Role of Hormones in Oocyte Maturation

ALLEN W. SCHUETZ

Johns Hopkins University, Department of Population Dynamics, School of Hygiene and Public Health, 615 N. Wolfe Street, Baltimore, Maryland 21205

In order for normal fertilization and embryonic development to occur, oocytes, during the course of meiosis, undergo a strict sequence of morphological and physiological transformations in the cytoplasm, nucleus, and at the cell surface. The nature of these maturational changes at various stages of meiosis and the role hormones play in them have been discussed primarily on the basis of in vitro data. Gametogenic and endocrine functions are performed by the gonads of most species, and evidence presented here leads to the general conclusion that certain aspects of these two processes occur as a result of an interaction between germinal and somatic cells. Typically, gametic and somatic endocrine cells are in physical juxtaposition within an ovarian follicle, and structural and functional changes which occur in these cells during the course of gametogenesis appear to be important for allowing oocyte maturation to proceed as well as for regulating the process. A central event in the oocyte maturational process is interruption of oocyte arrest as characterized by the disintegration of the nucleus or germinal vesicle. Oocytes, prior to this event, differentiate through phase I of meiosis and undergo the major portion of cytoplasmic and nuclear growth. Subsequent to nuclear breakdown, chromosomes proceed through the remaining meiotic events and differentiation of new functions occurs in the cytoplasm. Considerable evidence in nonmammalian species indicates that particular steroids or other low molecular weight substances mediate certain aspects of the cytoplasmic and nuclear maturation processes in oocytes. In mammals, a direct involvement of hormones (pituitary and/or ovarian) in initiating nuclear or cytoplasmic maturation has not been established, although the data are suggestive. Spontaneous oocyte maturation is a complicating factor in many species, and its relevance is discussed in terms of processes of follicular and oocyte differentiation. In amphibians, the nature of the steroids, the time at which they function, and the functions they perform vary in relation to particular stages of meiosis. Thus, ovarian estrogenic steroids are required (indirectly) for oocyte growth, their function being to stimulate hepatic synthesis of vitellogenin, the major cytoplasmic yolk platelet precursor. Subsequent to its release into the circulation, vitellogenin is sequestered into the oocyte by a micropinocytotic process at the oocyte surface and undergoes transformation into yolk within the oocyte. In contrast, reinitiation of the meiotic process (germinal vesicle breakdown) occurs in direct response to certain nonestrogenic steroids (progestational, adrenocortical, androgens) and proceeds in vitro to the typical in vitro stage of second metaphase arrest. Somatic follicle cells appear to be the cellular source of both the estrogenic steroids required for vitellogenesis or oocyte growth and the "progestational-like" steroids involved in nuclear and cytoplasmic maturation including and proceeding from nuclear breakdown. Presumably, follicle cells alter their steroidogenic function during the course of oocyte and follicle differentiation. Following and/or in synchronization with induced nuclear disintegration and meiosis, numerous time-dependent maturational changes occur in the nuclear contents, oocyte cytoplasm (including cortical granules), and in the relationship between the oocyte surface and associated membranes. These are important in establishing conditions for fertilization or activation, ovulation, block to polyspermy, cleavage, and embryogenesis. Although many of these maturational processes are responses to the same steroidal stimuli which initiate nuclear breakdown, mediation of these changes in many cases occurs in the cytoplasm independent of the nucleus.

Considerable evidence indicates that cytoplasmic "maturation" factors(s), released or synthesized within the oocyte in response to the steroids, can initiate nuclear disintegration.
in addition to many of the subsequent maturational changes in the oocyte cytoplasm in the meiotic process. A second, "cytostatic" factor, formed in the oocyte cytoplasm in response to steroid hormones and independent of the nucleus, has been implicated in the arrest of oocytes at the second meiotic metaphase. Although nuclear breakdown is stimulated by certain steroid hormones, the same steroids simultaneously have inhibitory effects on oocyte functions related to cytoplasmic and nuclear maturation processes. Incorporation of vitellogenin continues into oocytes which are capable of undergoing steroid-induced nuclear disintegration; however, macromolecular incorporation is essentially terminated following exposure to such a steroid. Inhibition of incorporation occurring after a lag of several hours, is associated with changes in the oocyte surface and cortex, and probably occurs as a result of the inhibition of micropinocytosis. These results suggest that the inverse relationship which exists between the slowdown in oocyte growth and onset of the capacity of oocytes to undergo nuclear disintegration may be an expression of a linked or coordinated process. Obviously, the events and processes associated with the development and maturation of the oocyte and its transition from intraovarian to extraovarian environment are complicated, highly integrated, and fundamental for normal embryonic development. On the basis of experimental data discussed here, it is evident that many aspects of oocyte maturation are induced by steroids independent of the nucleus and that the cytoplasm exerts a considerable controlling influence on the nuclear maturation process. Significantly, this separation of functions in the oocyte, as well as the asynchronies which arise between oocyte and follicle maturation, appears relevant to and provides a new basis for examining such problems as chromosomal abnormalities, overripeness, teratogenesis, and atresia. Clearly, the fact that, in amphibians, for the first time, the entire gamut of oocyte maturation events (vitellogenic growth, nuclear, and cytoplasmic maturation, fertilization and embryonic development) can be carried out and studied under

Species perpetuation is dependent primarily upon the capacity of its individuals to produce cellular vehicles of evolution, specifically the gametes. In the female, the primary ovarian function is to form and develop those cells (oocytes) endowed with the unique capacity to undergo embryonic development following fertilization or parthenogenetic activation. The capability to undergo embryonic development, however, is not achieved with the first appearance of the cells, but arises following a long complicated, sequential, integrated process of cytodifferentiation of the cell itself and the follicle in which it is normally contained. Oogenesis is the general term applied to describe these changes in the oocyte. Growth and differentiation of oocytes involve cytoplasmic and nuclear alterations, and the mechanisms by which these changes are brought about is the question to which this discussion is directed.

To a considerable extent, the endocrine system is a modulator and regulator of certain aspects of oogenesis; however, the precise cellular, hormonal, and molecular interactions are only now becoming amenable to experimental analysis. Significantly, the problems are concerned with identifying molecular and cellular processes which occur during oocyte and follicle development, establishing when they happen and are completed, and elucidating the role hormones play in mediating this differentiation. Recent advances in understanding these hormonal processes have been derived from studies of nonmammalian and mammalian species. A major emphasis of this discussion will be to summarize and correlate our present knowledge and understanding of the nature and problems of oocyte maturation, particularly in amphibians and mammals, and, to a limited extent, in starfish and other species.

It is becoming increasingly evident that many parallels exist in the types of changes which occur in oocytes of all species, as
Fig. 1. Examples of oocytes of different species with an intact germinal vesicle or nucleus. A—starfish, B—mouse, (from Sorensen, 1972, with permission), C—frog, cross section, D—starfish oocyte after germinal vesicle breakdown. Photos on top taken with Nomarski optics. Germinal vesicle (GV); follicle cells (FC); nucleolus (N); polar bodies (PB).
well as in the manner in which they are regulated (Biggers and Schuetz, 1972). From a structural point of view this similarity can be appreciated by examining the oocytes which are arrested at the germinal vesicle stage from starfish, mouse, and frog (Fig. 1). Figure 2 contrasts the differences in ovarian follicle and oocyte structure in amphibians and mammals. The complicated nature of the oocyte maturation process has been evident morphologically for a long time; however, experi-

Fig. 2. Contrast in ovarian and follicular structure in amphibians and mammals. (Top) frog (*Rana pipiens*) ovary (*×8*). (Bottom) rat ovary from prepuberal PMS-injected animal.
mental analysis of this process has been limited by the fact that a multitude of oocyte and follicle stages exist within the ovary at any one time. Clearly then, oogenesis or oocyte differentiation and maturation include to a large extent common processes which occur at different times in the various gametes. Thus, it would appear, and the experimental evidence suggests the idea, that understanding oocyte differentiation requires an understanding of local, intraovarian; and intrafollicular processes.

