory} \rightarrow \text{plasminogen} \]
\[ \text{follicles} \rightarrow \text{activator} \]

This action may be quite relevant, since plasmin can activate procollagenase (see Eq 45).

In inflammation and related processes. The basic metabolic pathways of prostaglandins are summarized in Fig. 1. Prostaglandins can be synthesized by essentially all cells since the fatty acids from which they are derived are a natural component of plasma membranes (Lewis, 1977; Vane, 1978). Although membrane phospholipids are the principal source of prostaglandin substrate, some cells (adipocytes) contain triglyceride deposits which can also liberate fatty acids for prostaglandin synthesis.

Most processes which disturb membrane function simultaneously activate phospholipase A\(_2\), which cleaves arachidonic acid from membrane phospholipids (Arrigoni-Martelli, 1977). Hydrolysis of arachidonic acid is normally the rate-limiting step in prostaglandin formation. Once this precursor is liberated, cyclooxygenase generates intermediate cyclic endoperoxides which are converted into a variety of prostaglandins with different biological activities (Lewis, 1977).

Metabolism of the endoperoxides into prostacyclin is the dominant pathway in vessel walls (Vane, 1978). Prostacyclin is a potent vasodilator. In contrast, thromboxane A\(_2\), which is an unstable metabolite of a cyclic endoperoxide, is probably a vasoconstrictor (Lewis, 1977).

Thromboxane A\(_2\) and prostacyclin (along with the precursor, prostaglandin G\(_2\)) may have significant roles in inflammation (Vane, 1976). In fact, these prostaglandins with rapid turnover rates may be even more important than prostaglandins E\(_2\) or F\(_{2\alpha}\) as proinflammatory agents (Parnham et al., 1977; Tam et al., 1977). However, these derivatives of arachidonic acid are so unstable (thromboxane has a half-life of only 30–40 sec) that it is difficult to analyze their production in situ. Because of this instability, a more accurate estimation of their activity in specific tissues is obtainable only by determining the concentration of their more stable metabolites, thromboxane B\(_2\) and prostaglandin 6-keto F\(_{1\alpha}\), respectively (Lewis, 1977).

The enzyme complex "prostaglandin synthetase" includes an isomerase and a reductase which generate PGE\(_2\) and PGF\(_{2\alpha}\), respectively, from arachidonic acid (Vane, 1978). These "primary" prostaglandins are more stable and therefore they have been investigated more thoroughly. There is no evidence of significant interconversions between prostaglandins E and F (Arrigoni-Martelli, 1977). Although these prostaglandins are relatively stable in various tissues including blood, they are not considered to be true circulating hormones because they are rapidly inactivated in the lungs and liver (Lewis, 1977). As much as 80–98% of prostaglandin in the blood is removed during a single passage through the pulmonary circulation.

It is widely accepted that prostaglandins (especially the E-type) are important mediators of inflammation (Vane, 1976; Higgs et al., 1976; Greaves et al., 1976; Bonta et al., 1976a; Smith, 1977; Ford-Hutchinson et al., 1977; Bray and Gordon, 1978; Bonta and Parnham, 1978). The E-type prostaglandins are effective vasodilators (Johnston et al., 1976; Williams and Peck, 1977; Lewis, 1977; Bonta and Parnham, 1978):

Eq 39:
\[ \text{PGE} \downarrow \]
\[ \text{inflamed tissue} \rightarrow \text{vasodilatation} \]

Prostaglandins E\(_1\) and E\(_2\) are also capable of potentiating the increased vascular permeability and functional hyperemia induced by histamine and bradykinin (Johnston et al.,
Prostaglandin metabolism. Abbreviated diagram of the metabolic pathways of prostaglandin synthesis, beginning with the release of arachidonic acid from cellular lipid deposits and ending with inactivation in the lungs and liver. PGX, prostacyclin; PGE₂, prostaglandin E₂; PGF₂α, prostaglandin F₂α; thromboxane A₂; 6-keto F₁α, 6-keto prostaglandin F₁α; TXB₂, thromboxane B₂. (Adapted from Vane, 1978; Lewis, 1977; Kuehl et al., 1976).

