

## The Regulation of the Mammalian Corpus Luteum<sup>1</sup>

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### I. Introduction

#### A. LH DEPENDENCY IN THE RAT

During roughly the last 10 years my colleagues and I have worked on an aspect of corpus luteum (CL) activity in the rat that we called LH dependency. The term arose as the result of studies that began to appear from about the mid-1960s, suggesting or demonstrating that LH, as well as prolactin, played a part in the luteotrophic process (Alloiteau and Bouhours, 1965; Kiracoff *et al.*, 1969; Loewit *et al.*, 1969; Raj and Moudgal, 1970; Chang *et al.*, 1971; Lawrence *et al.*, 1971; Moudgal *et al.*, 1972; Maneckjee *et al.*, 1973; Yoshinaga *et al.*, 1972). Raj and Moudgal (1970) showed that the need for LH was a crucial one between days 8 and 12 of pregnancy and Morishige, Pepe, and I showed that this need appeared quite suddenly between days 7 and 8 of pregnancy (Morishige and Rothchild, 1974), but that it also appeared in pseudopregnant rats of various types, usually about a day later (Rothchild *et al.*, 1974). LH thus seemed to be as important a luteotrophin in the rat as prolactin, and since it was also becoming clear at this time that LH probably was an even more widespread luteotrophin among the species than prolactin, its luteotrophic effect in the rat raised the question of how the rat's CL was related to those of other species.

Our own studies of the characteristics of LH dependency in the rat eventually led us to the inescapable conclusion that it was a developmental phenomenon of all luteal phase conditions in which the CL were exposed to LH. LH, in other words, induced the dependency on LH. We also found that the progestational uterus advanced, and that prolactin delayed the time of appearance of LH dependency; the uterus was not essential for its development, however, nor could prolactin delay its ap-

<sup>1</sup> This article is dedicated to the memory of my dear friend and colleague Jean-Jacques Alloiteau (d. 1968).

pearance indefinitely (Lam and Rothchild, 1977; Nanes *et al.*, 1980; Garris and Rothchild, 1980; Nanes and Rothchild, 1981; Garris *et al.*, 1981).

Many nagging questions accompanied our effort to define the characteristics of LH dependency. Why was LH suddenly needed when prolactin had been sufficient until then? Why was prolactin still needed after LH had become essential? Why did LH dependency shorten the CL's life span? How did the uterus promote LH dependency without being essential? Why was LH essential for the development of LH dependency? But the most nagging questions of all were: What did LH dependency mean? Could it help us understand the nature of the CL in general? Could LH dependency in the rat be an important clue to our understanding of CL physiology, because the rat's CL was an exception to, or an example of, the general nature of a CL?

The search for an answer to these questions finally led to the realization that one must *start* with a concept of the general nature of the CL in order to answer them. The definition of this general nature is the primary object of this article. It was not easy to achieve, because the CL is probably the wierdest endocrine gland in the body.

## B. THE PECULIARITIES OF THE CL

The CL is really an ovarian follicle in which the rapid regression that is otherwise the fate of all postovulatory follicles is temporarily arrested. In the mammalian CL progesterone secretion goes hand in hand with this postponement of regression. It is fairly certain that reptilian CL also secrete progesterone (Yaron, 1972; Browning, 1973; Callard and Lance, 1977; Lance and Callard, 1979; Cuellar, 1979; Callard and Ho, 1980). The CL of the other vertebrates probably also do so (Browning, 1973).

The mammalian CL is formed during the period around ovulation by a transformation ("luteinization") of the cells lining the cavity of the follicle (Harrison, 1962; Rothchild, 1965; Greenwald and Rothchild, 1968; Mossman and Duke, 1973). Luteinization affects the granulosa cells in all species, but in some, a similar or related process may also affect the theca interna cells, although the latter are never more than a small proportion of the CL (Mossman and Duke, 1973). Luteinization involves an enormous enlargement of the cell with a great increase in the nucleus–cytoplasm ratio and the formation of a very distinct cell wall, the development of an extensive capillary network enmeshing all the cells, marked proliferation of the endoplasmic reticulum, and its transformation from a predominantly rough to a predominantly smooth type, and of the mitochondria from a small round or rod shape type with lamelliform cristae into larger and more varied shapes with tubular and villiform cristae, and an increase in

complexity of the Golgi apparatus (Christensen and Gillim, 1969; Enders, 1973; Koering, 1974; Crisp *et al.*, 1970; Paavola, 1977). The CL's activity differs most from that of the follicle in how much rather than in what kinds of steroid it makes. The principal change is a striking increase in progesterone secretion, accompanied by a variable, but severe fall or even loss (among different species) of the capacity for estrogen and androgen secretion (Dorrington, 1977; Savard, 1973). The ability to make androgens and estrogens is probably directly related to the extent to which theca cells take part in the composition of the CL. A fall in the production of prostaglandins (PGs), which the ovary and especially the preovulatory follicle makes in large amounts (Channing and Tsafiriri, 1977; Behrman, 1979; Shemesh, 1979; Chasalow and Pharris, 1972; Espey, 1980; Plunkett *et al.*, 1975; Goldberg and Ramwell, 1975; Lindner *et al.*, 1977; Poyser, 1978), also accompanies the formation of the CL.

Since the CL secretes progesterone and since progesterone is necessary for the establishment and maintenance of pregnancy in mammals (Heap *et al.*, 1973; Amoroso and Perry, 1977), the physiologic connection between mammalian viviparity and the CL is easy to understand. But the CL apparently also occurs in all reptilian species (Miller, 1948), among which, however, only some, and these only among the squamates (snakes and lizards) are viviparous (Packard *et al.*, 1977; Yaron, 1972). The connection between the CL and viviparity in reptiles, therefore, is not as obvious as it is in mammals, although the fact that the CL may control the time of oviposition in the oviparous forms (Cuellar, 1979; Klick and Mahmoud, 1977; Roth *et al.*, 1973) suggests that its general effect in reptiles is to retain the egg in the oviduct (Callard and Ho, 1980; Cuellar, 1979). Among the fish and amphibian species which form CL the relation of the CL to viviparity is even more obscure than it is in the reptiles (Browning, 1973; Hisaw and Hisaw, 1959), although a connection with viviparity is again implied by the fact that the birds, all of which are oviparous, do not form CL.

In spite of the universal connection between CL activity and viviparity among the mammals, the relation of the period of CL activity to that of pregnancy varies enormously. CL activity in some species, with only minor exceptions, may last as long in the nonpregnant female as in the pregnant one, and as long as pregnancy itself, yet this duration can be as little as about 2 weeks as in many marsupials (Sharman, 1970, 1976; Tyndale-Biscoe, 1973) or about 2 months, as in many carnivores (Eckstein and Zuckerman, 1956; Asdell, 1964) or even as much as 10 months, as in the roe deer (Hoffmann *et al.*, 1978). Among marsupials pregnancy ends before or with the regression of the CL; in no case does pregnancy prolong the period of CL activity (Tyndale-Biscoe, 1973, 1979; Sharman, 1970, 1976), nor is pregnancy itself, considered from the time of

attachment of the embryo to the uterine wall until parturition, longer than about 2 weeks. This rule applies even to the bandicoots, which have a CL with an active life span of at least 6–8 weeks (Hughes, 1962; Gemmell, 1981) so that in them parturition occurs when progesterone secretion is at its peak (Gemmell, 1981). Among many eutherian species, however, the duration of pregnancy also varies greatly (e.g., 16 days in hamsters, and 280 days in the human) (Amoroso and Finn, 1962; Amoroso and Perry, 1977; Asdell, 1964) but in these species pregnancy itself prolongs the section of progesterone by the CL (Sauer, 1979; Heap *et al.*, 1973; Jöchle, 1969; Hilliard, 1973; Greenwald and Rothchild, 1968; Amoroso and Perry, 1977). In some cases the prolongation is for only a part of the duration of pregnancy, as in the horse (Amoroso and Perry, 1977), and in others, for the full duration of pregnancy, as in the human (Guraya, 1972; LeMaire *et al.*, 1968; Maqueo and Goldzieher, 1966; Weiss *et al.*, 1977), or the rat (Hilliard, 1973; Greenwald and Rothchild, 1968) (Fig. 15).

The mammalian CL seems to belong to the same class of endocrine glands to which the adrenal cortex and the thyroid belong; that is, the ability of the CL to secrete progesterone depends in general on trophic hormones of the pituitary. Yet among the marsupials—with the probable exception of the bandicoots—the CL is probably completely independent of such control, and among the eutherian mammals there are some species in which the CL, during at least the first part of its life cycle, is also independent of the pituitary but becomes dependent on it later, and others, in which the CL seems to always depend on the pituitary for growth and secretion (p. 194).

The CL differs from the other pituitary-dependent endocrine glands in other ways. In contrast to the adrenal cortex and the thyroid, for example, in which the trophic hormones are ACTH and TSH, respectively, for all species, in the case of the CL there is no such thing as a single trophic pituitary (or other) hormone for all species. In some it may be LH, in others prolactin, in still others, combinations of prolactin and LH or FSH (Rothchild, 1965, 1966; Greenwald and Rothchild, 1968; Hilliard, 1973; Niswender *et al.*, 1972; Hansel *et al.*, 1973; Knobil, 1973). Even in the same species, as exemplified by LH dependency in the rat, the CL may secrete progesterone in response to prolactin during the first week of its life, but only in response to both prolactin and LH during its second week (Morishige and Rothchild, 1974; Rothchild *et al.*, 1974). In the rabbit, estrogens secreted by neighboring follicles are the essential stimulus (Keyes and Nalbandov, 1967; Spies *et al.*, 1967b, 1968a,b; Rothchild 1965; Fuller and Hansel, 1971); although in some other species like the cow (Hansel, 1975) or the human (p. 210) estrogens prevent the CL from secreting progesterone. Even more striking is the contrast between this diversity of regulation of the CL's activity and the relative uniformity of

the way the follicle, from which it comes, is regulated; there are probably only minor exceptions to the generalization that the follicle's growth to maturity, its ability to ovulate, and its secretion of estrogens depend crucially on the combined effects of FSH and LH and very probably the estrogens themselves, throughout the vertebrate series (Licht *et al.*, 1977; Lance and Callard, 1979).

The CL differs from the other pituitary-dependent endocrine glands in yet another important way. The general level of activity of the adrenal and thyroid, for example, is determined mainly by a negative feedback control system. Such a control system is not typical of the CL. In fact, in some animals, as for example the rat, there is a positive feedback relationship between progesterone and prolactin secretion (Alloiteau and Vignal, 1958; Rothchild, 1960; Rothchild and Schubert, 1963; Everett, 1963; Rothchild and Schwartz, 1965; de Greef and Zeilmaker, 1976, 1978, 1979; van der Schoot *et al.*, 1978; Freeman and Sterman, 1978; Murakami *et al.*, 1979, 1980; Gilman *et al.*, 1980; Damassa *et al.*, 1980; Takahashi *et al.*, 1980). Furthermore the response of the adrenal cortex or thyroid to their respective trophic hormones does not change significantly with time; the trophins thus can maintain them at a constant level of activity. In the case of the CL, on the other hand, the response changes with time, so that, in spite of the presence of the trophic hormone, progesterone secretion inevitably regresses and stops. In the intact individual the negative feedback control of the adrenal cortex and thyroid allows the activity of these glands to oscillate around a given mean level. The CL's activity on the other hand is essentially nonoscillatory; that is, it is a single cycle or ephemeral system.

Its ephemerality is its most distinguishing and important characteristic but in this too there is variety in how it is expressed. The life span of all CL consists of three parts. For example, the pattern of progesterone secretion is made up of *a rising phase*, *a plateau phase*, and *a regression phase* (Fig. 1). Growth tends to accompany the rising and plateau phases closely in all species, but regression in size may lag behind regression of progesterone secretion in some (Bonnin-Lafargue *et al.*, 1972) or accompany it in others (Rothchild, 1965; Mossman and Duke, 1973; Harrison and Weir, 1977). Growth itself may be the result of only cellular hypertrophy in some species, of cellular hyperplasia in others, and of both hypertrophy and hyperplasia in still others (Mossman and Duke, 1973). The whole cycle in the nonpregnant animal can be as short as 3 days, as in rats (Uchida *et al.*, 1969, 1970b; Butcher *et al.*, 1974; Smith *et al.*, 1975; van der Schoot and de Greef, 1976; Nequin *et al.*, 1979) and mice (Michael, 1976), or as much as 10 months as in the roe deer (Hoffmann *et al.*, 1978). The rising and plateau phases together can occupy as little as 2 days, as in rats, are usually over 5 to 10 days in most polyestrous species,

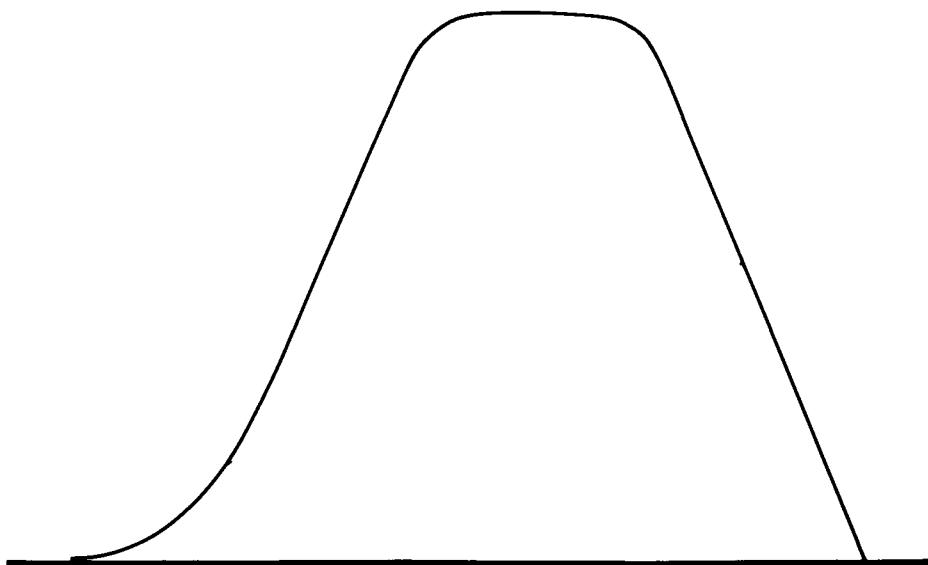


FIG. 1. An idealized CL life cycle. The curve describes the general pattern of change with time (abscissa), in size and progesterone secretion (ordinate), of all mammalian, and probably all vertebrate CL. The rising and regression phases are always easily visible, but a plateau phase may at times or in some species be nonexistent or very brief (see also Fig. 2).

or from about 10 days to 3 weeks in monestrous eutherian carnivores, as, e.g., ferrets (Blatchley and Donovan, 1972; Heap and Hammond, 1974), dogs (Christie *et al.*, 1971; Jones *et al.*, 1973a; Smith and MacDonald, 1974; Concannon *et al.*, 1975; Gräf, 1978), wolves (Seal *et al.*, 1979), cats (Shille and Stabenfeldt, 1979; Verhage *et al.*, 1976), skunks (Mead and Swannock, 1978), minks (Møller, 1973a; Papke *et al.*, 1980), and foxes (Møller, 1973b), but can be 6 months long as in the roe deer (Hoffmann *et al.*, 1978). The regression phase can also vary considerably. Even if we disregard species like the rat, regression occurs in about 3–4 days in sheep (Moore *et al.*, 1969; Stabenfeldt *et al.*, 1969; Pant *et al.*, 1978). Variations between these extremes are easy to find. Among the eutheria all the polyestrous species have only short-lived CL, that is, CL with a life cycle of about 2 weeks duration, while the monestrous species have only long-lived CL, that is, CL with a life cycle of between about 5 weeks and 2 months or more. The roe deer may be a minor exception to this rule since it has the longest lived CL known, yet this CL comes from the *second* ovulation of its breeding season (Hoffmann *et al.*, 1978). Almost all marsupials are monestrous but their “short-lived” CL have a more variable life

span than the eutherian CL; the bandicoots are also polyestrous but their CL secrete progesterone in a pattern remarkably like that of the long-lived CL of the monestrous eutherian carnivores (Gemmell, 1981) (Fig. 2).<sup>2</sup>

In some species even the basic pattern of the CL's life cycle has been modified by the appearance of a quiescent period before the rising phase begins. This can be associated either with an effect of season, as, for example, in the european badger (Canivenc and Bonnin-Lafargue, 1973), the western spotted skunk (Mead and Eik-Nes, 1969), the short-tailed stoat (Gulahusein and Thawley, 1974), or with lactation as well as season, as in the tammar wallaby (Hearn, 1973). This modification illustrates the general inverse relation between activity and life span, i.e., these CL may live for almost a year in the quiescent state, but for only about 2 weeks (wallaby) or months (eutherian species), once the rising phase begins. Although the roe deer, and apparently also the armadillo (Peppler and Stone, 1980), are exceptions in having CL that are active throughout a very long luteal phase, the general association reminds one of a similar one between the growth rate of ovarian follicles and the interval to atresia.

There is also an enormous variability in the rate at which the CL secretes progesterone and this seems to have little to do with taxonomic divisions. If we look at circulating levels of progesterone, for example, among rodents, the peak level in the guinea pig's cycle is only about 3 ng/ml (Blatchley *et al.*, 1976; Challis *et al.*, 1971) but is over 50 ng/ml in pseudopregnant rats (Bartosik and Szarowski, 1973; Pepe and Rothchild, 1974). Among some primates, as, e.g., the human (Cargille *et al.*, 1969; Neill *et al.*, 1967), baboon (Stevens *et al.*, 1970), chimpanzee (Graham *et al.*, 1972; Reyes *et al.*, 1975), or monkey (Atkinson *et al.*, 1975; Wilks, 1977), the level is between 5 and 15 ng/ml but is over 60 ng/ml in capuchin monkeys (Nagle *et al.*, 1979) and over 250 ng/ml in the squirrel monkey (Wolf *et al.*, 1977). Many other examples of such differences could be cited.