Recent progress has been primarily the result of (1) development and use of in vitro culture methods for maintaining ovarian tissue and oocytes; (2) experimentation on individual oocytes and follicles; and (3) selection of species in which particular aspects of the problem can be studied. For experimental studies there has been and continues to be a crucial need for the establishment of simple reliable criteria to classify individual follicles and oocytes according to their cytoplasmic or nuclear stage of differentiation. To a considerable extent, many of the difficulties in studying oocyte maturation, particularly in vivo, arise because it is not appreciated that oogenesis is a dynamic and continuous process which involves many cellular and molecular processes and interactions that may be simultaneously occurring. Selected aspects of oocyte maturation will be discussed here.

OOCTYE MATURATION—GENERAL CONSIDERATIONS

To consider oocyte maturation, we must define it, even though in general terms. Great difficulties arise because the term maturation has been applied to numerous processes occurring in the oocyte. To some, oocyte maturation consists of one particular event and to others it may be a gradual change in the composition or size of the oocyte. Thus, reduction divisions associated with meiotic spindle and polar body formation may be considered to be meiotic maturation, but neglected is a significant phase of meiosis, prophase I, during which the oocyte cytoplasm and nucleus undergo a major portion of their growth and differentiation. Furthermore, since most oocytes arrest at second meiotic metaphase, completion of the meiotic process or formation of the haploid gamete is not attained until after sperm-induced activation of the oocyte. The scope of oocyte maturation as considered in this discussion is outlined in Fig. 3.

Oocyte maturation is a sequential, programmed, time-dependent set of morphological, biochemical, and functional transformations in the nucleus and cytoplasm of a single cell which is uniquely endowed with the capacity to undergo the cytoplasmic partitioning associated with meiosis. Since events of oocyte maturation are closely tied to stages of meiosis, the study of maturation is essentially a study of the meiotic process. Maturation thus begins with oogonia formation and continues through cytoplasmic growth, nuclear changes associated with formation of haploid cells, and reconstitution of the diploid state subsequent to fertilization.

In general, oocytes of all species go through similar chromosomal and nuclear changes during meiosis and characteristically “arrest” at particular stages. Two points of oocyte arrest are of major significance for this discussion: arrest at the germinal vesicle stage and at second meiotic metaphase. During the time the oocyte nucleus (germinal vesicle) remains intact, chromosomal changes associated with prophase I of meiosis are completed and a major proportion of the oocyte cytoplasmic growth is completed.

SIGNIFICANCE OF GERMINAL VESICLE BREAKDOWN

Disintegration of the germinal vesicle marks a major turning point in oocyte function and composition and is approximately correlated, in most species, with the shift of the oocyte from an intraovarian to extra-
HORMONES AND OOCYTE MATURATION

Fig. 3. Diagrammatic representation of the scope of the oocyte maturation process. Figure illustrates the major structural (nuclear and cytoplasmic) changes which occur in oocytes from the time of oogonium formation through reconstitution of the two n or diploid state following fertilization. Based on the oocyte maturation process in amphibians and most mammals.

ovarian environment. Preceding this event, cytoplasmic accumulation of yolk and oocyte growth occur, chromosomes differentiate within the nucleus through the stages of prophase I of meiosis, and the process of generating genetic variation (chromosomal pairing and crossing over) has been completed. Following a short exposure to an inducing influence, disintegration of the nucleus occurs within short periods of time (minutes to several hours) and the oocyte differentiates new functions and properties which drastically affect its ultimate fate. Table 1 presents a list of structural and physiological changes which occur or are associated with nuclear disintegration. Significantly, the tetraploid nucleus is reduced to the haploid condition by the meiotic divisions and the cytoplasm undergoes changes in preparation for fertilization. Chromosomal changes which occur during maturation have been given major consideration in the past because of their primary significance to genetics; however, it is becoming increasingly clear that the nonchromosomal elements (cytoplasm and cortex) have important roles to play in the oocyte maturation process and even in chromosomal events. The questions: what maintains an oocyte in the germinal vesicle stage of arrest, what initiates disintegration, how is it accomplished and what are the consequences, are of major significance to understanding the oocyte maturation process.

HORMONAL REGULATION—GENERAL ASPECTS

Experimental evidence for the concept that hormones, and in particular gonadal hormones, can have a positive and direct influence on the process of oocyte maturation and differentiation is of recent origin and comes primarily from studies in starfish and amphibians (Schuetz, 1969, review). Experimental results in amphibians indi-
cate that hormones are involved in (1) oocyte growth via stimulation of vitellogenesis, (2) reinitiation of the meiotic process as distinguished by the breakdown of the nucleus or germinal vesicle, and (3) physiological and structural changes in the cytoplasm and oocyte cortex in association with and/or subsequent to germinal vesicle breakdown.

Induction of oocyte growth appears to be mediated by estrogenic steroids produced in the ovary which stimulate the synthesis and release of yolk precursors from the liver (Wallace, 1972; Redshaw, 1973). The role of estrogens in promoting oocyte growth therefore appears to be mediated indirectly through other tissues. At present, no evidence indicates that estrogens have a direct effect on the oocyte, although inhibitory effects within the ovarian follicle have been reported (Wright, 1961; Schuetz, 1972a).

Induction of nuclear disintegration in isolated starfish oocytes by an ovarian product was demonstrated by Schuetz and Biggers (1967), and Kanatani and Shirai (1967). Subsequently, this starfish ovarian substance was identified as 1-methyladenine (Kanatani, et al. 1969). In amphibian oocytes, in vitro initiation of germinal vesicle breakdown in response to certain steroid hormones was demonstrated (Schuetz, 1967a, b; Masui, 1967; Smith, Ecker, and Subtelny, 1968). Thus, in both starfish and amphibians, it has been shown that the meiotic maturation process is directly amenable to hormonal regulation independent of gonadotrophic hormones, follicular tissues, spawning, or ovulation. Significantly, steroids induced nuclear disintegration in the absence of follicle cells in vitro and such oocytes matured to and arrested at second meiotic metaphase as occurs in vivo. Induction of oocyte meiotic maturation by steroids has since been shown in a variety of amphibian species and fishes (Table 2). In amphibians, androgenic, progestational, and adrenal steroids stimulate the maturation process, whereas estrogenic steroids are strikingly ineffective (Schuetz, 1967a, 1972). In all species, the primary molecule which initiates the oocyte maturation process appears to be under some type of gonadotrophic regulation.