1976; Greaves et al., 1976; Messina et al., 1976):

Eq 40: $PGE_{\text{hyperemia}} \downarrow$

hyperemia induced by enhanced histamine and bradykinin hyperemia

Prostaglandin F₂α is considerably less effective in this regard (Johnston et al., 1976; Bonta and Parnham, 1978). The superior action of PGE may be due to its ability to liberate histamine (Hagermark et al., 1976; Dunn et al., 1976).

It is becoming increasingly clear that prosta-
glandins induce changes in cAMP levels in various cells. Of particular interest is the abundant evidence that prostaglandins increase the concentrations of cAMP in fibroblasts; in some cases this increase is as much as 500-fold (Johnson and Pastan, 1971; Peery et al., 1971; Peters et al., 1974; Manganiello and Breslow, 1974; Haslam and Goldstein, 1974; DeAsua et al., 1975; O’Neill and Hsie, 1975; Dixon-Shanies and Knittle, 1976; Fahey et al., 1977; Peters et al., 1977a; Peters et al., 1977b; Polgar and Taylor, 1977; Baum et al., 1978; Chlapowski et al., 1978; Rao et al., 1978):

\[
\text{Eq 41:} \quad \text{prostaglandin} \xrightarrow{\downarrow} \text{cAMP synthesis}
\]

Prostaglandin E₁ appears to be the most effective in this regard, followed by prostaglandins E₂, F₂α, B₁ and A (Peery et al., 1971). Other cells in which prostaglandins stimulate the production of cAMP include leukocytes (Dunn et al., 1976), macrophages (Gemsa et al., 1979) and even nerve cells (Kalix, 1979), but not epithelial cells (Rao et al., 1978).

The prostaglandin-induced changes in cAMP levels may be important in the regulation of fibroblast proliferation. In some populations of fibroblasts, prostaglandin E₁ and, to some extent, prostaglandins E₂, F₁α and F₂α, slows growth and proliferation in vitro (Johnson and Pastan, 1971; Ko et al., 1977; Peters et al., 1977b; Polgar and Taylor, 1977; Bonta and Parnham, 1978). In other types of fibroblasts, prostaglandin F₂α can initiate DNA synthesis and cell proliferation (DeAsua et al., 1975):

\[
\text{Eq 42:} \quad \text{PGF}_2\alpha (\text{cAMP}?) \xrightarrow{\downarrow} \text{quiescent} \rightarrow \text{proliferating}
\]

Fibroblasts The influence of prostaglandins on thecal fibroblasts in ovarian follicles has not been specifically examined; however, studies of the general ultrastructure of the follicle (Espey, 1978b) suggest that the preovulatory elevation in follicular prostaglandins may stimulate the proliferation of fibroblasts in the ovary.

The adenylate cyclase system in fibroblasts becomes refractory to rechallenge with prosta-

glandin, or at least to prostaglandin E₁ (Fahey et al., 1977). Yet cells which have become refractory to prostaglandin E₁ can still respond to bradykinin by producing significant amounts of cAMP. Therefore, fibroblasts probably have different receptors (or activator systems) for prostaglandin and bradykinin (Fahey et al., 1977). It would be interesting to know whether gonadotropin stimulated follicles with “desensitized” adenylate cyclase systems can respond to exogenous prostaglandins or bradykinin by producing additional cAMP.

It has not yet been settled whether prostaglandins do (Ford-Hutchinson et al., 1977; Till et al., 1979), or do not (Walker et al., 1976; Sims et al., 1979) possess chemotactic properties to attract leukocytes into inflamed tissue. Nor is it clear whether the leukocytes and macrophages, which migrate into inflamed tissue, contribute prostaglandins to the inflammatory reaction (Glatt et al., 1974; Walker et al., 1976; Lewis, 1977).

Turning to other functions, prostaglandins may have an important role in the degradation of connective tissue. Prostaglandins F₂α and E₂ stimulate collagenase activity in fibroblasts and similar types of cells (Dayer et al., 1976; Dowsett et al., 1976; Lupulescu, 1977; Pettigrew et al., 1978):

\[
\text{Eq 43:} \quad \text{prostaglandins} \xrightarrow{\downarrow} \text{collagenase}
\]

This initial stimulatory effect on collagenase activity may transform into inhibitory action during chronic inflammation (Dayer et al., 1977).