Prostaglandins (PGs) are probably an essential part of what causes the CL to die (p. 208) but even here there is variability. In some species (the sheep is the most completely documented example) uterine PGs are unquestionably the most important single factor that determines when the CL dies (Horton and Poyser, 1976). In a great many others, however, the uterus has only a minor say (e.g., rabbits, rats) or none at all (e.g., primates, marsupials, monestrous breeders) in determining how long the CL lives (see below: p. 244) in spite of the fact that in these species the uterus

<sup>2</sup> The monotreme CL is probably the same type as the marsupial CL, but too little is known about it (Hill and Gatenby, 1926; Eckstein and Zuckerman, 1956; Hughes and Carrick, 1978) to justify putting it into either the marsupial or eutherian category or into one of its own.

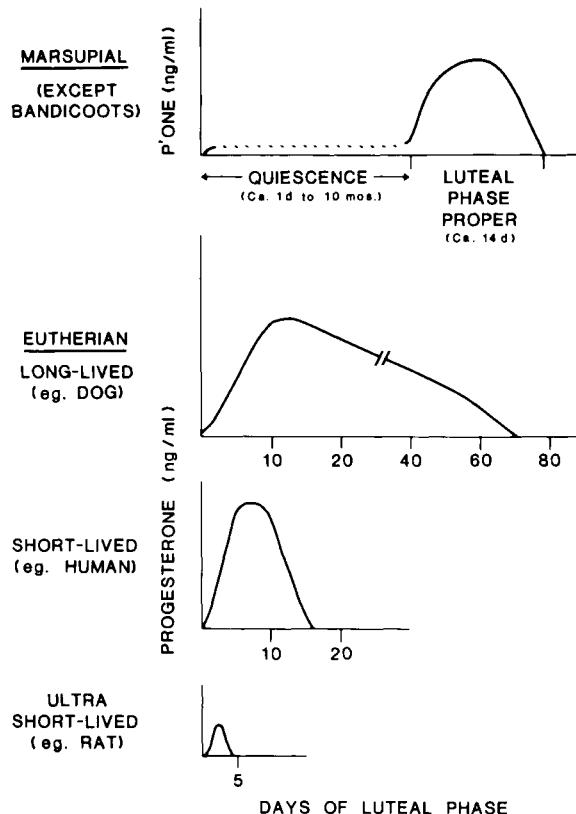


FIG. 2. The types of mammalian CL. (Activity is represented in terms of the pattern of progesterone concentration in the peripheral circulation.) *Marsupial CL*, with the exception of the bandicoots (Peramelidae) (Gemmell, 1981), have short-lived CL although in some species a quiescent phase may precede the luteal phase proper. Neither pregnancy, the uterus, nor the type of ovulation cycle affects the duration of the luteal phase proper. The three main varieties of *eutherian CL* differ in how they are affected by pregnancy etc. *Long-lived CL* are typical of monestrous breeders, and the duration of their activity is not affected by pregnancy or the uterus. *Short-lived CL* are typical of all polyestrous breeders and the duration of their activity is always increased by pregnancy. *Ultra-short-lived CL* are typical of a group of polyestrous rodents (possibly also some insectivores). The duration of their activity is increased by sterile mating and still further by pregnancy (see also Fig. 15). The peak of activity of the ultra-short-lived CL, in relation to that of other types of CL, can actually be much greater than shown here.

probably makes as much of the same kinds of PG that the sheep uterus does (Horton and Poyser, 1976; Thorburn and Challis, 1979; Abel and Baird, 1980).

### C. A COMMON SYSTEM OF REGULATION OF ALL MAMMALIAN CL?

The one thread of uniformity throughout this pattern of diversity is the CL's ephemeral nature. It is its most important characteristic precisely because it is universal, and if there is a basic, common system of regulation of all mammalian CL, the clue to its nature must be in what causes ephemeral nature. Three characteristics of the CL are important expressions of this ephemeral nature: *autonomy of progesterone secretion*, which appears to a variable extent among the mammalian species; *responsiveness to the luteolytic effect of PGs*, which does not seem to be but probably is a universal characteristic; and *the ability to make PGs*, which is probably also a universal characteristic. It is reasonable to assume that autonomy of progesterone secretion appears among the mammalian species to only an occasional extent because it is a sign of an older, once universal attribute of all CL, remnants of which persist today to varying degrees among the species; this variability is in no way different in principle from, for example, the extent to which hair, a specific mammalian characteristic, appears among the mammals. One may also assume that the luteolytic action of PGs and the CL's ability to make them represent universal and probably even more primitive attributes, not only of all CL, but of all vertebrate ovarian follicles, particularly during the period that follows ovulation.

When these characteristics of the CL were put together with the evidence that progesterone has effects which tend in general to postpone the CL's ability to make and release PGs, the idea emerged that the CL was an ephemeral gland because the basic system that regulates its activity consisted of two mutually opposing processes, each of which was regulated by positive feedback. In the rest of this article, I will first describe the evidence and ideas that led to this concept. I will then try to trace the path of evolution that gave rise to the great diversity in CL activity and its regulation. Finally, as an example of the usefulness of the theory, I will try to show the origin of the rat's (and similar mammals') peculiarly ultra-short-lived CL and its relation to LH dependency. Before beginning, definitions of a few terms that I will use throughout the article may be useful.

### D. DEFINITIONS

#### 1. *Luteotrophic Process*

In all its forms (e.g., luteotrophism, luteotrophin, etc.) this means the quality of promoting progesterone secretion (production and/or release) by

the CL. It should be kept apart from whatever term we will eventually use to describe the stimulation of progesterone secretion in nonluteal tissues, such as the follicular granulosa cells. A luteotrophic effect can be one that raises the rate of progesterone secretion, or one that maintains an existing rate, or even (as I will show) one that only reduces the rate of regression of progesterone secretion. The term does not include or imply the promoting of other activities in the CL such as estrogen production, for example, unless such activities specifically promote or stimulate progesterone secretion. The growth of the CL is not in itself evidence of a luteotrophic effect, but it is often (and justifiably) assumed to be so because a growing CL is almost always also secreting progesterone.

It may be convenient to think of two kinds of luteotrophic effects: permissive and stimulatory. A *permissive* effect is one which facilitates the CL's production of progesterone but does not itself determine how the process works or at what rate, even though it may be essential for such production. For example, an agent which facilitates the passage of cholesterol through the mitochondrial membrane might act as a permissive luteotrophin. Although this is not necessarily the way prolactin acts, many of its luteotrophic attributes suggest that prolactin is primarily a permissive luteotrophin.

A *stimulatory* luteotrophin is one which raises the rate of production and/or release of progesterone, usually in direct proportion to dose over a wide range of doses. It may or may not affect the basic process through which progesterone is made, and it may not maintain the increased rate of secretion which it induced, and it is also immaterial whether it induces the effect itself, or through another agent. Many of the effects of LH suggest that it is primarily a stimulatory luteotrophin.

## 2. *The Luteolytic Process*

The term in all its forms (e.g., luteolysis, luteolysin, etc.) means the exact opposite of luteotrophic, that is: the quality of stopping the secretion of progesterone by the CL. Luteolysis is sometimes described as functional or structural, but this helps only to describe what I mentioned above as the difference between the regression of progesterone secretion and of size. There should also be no distinction made between slow and rapid regression, as evidence of luteolysis, since both are variants of the same process.

## 3. *Antiluteolytic Effect*

It is sometimes convenient to use this term to describe a luteotrophic action which occurs primarily or exclusively by inhibiting a luteolytic one. It can be useful because under the right conditions it may imply the presence of an autonomous luteotrophic process.

#### 4. Luteal Phase and Related Terms

This term means the period of progesterone secretion by the CL during the rising, plateau, and regression phases of the CL's life span (Figs. 1 and 2). It thus excludes periods of quiescence mentioned above, which I will refer to, where necessary, as "quiescence." Long, drawn-out regression in size of the CL, after progesterone secretion has stopped, is also not included within the term "luteal phase." Some authors use the term for the endometrial changes induced by progesterone; I will not use it in this way. To some extent I will use the terms "long luteal phase," "short luteal phase," and "ultra-short luteal phase" interchangeably with "long-lived," "short-lived," and "ultra short-lived" CL, but the reasons for this will be self-evident.

#### 5. Dating System for the CL Life Cycle

Day 1 is the day of ovulation and, therefore, the first day of the CL's life cycle. I will use this system in all references to the age of the CL regardless of whatever system particular authors may have used.

#### 6. Polyestrous and Monestrous

These terms refer to patterns of ovulation cycles (Fig. 7). Monestrous cycles are those that occur only at long intervals, such as 6 months to a year. In a true monestrous ovulation cycle there is at least a month or two of anestrus between the end of the postovulatory period of one ovulation cycle, and the beginning of the preovulatory part of the next one. Polyestrous cycles are those that occur at frequent intervals with no anestrus between ovulations, and with each ovulation usually not much more than 2.5 to 5 weeks apart; in rats, mice, hamsters, voles, gerbils, however, the cycles can be as little as 4 or 5 days long. In both monestrous and polyestrous cycles, ovulation itself can be either monovular (ovulation of a single follicle) or polyovular (ovulation of several follicles).

#### 7. Eutheria, Metatheria, Prototheria, Pantothenia, Therapsida, Synapsida, Cotylosaura

*Eutheria* include all mammals except the metatheria and prototheria. *Metatheria* are the marsupials. *Prototheria* are a group of mammals, all of which are now extinct except for two genera of monotremes (egg-laying mammals) (Walker, 1968). The *pantothenia* were early therian mammals (200 million to 70 million years before present) which gave rise to several groups of now extinct mammals, as well as to the metatheria and eutheria. *Therapsids* were mammal-like reptiles from some of which the pantothenes and prototheres descended. The *synapsids*—which include the therapsids and other groups of mammal-like reptiles—were one of several lines of

reptile descendants of the stem reptiles (*cotylosaurs*) about 300 million years before present (Romer, 1966; Crompton and Jenkins, 1979).

## II. The Basic System of Regulation of the Mammalian CL

The three characteristics I will discuss here—autonomy of progesterone secretion, responsiveness to the luteolytic effect of PGs, and the ability of the CL to make PGs—do not at first glance appear to be universal properties of mammalian CL. The purpose of this discussion, however, is to summarize the evidence for their existence and to show that if certain assumptions are made, the lack of universality does not remain as important as it might at first appear to be.

### A. AUTONOMY OF PROGESTERONE SECRETION

The central issue in the search for a common luteotrophic process is not whether all mammalian CL depend on LH, or on prolactin, or on estrogens, or on any other extrinsic luteotrophin, or on combinations of any of these. The central issue is whether progesterone secretion is essentially autonomous. This is so because none of the extrinsic luteotrophins can satisfy the absolute requirement for commonality, which is that it must be necessary, not only in all species, but at all times during the life of the CL.

The idea of autonomy, in the sense that the CL can function independently of pituitary or other extrinsic controls is not new. Hisaw and Hisaw (1959) suggested that autonomy might account for the behavior of the CL among nonmammalian vertebrates. Yaron (1972) also suspected that the CL of viviparous reptiles may function independently of the pituitary. Armstrong *et al.* (1970) theorized that the rat CL could make progesterone from cholesterol autonomously, since prolactin, which is essential for prolonged progesterone secretion by the rat's CL (Rothchild, 1965; Greenwald and Rothchild, 1968; Hilliard, 1973) seemed to have only the effect of preventing the reduction of progesterone to  $20\alpha$ -pregn-4-en-3-one ( $20\alpha$ -OHP) (Wiest *et al.*, 1968; Armstrong *et al.*, 1969, 1970; Lamprecht *et al.*, 1969). Raj and Moudgal (1970) tried to explain the secretion of progesterone by the rat's CL, after day 12 of pregnancy, on the basis of what was essentially an autonomous process, and my colleagues and I did indeed find a limited capacity for autonomous progesterone secretion in the rat's CL between days 12 and 15 of pregnancy (Rothchild *et al.*, 1973; Rothchild, 1973). Brinkley *et al.* (1964b) and Cook and Nalbandov (1968) had suggested that the initial stimulus of ovulation was all that was necessary for the CL of the pig, or of marsupials, respectively, to function for

about 2 weeks. Knobil (1973) implied that the monkey CL functioned by itself during the luteal phase of the ovulation cycle, aided only by the "permissive" action of LH.

### 1. Evidence for Autonomy

Evidence for autonomy comes from several sources. The main ones are from effects of hypophysectomy and related procedures, and from the behavior of granulosa cells in tissue culture. Some of the other sources have to do with the response of the CL to treatment with a particular luteotrophin, but this is not inconsistent with the concept of autonomy, since autonomy should not be seen as an absolute quality. In the examples I will describe we should keep in mind that we are looking at differences among species in *how long* the CL secretes progesterone autonomously, not whether it can or cannot. As a general rule we can even say that no CL secretes progesterone autonomously for longer than about 2 weeks; beyond this time, it depends on either a permissive or stimulatory luteotrophin or a combination of both, to continue to secrete progesterone.

a. *Effects of Hypophysectomy.* CL of the sheep (Denamur *et al.*, 1973; Kann and Denamur, 1974), the pig (Anderson *et al.*, 1967; du Mesnil du Buisson, 1966), and the guinea pig (Perry and Rowlands, 1962; Heap *et al.*, 1967; Illingworth *et al.*, 1973) can grow and both by direct and indirect (Rothchild, 1965, p. 275) evidence, secrete progesterone for almost the full duration of a normal luteal phase (about 2 weeks) in the absence of the pituitary.

The CL of the tammar wallaby (*Macropus eugenii*), a marsupial, has a long quiescent period associated with both lactation and season (Tyndale-Biscoe, 1973; Tyndale-Biscoe *et al.*, 1974). Hypophysectomy during the quiescent period activates the CL almost exactly as does the change in season, or as does stopping lactation in the intact animal (Hearn, 1973, 1974).

The growth of the CL and its rate of progesterone secretion in the rabbit, hypophysectomized immediately after ovulation, are no different from that of intact rabbits during the first few days after ovulation (Yuh, 1980). This fits with other evidence that the rabbit CL does not depend on estrogens until it is about 4 or 5 days old (Miller and Keyes, 1975) and with the fact that LHAS treatment induces luteolysis in rabbits only when the CL are older than 3 days, an effect specifically preventable by estrogen treatment (Spies and Quadri, 1967).

CL induced in hypophysectomized women by treatment with FSH and LH will function for about 3 or 4 days, without any further treatment after the LH dose that induced ovulation and for the full length of a luteal phase (about 2 weeks) if ovulation was induced with hCG (Vande Wiele *et al.*,

1970); although the half life of hCG is much greater than that of LH, this probably does not alone account for the longer period of CL activity than with LH induction.

Hypophysectomy induces luteolysis in the dog, but the rate of regression of progesterone secretion after hypophysectomy on day 10 of the luteal phase is very much slower than after hypophysectomy on day 34 or later (Concannon, 1980). In the western spotted skunk the histologic signs of CL activity during the quiescent period of delayed implantation are not affected by hypophysectomy, and the operation induces only a very slow decline in the peripheral serum progesterone levels although it prevents the increase that normally accompanies implantation (Mead, 1975). There seems to be a similar lack of effect of hypophysectomy on CL histology in the short-tailed weasel (Matson, 1969, cited by Mead, 1975).

In the rat's estrous cycle the rising, plateau, and regression phases of a CL life cycle occupy only 3 days (Uchida *et al.*, 1970b; Butcher *et al.*, 1974; Smith *et al.*, 1975; van der Schoot and de Greef, 1976). Removal of the pituitary just after ovulation has no effect on the CL's pattern of progesterone secretion (Uchida *et al.*, 1969, 1970b; Smith *et al.*, 1975). Acker and Alloiteau (1968) also found biological evidence for such activity and suggested that it was due to autonomy of progesterone secretion. Uchida *et al.* (1969) came to the same conclusion.

Another group of findings related to those of hypophysectomy comes from treatment with inhibitors of a presumed essential luteotrophin. For example, progesterone treatment of pigs during the luteal phase of the ovulation cycle doesn't interfere with CL function (Sammelwitz *et al.*, 1961), but similar treatment of pigs with active CL older than about 15 days, as for example, during early pregnancy, causes luteolysis (Brinkley *et al.*, 1964a). The effects of treatment with a specific antiserum to LH (LHAS), which neutralizes the biological activity of LH in the circulation (Spies *et al.*, 1967a) or with methallibure, an inhibitor of gonadotrophin secretion (Schafer *et al.*, 1973), has essentially the same results. In the sheep LH and prolactin are needed to maintain activity of CL that are older than about 2 weeks (Denamur *et al.*, 1973; Kann and Denamur, 1974) but treatment with 2-Br- $\alpha$ -ergocryptine, which inhibits the pituitary's secretion of prolactin, during the middle of the luteal phase of the ovulation cycle has no apparent effect on its activity (Niswender 1974; Niswender *et al.*, 1976; Kann and Denamur, 1974), and treatment with LHAS reduces progesterone levels in the peripheral circulation by only about 50% (Niswender *et al.*, 1976). In the cow, LH is assumed to be an important, if not exclusive luteotrophin during the ovulation cycle (Hansel and Seifert, 1967; Hansel *et al.*, 1973), but treatment with LHAS does not shorten the

duration of the luteal phase (Hoffmann *et al.*, 1974) or reduce the concentration of progesterone in the CL (Snook *et al.*, 1969).