Principal cellular sources of intermediate molecules are the ovarian follicle cells in the starfish (Shirai and Kanatani, 1971; Cloud and Schuetz, 1973) and amphibians (Schuetz, 1967b; Masui, 1967, 1973; Smith, Ecker, and Subtelny, 1968). Interalten tissue appears to be involved in the maturation process in catfish (Goswami and Sun-
TABLE 2
REPORTS OF NONGONADOTROPHIC INDUCTION OF GERMINAL VESICLE BREAKDOWN IN OOCYTES IN VITRO

<table>
<thead>
<tr>
<th>Class</th>
<th>Effective stimulators</th>
<th>Pertinent reference</th>
</tr>
</thead>
<tbody>
<tr>
<td>Asterideae (starfish)</td>
<td>1-Methyladenine (ovarian factor)</td>
<td>Schuetz and Biggers (1967)</td>
</tr>
<tr>
<td>Asterias forbesi</td>
<td>1-Methyladenine (meiosis inducing factor)</td>
<td>Kanatani and Shirai (1967)</td>
</tr>
<tr>
<td>Asterina pectinifera</td>
<td>Progestational steroids</td>
<td>Dettlaff and Skoblina (1969)</td>
</tr>
<tr>
<td>Asteroidea (starfish)</td>
<td>Progestational steroids (not progestational or estrogenic)</td>
<td>Goswami and Sundararaj (1971)</td>
</tr>
<tr>
<td>Catfish</td>
<td>Progestational steroids (not estrogenic)</td>
<td>Sundararaj and Goswami (1971)</td>
</tr>
<tr>
<td>Sturgeon</td>
<td>Progestational steroids (not estrogenic)</td>
<td>Jalabert, Breton, and Bry (1972)</td>
</tr>
<tr>
<td>Rana pipiens (Frog)</td>
<td>Progestational and adrenal steroids (not estrogenic)</td>
<td>Schuetz (1967a,b)</td>
</tr>
<tr>
<td>Rana temporaria</td>
<td>Progesterone</td>
<td>Dettlaff and Skoblina (1969)</td>
</tr>
<tr>
<td>Xenopus laevis</td>
<td>Progesterone (not estrogenic)</td>
<td>Merriam (1971)</td>
</tr>
<tr>
<td>Bufo bufo</td>
<td>Progesterone (not estrogenic)</td>
<td>Brachet et al. (1970)</td>
</tr>
<tr>
<td>Discoglossus pictus</td>
<td>Progesterone</td>
<td>Thornton and Everett (1969)</td>
</tr>
<tr>
<td>Mammalia</td>
<td>Progesterone</td>
<td>Alanso-Bedate et al. (1971)</td>
</tr>
<tr>
<td>Rabbit</td>
<td>Spontaneous</td>
<td>Pineus and Enzmann (1935)</td>
</tr>
<tr>
<td>Mouse</td>
<td>Spontaneous</td>
<td>Tsafiriri et al. (1972)</td>
</tr>
<tr>
<td>Rat</td>
<td>Spontaneous</td>
<td>See Reviews</td>
</tr>
<tr>
<td>Guinea pig</td>
<td>Spontaneous</td>
<td>Schuetz (1969)</td>
</tr>
<tr>
<td>Pig</td>
<td>Spontaneous</td>
<td>Donahue (1972)</td>
</tr>
<tr>
<td>Cow</td>
<td>Spontaneous</td>
<td></td>
</tr>
<tr>
<td>Sheep</td>
<td>Spontaneous</td>
<td></td>
</tr>
<tr>
<td>Hamster</td>
<td>Spontaneous</td>
<td></td>
</tr>
<tr>
<td>Monkey</td>
<td>Spontaneous</td>
<td></td>
</tr>
<tr>
<td>Man</td>
<td>Spontaneous</td>
<td></td>
</tr>
</tbody>
</table>

dararaj, 1971), whereas in the sturgeon (Dettlaff and Skoblina, 1969), the ovarian follicular tissues can serve as an intermediate of gonadotropin-induced maturation. A diagrammatic representation of my present interpretation of the overall regulation of oocyte maturation is depicted in Fig. 4.

Whether there is a positive specific inducer responsible for oocyte maturation in mammals has not been resolved; however, there is little question that the gonadotropic hormones are involved in some way in the selective synchronization and induction of oocyte maturation in preovulatory ovarian follicles (Everett, 1961; Strauss and Meyer, 1962). Tsafiriri et al. (1972) have recently demonstrated, using in vitro cultures of preovulatory rat ovarian follicles, that oocyte maturation results following treatment with LH, prostaglandin E\(_2\), and high concentrations of injected cAMP. Whether these hormones directly induce oocyte maturation in immature oocytes has not been established, however, and is complicated by the fact that
most oocytes liberated from mammalian ovarian follicles undergo meiotic maturation divisions "spontaneously."

In addition, evidence for the presence of inhibitory influences within the ovarian follicles has been obtained in mammals. Channing (1970) has convincingly demonstrated that "spontaneous" luteinization and secretion of progestins occurred when granulosa (follicle) cells of horse, pig, monkey, and human were released from ovarian follicles and cultured in vitro. Removal of the oocyte from the follicle was shown to result in luteinization and steroid secretion (progestational) by the follicle (El-Fouly et al., 1970; Nalbandov, 1972). Suppression of follicle cell luteinization was also observed when isolated granulosa cells were incubated in the presence of oocytes (Nekola et al., 1972). These results suggest that the oocyte suppresses follicular cell function, possibly by the production of an anti-luteinizing factor. Thus, if the follicle cells are in some way functionally related to the oocyte maturation process, the possibility exists that inhibition of the effects of the oocyte could be a mechanism of gonadotropin action. Alternatively, experiments by Foote and Thibault (1969) (reviewed by Thibault, 1972) indicate that the granulosa cells within the follicles exert an inhibitory influence on the oocyte to keep it in arrested meiosis as originally hypothesized by Pincus and Enzmann (1935). Although these observations do not exclude the possibility of a positive maturational stimulus, they clearly indicate that the follicle cell--oocyte interactions play a central role in intrafollicular events. Further analysis of the oocyte--follicle cell changes during preovulatory follicular differentiation is required to resolve the roles of cAMP, pituitary hormones, and follicle cells in oocyte nuclear maturation.

**SPONTANEOUS AND NONSPONTANEOUS OOCYTE MEIOTIC MATURATION**

Pincus and Enzmann (1935) originally observed in mammals (rabbits) that mechanical release of oocytes from the ovarian follicle into various culture media resulted in "spontaneous" breakdown of the germinal vesicle and the continuation of meiosis to the metaphase II stage. "Spontaneous" maturation of oocytes has subsequently been described in numerous mammalian species (Table 2, Donahue, 1972) and occurs in chemically defined as well...
as biological media (Biggers, 1972). This phenomenon is not unique to mammals but has been recognized since the turn of the century as a common occurrence following liberation of the oocytes from the ovary in many marine species (see Wilson, 1925; Costello et al., 1957; Kume and Dan, 1968). Considerable confusion and a variety of physiological interpretations have resulted from this observation, particularly in attempting to explain why the oocyte remains in an arrested state within the ovary and how this is related to follicular development and the hormonal state of the animal.

Following are interpretations which have been suggested: (1) there are maturation inhibitors within the follicular cells or follicle; (2) oocyte maturation occurs independent of follicular development; (3) there are no specific inducers of maturation; (4) gonadotrophins are not involved in oocyte maturation; (5) ions act as inducers of oocyte maturation. That spontaneous maturation occurs in isolated oocytes eliminates the possibility of directly testing for the induction hypothesis. Table 2 summarizes the relationship between the type of maturation (spontaneous or nonspontaneous) which occurs in oocytes following their liberation from the follicle or ovary and evidence for specific inducers. In those species where direct hormone induction of oocyte maturation can be demonstrated, the oocytes typically do not undergo spontaneous maturation. Thus, the question arises of whether there is a fundamental difference in the oocytes of these species or the way in which they are regulated.