Prostaglandins may also be involved in the regulation of collagenase production by leukocytes (Weissman et al., 1976; Smith, 1977) and macrophages (Wahl et al., 1977b). However, it is not possible to decipher a pattern to the prostaglandin influence on collagenolytic activity in these cells.

It is well documented that antiinflammatory drugs can inhibit prostaglandin synthesis in most tissues (Vane, 1976; Higgs et al., 1976; Floman and Zor, 1976; Bonta et al., 1977b; Ferreira et al., 1977; Tam et al., 1977; Lewis, 1977; Arrigoni-Martelli, 1977; Vane, 1978). Nonsteroidal antiinflammatory agents, such as indomethacin, directly inhibit the biosynthesis of prostaglandins by interfering in the enzymic
conversion of arachidonic acid into prostaglandin, prostacyclin and thromboxane (Vane, 1976; Bonta et al., 1977a; Lewis, 1977; Vane, 1978). Steroidal antiinflammatory agents do not inhibit prostaglandin synthesis in all cells but, where they are effective, they appear to act by impairing the availability of arachidonic acid for prostaglandin synthesis and this is possibly achieved through membrane stabilization or by inhibition of phospholipase A₂ (Floman and Zor, 1976; Higgs et al., 1976; Tam et al., 1977; Vane, 1978). A more detailed account of the specific actions of various antiinflammatory agents is presented below in a separate section.

Serine Proteases

In ovulation. There is evidence that mature ovarian follicles contain proteolytic enzymes (Espey, 1974; Espey, 1975; Beers et al., 1975; Espey, 1976; Espey and Coons, 1976; Strickland and Beers, 1976; Morales et al., 1978). It is particularly interesting that fibrinolytic activity increases in the follicle during the hours preceding ovulation. This activity is associated with the production of plasminogen activator in the follicle during ovulation (Beers et al., 1975; Strickland and Beers, 1976):

Eq 44:

\[
\text{gonadotropin} \\
\text{cAMP and PGE} \\
\text{mature follicles} \rightarrow \text{plasminogen activator (plasmin)}
\]

Cyclic AMP and prostaglandins E₁ and E₂, but not prostaglandins F₁₀ and F₂₀, are also capable of stimulating follicular cells to produce plasminogen activator, which leads to plasm formation. This increase in plasminogen activator in the follicle may be related to the elevation in urokinase, the plasminogen activator found in urine, near the time of ovulation (Oster, 1978).

In inflammation and related processes. For more than a decade, proteolytic enzymes have been implicated in inflammatory processes (Bertelli et al., 1969). Of special interest is the evidence that plasmin and other serine proteases in inflamed tissues activate collagenase from its proenzyme (Werb et al., 1977; Birkedal-Hansen et al., 1976a; Eckhout and Vaes, 1977; Stricklin et al., 1978):

\[
\text{Eq 45:} \\
\text{plasmin and other} \\
\text{serine proteases} \\
\text{procollagenase} \rightarrow \text{collagenase}
\]

This means that plasminogen activator, which is responsible for plasmin formation, can lead to the activation of collagenase.

Fibroblasts and transformed fibroblasts are common sources of plasminogen activator (Ossowski et al., 1973; Werb et al., 1977; Werb and Aggeler, 1978; Goldfarb and Quigley, 1978; and see Eq 11). Trypsin and probably other serine proteases induce the secretion of plasminogen activator from fibroblasts (Werb and Aggeler, 1978). In addition, estrogens enhance plasminogen activator in some tissues (Ambrus et al., 1971; Katz et al., 1976); but apparently not in the ovary (Strickland and Beers, 1976).

The treatment of fibroblasts with serine proteases also releases the cells from contact inhibition and stimulates them to undergo mitosis and migration (Burger, 1970; Vassalli et al., 1976; Pohjanpelto, 1976; Zetter et al., 1977; and see Eq 9). Proteases which stimulate fibroblast proliferation include trypsin, plasmin and thrombin.

Macrophages are another source of plasminogen activator (Vassalli et al., 1976; Birkedal-Hansen et al., 1976a; Werb, 1978). Therefore, these cells also represent a potential source of serine proteases capable of activating collagenases in inflamed tissues.