Treatment with 2-Br- $\alpha$ -ergocryptine also has no effect on the short period of progesterone secretion during the first day of diestrus of the rat's estrous cycle (Döhler and Wuttke, 1974), although secretion of progesterone beyond this time is prevented by the absence of prolactin (Rothchild, 1965; Greenwald and Rothchild, 1968; Hilliard, 1973; Smith *et al.*, 1975; Day *et al.*, 1980b). Another possible indication of autonomy in rat CL is the finding that young CL remain responsive to prolactin, after hypophysectomy, for a much longer time (Malven, 1969; Acker and Alloiteau, 1968) than do older CL (Rothchild, 1965), and in either the intact or hypophysectomized rat the CL of diestrus do not respond to prolactin with an increase in progesterone secretion, until they are at least 48 hours old (Day *et al.*, 1980). In the ferret (Donovan, 1963; Murphy, 1979) as in the mink (Papke *et al.*, 1980) prolactin is almost certainly an important luteotrophin, but treatment of ferrets with 2-Br- $\alpha$ -ergocryptine during the first 10 days after ovulation has no noticeable effect on the growth or activity of the CL (Blatchley and Donovan, 1974). The bandicoot CL is probably also responsive to prolactin, but treatment with 2-Br- $\alpha$ -ergocryptine early in the luteal phase does not affect the CL (R. T. Gemmell, personal communication).

b. *Granulosa Cells in Tissue Culture.* In every species in which granulosa cells have been removed from the follicle before luteinization began, and maintained in tissue culture, the cells have luteinized and secreted progesterone spontaneously, that is, in the absence of added specific hormones (Table I). In spite of great differences in amounts and duration of such progesterone production the pattern of progesterone production was similar in general to that of intact CL *in vivo*. Accompanying ultrastructural (Crisp and Channing, 1972) and intracellular enzyme changes (Fischer and Kahn, 1972) fit very well with these signs of autonomous progesterone secretion.

In the monkey also, cells from mid-luteal phase CL maintained *in vitro* secreted progesterone, although in decreasing amounts, for at least 4 days in the absence of added hormones (Gulyas *et al.*, 1979; Gulyas and Hodgen, 1981). Rice *et al.* (1976) made a similar finding with human CL cells maintained in tissue culture; human CL maintained in organ culture, also continued to make progesterone for several days without added hormones (Sadler *et al.*, 1980).

This and the evidence summarized in Table I are especially important because several of the species listed have CL which do not seem to be able to secrete progesterone *in vivo* after hypophysectomy. The *in vitro*

TABLE I

*Species in Which Follicle Granulosa Cells (GC), Maintained in Tissue Culture, Have Luteinized Spontaneously and Secreted Progesterone for at Least Several Days in the Absence of Extrinsic Luteotrophins<sup>a</sup>*

Species	Source of GCs and related information	Reference
Rat	Preov. foll. of PMSG-treated immat.	Bernard (1975)
	Preov. foll. of PMSG-LH treated immat.	Crisp (1977); Centola (1979)
Hamster	Preov. foll. 1.5–2 hours after spontaneous LH surge	Makris and Ryan (1975)
Rabbit	Normal preov. foll.	Erickson <i>et al.</i> (1974); Erickson and Ryan (1975); Nicosia (1972)
	Preov. foll. of PMSG-treated immat.	Shirley and Stephenson (1973)
Sheep	Preov. foll. of cyclic sheep	Moor (1977)
Pig	Sl.h.: various sizes, incl. large preov.	Channing (1970); Channing and Crisp (1972); van Thiel <i>et al.</i> (1971); Stouffer <i>et al.</i> (1976); Henderson and McNatty (1977); Goldenberg <i>et al.</i> (1972)
	Large, med., sm. foll. from known stages o.c.	Channing <i>et al.</i> (1980)
Horse	Sl.h.: various sizes	Channing (1970); Channing and Crisp (1972)
Cow	Individual foll. of various size Sl.h. large foll.	Korenman <i>et al.</i> (1973)
Human	Late foll.ph. large foll. at lap. "Active" and "inactive" foll. at lap. Various size foll. from ov. wedge at lap.	Henderson and McNatty (1977) Henderson and McNatty (1977); McNatty <i>et al.</i> (1974) McNatty and Sawers (1975); McNatty <i>et al.</i> (1975); Channing (1970); Channing and Crisp (1972)
Monkey	Various sizes, incl. preov. foll.	Channing (1970); Channing and Crisp (1972); Gould <i>et al.</i> (1977)

<sup>a</sup> Abbreviations: foll., follicle(s); foll.ph., follicular phase of ovulation cycle; immat., immature animals; incl., including; lap., laparotomy; med., medium (size follicle); preov., preovulatory; o.c., ovulation cycle; ov., ovary; ovarian; sl.h., slaughter house material; sm., small (follicle).

evidence thus implies a potential capacity for autonomy which is unable to be expressed under the conditions accompanying hypophysectomy.

c. *Other Evidence.* In the Introduction I touched on another kind of indirect but important evidence, not so much for autonomy per se, but for the possibility that the rate of progesterone secretion is determined by intrinsic rather than extrinsic factors. This is the general lack of direct relation between the pattern of progesterone secretion and that of the

luteotrophin on which the secretion of progesterone is assumed to depend. Two very good examples are the following. In the monkey, LH is believed to be the essential luteotrophin, but there is no relation between the progesterone and the LH levels in the circulation in either the intact monkey (Karsch *et al.*, 1973), or in the median-edminence lesioned or stalk-sectioned monkey in which a constant low level of LH secretion was achieved through the pulsatile infusion of a fixed dose of GnRH (Knobil, 1980). In the rabbit estrogen is the essential luteotrophin (Greenwald and Rothchild, 1968; Hilliard, 1973) and there is a similar lack of relation between progesterone secretion in the hypophysectomized rabbit and the constant level of estrogen in the circulation, achieved through an estrogen containing Silastic implant (Bill *et al.*, 1980). Other examples are summarized in Table II. Additional evidence for this is the relative ineffectiveness of a presumed luteotrophin to stimulate progesterone secretion in the young CL of the sheep (Weiss *et al.*, 1978), cow (Schomberg *et al.*, 1967), pig (Channing *et al.*, 1980), human (Sadler *et al.*, 1980), monkey (Nutting *et al.*, 1980), and rabbit (Miller and Keyes, 1975).

Another manifestation of autonomy may be that CL formed either naturally or in response to hormone treatment during the life cycle of an existing set of CL tend to grow and regress independently of the original set. Some examples of this were mentioned in Rothchild (1965, p. 299), and have also been reported for the pig (Caldwell *et al.*, 1969), Uganda kob (Morrison, 1971), rat (Nakamura and Ichikawa, 1978; Takahashi *et al.*, 1979), rabbit (Scott and Rennie, 1970), and the brush-tailed possum (Pilton and Sharman, 1962).

During the last few years a group of findings imply that if CL *in vitro* are supplied with substrate (cholesterol) in the form of lipoproteins they cannot only increase the amount of progesterone made in response to a given dose of luteotrophin (Azhar *et al.*, 1980), but do not even need the "luteotrophin" to increase their progesterone production (Sadler *et al.*, 1980; Schreiber and Nakamura, 1980; Schuler *et al.*, 1980a,b). There is also a direct relation between progesterone and lipoprotein lipase activity in the cow's CL (Shemesh *et al.*, 1976). Adrenal cortical cells, however, require ACTH to respond to lipoproteins to an equivalent extent (Brown *et al.*, 1979). It is interesting, in this connection, that adrenal cortical mitochondria do not make corticosteroids *in vitro* except in response to ACTH, and that this response can be increased by lipoproteins, although the latter have no effect by themselves (Farese and Sabir, 1980; Carr *et al.*, 1980). CL mitochondria, however, can make progesterone *in vitro* even in the absence of luteotrophins or lipoproteins (Dimino and Campbell, 1976; Dimino, 1977).

TABLE II

*Absence of a Direct Relation between the Pattern of Progesterone Secretion<sup>a</sup> during the Ovulation Cycle, and That of a Known, Suspected, or Possible Extrinsic Luteotrophin*

Species	Pattern of "luteotrophin" <sup>b</sup> secretion	Reference
Human	LH: low and constant, or slightly inverse to progesterone	Neill <i>et al.</i> (1967); Cargille <i>et al.</i> (1969)
Monkey (intact)	LH: low and constant, or slightly decreasing	Kirton <i>et al.</i> (1970); Karsch <i>et al.</i> (1973); Monroe <i>et al.</i> (1970); Resko <i>et al.</i> (1974); Wilks (1977); Goodman <i>et al.</i> (1977); Hodgen <i>et al.</i> (1976)
Monkey (with denervated pituitary <sup>c,d</sup> )	LH: low and constant	Knobil (1974, 1980)
Chimpanzee	LH: as in human	Reyes (1975)
Baboon	LH: as in human	Stevens <i>et al.</i> (1970)
Sheep	LH: low and constant	Niswender (1974); Pant <i>et al.</i> (1977); Salamonson <i>et al.</i> (1973); Scaramuzzi <i>et al.</i> (1970)
	FSH? slightly parallels progesterone	Pant <i>et al.</i> (1977); Salamonson <i>et al.</i> (1973)
	PRL: mostly constant, but increases as progesterone decreases	Niswender (1974, 1976)
Pig	LH: generally low and constant or slightly inverse to progesterone	Niswender <i>et al.</i> (1970); Parvizi <i>et al.</i> (1976); Ginther <i>et al.</i> (1972)
	PRL: peaks during regression phase of progesterone	Dusza and Krzymowska (1979)
Horse	LH: generally inverse to progesterone	Nett <i>et al.</i> (1976); Evans and Irvine (1975)
Cow	FSH: broad peak in early luteal phase LH: low and constant	Evans and Irvine (1975) Henricks <i>et al.</i> (1970); Snook <i>et al.</i> (1971)
Dog	PRL: increases as progesterone decreases	Gräf (1978)
Rat <sup>e</sup>	PRL: 2 daily surges but of average constant value	Smith and Neill (1976); Freeman <i>et al.</i> (1974); de Greef and Zeilmaker (1978)
	Low and constant in <i>in vitro</i> medium of luteinized granulosa cells	Crisp (1977)
	LH (after day 8): low and constant, with tendency to rise toward end of luteal phase	Morishige <i>et al.</i> (1973); Welschen <i>et al.</i> (1975); Linkie and Niswender (1972)
Rabbit <sup>f</sup>	Estrogen: low and constant	Hilliard <i>et al.</i> (1973); Challis <i>et al.</i> (1973); Bill <i>et al.</i> (1980)

(Continued)

TABLE II (*Continued*)

Species	Pattern of "luteotrophin" <sup>b</sup> secretion	Reference
Rabbit <sup>a</sup>	Estrogen (per Silastic implant): low and constant	Bill <i>et al.</i> (1980)
Guinea pig	LH: generally inverse to progesterone low and constant FSH: low and constant	Blatchley <i>et al.</i> (1976); Croix and Franchimont (1976) Blatchley <i>et al.</i> (1976); Croix and Franchimont (1976)

<sup>a</sup> Progesterone secretion is assumed to parallel the level of progesterone in the peripheral circulation; it is expressed in all cases as a typical rise, plateau, and fall (see Fig. 2).

<sup>b</sup> During the luteal phase only.

<sup>c</sup> FSH pattern is generally like that of LH (e.g., Resko *et al.*, 1974).

<sup>d</sup> Denervation was by median eminence lesion, or stalk secretion. The animals were treated by pulsatile infusion of a constant average daily dose of GnRH.

<sup>e</sup> Pseudopregnant.

<sup>f</sup> Pregnant.

<sup>g</sup> Hypophysectomized immediately after ovulation.

Besides the ability of the tammar wallaby's CL to function independently of pituitary stimulation, several other characteristics of CL physiology among marsupials (with the exception of the bandicoots, which I will consider below in relation to the evolution of the eutherian CL) are indirect but provocative evidence for autonomy. Although the length of the luteal phase can vary among the species from about 12 days (in most) to as much as 5 weeks (in the large kangaroos) (Sharman, 1970; Tyndale-Biscoe, 1973; Tyndale-Biscoe *et al.*, 1974; Tyndale-Biscoe and Hawkins, 1977) the longer luteal phases probably all include a quiescent phase before the true rapid increase typical of a rising phase begins. This can be seen in the difference in progesterone levels between the brushtailed possum, which lacks a quiescent phase (Thorburn *et al.*, 1971), and the tammar (Lemon, 1972) and quokka (Cake *et al.*, 1980) which have one; the very slow increase in the size of the CL during the first few weeks of the luteal phase in the large kangaroos (Tyndale-Biscoe, 1973) probably is only a variant of this pattern. In other words, no true long-lived CL, of the type seen among eutherian monestrous breeders seems to occur among the marsupials (excepting the bandicoots), although at least one genus (*Antechinus*) is known to be a monestrous breeder (Lee *et al.*, 1977). This is significant in comparison with the eutheria, because among the latter, no polyestrous breeder has a luteal phase longer than 2.5 weeks, and no monestrous breeder has a luteal phase shorter than about 5 weeks, and this difference seems to depend, among other things, on the responsiveness of the eutherian CL to extrinsic controls (p. 235).

Pregnancy also does not affect the length of the luteal phase in any

marsupial species so far examined (Sharman, 1970, 1976; Tyndale-Biscoe, 1973, 1979; Cake *et al.*, 1980), although it is becoming more and more clear that the marsupial conceptus is not endocrinologically inactive, as was once thought to be the case. It has a specific growth stimulating effect on the uterus (Renfree, 1977) and evidence for *in vitro* production of progesterone by the placenta, for example, has been found in the quokka (Bradshaw *et al.*, 1975) and the tammar (Renfree, 1977).

The marsupial CL's life span is also unaffected by hysterectomy, whether the animal is pregnant or not (Sharman, 1970, 1976; Tyndale-Biscoe, 1973, 1979; Tyndale-Biscoe and Hawkins, 1977). Thus the lack of influence of pregnancy, the uterus, or the type of ovulation cycle on the life span of the marsupial CL implies that it is largely insensitive to extrinsic controls, and, therefore, is regulated primarily by intrinsic ones. It would not be surprising to find that the monotreme CL was similarly regulated.

## 2. *The Implications of Autonomy*

The first question raised by these manifestations of autonomy among representatives of at least five eutherian orders (ungulates, rodents, lagomorphs, carnivores, primates) and probably among most marsupials is: if autonomy of progesterone secretion is presumed to be a universal characteristic of the mammalian CL, why is it not present to the same extent among all mammalian species? The answer is that differences in the capacity to secrete progesterone autonomously could have arisen as the result of the selection process; I will discuss this later (p. 243). The other question raised by the possibility of autonomy is: what might be the stimulus to progesterone secretion within the CL? This will be considered now.

*The Role of Progesterone in the Regulation of Progesterone Secretion.* A rather assorted collection of findings, in addition to those described in a previous paper (Rothchild, 1965, p. 274), fit with or suggest the possibility that progesterone may have a stimulatory effect on its own secretion. One was already mentioned: the lack of relation between the pattern of progesterone secretion and that of its known or assumed luteotrophin (Table II). Even more significant is that the progesterone concentration within the CL is directly related to the rate of progesterone secretion (as reflected in changes in the level of progesterone in the peripheral circulation) (Table III). The rising phase is in part, of course, the result of growth of the CL, but if it was due entirely to growth the progesterone concentration within the CL would not increase as progesterone secretion increases. This conclusion fits with the general relationship between the pattern of growth and that of progesterone secretion. In the sheep, for example, the CL is fully grown by about day 6 after ovulation (Stabenfeldt *et al.*, 1969) while progesterone secretion continues to increase to between days 9 and 11

TABLE III  
*The Direct Relation between Changes in Progesterone (P) Secretion and Changes in the Intraluteal Concentration of Progesterone (P/CL)<sup>a</sup>*

Species	Remarks	Reference
Pig	P/CL changes in o.c. and experimental conditions parallel changes in CL size and P secretion	Anderson <i>et al.</i> (1967); Rathmacher and Anderson (1968); Rombauts <i>et al.</i> (1965); Restall (1964)
	P/CL and P in ov. vein directly related in o.c., pregnancy, after hysterectomy	Masuda <i>et al.</i> (1967)
Sheep	P/CL directly related to changes in CL size and/or P secretion in o.c.	Deane <i>et al.</i> (1966); Caffrey <i>et al.</i> (1979b)
Cow	Direct relation between growth and/or P secretion during o.c.	Shemesh <i>et al.</i> (1976); Hafs and Armstrong (1968); Kristofferson (1960)
	P/CL decreases slowly with duration of extended luteal ph. in hysterectomized cows	Brunner <i>et al.</i> (1969)
Monkey	P/CL decreases after day 6 luteal ph.	Butler <i>et al.</i> (1975)
Human	P/CL fell with P secretion after norgestrel treatment	Mukherjee <i>et al.</i> (1972)
Guinea pig	P/CL directly related to CL size and P secretion during o.c.	Heap <i>et al.</i> (1967)
Rabbit	P/CL directly related to P secretion throughout pseudopregnancy	Horrell <i>et al.</i> (1972)
Cuis	P/CL directly related to P secretion in o.c. and pregnancy	Tam (1973)
Tammar wallaby	P/CL parallels CL growth and P secretion during luteal ph.	Renfree <i>et al.</i> (1979) (P/CL); Lemon (1972) (P)
Rat	P/CL directly related to P secretion throughout pregnancy	Wiest <i>et al.</i> (1968); Wiest (1970)
	In pseudopregnancy also	Rothchild (unpublished)
	In pseudopregnancy on day 8, in connection with several experimental conditions	Terranova (1980)
Hamster	Direct relation P/CL and P secretion in o.c. and experimental conditions in pseudopregnancy	Terranova <i>et al.</i> (1978); Terranova and Greenwald (1978)
Garter snake	P/CL directly related to P secretion throughout gestation	Highfill and Mead (1975)

<sup>a</sup> Abbreviations as in Table I. Progesterone secretion is assumed to parallel changes in peripheral circulation levels of progesterone. Not all the references contain information about P secretion itself, but the latter information is available from many other sources. For references to same subject before 1965, see Rothchild (1965, p. 272).