Recent experiments, however, raise the question as to whether the difference between spontaneous and nonspontaneous maturing oocytes is indeed real since several factors are not generally considered in interpreting these observations. First, all oocytes within an ovary are not at the same stage of maturation. In mammals, it has not been satisfactorily demonstrated experimentally that oocytes, irrespective of their stage of development or that of the follicle in which they are contained, undergo spontaneous maturation. In fact, most oocytes mechanically liberated in such a manner are probably obtained from the larger follicles present in the ovary and usually less than 50 oocytes are thus collected. This number does not represent the total oocyte or follicle population within the ovary or assure that oocytes so collected are obtained in a random fashion. Furthermore, since all oocytes are in arrested meiosis from the time of birth, liberation of all oocytes should lead to maturation. Szybek (1972) has recently shown that oocytes liberated from 11- to 13-day-old mice do not undergo maturation at all following in vitro culture. After this period, a gradual increase in the incidence of oocyte maturation was observed. Histological examination furthermore indicated that no full-grown oocytes were present in the ovary even at 17 days of age. A comparable situation is seen in the starfish (Asterias forbesi). Liberation of the oocytes from the ovary into sea water results in "spontaneous" maturation in a high proportion of the oocytes, and the response has been specifically attributed to the presence of calcium ions in the water (Dalcq, 1924). However, this is to a large extent a seasonal phenomenon. A certain proportion of the oocyte population (smaller ones) does not undergo spontaneous maturation even at the normal breeding season. Other species of starfish, Asterina pectinaria and Pisaster ochraceous, exhibit little or no evidence of spontaneous maturation; yet, oocytes can be induced to undergo maturation in the ovary or following their release from it by 1-methyladenine. Thus, it appears in some mammalian and invertebrate species that both spontaneous and nonspontaneous oocyte maturation can occur in the same animal. In contrast to mammals, amphibian species are considered not to exhibit spontaneous maturation. This is generally true; however, data collected in this laboratory over several years demonstrate that germi-
nal vesicle breakdown occurs, to a limited extent, following incubation of whole follicles in vitro. This occurs in a few animals, is rarely seen in all incubated oocytes, is seasonally dependent, and is readily inhibited by EDTA treatment or removal of the outer layers of the follicular wall (Schuetz, unpublished).

What appears to be significant is the fact that the condition, competence, or maturation of these oocytes and/or its associated cells change in some way.

A second factor which has not generally been considered is that in starfish (Asterias forbesi), as well as mammals, oocytes when released from the ovary or follicle are surrounded by a single or several layers of somatic follicle cells. Although the follicle cells appear to play a central role in mediating the oocyte maturation response in amphibians and certain fish, their role in spontaneous maturing species has been unclear. Recent studies in the starfish indicate these cells may play an important role in the induction of spontaneous maturation. It has been known that treating ovaries with calcium-free seawater (CaFSW) or releasing oocytes into CaFSW inhibits spontaneous maturation, as well as causing the follicle cells to detach from the oocyte (Dalcaq, 1924; Kanatani, 1964; Schuetz and Biggers, 1968). Even though these changes occur, oocytes retain their sensitivity to 1-methyladenine and undergo nuclear maturation. Cloud and Schuetz (1973) recently demonstrated that by isolating follicle cells in CaFSW and subsequently incubating them with immature oocytes devoid of follicle cells and calcium-containing seawater, maturation of oocytes was induced. Oocytes denuded of follicle cells in CaFSW and subsequently returned to calcium-containing seawater did not mature. Thus calcium ions appear to induce “spontaneous” maturation by causing the synthesis and/or release of a meiosis-inducing substance from the follicle cells (Fig. 5). Significantly, a few minutes’ exposure to calcium was sufficient to induce release of biological activity from the follicle cells. These data clearly indicate that follicle cells, calcium ions, and time are exceedingly important variables in studying the process of spontaneous maturation and may be equally important in mammalian species where removal of follicle cells from oocytes has not been shown to interfere with maturation to any significant extent.

Follicle cells, however, can play a role in mammalian oocyte metabolism which is directly involved in the maturation process. Mouse oocytes cultured in vitro do not mature with certain specific energy sources (phospho-enol pyruvate, lactate, glucose); however, when follicle cells are incubated with oocytes, and these substrates, maturation occurs (Biggers, Whittingham, Donahue, 1967). Donahue and Stern (1968) subsequently showed that follicle cells in vitro converted ineffective substrates to pyruvate, which allowed

---

**Fig. 5.** Release of meiosis inducing substance (MIS) from starfish ovarian follicle cells: relationship between amount of follicle cells and the presence or absence of calcium free seawater. Various ovarian tissue equivalents of follicle cells were diluted in 1 ml of normal or calcium free seawater. Immature oocytes were added to the incubation mixture and the incidence of oocyte maturation assessed after 90 min. The experiment was replicated three times. Each point represents the mean and the vertical bars represent the standard error of the mean (from Cloud and Schuetz, 1973).
maturation to proceed in the oocytes. Changes in the follicle cell-oocyte relationship during the course of in vitro incubation of immature (germinal vesicle) oocytes have been described (McGaughy and Polge, 1971); however, the significance to the maturation process is unknown.

Thus, with respect to oocyte maturation in mammals it can be stated that neither the absence of induction nor the presence of inhibition has been experimentally demonstrated on denuded oocytes. Likewise, follicle cells have not been eliminated as an important influence on the oocyte maturation process. The fact that cAMP stimulates in vitro steroidogenesis in mammalian follicle cells, particularly those taken from smaller follicles, (Channing and Seymour, 1970) as well as induces oocyte maturation in isolated follicles in vitro (Tsafri et al., 1972) suggests a possible common link involving these somatic cells.

The question remains, however, as to why mammalian oocytes, in particular, undergo spontaneous maturation when released from follicles at times other than the normal preovulatory period. One possibility is that oocyte and follicle maturation can occur independently of the normal reproductive cycle. This is suggested by the fact that precocious ovulation can be induced within 24 h of gonadotrophic stimulation. Such atypical ovulation has been observed in response to gonadotropins in mature mice (Burdick and Whitney, 1941; Stern and Schuetz, 1970), immature prepuberal PMS-primed rats (Schuetz, 1971), or following progesterone treatment of PMS-primed rats (Zarrow and Gallo, 1969; Gallo and Zarrow, 1970). In addition, stimulation of follicle growth and even maturation by gonadotropins without ovulation is a common feature in the mammalian ovary (Ingram, 1962; Schuetz, 1969; Donahue, 1972). Thus, if development of follicles and their enclosed oocytes is sufficiently complete, oocytes liberated from them could contribute to the spontaneous maturation observed. The fact that increasing numbers of such liberated oocytes can be fertilized and undergo embryonic development (Cross and Brinster, 1970; Mukherjee, 1972; Kaufman and Whittingham, 1972) indicates that some of these oocytes are essentially normal.