A number of serine proteases, including trypsin and plasmin, can split complement components from the parent molecule (Arri-goni-Martelli, 1977). Since complement components are functional in inflammatory reactions (by promoting bacterial lysis, it would be interesting to know whether they increase in the follicle during ovulation.

Collagenolytic Activity

In ovulation. In the final stages of the ovulatory process the collagenous connective tissue in the follicle wall becomes flaccid and distensible (Espey, 1978b):

\[
\text{Eq 46:} \\
\text{gonadotropin} \\
\text{mature follicles} \rightarrow \text{collagen degradation}
\]
The decomposition of follicular connective tissue accelerates rapidly during the final hour preceding ovulation. This degraded condition is probably the result of action of collagenolytic enzymes (Espey, 1976; Espey and Coons, 1976). The follicular collagenolytic activity has properties of a true collagenase (Morales et al., 1978).

During the past decade, a great deal has been learned about the regulation of collagenase secretion in mammalian tissues (Espey and Coons, 1976; Espey, 1978b). On the basis of present information, collagenase should not be expected to accumulate within the follicle during the ovulatory process, because this type of enzyme is not stored in tissues in any appreciable amount. Instead, it is synthesized de novo as required for collagen degradation or remodeling. When the enzyme is secreted, it becomes bound up in the collagen matrix and/or it is inactivated by serum antiproteases.

In inflammation and related processes. Fibroblasts (Bauer et al., 1975; Werb and Reynolds, 1975; Birkedal-Hansen et al., 1976a; Birkedal-Hansen et al., 1976b; Wahl et al., 1977; Stricklin et al., 1978; Werb and Aggeler, 1978) and similar types of cells (Dayer et al., 1976; Dowsett et al., 1976; Werb et al., 1977; Eeckhout and Vaes, 1977; Pettigrew et al., 1978) are well established sources of collagenase. Therefore, since serine proteases themselves can stimulate fibroblasts to secrete serine proteases and procollagenase (see Eqs 10, 11), inflammatory stimuli may induce fibroblasts to secrete a “cascade” of enzymes which rapidly increase the extracellular collagenase activity to proportions that cause substantial degradation of local collagen (Eeckhout and Vaes, 1977). A similar “cascade” of enzymes is probably responsible also for the rapid decomposition of follicular collagen during the final stages of the ovulatory process. Prostaglandins might contribute to the formation of this “cascade” since these mediators of inflammation are known to stimulate collagenase activity in fibroblasts and related cells (see Eq 43).

Macrophages also produce collagenolytic enzymes (Birkedal-Hansen et al., 1976a; Wahl et al., 1977; Werb, 1978). These migrating cells may contribute collagenolytic enzymes to inflamed tissues during the more advanced stages of the process.

The manifestation of collagenolytic activity in various tissues is also influenced by sex steroids. Diminished levels of progesterone and estrogen appear to be a prerequisite for collagen breakdown in the uterus (Jeffrey et al., 1971; Koob and Jeffrey, 1974; Jeffrey et al., 1974; Woessner, 1976; Tansey and Padykula, 1978). It may be that low levels of estrogen and progesterone are also essential for collagen degradation in the follicle during ovulation (LeMaire and Marsh, 1975; Hunzicker-Dunn, 1979). This comparison of collagen degradation in the follicle to involution of the postpartum uterus becomes even more interesting in light of a recent description of uterine regression in terms of an inflammatory reaction (Tansey and Padykula, 1978).

**CHARACTERISTICS OF ANTIINFLAMMATORY AGENTS**

Antiinflammatory agents can be divided into a number of categories: 1) the steroidal antiinflammatory agents include dexamethasone, prednisolone, hydrocortisone and other corticosteroids; 2) the nonsteroidal antiinflammatory drugs are a group of aspirin-like compounds including indomethacin, phenylbutazone, diclofenac, meclofenamic acid, flufenamic acid, naproxen and similar drugs; 3) the antineoplastic drugs with antiinflammatory properties include colchicine, vinblastin, 6-mercaptopurine and other immunosuppressants which commonly inhibit the metaphase stage of cell division; and 4) a wide variety of other agents that suppress inflammation to some extent exist, including levamisole (an antihelminthic agent), allopurinol (an antigout agent), chloroquine (an antimalarial agent) and acetaminophen (an antipyretic agent). This section summarizes current information on the mechanism of action of some of these agents as they exert their antiinflammatory effects in various biological systems.