(Pant *et al.*, 1977; Plotka and Erb, 1967; Stabenfeldt *et al.*, 1969; Thorburn and Mattner, 1971). The rate of conversion of pregnenolone to progesterone by the horse CL *in vitro* (Evans *et al.*, 1975) also increases with the

increase in progesterone secretion, as does acetate incorporation into progesterone by the rabbit CL *in vitro* (Suzuki *et al.*, 1977). Progesterone secretion increases during the rising phase, in other words, not just because the amount of producing tissue increases, but because each unit of tissue increases its rate of progesterone production. This direct relationship between the intraluteal progesterone concentration and the rate of progesterone secretion is such a dramatic contrast to the lack of relation between progesterone secretion and that of its presumed extrinsic stimulus (Table II) that it points to the possibility that an intrinsic stimulus increases the rate of autonomous progesterone secretion, a conclusion which is almost self-evident from the typical S-shaped pattern of the curve of progesterone secretion during the rising phase.

The following findings suggest that this stimulus may indeed be progesterone itself. The CL makes progesterone from free cholesterol through the action of the cholesterol side chain cleavage enzyme (Chol SCC) and the  $3\beta$ -hydroxysteroid dehydrogenase ( $3\beta$ -OHSDH) and  $\Delta^{4-5}$ -isomerase enzymes. Esterified cholesterol must first be hydrolyzed before it can be converted to progesterone, and one of the actions of progesterone is to activate cholesterol esterases (Caffrey *et al.*, 1979b; Dorrington, 1977). Progesterone also depresses the activity of the cholesterol ester synthetases in human cancer cells, but only in the presence of low density lipoproteins (LDL) (Gal and Simpson, 1980). The general inverse relation between intraluteal cholesterol concentration and progesterone secretion (Rothchild, 1965; Spies *et al.*, 1968a; Guraya, 1975) is also consistent with a stimulating effect of progesterone on the conversion of cholesterol.

In the rat the neutralization of circulating LH by LHAS treatment between days 8 and 12 of pregnancy results in luteolysis and abortion (Raj and Moudgal, 1970; Morishige and Rothchild, 1974), accompanied by a marked increase in the production of  $20\alpha$ -OHP by the CL. This is reflected in increased activity of the enzyme  $20\alpha$ -hydroxysteroid dehydrogenase ( $20\alpha$ -OHSDH) (Loewit and Zambelis, 1979). Treating the rats with progesterone, after the injection of LHAS prevented the increase in  $20\alpha$ -OHSDH, even when the pregnant uterus was removed (Loewit and Zambelis, 1979); this suggests that the progesterone may have affected the CL directly.

The lack of an inhibiting effect of large doses of progesterone on the CL during the first 2 weeks of its life span in rats (Rothchild, 1965), sheep (Woody *et al.*, 1967; Thwaites and Edey, 1970), and pigs (Spies *et al.*, 1967a) is one expression of the absence of negative feedback control of its activity (Rothchild, 1965). Progesterone treatment around day 35 of pregnancy also has no effect on the cow's CL (Menge and Verville, 1969); treatment of pregnant rats at a dose calculated to be 5 times the estimated

daily production rate also had no effect on the rat's CL (Bartholameusz and Bruce, 1976). In the guinea pig also progesterone treatment did not affect the CL (Bland and Donovan, 1970). Related to these findings is the direct relationship between the number of CL and the level of progesterone in the circulation in the rat (de Greef and Zeilmaker, 1974; Elbaum *et al.*, 1975; Nuti and Meyer, 1975) and cow (Lemon and Saumande, 1972) (in the latter different numbers of CL were induced by gonadotrophin treatment). The absence of negative feedback control does not of course prove that progesterone facilitates its own secretion, but it is consistent with such a possibility. The presence of a negative feedback control of estrogen secretion by the ovary, in fact, hindered the recognition of the fact that estrogens can also stimulate their own production within the follicle. Perhaps the findings of Uchida *et al.* (1970a, 1972) that treatment of proestrous rats with progesterone raised the rate of progesterone secretion by the preovulatory follicle, without affecting the level of LH secretion, is another indication that progesterone may potentiate its own secretion.

Alloiteau's (1957) suggestive finding of a direct trophic effect of progesterone on the rat's CL (Rothchild, 1965, p. 275) is supported not only by those of Loewit and Zambelis (1979) mentioned above, but by others. Ahmad (1971) treated pregnant rats with a combination of progesterone and estrogen only until day 12, after hypophysectomy on day 8, and found that this allowed pregnancy to continue to term. Raj *et al.* (1979) found a similar result after treating pregnant rats with LHAS on day 8, together with dydrogesterone from day 8 to 12. Dydrogesterone is a synthetic progestogen that does not have the luteolytic effect typical of many other synthetics (Raj *et al.*, 1979; see below also), and, at this time in pregnancy, LHAS by itself always induces luteolysis and abortion. The significance of these findings comes from the fact that the CL are needed to maintain pregnancy after day 12, that placental hormones alone are needed to maintain CL activity after day 12, but that only the *combined* effect of the pregnant uterus and LH will maintain the CL between days 8 and 12 (Morishige and Rothchild, 1974). Since the CL remained alive in the absence of the pituitary in the one experiment, and in the absence of LH in the other, the findings suggest that progesterone and dydrogesterone had a direct trophic action on the CL. The finding of Pal *et al.* (1976) that progesterone treatment not only prevented the aborting action of PMSG treatment in the rat, but also the luteolysis that caused the abortion, suggests a similar conclusion.

In guinea pigs injected with a luteolytic dose of PGs, progesterone secretion fell very soon after treatment but pregnenolone production fell much more slowly (Dwyer and Church, 1979). If the primary effect of PGs

was on the biosynthesis of cholesterol or pregnenolone, one would expect pregnenolone production to fall at least as quickly, or even sooner than that of progesterone. This finding suggests, therefore, that the guinea pig CL could not maintain its production of pregnenolone in the absence of progesterone.

The idea that progesterone may facilitate its own secretion seems to disagree with some findings. For example, active immunization of rabbits against progesterone markedly increased the progesterone level in the circulation (French, 1977; Surve *et al.*, 1976), but since the binding of progesterone to an immune globulin undoubtedly prolonged its stay in the circulation without prolonging its biological activity at all [the treatment had no effect, for example, on the length of the luteal phase (Surve *et al.*, 1976)], this finding probably has no significance for the effect of progesterone on its own secretion.

Although a limited period of progesterone treatment does not affect CL activity, a prolonged one may. The effect, however, can almost always be identified as the result of an action of progesterone outside the CL. For example, Rothchild and Schwartz (1965) found that after 4 weeks of progesterone treatment of intact rats, the CL had markedly regressed. The progesterone treatment did not reduce pituitary LH concentration, but when the same progesterone treatment was combined with estrogen treatment, pituitary LH concentration was reduced and the CL did not regress. In the sheep, prolonged progesterone treatment has no inhibiting effect on the CL in the absence of the uterus (Woody and Ginther, 1968).

Treatment of women with synthetic progestins during the luteal phase of the cycle slightly but definitely shortens its duration and reduces the peak progesterone level in the circulation (Johannsson, 1971; Mukherjee *et al.*, 1972). The effect may be a direct one since in *in vitro* experiments Schreiber *et al.* (1981) found that the synthetics prevented FSH and androgen from stimulating progesterone production in rat follicular granulosa cells. In cows, however, such treatment increased the progesterone concentration in the CL (Johnson and Erb, 1962). In any case, the synthetics may not act as progesterone does and in fact their ability to bind to the same receptor that binds progesterone (Schreiber *et al.*, 1981) may explain their inhibiting effect if they do not act on the CL as progesterone does. Dydrogesterone, as I have already mentioned, not only does not inhibit progesterone secretion, but may enhance it (Raj *et al.*, 1979).

In both the sheep (Caffrey *et al.*, 1979a) and the human (Shinada *et al.*, 1978) progesterone inhibits the conversion of pregnenolone to progesterone by CL *in vitro*. It also inhibits this step in progesterone biosynthesis in human placental tissue *in vitro* (Saure *et al.*, 1977). Serum in the medium

prevents this effect on the sheep's CL, presumably because it contains a progesterone-binding protein, and only the free progesterone had this effect. Progesterone (Cook *et al.*, 1968) or pregnenolone (Haksar *et al.*, 1967) may also inhibit one or more of the biosynthetic steps from acetate to cholesterol and progesterone by CL *in vitro*. However, regardless of whatever differences there may have been between the effective concentration of progesterone under these conditions and the ones that would be expected in the CL or placenta *in vivo*, and regardless also of the intrinsic interest of such findings, the fact is that the CL and the placenta keep making progesterone in large amounts *in vivo* in spite of intraluteal concentrations which in the *in vitro* conditions prevented progesterone production.

The findings suggesting the idea that progesterone, by whatever means, promotes its own secretion do not prove that it does so, but that they exist at all in an ephemeral organ like the CL must be more than just a coincidence. The instability of the graafian follicle, i.e., the overwhelming tendency for most of them to become atretic, is almost certainly also an expression of the self-stimulating quality of follicular estrogen production (Rothchild, 1965, p. 275) and the dependency of follicle growth on the stimulating effects of estrogen (e.g., Richards, 1979). Ephemerality means that the essence of the system that controls the CL's ability to make progesterone is instability; i.e., it lacks intrinsic mechanisms that allow it to maintain a constant level of activity, and what, indeed, could be more essentially unstable than a positive feedback system of regulation? Everything about the pattern of progesterone secretion during a typical luteal phase fits with such an interpretation and justifies the idea that progesterone is probably the intrinsic factor which facilitates the CL's production of progesterone. To a significant extent, the CL's ability to maintain progesterone secretion may depend on the presence within the CL of progesterone at some concentration above a critically *low* one. Having said this, I can add that progesterone has precisely two qualities that one would expect to find in a universal luteotrophin that has defied identification for a long time. One is that it has always been right under our noses and therefore invisible. The other is that all CL will be exposed to it.

The dependency of progesterone secretion on itself, while it is an important element contributing to the CL's limited life span, does not by itself guarantee a limited life span, for if nothing ever disturbed it, the system could continue to work indefinitely. The inevitability of regression of the CL, therefore, must be the result of another process, with the effects of progesterone on it, and the self-dependency of progesterone secretion, operating to determine *when* this process brings the life of the CL to an

end. In the following discussion I will summarize the evidence that it is the CL's production of PGs which is ultimately responsible for its ephemerality.

## B. PROSTAGLANDINS IN RELATION TO THE BASIC SYSTEM OF REGULATION OF THE CL

### *1. The Luteolytic Action of PGs*

The most thorough body of evidence that PGF<sub>2 $\alpha$</sub>  is the natural cause of luteolysis comes from studies of the sheep (Horton and Poyser, 1976; Goldberg and Ramwell, 1975; McCracken *et al.*, 1972; Ginther, 1974; Hansel, 1975; Ramwell *et al.*, 1977). That PGs are an important element in luteolysis in other species, however, although based on a less extensive body of evidence, is an inescapable conclusion, since they induce luteolysis in rats, mice, rabbits, guinea pigs, hamsters, cows, pigs, horses (Horton and Poyser, 1976; Goldberg and Ramwell, 1975; Pharriss and Shaw, 1974; Ramwell *et al.*, 1977), cats (Nachreiner and Marple, 1974; Shille and Stabenfeldt, 1979), dogs (Concannon and Hansel, 1977; Vickery and McRae, 1980), and the swamp buffalo (Kamonpatna *et al.*, 1979). They almost certainly do so also in primates (discussed below).

The PGs as a group are perhaps ideal candidates for the final common agent of luteolysis. Although no one knows exactly *how* they stop progesterone secretion, hardly anyone denies that they do, and their ability to labilize lysosomal membranes (Schwarz *et al.*, 1977; Liggins, 1979; Thorburn and Challis, 1979) allows one to see how easily their action could also lead to structural regression of the CL. Arachidonic acid, their obligate precursor (Lands, 1979; Bergstrom *et al.*, 1968; Hinman, 1972; Pong and Levine, 1977; Ramwell *et al.*, 1977), is universally distributed throughout tissues as one of the most frequently found fatty acid in the phospholipids and as a less frequent but important fatty acid in the cholesterol esters (Sabine, 1977; Vogt, 1978; Kuehl, 1974). The enzymes that free arachidonic acid from the phospholipids or cholesterol esters are also in rich supply in the CL and neither the cyclooxygenases, which convert arachidonic acid to the endoperoxides (PGG<sub>2</sub> or PGH<sub>2</sub>) in the presence of molecular oxygen (Lands, 1979), nor the enzymes which transform PGH<sub>2</sub> into PGs of the E or F series (Lands, 1979; Bergstrom *et al.*, 1968; Hinman, 1972) are limiting factors in PG synthesis. Furthermore, the effect of PGs themselves on the liberation of the acyl hydrolyases from lysosomal membranes, for example, but also from other intracellular membranes, sets up ideal conditions for a self-propagating process of PG formation

(Hinman, 1972; Kuehl, 1974; Nathanielsz, 1978; Pong and Levine, 1977; Schwarz *et al.*, 1977; Lands, 1979; Thorburn and Challis, 1979).

In spite of their ability to cause luteolysis in the species mentioned above and the general inverse relation between signs of PG production and progesterone secretion in most of this group of mammals (see Table V), the main reason for hesitating at all to look on them in the role of a final common agent of luteolysis is that their luteolytic effect among the primates is not as easy to see as it is among the other mammals. This is because of the inconsistency with which attempts to induce luteolysis by PG treatment have met with success. Failures are well-recognized (Goldberg and Ramwell, 1975; Horton and Poyser, 1976; Ramwell *et al.*, 1977; Swanston *et al.*, 1977) and continue to be reported (e.g., Gould *et al.*, 1977; Kajonoja *et al.*, 1978; Rao, 1979).

Negative findings, however, do not mean as much as positive ones. One reason is that, although PGs can act as hormones in the classical sense, in some mammals, under some conditions, PGs have their typical effects at the site of their production (Lands, 1979) and their half-life in the circulation is extremely short (Bergstrom *et al.*, 1968; Hinman, 1972; Lands, 1979). I am going to develop the point in this section that *intraluteal* PGs are probably the final common agent of luteolysis in all mammals, and that they have remained the *sole* source of PGs among some (or all?) of the primates; while among some of the other species, uterine PGs became incorporated into the process that switches on the intraluteal ones. There may be reasons peculiar to primates, therefore, for the failure of *in vivo* administered PGs or even PG synthesis inhibitors to affect the CL consistently. The negative findings in primates should be judged not only in comparison with whatever direct evidence there is for success, but also in relation to the connection between PGs and luteolysis as a whole. This includes at least the following: the luteolytic action of estrogens in monkeys and humans; the effect of estrogens on the release of PGs; the possibility of synergism between estrogens and PGs in inducing luteolysis; the inverse relationship between progesterone and PG production by the CL; and the influence of age of the CL and of its vasculature on the responsiveness to PGs.

There is suggestive evidence for *in vivo* luteolytic effects of PGF<sub>2 $\alpha$</sub>  treatment in women (Lehmann *et al.*, 1972; Arata and Chatterton, 1974; Korda *et al.*, 1975) and monkeys (Kirton *et al.*, 1970; Kirton and Koering, 1973; Spilman *et al.*, 1977) and somewhat more definite evidence from treatment with analogs of PGF<sub>2 $\alpha$</sub>  in monkeys (Russell, 1975; Spilman *et al.*, 1977; McCracken *et al.*, 1979; Wilks, 1979). In *in vitro* experiments with human or monkey CL or luteinized granulosa cells in tissue culture,

$\text{PGF}_{2\alpha}$  or its analogs (McNatty *et al.*, 1975; Henderson, 1976), or PGE<sub>2</sub> (Salazar and Archer, 1974) unquestionably induces luteolysis.

Hoffmann's (1960) suggestion that estrogens can cause luteolysis in women has been confirmed in women (Board *et al.*, 1973; Johannsson, 1973; Lehman *et al.*, 1975; Williams *et al.*, 1979), monkeys (Karsch *et al.*, 1973; Butler *et al.*, 1975; Karsch and Sutton, 1976; Stouffer *et al.*, 1977; Auletta *et al.*, 1978; Balmacida *et al.*, 1980), and baboons (Westfahl and Kling, 1981). The effect is quite clearly directly on the CL (Karsch and Sutton, 1976; Stouffer *et al.*, 1977; Williams, 1979), and probably through an effect on PGs. Auletta *et al.* (1978) found that an increase in intraluteal PG concentration accompanied the luteolytic action of estrogen treatment in monkeys and that indomethacin treatment prevented both the luteolytic action of the estrogen and the increase in PG concentration. The finding that neither  $\text{PGF}_{2\alpha}$  nor estradiol treatment alone induced luteolysis in monkeys, while the combined treatment did (Shaikh, 1972) is probably related to the findings of Auletta *et al.* Another is that estrogen treatment of monkeys during the first 7 days of the luteal phase induced a suggestive increase in the intraluteal PG/PGE concentration ratio and eventually an earlier luteolysis than in the controls (Balmacida *et al.*, 1980). In sheep estrogens may facilitate (although they are probably not essential for) the luteolytic effect of  $\text{PGF}_{2\alpha}$  (Hansel, 1975). A similar or related kind of interaction may be a necessary part of the way PGs and estrogens control luteolysis in the primate CL.