STEROIDOGENESIS AND Oocyte MATURATION IN AMPHIBIANS

Isolation and identification of an intrafollicular inducer of oocyte maturation has not been achieved in amphibians. Experimental evidence continues to indicate, however, that steroids are synthesized by the amphibian ovary, under gonadotropin stimulation, and are involved in intra- and extraovarian processes related to follicular and oocyte maturation. Electron microscopic studies of toad (Bufo bufo) ovarian follicles following in vivo HCG stimulation indicated there was loss of stored lipid material from follicle cells some 10-14 h after HCG injection and 10-20 h before ovulation occurred (Thornton and Evennett, 1969, 1973). Accumulation of membranous whorls or myelin figures in the follicle cells was associated with the loss of lipid which may represent secretion of a steroid-like hormone or its precursor. A progesterone-like factor, as detected by bioassay using oocytes of Xenopus laevis, furthermore was found in blood of the toad some 4-14 h after HCG injection (Thornton, 1972). Biochemical evidence for steroid synthesis in the ovarian follicle in vivo and in vitro was obtained by Polzonetti-Magni et al. (1970) in Rana esculenta, by Redshaw and Nicholls (1971) in X. laevis, and by Snyder and Schuetz (1973) in Rana pipiens. Synthesis of small amounts of progesterone and other metabolites occurred following incubation of whole R. pipiens follicles with radioactive pregnenolone. In Xenopus, in vitro synthesis of estrone and estradiol 17-β from steroid precursors was increased following stimulation of follicle cells in vitro with PMS. Histochemical studies localized steroidogenic enzymes (hydroxy steroid de-
hydrogenases 17-\(\beta\), 17-\(\alpha\), 3-\(\beta\), 3-\(\alpha\), and the diaphorase enzymes) in follicle cells, and their presence was correlated with the stage of follicle development. Previtellogenic follicles exhibited no enzymatic reaction, whereas vitellogenic and "mature" ones did. Thus, evidence with respect to steroidogenesis in the ovarian follicle indicates that both estrogenic and progesterational compounds can be synthesized, and that the follicle cells are intimately involved in production of both.

Crucial questions remain including: (1) What constitutes a mature follicle with respect to steroidogenesis? (2) Are estrogenic and progesterational compounds synthesized at similar rates during all stages of follicle development? (3) What are the relative roles of the steroids at different stages of oocyte and follicle maturation? The role of estrogen synthesis is generally considered in terms of the vitellogenic response rather than nuclear maturation since these steroids are ineffective in stimulating oocyte maturation (Schuetz, 1967a, 1972a; Wallace, 1972). Furthermore, it appears that one cell, the follicle cell, is involved in the synthesis and secretion of both types of steroid. Simultaneous secretion of both types of steroid does not appear likely, because induction of germinal vesicle breakdown and separation of the vitelline envelope from the oocyte resulted when follicles were incubated with both estrogenic and progesteronal steroids (Schuetz, 1972). If, as some believe, the ovarian follicle and its oocyte are undergoing a process of differentiation, the ability of follicle cells or other cells in the follicle wall to synthesize both types of steroid is dependent upon a change in their function. If this is so, questions arise as to what regulates the shift in the functional capacity of the follicle cells, whether this involves a change at the genome level and what role does the oocyte or the type of gonadotropic hormone which stimulates the follicle cells play (Schuetz, 1971)? There is a need for characterization of the steroidal products of individual or synchronized follicles at well-defined stages of follicular and oocyte development. The fact that, in a species such as Xenopus, oocyte nuclear maturation, ovulation, and vitellogenesis occur simultaneously in different follicles in response to gonadotropin stimulation, strongly suggests that the follicles are at different stages of competence and respond to similar hormones in different ways.

Evidence for involvement of steroidalogenic enzymes (3-\(\beta\) hydroxy-steroid dehydrogenase enzyme) in gonadotropin-induced oocyte nuclear breakdown has come from studies on the biological and biochemical effects of a metabolic blocker of the steroidalogenic enzyme (cyanoketone) (Snyder and Schuetz, 1973). Inhibition of nuclear maturation and ovulation was observed when whole follicles of R. pipiens were incubated with pregnenolone. Inhibition, however, was apparent only at low doses of steroid (Fig. 6). In contrast, no inhibitory effects of cyanoketone were observed when follicles from the same animals were incubated with progesterone, the normal product of the enzymatic reaction (Fig. 7).

Cyanoketone furthermore inhibited pituitary-induced germinal vesicle breakdown

![Fig. 6. Effect of cyanoketone on in vitro pregnenolone-induced germinal vesicle breakdown on Rana pipiens ovarian follicles. Each point represents the mean of three incubation flasks (one from each of three frogs) 20 follicles/incubation (from Snyder and Schuetz, 1973).](https://academic.oup.com/biolreprod/article-abstract/10/2/150/2841274/102160381274)
and conversion of radioactive pregnenolone which had been incorporated into oocytes. Gonadotropins had little effect on the amount of pregnenolone converted by the follicles; however, stimulation of steroid uptake into the follicles was observed. Effects of cyanoketone on follicular and oocyte responses to gonadotropins and steroids resembled those of estrogenic steroids and suggest that a common mechanism may be involved (Wright, 1961, Schuetz, 1972).

In mammalian species, there is no question concerning the fact that follicles are involved in the synthesis and secretion of steroids, but their roles with respect to intrafollicular processes and oocyte maturation have not been resolved. No stimulatory effects of progesterone, 20α-dihydroprogesterone, or estradiol-17β on oocyte meiotic maturation were observed following steroid addition to isolated rat follicles in vitro (Tsafiri et al., 1972). Cyanoketone under these conditions did not interrupt LH-induced oocyte maturation. The possibility exists that the ovarian follicle is not permeable to steroids, as is the case with cAMP, which induces oocyte maturation when injected into the follicles.

The question arises as to whether induction of oocyte maturation by gonadotropins in vitro results from the stimulation of both steroid synthesis and release or simply the release of preformed steroid. A time lag difference in the induction of oocyte maturation following gonadotrophin and steroid treatment has been observed in R. pipiens (Schuetz, 1967a, 1972b), catfish (Sundararaj and Goswami, 1971), and sturgeon (Dettlaff, and Skoblina, 1969). This suggests additional intermediate processes are involved in gonadotrophin-stimulated maturation. In X. laevis, Merriam (1972) found little or no difference in the lag period between these two hormones and suggested that release of hormone may be occurring in this species in response to gonadotrophins. The occurrence of a low rate of spontaneous maturation in R. pipiens follicles is consistent with hormone release (Schuetz, unpublished). Clearly, though, follicles and oocytes of R. pipiens in vitro are essentially "refractory" to gonadotropic hormones over a considerable portion of the hibernation period, when they are responsive to steroid hormones (Smith, Ecker, and Subtelny, 1969; Schuetz, 1967, 1971). Thus, the physiological state of the follicle rather than the oocyte appears to be involved in gonadotropic unresponsiveness. In addition, the steroid hormones may not have ready access to the oocyte. Snyder and Schuetz (1973) demonstrated that gonadotropins increased the amount of pregnenolone incorporated into follicles of R. pipiens in vitro. Resolution of this release-synthesis problem awaits characterization of the intermediate processes and their time course following gonadotropin stimulation of follicles at difference stages of development.