**Effects on Prostaglandin Synthesis**

In 1971, it was discovered that aspirin inhibits prostaglandin biosynthesis (Vane, 1971). Since that time, it has been demonstrated that most antiinflammatory agents exert their effect by blocking the synthesis of prostaglandins (Flower, 1974; Vane, 1978). With few exceptions, any drugs that inhibit prostaglandin synthesis will normally exhibit antiinflammatory properties in vivo. This action has now been confirmed in numerous species and in many biological preparations.
Steroidal antiinflammatory agents. The antiinflammatory steroids act by blocking the deacylation of phospholipids which otherwise would lead to the formation of arachidonic acid, the principal endogenous substrate for prostaglandin synthesis (Tam et al., 1977; Floman and Zor, 1976; Greaves and McDonald-Gibson, 1972; Vane, 1978). Since steroids act at this site, the suppression of prostaglandin synthesis by this group of antiinflammatory agents can be overcome by the addition of exogenous arachidonic acid to the experimental system (Gryglewski et al., 1975; Floman and Zor, 1976). It has also been suggested that corticosteroids might reduce the amount of prostaglandin in inflamed tissues: 1) by interfering with cyclooxygenase activity (Chandrabose et al., 1978); 2) by “stabilizing” plasma membranes, which would otherwise release phospholipids (Vane, 1978); and/or 3) by inhibiting the migration of leukocytes, which are another source of prostaglandins, into the inflamed tissue (Higgs et al., 1976).

Nonsteroidal antiinflammatory agents. The acidic nonsteroidal antiinflammatory agents appear to be most effective in reducing prostaglandin synthesis (Brune et al., 1976). There is general agreement that these agents inhibit cyclooxygenase, the enzyme which converts arachidonic acid into the endoperoxides that produce prostaglandins and their derivatives (Vane, 1976; Lewis, 1977; Chandrabose et al., 1978; Vane, 1978). Therefore, in contrast to the steroidal drugs, the inhibitory effect of aspirin, indomethacin and similar agents cannot be overcome by the addition of exogenous arachidonic acid to the experimental system (Floman and Zor, 1976).

Antineoplastic drugs and other agents. The antiinflammatory action of antineoplastic drugs has been less thoroughly investigated. At least one of these drugs, colchicine, reportedly enhances prostaglandin synthesis even though it exerts an antiinflammatory effect in vivo (Ferreira, 1976; Bray and Gordon, 1978). In contrast, the antipyretic agent, acetaminophen, inhibits prostaglandin synthesis (at least in brain tissue) but lacks the antiinflammatory properties of other inhibitors (Lewis, 1977).

Effects on Fibroblasts and Other Cells

It has been recognized for many years that fibroblasts are highly sensitive target cells for antiinflammatory agents (Berliner et al., 1967). Many different kinds of steroidal (Dayer et al., 1976; Hong and Levine, 1976; Tam et al., 1977; Chandrabose et al., 1978) and nonsteroidal (Dayer et al., 1976; Newcombe and Ishikawa, 1976) antiinflammatory agents inhibit prostaglandin synthesis by fibroblasts. The antiinflammatory agents also inhibit prostaglandin synthesis in other types of cells, including leukocytes and macrophages (Brogden et al., 1975; Fitzpatrick and Wynalda, 1976; Taylor and Salata, 1976; Floman and Zor, 1976; Walker et al., 1976; Adams et al., 1977; Bonta et al., 1977B; Platshon and Kaliner, 1978; Oliw et al., 1978; Nozu, 1978; Bray and Gordon, 1978).

Effect on Vascular System

Antiinflammatory compounds reduce the swelling of irritated tissues by suppressing the vasodilatation which usually occurs during inflammation (Williams, 1977B; Williams and Peck, 1977; Ferreira et al., 1977). This effect is presumable due to inhibition of prostaglandin E formation.