Another way through which estrogens may be involved in PG-induced luteolysis is by initiating an increase in the release of intraluteal PGs. They have a similar effect, especially after a priming one by progesterone, in the endometrium, where this is an important part of the chain of events that leads eventually to parturition in most if not all mammals (p. 214).

In rats, rabbits, guinea pigs, pigs, and cows, an inverse relationship has been described between some measure of progesterone secretion and one of PG production within the CL. This varies in clarity among these species, but it is at least suggestive in all of them. It occurs between the concentration of progesterone and of  $\text{PGF}_{2\alpha}$  in the guinea pig ovary (Sharma *et al.*, 1976) during the luteal phase of the ovulation cycle, between the progesterone and PG concentrations in the ovarian vein of pseudopregnant rats (Hall and Robinson, 1978), between the rates of progesterone and PG production by luteinized rabbit granulosa cells in tissue culture (Challis *et al.*, 1974), between the progesterone and PG concentrations, and their rates of production *in vitro*, in pig CL (Patek and Watson, 1976), and in the cow's CL between the  $\text{PGF}_{2\alpha}$  concentration and the rate of progesterone production *in vitro* (Lukeszewska and Hansel, 1979).

A similar inverse relationship in the human and monkey CL seems to

exist. Although Swanston *et al.* (1977) found that the PG concentration in the human CL did not rise during the late luteal phase, Downie *et al.* (1974) found that the concentration of PGEs and PGFs increased during the late luteal phase, the PGEs increasing more than the PGFs. Challis *et al.* (1976) found that the production of PGFs and PGEs similarly increased. This does not argue against a role of PGs in luteolysis in primates, although some PGEs can stimulate progesterone production by human CL *in vitro* (Channing, 1972; Henderson, 1976; Gould *et al.*, 1977) and they can even prevent the luteolytic effect of PGF<sub>2α</sub> in the human (McNatty *et al.*, 1975) and the sheep (Henderson *et al.*, 1977). However, PGF<sub>2α</sub> will sometimes stimulate progesterone production under *in vitro* conditions even in CL of some species (e.g., cow) (Hixon and Hansel, 1979) in which it readily induces luteolysis *in vivo* (Pharriss and Shaw, 1974; Goldberg and Ramwell, 1975). Furthermore, PGEs are not always luteotrophic (e.g., Weems *et al.*, 1979) and a very important determinant of how they act could be the enzyme PGE<sub>2</sub>-9-keto reductase, which converts PGE<sub>2</sub> to PGF<sub>2α</sub>. Its presence in human, pig, and rat CL (Watson *et al.*, 1979) may be a clue to the eventual solution of many of the peculiarities of the PG-CL relationship among mammals.

Henderson and McNatty (1977) found that the binding of PGFs to human CL cells was inversely related to their progesterone production. Shutt *et al.* (1975) first described a suggestive and later a more definite inverse relationship (1976) between progesterone and PG concentrations in the human CL; they also showed that the intraluteal PGF concentration did not increase in early pregnancy (Shutt *et al.*, 1976), a finding consistent with the continued and somewhat increased rate of progesterone secretion by the CL of early pregnancy (Johansson, 1969; Mishell *et al.*, 1973).

The PGF<sub>2α</sub> levels in ovarian vein blood during the first trimester of pregnancy in women were also found to be less than during the ovulation cycle; in contrast to the latter condition, in which the PGF<sub>2α</sub> level is higher in the blood draining the CL-bearing ovary than in that from the other ovary, there was no difference in the blood PG level between the two ovaries in pregnancy (Aksel *et al.*, 1978). The same authors also found that in one patient with a persistent CL, there was less PGF<sub>2α</sub> in the blood draining her CL-bearing ovary than in that from the other ovary, and that the levels from both ovaries were below those found in normal women during the ovulation cycle. The intraluteal PGF/PGE concentration ratio and production rates of each PG increased during the late luteal phase in monkeys (Balmeida *et al.*, 1979), and the levels of PGF and of progesterone in monkey ovarian vein blood were inversely related to each other during the luteal phase of the ovulation cycle (Fernandez *et al.*, 1980).

During the rising phase of the CL life cycle, the CL do not respond to the luteolytic effect of PGs, while older ones do (Table IV). This phenom-

TABLE IV  
*Evidence That Young CL Are Less Sensitive to the Luteolytic Effect of PGs  
 Than Are Older CL*

Species	Earliest time in luteal phase at which response to PG appears <sup>a</sup>	Reference
Cow	After day 4-5 and before day 9	Donaldson <i>et al.</i> (1965) <sup>b</sup> ; Harms <i>et al.</i> (1969) <sup>b</sup> ; Rowson <i>et al.</i> (1972); Aulettta <i>et al.</i> (1972) <sup>b</sup> ; Henricks <i>et al.</i> (1974); Henderson and McNatty (1977)
Sheep	After day 3, 4, or 5	Hawk and Bolt (1970) <sup>c</sup> ; Hearnshaw <i>et al.</i> (1973); Mellin and Busch (1976); Acritopolous and Haresign (1980)
Horse	After day 4 or 5	Allen and Rowson (1973); Neely <i>et al.</i> (1975)
Pig	Late in luteal ph., i.e., about day 10	Hallford <i>et al.</i> (1975); Lindloff <i>et al.</i> (1976); Moeljono <i>et al.</i> (1976); Watson and Maule Walker (1977); Krzymowski <i>et al.</i> (1978)
Human	Young CL less responsive than older; after day 5 of luteal ph. <sup>d</sup>	Turksoy and Safaai (1975); Henderson and McNatty (1977); Hamberger <i>et al.</i> (1980)*
Rabbit	CL of mid-pregnancy much more responsive to PGF <sub>2α</sub> than are younger CL	Koering and Kirton (1973)
Rat	After day 3, before day 7	Lamprecht <i>et al.</i> (1975)*; Mercier-Parot and Tuchmann-Duplessis (1976); Khan <i>et al.</i> (1979)*; Hamberger <i>et al.</i> (1980)*
Cat	After day 11 Not in early luteal ph. (?)	Shille and Stabenfeldt (1979) Wildt <i>et al.</i> (1979)
Dog	After day 8 After day 20	Concannon and Hansel (1977) Vickery and McRae (1980)
Ferret	After day 30 (?)	Heap <i>et al.</i> (1977)

<sup>a</sup> In some studies (e.g., Khan *et al.*, 1979) there was a clear relationship between the luteolytic effect of PGs and their ability to prevent adenyl cyclase stimulation by LH, or to increase 20α-OHSDH activity. Such information has also been used here to show a lack of effect of PG on young CL. These are marked by an asterisk.

<sup>b</sup> In relation to luteolytic effect of oxytocin, which probably acts through uterine PGs (Horton and Poyser, 1976).

<sup>c</sup> In relation to effect of estrogen, the luteolytic effect of which is probably through uterine PGs (Horton and Poyser, 1976; Thorburn and Challis, 1979).

<sup>d</sup> In the monkey, the luteolytic effect of estrogens, which is probably a PG-mediated effect, is much greater in older than younger CL (Stouffer *et al.*, 1977).

enon has not gone unnoticed (Henderson and McNatty, 1975), but Table IV makes it very obvious that the age of the CL must be considered in testing the responsiveness of a given species' CL to the luteolytic effect of a PG. It's very difficult to know exactly how much this relationship accounts for the reported failures of PG treatment to induce luteolysis in primates, but that it could account for at least some is certain, and it is also possible that it could account for all. The pig's CL, for example, does not become sensitive to the luteolytic effect of administered PGs until late in the luteal phase (Table IV), and it is much easier to determine the exact age of the CL in pigs than it is in women or monkeys. One indication of the importance of age is that 9-day-old human CL were much more sensitive to the luteolytic effect of PGs *in vitro* than were 7-day-old CL (Nutting *et al.*, 1980).

The inconsistency of the primate CL's response to PGs may be due not only to inaccuracy in determining how old the CL was at the time of testing, but perhaps also to a difference from other species in the vasculature of the CL. It is at least worth remarking that in all the species in which PGs readily induce luteolysis the CL receives a significant part of the luteolysis-inducing PGs from the uterus. This could mean that these CL are adapted to receive *extrinsic* PGs in a way which does not exist in the primates, in which *intraluteal* PGs may be solely responsible for the changes leading to regression. Hamberger *et al.* (1980) have shown that the ability of PGs to inhibit hCG-induced stimulation of adenyl cyclase in human CL *in vitro* is absent in young CL, and appears only in late luteal phase ones; the relation of the PG effect to age, thus, is exactly the same as that shown in Table IV between age and the luteolytic response to PGs. What is even more interesting is that they also showed that the appearance of responsiveness to PGs in the older CL was associated with the appearance of norepinephrine in their blood vessels, and that the addition of  $\beta$ -adrenergic blocking agents to the *in vitro* medium blocked the inhibiting effect of PGs on hCG stimulation of adenyl cyclase.

In the monestrous species, as in primates, the uterus has no influence on how long the CL function (p. 236). If the cat and the dog are typical of these species, the luteolytic response to PGs also appears in them only relatively late in the luteal phase (Concannon and Hansel, 1977; Shille and Stabenfeldt, 1979; Vickery and McRae, 1980). In the ferret PG treatment on day 30 or earlier of pregnancy did not induce abortion (Heap *et al.*, 1977), but I know of no studies of the effect of PG treatment on progesterone secretion later in the luteal phase.

## 2. Production of PGs by the CL

A few studies show directly that the CL can make PGs. In addition several others show that the CL contains PGs. Since PG metabolism is

very rapid (Bergstrom *et al.*, 1968; Hinman, 1972; Lands, 1979) it is likely that their presence in the CL means that they were made there. This should not be surprising since the follicle makes PGs very well (p. 185).

In addition to the evidence mentioned above for the inverse relationship between PG and progesterone productions by the CL, several related findings indicate that the CL can make PGs. The human CL contains large amounts of PGs (Henderson and McNatty, 1975) and makes them *in vitro* (Henderson and McNatty, 1975); their metabolites also appear in the blood draining the human (Aksel *et al.*, 1977, 1978) and monkey ovary (Fernandez *et al.*, 1980). The cow's CL makes PGs from arachidonic acid (Hansel, 1975; Shemesh and Hansel, 1975b,c) but it can also make PGs without added precursors, although in somewhat smaller amounts (Shemesh and Hansel, 1975c). The luteolytic synergism between estrogens and PGs, as for example, in the sheep (Hansel, 1975; Hixon *et al.*, 1975) or cow (Gengenbach *et al.*, 1977), most likely depends on intraluteal PG production, since the sheep's CL can also make PGs *in vitro* (Rexroad and Guthrie, 1979). Guthrie *et al.* (1978, 1979), like Watson and his co-workers, have shown that the pig CL makes PGs *in vitro*. The rat's CL contains PGs and can make them *in vitro* (Demers *et al.*, 1973; Strauss and Stambaugh, 1974), and indomethacin prevents PMSG-induced luteolysis in hysterectomized rats (Basu and Chatterton, 1978). Luteinized rabbit granulosa cells in tissue culture (Erickson *et al.*, 1977) and rabbit CL *in vitro* (Wilks *et al.*, 1972) also make PGs; this ability probably explains the readiness with which arachidonic acid treatment induces luteolysis in the rabbit (Hoffman, 1974; Carlson and Gole, 1978).

### *3. The Effect of Progesterone on the Production and/or Effect of Luteolytic PGs*

Even in species (e.g., sheep, pig) in which the effect of *uterine* PGs on the CL is the most important single factor that determines when luteolysis begins, the CL's ability to make progesterone seems to be inversely related to its ability to make PGs (Patek and Watson, 1976). Although such a relationship could be the result of only the luteolytic effect of PGs, it is also possible that it is an expression of the inhibiting effect of progesterone on PG production. Although there is almost no information about the influence of progesterone on intraluteal PG production, the fairly large amount of information about its effect on endometrial and related PG production suggests that progesterone probably suppresses the CL's ability to make PGs.

The pregnancy- and, particularly, the parturition-related information on this point is especially clear. From the analyses and summaries of findings made in recent years (Liggins, 1973, 1979; Goldberg and Ramwell, 1975;

Karin and Hillier, 1975; Currie and Thorburn, 1977; Csapo, 1977; Flower, 1977; Heap *et al.*, 1977; Schwarz *et al.*, 1977; Nathanielsz, 1978; Thorburn and Challis, 1979), two main points become very clear: progesterone increases the tissue's potential ability to make PGs and, at the same time, it suppresses their actual production and/or release, and reduces their effect. The suppressing effect is not well understood, but may depend in part on stabilizing various intracellular membranes (Liggins, 1979) within which are stored both the arachidonic acid-rich phospholipids and the phospholipases which hydrolyze them from the parent compounds. The potentiating effect may include, among other things, the building up of stores of arachidonic acid-rich phospholipids (Thorburn and Challis, 1979), the formation of lysosomes (Henzl *et al.*, 1972), the richest source of phospholipases, and increasing cathepsin D synthesis and storage in the lysosomes (Elangovan and Moulton, 1980). How the changes in progesterone, estrogens, and intrauterine PG production eventually lead to parturition need not concern us here, except to say that, almost beyond question, the final common pathway is through a switch-on of an increase of intrauterine PG production, arising in part from the removal or bypassing of the inhibiting effect of progesterone, and in part from the self-propagating quality of PG production and the ability of PGs to reduce progesterone secretion, and to stimulate myometrial activity (Currie and Thorburn, 1977; Csapo, 1977; Flower, 1977; Schwarz *et al.*, 1977; Nathanielsz, 1978; Lands, 1979; Liggins, 1979; Thorburn and Challis, 1979, 1980).

In general, progesterone seems to have a similar influence on endometrial PGs during the ovulation cycle. The same kind of inverse relationship we have already seen between intraluteal progesterone and PG production exists between progesterone secretion during the ovulation cycle and various measures of endometrial PG activity (Table V). More direct evidence is that, although progesterone may under some conditions of prolonged treatment be associated with an increase in uterine PG production (e.g., sheep: Louis *et al.*, 1978), in general it suppresses uterine PG production but sets up conditions which allow estrogen to stimulate PG production and release (*human*: Abel and Baird, 1980; Kelly and Abel, 1980; *monkey*: Demers *et al.*, 1974; *rat*: Castracane and Jordan, 1975; Kelly and Abel, 1980; *mouse*: Saksena and Lau, 1973; *sheep*: Caldwell *et al.*, 1972; Wilson *et al.*, 1972; Murdoch *et al.*, 1978; *guinea pig*: Blatchley and Poyser, 1974) (see also Horton and Poyser, 1976).

In a few conditions, however, the inverse relationship between progesterone secretion by the CL and PG production by the uterus either does not hold or cannot be readily seen. In the sheep, for example, progesterone secretion during early pregnancy remains at a level equivalent to

TABLE V

*The General Inverse Relation between the Progesterone Level in the Circulation and PG Production by and/or Release from the Uterus<sup>a</sup>*

Species	Activities compared	Reference
Guinea pig	PGs/ut.ov.vein increase after day 11, o.c. as P/ut.ov.vein falls	Earthy <i>et al.</i> (1975)
	Inverse relationship P secretion and PG/circ. on day 12–15 of o.c.	Blatchley <i>et al.</i> (1975)
	PGs/uterus increase toward end luteal ph.	Poyser (1972)
	PG production by uterus does not increase in early preg. as P stays high	Maule Walker and Poyser (1973); Blatchley <i>et al.</i> (1975)
Rat	PG/uterus and in ut.ov.vein increase toward end of psp.	Weems <i>et al.</i> (1975); Castracane and Shaikh (1976); Weems (1979)
Sheep	PGs/uterus increase toward end of luteal ph. PGF/ut.vein increases at end luteal ph.	Wilson <i>et al.</i> (1972b)
Cow	Cyclooxygenase/uterus maximum by day 14 of o.c.	Several authors (see Horton and Poyser, 1976)
	Surges of PG release into ut.vein begin as P falls in late luteal ph.	Huslig <i>et al.</i> (1979); Smith <i>et al.</i> (1980)
	Arachidonic acid/endometrium increases with fall in progesterone at end luteal ph.	Kindahl <i>et al.</i> (1980)
Pig	PG/uterus and ut.vein as for arachidonic acid	Hansel <i>et al.</i> (1975)
	Spikes of PG release into ut.vein as P falls in late luteal ph.	Shemesh and Hansel (1975a)
	PGF/circ. increase after day 12	Gleeson <i>et al.</i> (1974)
Horse	Luteolytic effect endometrial extracts <i>in vitro</i> at max. at end luteal ph.	Moeljono <i>et al.</i> (1977)
	Endometrial production PGs <i>in vitro</i> increase from mid- to late luteal ph.	Watson and Maule Walker (1977)
	PGs/ut.vein increase to max. by middle of CL regression ph.	Patek and Watson (1976)
	Inverse relation P and PG metabolites/ circ. throughout o.c.	Douglas and Ginther (1976)
Monkey	PG/ut. fluid increases to max. by day 20–25 o.c.	Neely <i>et al.</i> (1979)
Human	Inverse relation P and PG metabolites/ circ. during luteal ph. Activity of 15-OHPGDH/endometrium parallels P secretion pattern PGs/endometrium increase at end luteal ph. PGF/endometrium decreases in early pregnancy as P stays high	Demers <i>et al.</i> (1974) Koullapis and Collins (1980) Casey <i>et al.</i> (1980) Downie <i>et al.</i> (1974); Singh <i>et al.</i> (1975) Willman and Collins (1976)

<sup>a</sup> Abbreviations as in Table I, plus these: max., maximum; P/ut.ov.vein, level of progesterone in the utero-ovarian vein; PG/circ., level of PG in the peripheral circulation; PG/uterus, level of PG in the uterus; preg., pregnancy; psp., pseudopregnancy; ut., uterine.

the peak reached during the ovulation cycle (Bassett *et al.*, 1969; Stabenfeldt *et al.*, 1970; Thorburn and Mattner, 1971), but the concentration of PGF in the uterine vein (Pexton *et al.*, 1975; Lewis *et al.*, 1978; Ellinwood *et al.*, 1979) and the apparent rate of PG production by the endometrium (Lewis *et al.*, 1977; Ellinwood *et al.*, 1979) are as high during this time as during the ovulation cycle. A similar situation exists in the cow (Lukaszewska and Hansel, 1979). The pulses of PGF released into the uterine vein, however, which typically occur at the end of the luteal phase of the ovulation cycle in sheep, do not occur at the equivalent time in early pregnancy (Peterson *et al.*, 1976; Niswender, 1981). In the rat also the process through which decidual tissue (DT) formation prevents the luteolytic action of the uterus (Bradbury *et al.*, 1950; Rothchild, 1965, p. 283; Anderson *et al.*, 1969) does not involve a decrease in uterine PGF production (Anteby *et al.*, 1975; Weems *et al.*, 1975; Weems, 1979; Castracane and Shaikh, 1976).