**MECHANISMS OF STEROID INDUCTION OF MATURATION**

A complex series of physiological and biochemical processes and structural changes are initiated and occur in the nuclear and cytoplasmic components of the oocyte when this cell alone or within its ovarian follicle is exposed to a single steroid such...
Fig. 8. Ultrastructure of the ovarian follicle wall-oocyte surface of a preovulatory follicle of *Rana pipiens* (×5000). A—higher power photomicrograph of oocyte microvillous projections into the perivitelline space and vitelline membrane or envelope (×9700). B—change in oocyte-vitelline membrane interconnections following 24-h exposure of follicles to progesterone in *vitro* (×14,500). Vitelline membrane (VM) perivitelline space (PVS); macrovilli (MAV); microvilli (MV); follicle cells (FC); theca (TH); oocyte (OO). Courtesy, Dr. Gertrude Hirsch.
as progesterone (Table 3; Schuetz, 1972b, c). Ultrastructural changes are also produced in the follicular wall and between vitelline envelope and oocyte, following exposure to progesterone. As a result of the structural rearrangement at the oocyte surface (Fig. 8), vitelline envelopes were readily isolated (Fig. 9). Early studies in *Rana* showed or suggested that protein synthesis was stimulated by progesterone in oocytes and was probably required for nuclear breakdown and subsequent meiotic maturation divisions (See review of Smith and Ecker, 1970; Smith, 1972). Synthesis of new RNA does not appear to be required for progesterone induced nuclear disintegration (Schuetz, 1967; Smith and Ecker, 1969). Synthesis of proteins during spontaneous meiotic maturation in mouse oocytes was also observed and may be a general phenomenon in all oocytes (Stern, Rayyis, and Kennedy, 1972). Ions and, in particular, calcium plays a significant but unidentified role in the maturation and other cell processes (Schuetz, 1967a, 1972d; Ecker and Smith, 1971; Merriam, 1971a, b; Morrill, 1971).

Uptake of radioactive steroids by oocytes has been demonstrated and appears to be time and concentration dependent (Smith and Ecker, 1971; Schuetz, 1972c). Non-specific uptake was observed, however, since nonphysiological estrogens, as well as physiologically active progesterone, were incorporated by the oocytes. Addition of progesterone to the media containing oocytes typically initiated oocyte maturation, whereas injection of equal or larger amounts of progesterone directly into oocytes did not (Smith and Ecker, 1969; Masui and Markert, 1971). This result has been interpreted to mean that steroids exert their influence through the surface of the oocyte, although it is possible that the steroid may act within the oocyte, but is not readily accessible to an active site when injected. The metabolic fate of radioactive progesterone incorporated into *R. pipiens* oocytes was studied by Reynhout and Smith (1973). Rapid conversion of steroid

![Fig. 9. Vitelline envelope removed from *Rana pipiens* oocyte after 24 h incubation with progesterone (×50).](https://academic.oup.com/biolreprod/article-abstract/10/2/150/2841274/102/10/2841274)
occurred during the course of in vitro incubation, and three major metabolites were identified by thin-layer chromatography as 5α reduced derivatives: 5α-pregnanedione, 5α-pregnan-20α-ol-3-one and 5α-pregnan-3β 20α-diol. Similar metabolites apparently were also formed when progesterone was directly injected into the oocytes, and these metabolites induced maturation when added to media containing immature oocytes. It was suggested that steroid metabolism was probably not required to induce maturation since injected progesterone does not appear to induce maturation; the question of how progesterone initiates the physiological changes in the oocyte remains unresolved. Studies on a variety of systems indicate that binding to specific proteins may be involved in the initial effects of a steroid. Binding of steroids by intact oocytes clearly occurs (Smith and Ecker, 1971), and we have recently demonstrated a small amount of binding of radioactivity to high molecular weight material obtained from whole ovarian homogenates of R. pipiens incubated with progesterone (Ozias and Schuetz, unpublished). Its significance in the maturation process, however, is unclear.

ROLE OF HORMONES IN CYTOPLASMIC NUCLEAR INTERACTION

Although the nature of the initial hormone-oocyte interaction is not resolved, evidence for localized effects of the steroids within the oocyte and for the presence of intracellular secondary mediators has been obtained. Three techniques which have greatly aided in elucidating these hormone-nuclear-cytoplasmic interactions have been those of oocyte microinjection, enucleation, and nuclear transplantation (Detzlaff et al., 1964; Smith, Ecker, and Subtelny, 1969; Masui, and Markert, 1971; Gurdon et al., 1971). By showing that enucleated oocytes (germinal vesicle stage) subsequently exposed to progesterone underwent activation in response to pinching, it was demonstrated that cytoplasmic maturation occurred independently of the nucleus (Smith, Ecker, and Subtelny, 1968).

The presence of oocyte cytoplasmic factors which cause germin al vesicle breakdown was demonstrated following injection of cytoplasm from hormone-treated or control oocytes into immature oocytes. Detzlaff et al. (1964) originally obtained evidence of cytoplasmic maturation factors in pituitary stimulated oocytes and such activity was convincingly demonstrated in progesterone-treated oocytes by Masui and Markert (1971) and Smith and Ecker (1971). Cytoplasm removed from nonhormone-treated or from steroid-treated oocytes during the early postprogesterone treatment period did not induce germinal vesicle breakdown. Injected cytoplasm became effective in inducing maturation by 12 h after progesterone treatment and appeared to be maximally effective by 20 h. The incidence of maturation induction was dependent upon the volume of cytoplasm injected into immature oocytes. Similar results were obtained when oocytes were enucleated prior to progesterone treatment, indicating that the "maturation promoting factor" was cytoplasmic in origin. Maturation of injected oocytes proceeded to the stage of second meiotic arrest and the cells underwent a normal response to artificial activation.

Evidence was also obtained which suggested that arrest at second meiotic metaphase was under control of a cytoplasmic "cystostatic" factor (Masui and Markert, 1971). Cytoplasm from arrested oocyte suppressed mitosis and cleavage when injected into blastomeres of the two-cell embryo. This "cystostatic" activity was also present in oocyte cytoplasm of enucleated progesterone-treated oocytes and decreased with activation or fertilization. Masui (1972) subsequently demonstrated that cytoplasmic maturation factor activity was higher in the animal than vegetal hemisphere of the oocyte and that highest maturation activity was present in the
hyaline ooplasm following centrifugation. It is not known whether the cytoplasmic maturation and cytostatic factor are the same or different entities, although they appear and disappear at different times or under different conditions. Significantly, these data present direct evidence of cytoplasmic control over nuclear activity and the maturation process by the release and/or synthesis of biologically active components in response to hormone stimulation. Possibly the inhibition of progesterone-induced germinal vesicle breakdown by protein inhibitors is a result of an interference with formation of cytoplasmic maturation factor (Schuetz, 1967b; Smith and Ecker, 1969).

Although certain aspects of cytoplasmic maturation occur independently of the germinal vesicle, nuclear components, including chromosomes, play a significant role in mediating oocyte maturation and embryonic development following nuclear breakdown. Whether changes in the nuclear components are a result of interactions with cytoplasm or merely the result of being released from the nucleus is, in many cases, not clear. Experiments of Smith and Ecker (1969), as well as those of Dettlaff et al. (1964), indicate that a cleavage factor released from the oocyte germinal vesicle is necessary to prepare the oocyte for cell division. Cleavage of normal or enucleated oocytes, treated with progesterone, was not obtained following artificial activation with a glass needle. However, injection of somatic nuclei into oocytes which have undergone normal maturation induced cell division, whereas if injected into enucleated progesterone-treated oocytes, no cleavage occurred. Transplantation of germinal vesicle contents into enucleated oocytes restored the capacity of such oocytes to undergo cleavage to a very limited extent.