Effect on Cell Proliferation

Antiinflammatory agents, including the metaphase inhibitors, prevent the growth and proliferation of fibroblasts (Berliner et al., 1967; Ruhmann and Berliner, 1967; Vasiliev et al., 1970; Ponec et al., 1977; Hial et al., 1977). These agents also inhibit the migration of leukocytes and macrophages into inflamed tissue (Walker et al., 1976; Meacock and Kitchen, 1976; Vassalli et al., 1976; Adams et al., 1977). Thus, it appears that antiinflammatory drugs have an antiproliferative effect on a variety of cell types (Hial et al., 1977).

Effect on Proteolytic Activity

Antiinflammatory agents have the ability to inhibit the production of proteolytic enzymes, including plasminogen activator and collagenase, by fibroblasts (Jeffrey et al., 1974; Werb et al., 1977), by leukocytes and macrophages (Vassalli et al., 1976; Wahl, et al., 1977B; Smith, 1977; Werb, 1978) and by several other types of cells (Bertelli et al., 1969; Brown and Pollock, 1970; Ito et al., 1974; Suzuki et al., 1976; Arrigoni-Martelli, 1977). However, under some circumstances, antiinflammatory agents reportedly stimulate proteolytic activity (Houck et al., 1968; Brown and Pollock, 1970; Dayer et al., 1976). Collage-
nolytic activity may (Brown and Pollock, 1970), or may not (Dowsett et al., 1976), be inhibited by nonsteroidal antiinflammatory agents.

There is only sparse information on the effect of antiinflammatory agents on ovarian proteolytic enzymes. There is some indication that dexamethasone has no effect on the production of plasminogen activator by follicular tissue (Strickland and Beers, 1976). On the other hand, betamethasone, prednisolone, indomethacin and chloroquine all show some degree of inhibition of collagenolytic activity in cultured ovarian follicles (Espey and Coons, 1976).

Effects on the Ovulatory Process

**Indomethacin.** It is now well established that prostaglandins are important in ovulation and that indomethacin inhibits this process by impeding the synthesis of prostaglandins (Orczyk and Behrman, 1972; Armstrong and Grinwich, 1972; Grinwich et al., 1972; Behrman et al., 1972; O’Grady et al., 1972; Tsafriri et al., 1972b; Tsafriri et al., 1973; Marsh and LeMaire, 1973; LeMaire et al., 1973; Yang et al., 1973; Lau et al., 1974; Parr, 1974; Wallach et al., 1975; Bowring et al., 1975; Rigler et al., 1976; Hamada et al., 1977; Hamada et al., 1978). The inhibitory action of indomethacin does not prevent the initial rise in cAMP and the subsequent steroidogenesis and luteinization that normally occur in the follicle after gonadotropin stimulation (O’Grady et al., 1972; Armstrong and Zamecnik, 1975; Goff and Major, 1975; Bowring et al., 1975; Armstrong et al., 1976; Maia et al., 1978; YoungLai, 1978; Lee and Noy, 1978).

Indomethacin inhibits the ovarian hyperemia that develops in response to an ovulatory surge of gonadotropin (Lee and Noy, 1978). This effect is comparable to what happens in other inflamed tissues when indomethacin inhibits prostaglandin-induced vasodilatation. Collectively, this information, along with the rest of the data that have been discussed throughout this review, indicates that **indomethacin probably inhibits ovulation by suppressing an inflammatory reaction mediated by prostaglandins.**

**Aspirin.** There are several brief accounts of aspirin blocking ovulation in rats (Orczyk and Behrman, 1972; Parr, 1974), supposedly by acting at the hypothalamic level (Behrman, 1972; Parr, 1974), supposedly by acting at the hypothalamic level (Behrman et al., 1972). On the other hand, there are also reports that therapeutic doses of aspirin do not prevent ovulation in humans (Chaudhuri and Elder, 1976; Greenway and Swerdloff, 1978). However, a closer assessment of these latter studies reveals that follicular rupture and ovum release were not actually confirmed. Instead, the reports merely show that LH release, steroidogenesis and luteinization occurred in the aspirin treated women. Yet, these three events usually proceed without impairment in animals in which ovulation has been inhibited by nonsteroidal antiinflammatory agents.

**Dexamethasone.** There is a single report that dexamethasone inhibits both ovulation and luteinization in PMSG treated immature rats by a mechanism which might involve the central nervous system (Soliman and Walker, 1977). There is no information on the effect of this steroid antiinflammatory agent on ovarian prostaglandin levels.