Although the reduced luteolytic action of the uterus under these conditions in rat and sheep may be connected with an increased production of PGEs (Anteby *et al.*, 1975; Castracane and Shaikh, 1976; Ellinwood *et al.*, 1979), some of which can prevent the luteolytic effect of PGF<sub>2α</sub> (Henderson *et al.*, 1977; Weems *et al.*, 1979), the reason why the continued secretion of progesterone at a high level does not suppress the production of uterine PGFs is not easy to see. It may be that only a *rising* rate of progesterone secretion, i.e., *not* a constant one, can stop or reverse an already started increase in PG production.

#### 4. The Self-Stimulating Quality of PG Production

Although inhibitory control over intracellular PG production exists as, for example, cyclooxygenases can catalyze their own destruction (Lands, 1979), most if not all tissues contain the elements needed for the self-stimulation of PG production. This positive feedback quality of PG production has been remarked on in most reviews dealing either with the control of PG biosynthesis or related topics (Hinman, 1972; Pong and Levine, 1977; Ramwell *et al.*, 1977; Lands, 1979; Thorburn and Challis, 1979). Among other things, the ubiquity of arachidonic acid esters and of acyl hydrolases within various kinds of cell membrane, the facts that cyclooxygenases are rarely a limiting factor in PG biosynthesis, and that molecular oxygen has to be almost nonexistent for there to be too little to maintain PG biosynthesis, and that PG metabolizing enzymes are more easily suppressed than are those that stimulate PG biosynthesis (Blackwell *et al.*, 1975) are the most obvious reasons for it. The autocatalytic aspects of PG production are also due to the ability of PGs to stabilize cellular membranes (Vogt, 1978; Buhr *et al.*, 1979) and thus free

the enzymes and substrates needed for PG biosynthesis (Morita *et al.*, 1979; Murota *et al.*, 1978).

These processes can occur in the CL. PGE treatment of the rat CL *in vitro* increased their production of PGF<sub>2α</sub> (Demers *et al.*, 1973). A similar effect of colprostенол (a synthetic PGF analog) occurs in pig (Guthrie *et al.*, 1979) and sheep CL (Rexroad and Guthrie, 1979). The description by Salazar *et al.*, (1976) of the EM changes that occur in rat CL in response to a single treatment with colprostенол implies that the treatment increased the production of intraluteal PGs. The fact that phospholipase A<sub>2</sub> concentrations reach a peak in the cow's CL just before regression begins (Shemesh *et al.*, 1976) and that lysosomal membrane fragility is at its peak at this time (Dingle *et al.*, 1968) are further indications that an initiating stimulus may be all that is necessary to set PG synthesis in motion. For example, although LH can stimulate intraluteal PG production (p. 240), the increase in PG production and decrease in progesterone secretion started by arachidonic acid treatment in the cow's CL (Hansel *et al.*, 1973; Hansel, 1975) can proceed without any change in the level of LH in the circulation (Shemesh and Hansel, 1975b). I have already mentioned that the same authors showed that even without added arachidonic acid the cow's CL can make PGs *in vitro*.

Another factor peculiar to the CL that probably contributes to the self-propagation of PG production is that progesterone stimulates the activity of the 15-hydroxy PG dehydrogenase (15-OHPGDH) (Flower, 1977; Liggins, 1979), the most important of the enzymes that metabolize PGs and reduce their biological activity (Hinman, 1972; Lands, 1979). Thus, the fall in progesterone concentration induced by intraluteal PGs would itself increase the concentration of biologically active PGs. Essentially the same interrelationship between progesterone and PGs is an important part of events leading to parturition.

### C. A THEORY OF THE BASIC AND COMMON ELEMENTS OF REGULATION OF ALL MAMMALIAN CL

The unique attribute of all vertebrate CL is ephemerality. The ability to secrete progesterone and the connection to viviparity are not unique, since other tissues can secrete progesterone and a one-to-one relationship between the CL and viviparity is true only of the mammalian species. The connection between CL activity and viviparity in its many forms, however, is related to the cause of the CL's ephemerality, as I will try to show.

No explanation of the CL's ephemerality can depend crucially on how an extrinsic luteotrophin acts or on how an extrinsic luteolysin acts, or on

how widespread a dependency on either of such extrinsic controlling agents may be among the species, because ephemerality is common to *all* species' CL, and the dependency on either an extrinsic luteotrophin or luteolysin is not. Earlier explanations for one or another aspect of the CL's ephemerality (whether they were expressed as such or not), as for example, those of Caldwell *et al.* (1972), McCracken *et al.* (1972), Henderson and McNatty (1975), Kuehl (1974), and my own (Rothchild, 1960), although they grasped part of the relationship of the CL's ephemerality to the instability of the system that regulated CL activity, failed to see how important it was to differentiate between intrinsic and extrinsic controls in the construction of a theory that could apply to *all* species. In a word: since the CL can go through its life cycle in the absence of extrinsic controls in at least *some* species, and in others, under at least some conditions, the cause of its ephemerality, which is to say, the basic and common elements of regulation that determine the CL's ability to secrete progesterone, and that bring this secretion and with it the life of the CL as a whole to an end, *must lie within the CL itself*, in *all* species. But what the intrinsic causes are must also be common to all CL, and therefore, they cannot depend crucially on such things as estrogens, androgen, relaxin, inhibin, etc., since these are not common to all CL at all times.

I pointed out above that an essential quality of regulation of an ephemeral system like the CL must be instability, i.e., the absence of controls that maintain constancy. Ideal conditions for ephemerality, therefore, would be a positive feedback regulation of progesterone production and either ubiquity of factors that could interfere with progesterone production, or the potentiation, by progesterone, of processes that could reduce progesterone production (Rothchild, 1964, 1965; McCracken *et al.*, 1972). Autonomy of progesterone secretion, the possibility that progesterone stimulates its own secretion, the luteolytic effect of PGs, the production of PGs by the CL, the self-stimulating quality of PG production, and the way progesterone affects PG production, are all qualities of the CL that admirably satisfy the requirements for a regulating system that could account for ephemerality. The summary of information about each of these does not establish beyond any question that they are attributes of CL physiology. There is enough evidence for each of them, however, to confirm in me the belief that they are more than a collection of unrelated attributes of the CL, but rather indications of what the basic system of regulation of the CL actually consists of. I will theorize, therefore, that the system is put together according to the following five postulates.

1. *Progesterone production is an intrinsic property of the luteinized granulosa cell.* This means that the production of progesterone by the

CL is essentially autonomous, in the same sense that production of prolactin by the pituitary lactotroph is autonomous. The follicular granulosa cells probably develop this property as they mature but their ability to express it is inhibited by the intrafollicular environment. Removal from this environment or the change in the environment induced by LH removes this inhibition and allow the intrinsic ability to be expressed. Progesterone secretion, although essentially autonomous, can be influenced by extrinsic luteotrophins, and under certain conditions, even become dependent on such extrinsic controls.

2. *Progesterone is the primary stimulus to its own secretion.* How it does this is unknown and for the moment, at least, immaterial. The importance of the postulate is that it helps to explain at least one aspect of ephemerality, since all systems regulated by positive feedback are ephemeral. The system can work to maintain progesterone secretion permanently, therefore, only as long as it is undisturbed, but since it is self-dependent, any disturbance that causes progesterone secretion to fall must eventually induce a further fall and the eventual disintegration of the system as a whole.

3. *The CL makes PGs, but progesterone suppresses their production.* The action of progesterone as previously described (p. 214) is to set up the conditions for an eventual high rate of PG production, at the same time that it suppresses their actual production, and/or increases their inactivation. The suppressing effect is strongest during the rising phase of the CL's life cycle; it becomes weaker during the plateau phase, and it progressively diminishes to the point of complete disappearance during the regression phase.

4. *PG production is a self-stimulating intrinsic property of the CL, and PGs inhibit progesterone secretion.* This and the first three postulates imply that the essence of the CL is that it is a postovulatory vertebrate follicle whose regression has been temporarily arrested by its ability to make progesterone. The ultimate cause of its regression comes from its ability to make PGs, and the effects of PGs on progesterone secretion and the integrity of the CL cell. The CL cannot secrete progesterone permanently because this secretion is self-dependent and because progesterone builds up the potential for PG production. PGs, by contrast, regardless of how they suppress progesterone secretion, do so without potentiating future progesterone secretion. Once PGs begin to reduce progesterone secretion, therefore, the CL's potential ablity to secrete progesterone is also reduced. The self-stimulating quality of PG production and the inhibiting effect of PGs on progesterone secretion thus eventually become meshed with the progesterone secretion process in a way which leads to inevitable and irreversible regression of progesterone secretion.

5. An increase in the intraluteal concentration of progesterone above a critical level initiates the regression phase of the CL life cycle. Since the intraluteal concentration of progesterone rises as the rate of progesterone secretion rises, it is obvious that the CL would eventually consist of nothing but progesterone if the process continued indefinitely. Some event, therefore, initiated by an increase in the intraluteal progesterone concentration above a critical level must act to decrease the rate at which progesterone secretion rises. Once this begins, it sets in motion the processes that eventually lead to regression, because it will tend to reduce both the suppressing effect of progesterone on PG production and the stimulating effect of progesterone on its own production. The plateau phase is the first symptom of these changes, and, therefore, of regression and it is the most critical period of the CL life cycle. Extrinsic factors, such as pituitary and placental hormones, estrogens, uterine PGs, may affect the system as a whole by acting either to raise the rate of progesterone secretion or of PG production, or by interfering with the effect of progesterone on PG production or vice versa, or by inhibiting the production of either progesterone or PGs. They begin to have their major effects during the early part of the plateau phase.

#### *Summary of the Theory*

The CL is ephemeral because of a Yin/Yang type of interaction between the process that stimulates progesterone secretion and the one that stimulates PG production. Each process is self-propagating and tends to suppress the other. Progesterone, however, potentiates the CL's ability to make PGs while PGs only reduce the CL's ability to make progesterone. The CL begins its life cycle with a high potential ability for progesterone secretion, and therefore for suppressing PG production. As progesterone secretion rises so does the intraluteal progesterone concentration, and when the latter rises above a critical level, it initiates the CL's eventual regression by reducing the suppressing effect of progesterone on PG production. Once the balance swings toward PG production regression becomes inevitable (Figs. 3 and 4).

#### D. OBJECTIONS OR ALTERNATIVES TO THE THEORY

Can the ephemerality of the CL be explained solely on the basis of a self-destruct system due to an inhibiting effect of progesterone on its own secretion? For example, since the concentration of progesterone within the CL rises as the rate of secretion of progesterone rises, the critical intracellular concentration could be said to be the only factor required to shut off progesterone secretion, possibly by inhibiting the activity of  $3\beta$ -

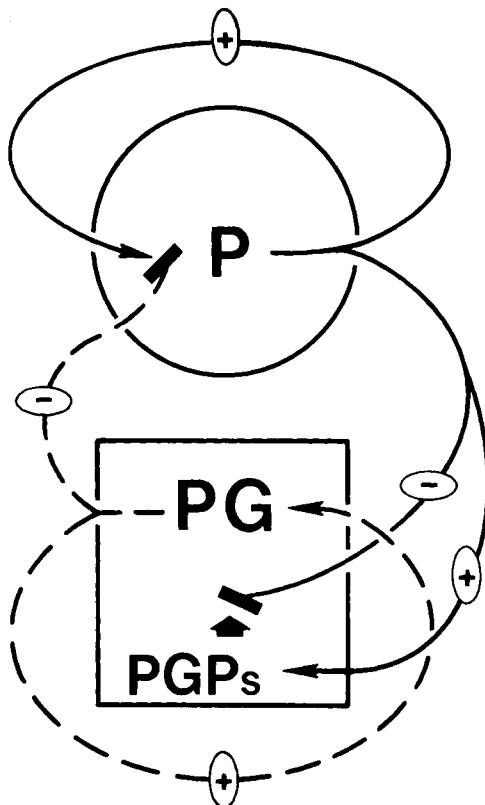


FIG. 3. A schematic representation of the essence of the intrinsic factors that regulate CL activity. P, Progesterone secretion; PG, prostaglandin production; PGPs, prostaglandin precursors;  $\oplus$ , stimulation;  $\ominus$ , inhibition. The diagram represents the concept that the CL's two basic activities, secretion of progesterone and production of PGs, are each self-stimulating (i.e., regulated by positive feedback) and are mutually inhibitory. Progesterone, however, increases the CL's potential ability to produce PGs (e.g., by stimulating the storage of PG precursors), as it suppresses their production (e.g., by preventing the use of PG precursors for PG synthesis), while the effect of PGs on progesterone secretion is purely inhibitory (see also Fig. 4).

OHSDH (p. 206). If this was so, why is it necessary to invoke a PG effect as the cause of regression?

If the only effect of a critically high intraluteal progesterone concentration was to reduce the rate of progesterone secretion, the system would tend to work like an oscillating, self-correcting one (perhaps the way pituitary lactotrophs secrete prolactin when removed from the inhibiting effect of the CNS) because the reduction in progesterone secretion would also reduce the intraluteal progesterone concentration below the critical

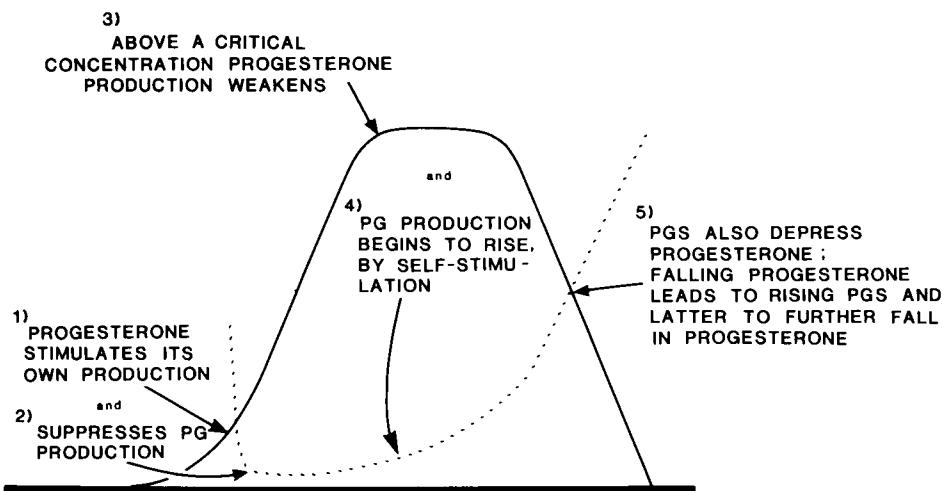


FIG. 4. A CL life cycle expressed in terms of how progesterone secretion and PG production are related to one another. Solid line, progesterone secretion; broken line, PG production (1 and 2) The *rising phase* reflects the self-stimulating quality of progesterone secretion; during this period, progesterone strongly suppresses PG production. (3 and 4) The *plateau phase* reflects the change in the rate of increase in progesterone secretion that arises from the effects of a critically high intraluteal concentration of progesterone. Exactly what this effect is is unknown, but it is postulated to reduce progesterone secretion and switch on an increase in production of PGs. (5) The *regression phase* is the result of the steadily increasing production of PGs, and their suppression of progesterone secretion. Once PG production begins to increase, regression is inevitable because of the self-stimulating quality of its production, and because, as progesterone secretion falls, it reduces the stimulus to its own secretion, and the suppression of PG production (see text, p. 219).

level, and so remove its inhibiting effect. The importance of self-stimulation as a cause of ephemerality is not just that the system disintegrates as soon as *any* reduction in the product occurs. The reduction must gather a certain momentum before disintegration becomes inevitable. The ability of PGs to reduce progesterone secretion means that with each such oscillation, the amount of progesterone produced during each upswing would be slightly less than that produced during the previous one. This is why the ability to make PGs and the way they affect progesterone production fit so much better with the facts of the CL's life cycle than would only the possible inhibiting effect of a critically high progesterone concentration on progesterone secretion.