Significantly, the germinal vesicle contains a substance which is involved in the morphogenetic process of gastrulation in axolotl embryos (Briggs and Cassens, 1966). The substance is proteinaceous in nature, synthesized during oocyte growth (Briggs and Justus, 1968), and does not appear to be species-specific in its origin or biological effects (Briggs, 1972). Experiments of Humphrey (1966) established that the presence of this material was genetically regulated and indicates that the oocyte genome is functional during early meiotic phases of oogenesis. In general, the role of the oocyte genome in the process of oocyte cytodifferentiation, other than RNA synthesis, is largely unexplored (Macgregor, 1972). On the basis of X-irradiation studies, Masui (1973a, b) concluded that the oocyte genome is relatively inactive during hormone-induced maturation. Other physiological or biochemical changes which occur in the cytoplasm as a result of germinal vesicle disintegration include induction of sperm head swelling and pronuclear formation, as well as DNA synthesis (Gurdon, 1967; Gurdon and Speight, 1969) (Table 2). High concentrations of RNA polymerase in the germinal vesicle have been recently reported (Wassarman, Hollinger, and Smith, 1972) and are presumably released into the cytoplasm at the time of germinal vesicle breakdown. In sea urchins, recent experiments indicate that the messenger RNA, required for embryonic synthesis of microtubular proteins, originates during the oocyte maturation process (Raff et al., 1972). Alterations in the types of ATPase activity in R. pipiens oocytes during maturation were demonstrated by Morrill et al. (1971).

The nature of cytoplasmic-nuclear interactions in mammalian oocytes or the roles of the hormones are essentially unknown, although the basic processes are the same as those seen in amphibians (Donahue, 1972). Iwamatsu and Chang (1972) studied the fertilization process at various stages of meiosis in the PMSG, HCG-injected adult mouse. Penetration of sperm through the zona pellucida occurred at any time of nuclear maturation; however, the capacity of the oocyte to be fertilized increased
as meiosis progressed and was fully developed by the completion of the first maturational division. The authors suggested that maturation changes in the cytoplasm as well as the nucleus were important factors in the fertilization process.

Even though normal meiotic maturation of amphibian oocytes occurs in vitro in response to steroid treatment, this does not ensure that normal fertilization and development can take place. Evidence indicates that "maturational" changes in the vitelline envelope must occur before fertilization is possible. Body cavity oocytes in ovulated females or progesterone-treated oocytes typically are at second metaphase arrest, can be artificially activated, but are not normally fertilized by sperm. Transplantation of somatic nuclei or of cleaving inducing factor (Fraser, 1972) furthermore will initiate normal or parthenogenetic development in such oocytes and indicates that the cytoplasm is physiologically mature (Smith, Ecker, 1969; Masui, 1967; Dettlaff and Skoblina, 1969). With the addition of jelly coats in the oviduct, fertilization can take place on exposure to sperm (Elinson, 1971, 1973a, b). Elinson (1973) further demonstrated that the vitelline envelope on oocytes without jelly coats, does not interact with the sperm in a normal way, and thus acts as a block to fertilization. When vitelline membranes were weakened by pretreatment with enzymes or mechanically punctured, fertilization occurred in the presence of egg water. Addition of jelly coats to the oocytes apparently alters the vitelline envelope such that it will interact with sperm.

Hartmann et al. (1972) have examined the initial interactions of sperm with hamster oocytes and have evidence that the sperm-egg interaction consists of two phases—attachment followed by binding. Possibly the amphibian vitelline membrane requires similar changes and does not acquire such until the jelly coats are added. On the basis of the difference in sperm binding to zonae pellucidae of intact oocytes and isolated membranes, Hartman et al. (1972) further suggested that the oocyte had some influence on the sperm binding process.

When an oocyte is exposed to fertilizing sperm or is artificially activated, physiological, biochemical, and structural changes occur in the oocyte cortex and adjacent vitelline envelope, all of which appear to be related to the expulsion of the contents of the cortical granules into the perivitelline space. Artificial activation can be induced in amphibians and many other species by prickling the egg with a glass needle and, in mammals, can be induced by electrical shock (Tarkowski et al., 1970) or hyaluronidase treatment (Graham, 1970). Changes occurring in the cortex include in addition to cortical granule breakdown, formation of the perivitelline space, formation or hardening of the fertilization membrane (zone pellucida, vitelline envelope), hyaline layer formation, block to polyspermy, and the zona reaction (Zotin, 1958; Ginsburg, 1961; Kemp and Istock, 1967; Vacquier et al., 1972; Barros and Yanagimachi, 1971; Cholewa-Stewart, and Massaro, 1972).

In Rana, these cortical changes are initiated after germinal vesicle breakdown and the structural changes associated with the separation of the vitelline envelope from the oocyte (Schuetz, 1972b, c). Cortical changes are not elicited in nonhormone-treated amphibian oocytes and therefore are clearly a hormone-dependent phenomenon. Significantly, progesterone induces cortex and cortical granule changes independent of the nucleus (Smith and Ecker, 1969), since enucleated progesterone-treated oocytes are capable of being artificially activated. Effects of the hormone do not appear to be directly upon the cortical granules, since Masui and Markert (1971) demonstrated that the transfer of the cytoplasmic maturation factor to nonsteroid-treated oocytes induced germinal vesicle breakdown, and that an activation response could be elicited in
such oocytes. This experiment clearly suggests that there may be a common mediator within the cytoplasm which (1) alters the vitelline membrane-oocyte relationship and (2) prepares the cortical granules in such a manner that they can be discharged. Protein synthesis appears to be involved in these steroid-induced events on the basis of inhibitor studies (Schuetz, 1967, 1972b, c; Smith and Ecker, 1969). Species vary in the time at which cortical granule formation occurs during oocyte maturation. In amphibians, cortical granules appear to be formed long before germinal vesicle breakdown and fertilization occur, whereas, in the mouse, their formation is not completed until after ovulation of the oocyte (Zamboni, 1970).

The nature of the components within the cortical granules is thus significant to the oocyte maturation process and have received increased attention. Experiments of Ginsburg (1961) directly showed that material discharged from cortical granules and present in the perivitelline space was effective in blocking polyspermy and causing changes in the vitelline envelope. She considered the material to be enzyme-like, and recent evidence clearly substantiates this in other species (Vacquier et al., 1972). Barros and Yanagimachi (1971) demonstrated that isolated cortical granule material would induce the zona reaction in hamster oocytes.

In mammals abnormalities in the sequence and nature of oocyte maturation have been observed which appear to be related to alterations in cytoplasmic and nuclear components. Thibault (1972) observed failure of the sperm head to swell during fertilization of rabbit oocytes matured in vitro. Sorensen (1973) reported that the zona pellucida became detached from the mouse oocyte during "spontaneous" maturation in vitro much sooner than under in vitro conditions. Mintz and Gearhart (1973) observed that the zona pellucida of the parthenogenetically activated oocyte was subnormal in its response to enzyme digestion and suggested that this resulted from incomplete release of cortical granules. Anomalies of first polar body formation following in vitro spontaneous maturation of mouse oocytes were described by Donahue (1970).

ROLE OF HORMONES AT THE TRANSITION FROM GROWTH TO MATURATION

Factors which determine oocyte size and are involved in the cessation of oocyte growth are poorly understood. In amphibians, cytoplasmic accumulation of yolk proteins in the form of yolk platelets is a major factor contributing to oocyte growth (Figs. 10, 11). Yolk platelets contain more than 80% of the protein phosphorus and protein nitrogen in the oocyte. Approximately 98% of yolk platelet composition is in the form of two proteins—phosvitin, a phosphoprotein containing a high proportion of serine residues, and lipo-vitellin, a large lipophosphoprotein (Wallace, 1968, 1972).