CONCLUSIONS

**The "Working Model"**

The principal conclusions of this review are outlined in Figs. 2 and 3. These figures project the steps by which luteinizing hormone might induce inflammation in a mature ovarian follicle and cause ovulation.

Figure 2 is a simplified scheme of the basic reactions that take place during the ovulatory process. As illustrated, the immediate responses to an ovulatory surge of LH include cAMP production, steroidogenesis and the release of histamine and related compounds which mediate the initial phase of the inflammatory reaction. During the intermediate stages of the ovulatory process, prostaglandins enhance the inflammatory reaction and activate thecal fibroblasts. In the advanced stages of the process, a phlogistic follicle secretes serine proteases that activate local collagenase and produce a cascade of proteolytic enzymes that degrade the follicular connective tissue and cause ovulation.

Figure 3 is an expanded version of the model presented in Fig. 2. This scheme attempts to integrate all of the equations which were established during the course of this review. Some of the relationships may not actually be as direct as illustrated. Others may need further
FIG. 2. Simple model of the ovulatory process. Broken lines indicate the reactions which take place during the first hours of the ovulatory process. Thick, solid lines indicate the hypothetical reactions during the intermediate stages of the process. Thin, solid lines indicate the final reactions which culminate in ovulation.
DETAILED MODEL OF THE OVULATORY PROCESS

FIG. 3. Detailed model of the ovulatory process. Solid lines duplicate the reactions outlined in Fig. 2. Broken lines represent additional, more speculative information. Numbers correlate with the equations in the text to the approximate position where they might enter into the working model.

verification. Still, the overall projection should be useful as a "working model" for future studies of the ovulatory process.

Additional Thoughts

In the course of organizing a review such as this, some ideas come to mind that extend beyond the central theme of the text. Although such thoughts are often little more than speculations arising from circumstantial evidence, some of them seem to have sufficient potential value to be worth citing. Three such thoughts are presented below.

Comparing ovulation to an immune response. Immune responses have characteristics similar to inflammatory reactions (Van Arman, 1976; Arrigoni-Martelli, 1977); in fact, inflammation is frequently of immunological origin. This review has established that the ovulatory
process resembles an inflammatory response. All three of these processes, ovulation, inflammation and immune responses: 1) require specific cellular recognition of some extrinsic substance; 2) utilize common mediators such as cAMP and histamine; and 3) result in cell proliferation and differentiation. Therefore, it can be deduced that ovulation is comparable to an immune response—not necessarily identical with, but still, in some respects, comparable to such a response.

An immune response is initiated by the binding of antigen to specific receptors on sensitive cells. Similarly, the ovulatory response is initiated by the binding of protein hormone to specific receptors on target endocrine cells. This correlation raises the question of whether follicle cells become sensitized in such a way that they react to gonadotropins as if they were antigens. In this regard, it is interesting to note how common it has become to refer to follicles as being either sensitized or “desensitized” to gonadotropins (Hunzicker-Dunn et al., 1979).

This comparison raises a number of other pertinent questions: For example, do components of the complement system appear before, or after, follicle rupture? During luteinization, are luteal cells comparable to immunoresponsive cells that have undergone morphological transformation into characteristically larger “immunoblasts”? If so, what is the “antibody equivalent” produced by the corpus luteum? Could it be the steroid hormones? Although such hormones do not directly impair the circulating gonadotropins, could their inhibitory action through the hypothalamus suffice as a corollary to antibody action? Since immunosuppressive agents can modify inflammatory processes (Arrigoni-Martelli, 1977), can they also suppress ovulation? Even if they should fail to inhibit ovulation, might they still impair the postovulatory proliferation of lutein tissue? In support of some such contention, there is scattered evidence that immunosuppressive agents inhibit fertility (Arrigoni-Martelli, 1977, p. 276). Lastly, could the major difference between the immune response and the ovulatory process merely be that, whereas immunological responsiveness is one of the fundamental mechanisms by which higher organisms react to their external environment, the ovulatory response represents a method by which normal tissues respond to specific changes in their internal environment?