One may also question the necessity to include the self-stimulating quality of progesterone secretion as one of the important causes of ephemerality in a theory which postulates that PGs are what cause the inevitable regression of the CL. The answer to this has two parts. The first

is that progesterone secretion could theoretically continue indefinitely, if it was not disturbed (as, e.g., in the experiment of Korenman *et al.*, 1973). The other is that, while PGs are the inevitable cause of death of the CL, the ability to secrete progesterone determines *when* that event occurs, because progesterone can suppress the production and/or release of PGs. The dependency of progesterone secretion on progesterone, thus ensures that this suppression will not be permanent.

### III. The Evolution of Diversity in the Regulation of the Mammalian CL

Even if one granted that there is some truth in this concept of how the CL in general is regulated, the connection between it and how the CL of, for example, a platypus, kangaroo, sheep, dog, woman, rat, or rabbit might be regulated is certainly not self-evident. I believe the connection is in the evolution of responsiveness of the CL to factors that first modified the plateau phase of the CL's life cycle and then the other phases as well. These factors, therefore, could then either prolong, or even as in the case of the rat, shorten the duration of the CL's life span. The first and most important of such factors were probably pituitary hormones, and the CL's ability to respond to them made it possible for the later evolution of responsiveness to other factors, such as placental hormones, ovarian estrogens, and uterine PGs, which could modify the basic system of regulation. A good part of what I will now describe has to include speculations based on what we know about the CL of present day species, but how else can one describe the evolution of things that leave no fossil records?

#### A. THE METATHERIAN-EUTHERIAN DICHOTOMY

The essential difference between marsupial and eutherian viviparity is in its duration. In no known marsupial species is pregnancy longer than 35 days (Tyndale-Biscoe *et al.*, 1974) and it is only during roughly the last week or two of this time that the embryo attaches to the uterine wall and develops rapidly (Hughes, 1974; Lyne and Hollis, 1977). It is born, therefore, as an embryo and it completes its development, which may occupy many months, during lactation (Sharman, 1970, 1976; Tyndale-Biscoe, 1973). Among the eutherians, however, intrauterine gestation is in all known species long enough for the embryo to progress far beyond where even the most advanced marsupial young is at birth (Parker, 1977); even though many species of eutheria give birth to altricial young. The formation of these two main branches of mammalian descendants from a common ancestor is often referred to as the metatherian-eutherian

dichotomy, and it is usually explained as arising from a difference between them in the kind of placenta the embryo formed (e.g., Lillegraven, 1979). It is more probable that the difference between them in their placentas, which is profound, arose first from a difference in the kind of CL they had, and in the responsiveness of their uteri to progesterone. The first change was probably the appearance of responsiveness to the luteotrophic action of prolactin in one of the descendants of the common ancestor.

The modern marsupial CL, as I have indicated above, seems to be almost or completely autonomous (except in the bandicoots), and is probably in a direct line of descent from the CL of the stem reptiles (the cotylosaurs) which first appeared about 300,000,000 years ago (Romer, 1966). These early reptiles, like their modern reptilian and avian descendants, probably made megalecithal eggs. Whether they laid these about a day or so after ovulation, as birds do (Fraps, 1954), or retained them in the oviduct for at least a week before laying them as an entire clutch as modern reptiles do (Callard and Lance, 1977; Cuellar, 1979) may never be known. However, the fact that the postovulatory follicle in birds is not luteinized (Jones, 1978) while in all modern reptiles it forms a true CL (Miller, 1948) suggests that when the CL first appeared in the stem reptiles or in their immediate descendants, its activity was associated with the retention of the egg in the oviduct.

It may make it easier to grasp the significance of this change by comparing the activity of the bird's postovulatory follicle with that of the reptile CL, in relation to the passage of the egg through the reproductive tract. In the chicken the secretion of progesterone by the postovulatory follicle falls off so rapidly that by 1 to 2 days after ovulation, it is less than 1/30 the amount secreted before ovulation (Dick *et al.*, 1978) (Fig. 5). In contrast, beginning about 10 hours after ovulation, it produces PGFs at a steadily rising rate until at least the time of the next ovulation (Day and Nalbandov, 1977) (Fig. 5), which occurs as a rule within each clutch about 24+ hours after ovulation (Fraps, 1954). The avian postovulatory follicle determines when the egg is laid, since if it is removed (Rothchild and Fraps, 1944; Rothchild, 1946; Tanaka and Nakada, 1974) or its blood supply cut off (Wood-Gush and Gilbert, 1964) or its granulosa cells removed or destroyed (Gilbert *et al.*, 1978) oviposition is delayed for about 1 to 5 days. Oviposition can also be delayed in intact birds by indomethacin treatment (Hertelendy, 1973; Hertelendy and Biellier, 1978b) and it can be induced in those whose postovulatory follicle has been removed, by treatment with PGs (Hertelendy and Biellier, 1978a) or oxytocin, the effect of which can be prevented by indomethacin (Hertelendy, 1973). The changes in PG levels in the circulation fit with those in the postovulatory follicle and with the time of oviposition (Hertelendy and Biellier, 1978a,b).

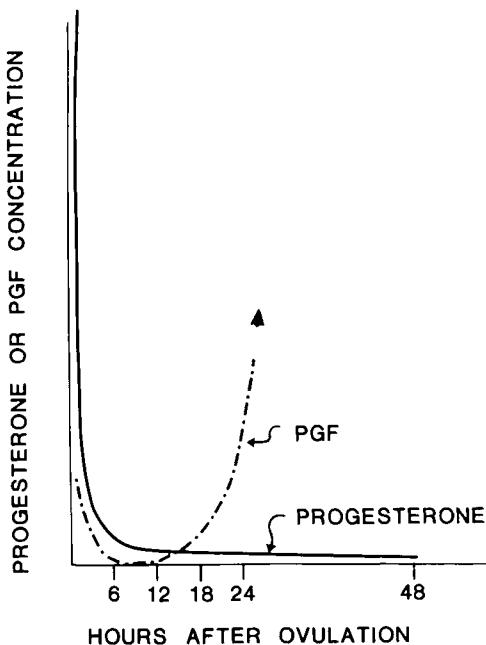


FIG. 5. The qualitative relation between PG and progesterone production in a non-luteinized postovulatory follicle (POF). The progesterone pattern is adapted from the findings of Dick *et al.* (1978), and the PG pattern from the findings of Day and Nalbandov (1977), for the domestic hen. The point of the relationship is that in the absence of luteinization—which is an expression of the prolongation of progesterone secretion by the POF—PG production begins very soon after ovulation and is probably responsible for the rapid regression of the POF, as well as for the relatively short stay of the egg in the reproductive tract (see text, p. 225).

These findings, taken together with the more prolonged stay of the egg in the reptile's than in the bird's oviduct (Cueller, 1979), are arguments in favor of the idea that luteinization, when it first appeared in the stem reptiles' postovulatory follicle, postponed its production of PGs. The fact that removal of the bird's postovulatory follicle delays but does not prevent oviposition suggests that the follicular PGs switch on the intrauterine PGs, and that, in the absence of the former, the latter switch themselves on, but at a slower rate. The increase in the postovulatory follicle's PGs does not depend on pituitary hormones, as Day and Nalbandov (1977) implied, since oviposition occurs on time in the vast majority of birds, in the absence of the pituitary (Rothchild, 1946). The switch-on of intrauterine PGs is probably also an autonomous process, although a slower one. Luteinization, therefore, by indirectly postponing the production of

intraoviductal PGs, also postponed the expulsion of the egg from the reproductive tract. I suggest that this is how viviparity among reptiles and mammals began, and is the mechanism behind the idea of Amoroso (1955) and of Hisaw (1959) that the first effect of the CL, which led toward viviparity, was to retain the egg in the reproductive tract. If so, I think the first effect of progesterone may not have been directly on the reproductive tract itself but on the production of ovarian PGs. I would also suggest that vertebrate follicles may have been making PGs long before they started making steroids, and that the PGs acted primarily as the agents of follicular rupture, postovulatory regression of the follicle, and the rapid passage of the eggs through the reproductive tract to the outside world.

The basic system of regulation of the stem reptile's CL was probably essentially what I have described above, and it probably also remained unchanged in any important detail, through the line of descent from the stem reptiles, until the metatherian-eutherian dichotomy of about 100,000,000 years ago.

The stem reptiles threw off several lines of descendants. Among them, one led to the modern birds, another led to the modern reptiles, and a third to the mammal-like reptilian ancestors (therapsids) of early mammals (Romer, 1966; Crompton and Jenkins, 1979) (Fig. 6). The therapsids presumably remained oviparous, with a long interval between ovulation and oviposition. The prototheria are generally assumed to have arisen from them and they eventually progressed to an ovoviviparous form of reproduction, which we still see today in their only surviving descendants, the monotremes (Hill and Gatenby, 1926; Hughes and Carrick, 1978). It was formerly believed that the eutherian line came off directly from a similar therapsid ancestor, but a more likely course of descent (Hopson and Crompton, 1969; Hopson, 1973; Lillegren, 1975, 1979; Marshall, 1979) is from a primitive mammalian ancestor (pantothere) which progressed from the ovoviviparity of the therapsids and prototheria to a kind of limited viviparity not essentially different in its basic characteristic from what we see today in marsupials like the American opossum. These early mammals probably had a CL still regulated entirely by intrinsic processes; it could secrete progesterone and/or postpone its production of PGs only long enough to allow for the initial stages of embryonic development in the uterus, the rest of development taking place during lactation. It was a slightly more advanced form of viviparity than the ovoviviparity practiced by the monotremes, and one which the modern marsupials modified to only a minor degree.

The modern marsupials became fixed on this method of reproduction, in spite of the fact that there are at least two groups among them with CL that are responsive, apparently, to two quite different effects of prolactin; one of

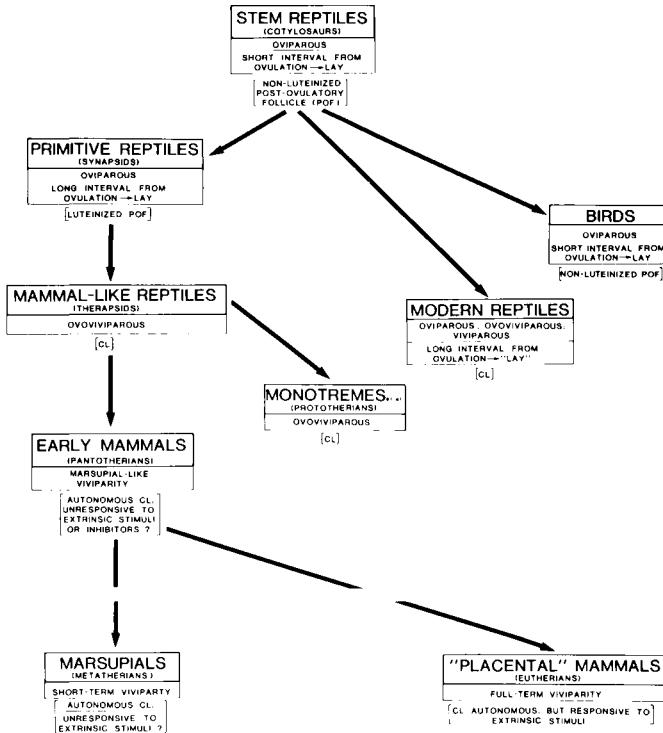


FIG. 6. The descent of the mammalian CL. The stem reptiles (cotylosaurs) arose about 300 million years ago. Among their many descendants are the three lines shown here, in the perspective of the postovulatory phase of reproduction. The birds either never evolved a CL, or else lost it; their postovulatory follicle (POF) regresses very quickly, and the egg passes through the oviduct within a day or two after ovulation. The modern reptiles, and presumably also the primitive reptiles (synapsids) which led to the mammals, evolved a CL from the POF; even in those with an oviparous form of reproduction, the egg was retained in the oviduct until all the eggs of a clutch had been ovulated, i.e., for about 2 weeks. Among the modern reptiles ovoviparity and true viviparity also evolved, and are related to more prolonged periods of CL activity. The CL in the descendants of the synapsids remained presumably an essentially intrinsically controlled organ until about 100 million years ago, and postovulatory reproduction changed only to the extent that oviparity progressed to ovoviparity (still seen today in the monotremes) and in the pantotheres, to the limited form of viviparity similar to that of modern marsupials. The evolution of the CL of the "placental" (eutherian) mammals began about 100 million years ago, with the appearance of responsiveness to pituitary luteotrophin hormones (see text, p. 230, and Fig. 8).

these effects actually prolongs the life span of the CL well beyond the limits of the typical short luteal phase of most marsupials (see below). This implies that the failure of marsupials to evolve a eutherian type of viviparity (i.e., an intrauterine gestation long enough for most of embryogenesis) was the result of more than just the very short life span of their CL or a failure to evolve CL responsive to extrinsic factors. Although the short-lived CL

appears to be, and may indeed be the major reason for the short gestation among the polyprotodont marsupials (e.g., the American opossum, the brush-tailed possum, etc.; see Tyndale-Biscoe and Hawkins, 1977) it cannot be the only reason, because the bandicoots, which are also polyprotodonts, also have a gestation of only 12 days, in spite of having long-lived CL (Hughes, 1962; Gemmel, 1981); the long luteal phase (about 6 weeks) is probably because the CL have become responsive to the luteotrophic effects of prolactin. The representative of the other type of responsiveness is the tammar wallaby (a macropodid; the latter include the kangaroos, and are part of the second major division of the marsupials, the diprotodonts). In the tammar, the CL have evolved responsiveness to the "luteostatic" effect of prolactin, one which probably accounts for the long period of CL quiescence during lactation and anestrus (Hearn, 1973, 1974; Tyndale-Biscoe and Hawkins, 1977; Tyndale-Biscoe, 1979). Thus although the CL of most marsupials probably remained unresponsive to extrinsic controls, it did not remain so in all, and one may wonder, therefore, what it was about the marsupials that fixed them on a short gestation and a long lactation as a way of reproducing.

These facts and the one that removal of the CL does not interrupt pregnancy once the embryo has attached itself to the uterine wall (Tyndale-Biscoe, 1973; Renfree, 1974, 1977; Sharman, 1976; Tyndale-Biscoe and Hawkins, 1977; Young and Renfree, 1979) imply that the primary function of the progesterone secreted by the CL in marsupials is not its pregnancy-maintaining action on the uterus, but its PG-suppressing action on the ovary. The marsupial CL may function mainly as it did in the first oviparous reptiles, as a device that delayed the production of ovarian PGs and so delayed the switch-on of oviductal and uterine PGs, and thus allowed the embryo to be retained in the uterus long enough for the embryo itself to take over the control of its further stay there (Renfree and Tyndale-Biscoe, 1973). To come back to the point I started with: the essential difference between marsupial and eutherian viviparity is in the duration of intrauterine gestation; regardless of whether the marsupial embryo controls its intrauterine stay through the secretion of progesterone by its placenta (Bradshaw *et al.*, 1975) or by other means, one of the essential changes that prolonged intrauterine gestation in the eutheria was the evolution of responsiveness of the uterus to the progesterone secreted by the CL, which reached the uterus through the circulation.

It would be logical to expect, from a familiarity with eutherian viviparity, that a prolonged luteal phase would not only be a necessary, but a sufficient, condition for the transition from marsupial to eutherian viviparity. The peculiarity of the bandicoot's CL and its relation to pregnancy, in comparison with the general relation between the CL and pregnancy in marsupials, tell us very clearly that this transition was the result of more

than *just* the prolongation of the luteal phase. The change that accompanied the prolongation of CL activity and that made the transition possible must have been one that prolonged the stay of the embryo in the uterus, i.e., a change in the responsiveness of the *uterus* to progesterone.

The bandicoot seems to have crossed the metatherian-eutherian line with the change that prolonged its CL's life span, but retained the marsupial form of viviparity, because its uterus has not undergone the change that would make it respond to progesterone. The relation between the CL and pregnancy in the bandicoot tempts me to suggest the heretical idea that the first eutherian descended from a bandicoot-like *marsupial* ancestor, rather than from a pantothere ancestor common to both marsupials and eutherians, as is generally believed (Romer, 1966; Lillegraven *et al.*, 1979). Regardless of its parentage, however, the ancestor of the eutherian mammals must very probably have been the first of the primitive mammals to evolve *both* a long-lived CL and a uterus that could respond to progesterone in a way that prolonged the embryo's stay within it. By prolonging the duration of gestation these changes made the further steps toward eutherian viviparity possible, since they provided conditions for the evolution of trophoblast far beyond where it could go among the marsupials (see Taylor and Padykula, 1978; and Lillegraven, 1979), and this in turn made the enormously diverse forms of eutherian viviparity possible.<sup>3</sup> The very first step, in any case, was the prolongation of the CL's life span, and I believe it occurred as it probably did in the bandicoot, by the appearance of responsiveness to the luteotrophic effects of prolactin.

## B. THE EVOLUTION OF THE EUTHERIAN CL

### 1. *The Pivotal Role of Prolactin*

There are several intuitive reasons for speculating that responsiveness to prolactin's luteotrophic action was the *first* change that led to the evolution of the eutherian CL. Its luteotrophic effects prolong rather than raise the rate of progesterone secretion. Since the pantotheres depended on lactation to complete embryogenesis, prolactin secretion must have begun to rise soon after ovulation, and have reached a fairly high level by about the time of the plateau phase of the CL's life cycle, so it would have been more readily available than, for example, a hormone like LH, which

<sup>3</sup> Regardless of whether eutherian viviparity had no or a great selective advantage over metatherian viviparity (see Lillegraven, 1975, 1979, Parker, 1977, and Kirsch, 1977, for examples of arguments pro and con) the fact is that it did evolve, through its own momentum, I suspect, if indeed it had no selective advantage over the metatherian type.

would be secreted only at basal levels between each ovulation. Prolactin is also an extremely versatile hormone (Nicoll and Bern, 1972; Nicoll, 1980) with effects throughout the vertebrates that seem to be more related to postovulatory than to preovulatory aspects of reproduction (Riddle, 1963).