Yolk platelet proteins, however, are formed from precursor protein molecules (vitellogenin) which are synthesized external to the ovary, in the liver, in response to estrogenic steroids. Following its secretion into the bloodstream, vitellogenin is selectively sequestered into the oocytes by a process of micropinocytosis (Wallace and Dumont, 1968; Dumont, 1972) (Fig. 12), which occurs at the oocyte surface and in the cortex. The rate of vitellogenin incorporation is determined in some way by the physiological condition of the oocytes. Gonadotropin (HCG) injections into intact animals stimulated oocytes within a certain size range to exhibit increased vitellogenin incorporation in vitro (Wallace, Jared, and Nelson, 1970).

Recently, it was demonstrated that individual follicles freed of the outer follicular epithelium and theal layers, incorporated radioactively labeled vitellogenin in vitro (Jared and Wallace, 1969; Wallace et al., 1970). This in vitro system has formed the
Fig. 10. Illustration of change in cytoplasmic composition of *Rana pipiens* oocytes before (top) and during vitellogenesis (bottom) (×1000). Yolk platelets (YP).

basis for a systematic analysis of the cellular and molecular mechanisms involved in the process of macromolecular protein incorporation by oocytes (Wallace *et al.*, 1972, 1973a, b; Wallace and Ho, 1972; Dumont and Wallace, 1972). Wallace *et*
Fig. 11. A diagrammatic representation of the six stages of oocyte development. Stage I oocytes are characterized by a very thin follicle cell covering and by a large mitochondrial mass, a few lipid droplets (light spheres), and small Golgi complexes. During Stage II, the follicle cells increase in thickness and arch over the oocyte surface which now possesses microvilli. The vitelline envelope forms beneath the arches of the follicle cells. Cortical granules, premelanosomes (small dark spheres), and some yolk appear. Vitellogenesis begins in Stage III, where pigment and cortical granules increase and the vitelline envelope forms a continuous layer over the oocyte. Irregularly shaped primordial yolk platelets are present in the peripheral cytoplasm of Stage IV oocytes. Microvilli are large and numerous. During Stage V, the accumulation of yolk gradually ceases, and the microvilli become shorter and less numerous. During Stage VI, many of the microvilli are lost (retracted?) and the follicle cells decrease in thickness. From Dumont (1972).

al. (1972) further demonstrated that vitellogenin incorporated in vitro underwent conversion to yolk platelet lipovitellin and phosvitin. Sequestering of vitellogenin by oocytes in vivo or in vitro typically occurs or is carried out while the oocyte is in the germinal vesicle stage. Major questions are whether incorporation occurs in oocytes which are sufficiently mature to undergo nuclear maturation and to what extent hormones alter this process. Inhibitory effects of progesterone on oocyte incorporation of vitellogenin were observed by Wallace and Ho (1972). The relationship between vitellogenin incorporation and maturation was
directly examined by studying the effects of hormones on the incorporation of radioactively labeled vitellogenin (X. laevis) into isolated denuded oocytes of R. pipiens in vitro (Schuetz et al., 1973). Oocytes simultaneously exposed to steroid hormone (DOCA) and labeled vitellogenin exhibited inhibition of protein incorporation...
as well as stimulation of nuclear maturation and cortical changes. Time course studies demonstrated that inhibition of protein incorporation was observed after approximately 9 h of incubation and was closely associated with the time of nuclear breakdown and the separation of the vitelline envelope from the oocyte. Preincubation of oocytes in steroid for 9 h essentially terminated labelled vitellogenin incorporation, apparently independent of follicle cells. The results demonstrate that steroid hormones can directly regulate the macromolecular transport system in the oocyte. Electron microscopic evidence suggests that the mechanism of steroid inhibition is a result of inhibition of the micropinocytic process in the oocyte cortex (Fig. 12). Significantly, it appears that oocytes incorporated vitellogenin even when "fully" grown.

**Perspectives**

Steroid inhibition of vitellogenin incorporation in *vitro* represents a marked alteration in the physiological function of the oocyte. Clearly, this change and its association in time to other cytoplasmic events and structural alterations resulting from steroid treatment have major implications concerning the normal physiology of the ovarian follicle and oocyte differentiation. (Schuetz, 1972b, c). Ovulation and oocyte maturation are normally synchronized in the ovarian follicle, result from the same gonadotropic hormone stimulation of the ovarian follicle and occur after a lag period of some hours. Obviously, if exchange between the extra- or intra-ovarian environment and oocyte is of importance for oocyte function, it would be advantageous for interruption of this process to occur close to the time of actual expulsion of the oocyte. Although vitellogenin incorporated into the oocyte is generally considered to be stored in yolk platelets for use during embryogenesis, it may possibly be used for general metabolic functions in the ovarian oocyte. In fact, Wallace et al. (1972) have recently demonstrated that a small portion of vitellogenin incorporated into oocytes of *X. Laevis* is hydrolyzed to small molecular weight constituents.

Alterations in the normal integrated preovulatory processes could furthermore be expected to have deleterious effects on follicular functions. It has previously been demonstrated that asynchrony of ovulation and oocyte maturation occur to a large extent in ovarian follicles in *vitro* (Schuetz, 1967a, b, 1971), and this has been suggested to be a causative factor for the processes of oocyte and follicular atresia (Schuetz, 1972a, b, Fig. 13). Similar asynchronies are observed in numerous mammalian species (Ingram, 1961). Thus, a plausible mechanism for degenerative changes associated with oocyte atresia or other abnormalities of oocyte function may be the absence of ovulation of oocytes which have undergone nuclear maturation and in which ovarian-oocyte exchange has been inhibited. In particular, these data may relate to the problem of overripeness of uterine and intrafollicular oocytes (Witschi, 1952; Witschi and Laguens, 1963; Mikamo, 1968). The term overripeness describes the fact that degenerative changes occur in oocytes under certain physiological conditions in the uterus or ovary. A rational explanation for these changes has not been evident. Smith and Ecker (1972) have suggested that exhaustion of oocyte components due to the accelerated rate of protein synthesis following germinal vesicle breakdown may be involved. Overripeness is associated with abnormalities of development or teratogenesis following fertilization. Chromosomal alterations have been implicated as a major factor in the etiology.

In view of cellular processes involved in oocyte maturation discussed here, several features are striking: (1) Many aspects of oocyte maturation in the cortex and cytoplasm can be induced independently of the nucleus; (2) cytoplasmic factors exert an influence on the nucleus; and (3)
cytoplasmic factors in fact mediate or produce certain aspects of oocyte maturation. Obviously, then, if abnormalities or dysfunction in cytoplasmic components of the oocyte occur, one has alternatives, other than just chromosomal ones, by which to explain normal development and congenital malformations. Clearly, to ensure that a normal maturation of the oocyte occurs is of central importance for perpetuation of any species. The complexity of this process and our absence of a basic understanding of it has been and remains truly striking.

REFERENCES


Biggers, J. D. (1972). Metabolism of the oocyte.


Channing, C. (1970). Influences of in vivo and


Hartmann, J., Gwatkin, R., and Hutchinson, C. (1972). Early contact interactions between mammalian gametes in vitro: Evidence that


genetics 7, 212.