Of course, both of these statements are well established facts by themselves, but the intended question is whether the endocrine system might be operating, at least in those instances where gonadotropins stimulate their specific target tissues, by “taking advantage” of the immunoresponsive capacity inherent in all cells in mammalian systems. This is not to imply that the ovulatory process is identical to an immune reaction, only that the two conditions may have sufficient features in common to warrant a comparison for the purpose of increasing the current level of knowledge of the mechanism of ovulation.

Comparing the corpus luteum to a granuloma. A chronically inflamed lesion often transforms into a small, firm, nodular mass called a granuloma, which persists for an extended period of time. Prostaglandins may regulate the formation of a granuloma through their action on fibroblast growth and collagen synthesis (Bonta and Parnham, 1978).

Since corpora lutea are also small, firm, nodular masses, the correlation of ovulation (and simultaneous luteinization) to an inflammatory response also introduces the possibility that the corpus luteum may be comparable to a granuloma. It is well known that gonadotropins are necessary to stimulate the formation of a corpus luteum. It is also known that the circulating levels of gonadotropins usually influence the output and longevity of a corpus luteum. However, continuous gonadotropic action is not always required for the maintenance of lutein tissue. In fact, corpora lutea can persist for the normal duration of pseudopregnancy in the absence of gonadotropins (Perry, 1972). This independence raises the question of what keeps a corpus luteum viable for such a long period of time. Its persistence could be explained, at least in part, if the corpus luteum is, indeed, comparable to a “granuloma” which develops as part of an “inflammatory” response to an “immunological” stimulus.

If the corpus luteum is similar to a granuloma, then steroid production should be one of the basic characteristics of a granuloma. In this connection, it may be relevant that granulomas which develop in inflamed middle ear tissues produce substantial amounts of cholesterol (Friedman and Graham, 1979) and tuberculosis granulomas produce significant amounts of lipids (Warren, 1972).

However, if the corpus luteum is comparable
to a granuloma, then it is somewhat puzzling why the antiinflammatory agent, indomethacin, which inhibits ovulation, does not also inhibit the formation of lutein tissue. One possible explanation might be that a single dose of indomethacin diminishes the ovulatory "trauma" just enough to prevent follicular rupture, without completely extinguishing the inflammatory reactions initiated by the gonadotropin surge. In support of this explanation, there is evidence that even the chronic administration of indomethacin causes only a partial suppression of granuloma formation (Suzuki et al., 1976).

Relating gonadotropin output to chronic inflammatory diseases. The conclusions of this review indicate that gonadotropic hormones induce ovarian follicles to produce the kinds of proteolytic enzymes that are commonly associated with connective tissue diseases. Therefore, it may be relevant that the incidence of chronic inflammatory diseases (such as arthritis and rheumatism) is greater in postmenopausal women (Morris, 1975; Ahlqvist, 1976), at a time when the circulating levels of gonadotropins increase significantly (Botella-Llusia, 1973; Sommerville et al., 1976; Monroe and Menon, 1977). This information suggests that the chronic elevation of gonadotropins during later life might somehow aggravate inflammatory diseases.

It may be equally relevant that the incidence and/or symptoms of rheumatoid arthritis decrease significantly in women taking oral contraceptives (Wingrave and Kay, 1978; Linos et al., 1978) and in women who have become pregnant (Neely and Persellin, 1977; Persellin, 1977). In both of these situations it is well known that the circulating levels of gonadotropins also decrease significantly. Thus, it appears there may indeed be a correlation between gonadotropin levels and connective tissue disorders. Such a relationship could exist, for example, if certain metabolic conditions were to cause synovial fibroblasts to become "sensitized" to gonadotropins. In any event, this potential correlation seems to be worthy of further attention.

Final Conclusion

By describing ovulation as an inflammatory process it is possible to integrate an omnium-gatherum of factual material on the physiology of ovulation that has been, heretofore, disunited. In particular, the model provides a logical role for prostaglandins in ovulation by suggesting that this group of inflammatory mediators stimulates the proliferation of thecal fibroblasts and the production of proteolytic enzymes capable of disrupting the follicle wall during ovulation. The hypothesis also encourages the evaluation of antiinflammatory agents, along with antihistamines and immuno-suppressive drugs, as potential antifertility agents.

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OVLATION AS AN INFLAMMATORY REACTION


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