It has luteotrophic effects which, as has been noted earlier (Rothchild, 1966; Short, 1967), are more widely distributed than only among the species with ultra-short-lived CL. It is an essential luteotrophin not only in the latter, but probably also for the prolonged luteal phase of monestrous breeders. It also has luteotrophic effects in several polyestrous breeders with short-lived CL (Table VI). In most or all of the latter it is not an essential luteotrophin, but the absence of essentiality is probably a late stage in the evolution of the polyestrous eutherian CL (p. 246) and in at least one of these species, the sheep, the need for it during the period of early pregnancy has persisted (Denamur *et al.*, 1973; Kann and Denamur, 1974).

One of the peculiar things about the luteotrophic action of prolactin is that in the rat, in which it is an essential luteotrophin, it has very little dose-related effect *in vitro* (Crisp, 1977; Wu and Wiest, 1978; Shiota and Wiest, 1979), while in the goat (Mohini *et al.*, 1980) and pig (Hammond *et al.*, 1980) in which it is not apparently essential [except possibly in mid to late pregnancy in the pig (du Mesnil du Buisson, 1973)], there is clear evidence of a dose-related stimulatory effect over a wide range of doses *in vitro*. Crisp (1977), for example, found that in luteinized rat granulosa cells in tissue culture, 0.10 µg of prolactin increased progesterone production more than did 0.01 µg, but 1.0 µg was no more effective than 0.10 µg. Wu and Wiest (1978) and Shiota and Wiest (1979) studied perfused rat CL cells and found that the major effect of prolactin was to preserve the cell's ability to produce progesterone at a low rate and to respond to the stimulating effect of LH on progesterone production. In human luteinized granulosa cells in tissue culture prolactin even had a dose-related inhibiting effect on progesterone production (McNatty *et al.*, 1974), although neutralization of the prolactin in the tissue culture medium reduced progesterone production (Table VI). In spite of the dose-related effect on pig and goat CL *in vitro*, prolactin has, in general, no effect at all on progesterone secretion by CL *in vitro* under conditions in which LH would have a clear dose-related stimulatory effect (Rothchild, 1966).

*In vivo* also one may see only a slight (Everett, 1944; Lam and Rothchild, 1977) or no (Yoshinaga *et al.*, 1967) indication of a dose-related effect on progesterone secretion; its action seems to be primarily all-or-none although there may be a dose-related effect on the duration of progesterone secretion (Malven *et al.*, 1967; Macdonald *et al.*, 1971).

TABLE VI  
*Various Direct and Indirect Indications of the Luteotrophic Action of Prolactin in Species Other Than the Rat*

Species	Type of effect or relation to CL function	Reference
Mink	PRL induces active phase of P secretion; 2-Br- $\alpha$ -ergocryptine prevents appearance of active ph.	Papke <i>et al.</i> (1980)
Ferret	Stalk section does not interrupt luteal ph. PRL treatment maintains P secretion after hypophysectomy	Donovan (1963) Murphy (1979)
Bandicoot	Only marsupial known with CL that secretes progesterone during lactation	Gemmell (1981)
Cow	Stalk section does not prevent maintenance of CL CL in whole organ perfusion increased progesterone production in presence of PRL	Henricks <i>et al.</i> (1969) Bartosik <i>et al.</i> (1967)
Sheep	Stalk section compatible with maintenance of CL, in hysterectomized sheep, beyond normal luteal ph.; hypophysectomy is not PRL plus LH (neither alone) maintain CL after hypophysectomy and hysterectomy PRL decreased luteolytic effect PGF LH alone did not maintain CL after complete hypophysectomy, and only in presence of PRL after incomplete hypophysectomy PRL increased progesterone release after hypophysectomy in mid luteal ph.	Denamur <i>et al.</i> (1973); Kann and Denamur (1974) Kann and Denamur (1974) Chamley <i>et al.</i> (1973) Schroff <i>et al.</i> (1971) Hixon and Clegg (1969)
Pig	Stalk section on day 70 of luteal ph. of hysterectomized pigs did not prevent maintenance of CL; PRL treatment after hypophysectomy on day 70 maintained CL $\times 10$ days PRL increased P production by GC luteinized in tissue culture Pituitary autotransplanted to kidney may have maintained CL longer than after hypophysectomy	du Mesnil du Buisson (1966, 1973) Hammond <i>et al.</i> (1980) Kraeling (1970)
Goat	PRL increased P production by CL cells <i>in vitro</i>	Mohini <i>et al.</i> (1980)
Rabbit	PRL in presence of estrogen or FSH increased P production	Spies <i>et al.</i> (1968b); Hilliard <i>et al.</i> (1968)
Human <sup>a</sup>	2-Br- $\alpha$ -ergocryptine decreased P levels in cyclic women Antiserum to PRL decreased P production by luteinized GC in tissue culture	Schulz <i>et al.</i> (1976, 1978) McNatty <i>et al.</i> (1974)

(Continued)

TABLE VI (Continued)

Species	Type of effect or relation to CL function	Reference
Monkey	P/circ. higher in lactating than nonlactating postpartum monkeys	Weiss <i>et al.</i> (1973)
	Evidence for 1' trophic effect summarized	Walsh <i>et al.</i> (1977)
	Bromocryptine + estrogen treatment induced luteolysis in cyclic monkeys	Castracane and Shaikh (1980)
	PRL treatment of postpartum monkeys increased P/circ.	Maneckjee <i>et al.</i> (1977)
	TRH treatment during luteal ph. increased P/circ.	Hagino and Kayama (1979)
Guinea pig	CL grow and secrete P after stalk section; PRL maintains CL growth after hypophysectomy	Illingworth and Perry (1971)
Dog	Treatment with 2-Br- $\alpha$ -ergocryptine in mid- to late luteal ph. depressed P secretion	Conconnon (see discussion)

" The human CL's response to prolactin is peculiar, because other evidence indicates that exposure to high levels of prolactin may depress progesterone secretion.

A variety of other CL-related effects of prolactin (Table VII), taken together with these, suggest that it is a *permissive* luteotrophin; that is, it permits the CL to secrete progesterone at a rate determined either by its intrinsic ability, or by whatever extrinsic luteotrophin it may have become sensitive to. This permissive action [which is implied in my earlier characterization of its effect as one that depresses the "activity of the enzymes that convert progesterone to androgens and estrogens" (Rothchild, 1965, p. 283), as well as in Lindner's (1979) description of it as an "inhibitory" hormone, and of Armstrong's (1969) and Raj and Moudgal's (1970) view of its principal action as that of depressing the activity of 20 $\alpha$ -OHSDH] may include protection of the CL against the luteolytic action of PGs (Table VII), or reduction in intraluteal PG production directly (a theoretical possibility) or through the maintenance of an optimal intraluteal progesterone concentration (Table VII). Blunting of PG induced luteolysis, however, may not be the only way its permissive (or other?) effects work. For example, we have recently found that indomethacin treatment does not prevent 2-Br- $\alpha$ -ergocryptine-induced luteolysis in hysterectomized pseudopregnant rats (Sanchez-Criado and Rothchild, 1981). This implies that (in the rat, at least) one of the steps in the biosynthetic pathway may have become completely dependent on prolactin, and will not proceed in its absence, even when PG synthesis is inhibited.

The idea that part of the luteotrophic effect of prolactin may be due to an ability to suppress PG production may seem to disagree with evidence that prolactin stimulates PG production in the mammary gland (Rillema,

TABLE VII  
*Some Effects of Prolactin Which May Help to Explain its Luteotrophic Effect*

Description of the effect	Reference
Prevents reduction of progesterone to 20- $\alpha$ OHP (rat)	Armstrong <i>et al.</i> (1969); Hashimoto and Wiest (1969); Lamprecht <i>et al.</i> (1969)
Prevents 5 $\alpha$ -reduction of progesterone (rat)	Armstrong <i>et al.</i> (1975); Dorrington (1977); Lahav <i>et al.</i> (1977a)
Prevents 17 $\alpha$ -hydroxylation of progesterone (rat)	D. C. Johnson (personal communication)
Increases synthesis of cholesterol (rat)	Everett (1947); Armstrong <i>et al.</i> (1969); Hashimoto and Wiest (1969); Behrman <i>et al.</i> (1971)
Prevents cholesterol accumulation (rat)	Everett (1947); Behrman <i>et al.</i> (1971); Guraya (1975)
Prevents cholesterol accumulation (rabbit)	Hilliard <i>et al.</i> (1968); Spies <i>et al.</i> (1968b)
Permits LH to increase progesterone production by CL <i>in vitro</i> (rat)	Armstrong <i>et al.</i> (1969)
Prevents PGF <sub>2<math>\alpha</math></sub> -induced luteolysis, increase in 20 $\alpha$ -OHSDH, and loss of LH receptors (rat)	Strauss and Stambaugh (1974); Lamprecht <i>et al.</i> (1975); Grinwich <i>et al.</i> (1976); Behrman <i>et al.</i> (1978)
Reduces but does not prevent luteolytic effect PGF <sub>2<math>\alpha</math></sub> in sheep	Chamley <i>et al.</i> (1973); Sasser <i>et al.</i> (1977)
Inhibits PMSG-, hCG-, or FSH-induced increase in PGF production by follicular GCs (rat)	Knazek <i>et al.</i> (1980)
Prevents PG-induced abortion in early pregnancy (rat)	Chatterjee (1973, 1976); Saksena and Lau (1978)
Prevents luteolytic effect of PMSG treatment (rat)	Hixon and Armstrong (1974)
Prevents luteolytic effect of LH (hamster)	Choudhary and Greenwald (1968)
Prevents luteolytic effect of LH (rat)	Krey and Everett (1973); Krey <i>et al.</i> (1973); Day and Birnbaumer (1980)
Prevents release of cathepsin D but increases amount bound to lysosome membranes (rat) <sup>a</sup>	Lahav <i>et al.</i> (1977b)
Prevents estrogen-induced luteolysis (rat)	Gibori and Keyes (1980)
Prevents estrogen production by luteinized GC in tissue culture (rat)	Crisp (1977)

<sup>a</sup> Effect may be mediated by progesterone.

1980) and possibly the mesentery (Horrobin, 1978). Even if such findings are confirmed, the fact that PGs induce luteolysis in rats, in which prolactin is very clearly an essential luteotrophin, the basic question is: what prevents prolactin from stimulating PG production in the CL? I can offer only the speculation that it is the very high concentration of progesterone in the CL that does this, and that probably also transforms the effect of prolactin on PG production into an inhibiting one either on

production or effect or both. The fact that prolactin *has* a luteolytic effect in the rat, but only on CL that cannot secrete progesterone (Desclin, 1949; Malven, 1969; Wuttke and Meites, 1971; Grandison and Meites, 1972; Lam and Rothchild, 1973) tends to support this supposition. If this is so, the action of prolactin on intraluteal PGs also differs from that of LH, because the latter can stimulate PG production in CL that are actively secreting progesterone (p. 240).

*The First Eutherian CL Was a Long-Lived CL.* Regardless of exactly how prolactin acts on the CL, it seems in general to protect the CL's ability to make progesterone, and thus slows down the rate of regression of progesterone secretion. When responsiveness to this action first appeared, therefore, it provided the progesterone that would be needed for a long intrauterine gestation. The accompanying change that made such a long gestation possible—uterine responsiveness to this progesterone—probably evolved very slowly, with the resulting increases in gestation length taking place in small steps; the tantalizing finding of a fossil *eutherian* with a pelvic girdle that seems to have articulated with marsupial epipubic bones (Kielan-Jaworowska, 1975) would support this interpretation. As intrauterine gestation increased in duration, it in turn provided conditions that facilitated the evolution of the trophoblast. But this could not have happened, as I have tried to show in the preceding discussion, without the prolongation of progesterone secretion, and the acquisition by the uterus of responsiveness to progesterone. The third change (possibly part of the latter one) that made a prolonged intrauterine gestation possible was one that protected the embryo against immunologic rejection by the mother. Evidence and arguments for the importance of trophoblast in this change are impressive (Lillegraven, 1975, 1979; Amoroso and Perry, 1975; Amoroso, 1979; Heap *et al.*, 1979; Beer and Billingham, 1979); I add only the essential point that the changes in progesterone secretion and in uterine responsiveness to progesterone must have occurred first and so must have set up the conditions that made the evolution of the highly developed trophoblastic placenta possible. The possible immunosuppressive effects of progesterone (e.g., Siiteri *et al.*, 1977; Beer and Billingham, 1979; Kincl and Ciacco, 1980), the ability of progesterone to also suppress PGs, the role of PGs in inflammation (Espey, 1980), and the connection between immunologic rejection and inflammation (Beer and Billingham, 1976) suggest that perhaps one of progesterone's effects on the uterus served as a bridge toward the evolution of the trophoblastic placenta.

If indeed the first change in what was until then a completely autonomous CL was responsiveness to prolactin, the first eutherians would have tended to evolve long-lived CL. As long as these CL remained otherwise autonomous, which, in this case, means unresponsive to any of the effects of LH, their life span would have been determined primarily by how

efficiently prolactin reduced the rate of regression. If the changes had begun in species that were already monestrous breeders, they would have tended to remain as such; if they began in polyestrous breeders, the lengthened luteal phase might have tended to transform them into monestrous breeders, but the potential for polyestrous breeding could have been retained, and may have been one of the factors that eventually led to the return to polyestry (see next section). In either case, the effect of prolactin would have led eventually to the evolution of a CL that secreted progesterone long enough for the embryo to complete the major part of its development within the uterus. In this evolution toward the pattern of viviparity among present day monestrous eutheria, I am assuming that if the trophoblast evolved into a hormone-secreting tissue, its hormones did not determine how long the CL secreted progesterone, and even if they raised its rate of secretion, as seems to be true, for example of the dog (Concannon *et al.*, 1977; Smith and MacDonald, 1974), the elevated levels were not essential for the maintenance of pregnancy (Concannon, 1980).

The evolution of the monestrous breeder's long-lived CL was probably facilitated by the relation of their CL to LH secretion, and to the uterus. During the course of evolution these CL probably eventually became responsive to other hormones as well as to prolactin; the most important of these was LH since it has both luteolytic and luteotrophic effects (p. 238). However, monestrous ovulation cycles are almost certainly associated with a relative deficiency of gonadotrophin secretion during the postovulatory period (e.g., Seal *et al.*, 1979) (Fig. 7), so these CL would not have been exposed, presumably, to enough LH to have shortened their life span. The dog's long luteal phase, for example, can be shortened considerably by treatment that raises the level of LH in the circulation (Jones *et al.*, 1973b). In a seasonal polyestrous breeder, as, e.g., the sheep, by contrast, the basal level of gonadotrophins in the circulation remains as high during the "anestrus" as during the breeding season (Yuthasastrakosol *et al.*, 1975; Walton *et al.*, 1977; Karsch *et al.*, 1980), and the CL formed at the last ovulation of each season, therefore, are still short-lived CL.

Various kinds of evidence indicate that the uterus does not have the luteolytic effect among the monestrous eutheria that it has in several of the polyestrous eutherian species. For example, in the dog and cat there is no connection between the uterine vein and ovarian artery of the kind the sheep has (Del Campo and Ginther, 1974) and hysterectomy of the dog, if anything, may shorten the luteal phase (Hadley, 1975). Hysterectomy has no effect on the histology and size of the CL during the luteal phase of the ferret (Deansley and Parkes, 1933; Deansley, 1967) or the European badger (Canivenc *et al.*, 1962), and it does not change the duration or pattern of progesterone secretion in the western spotted skunk

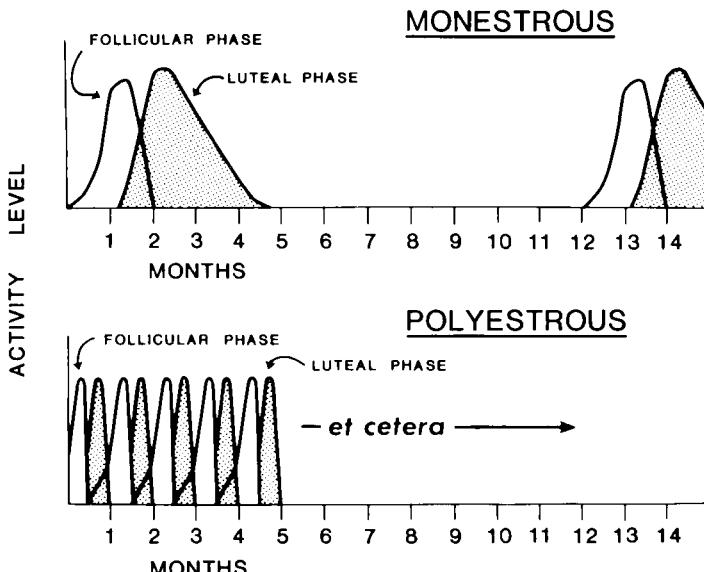


FIG. 7. Comparison of monestrous and polyestrous ovulation cycles among eutherian mammals. A major difference between the two types of ovulation cycles is in CNS control of the basal level of secretion of the gonadotrophins. Among monestrous breeders in general, seasonal and/or hereditary factors are responsible for a relatively long interval (6 months to over a year) between each period of increase in gonadotrophin secretion that leads to ovulation, and the anestrus between each ovulation is almost certainly due to a very low basal level of gonadotrophin secretion. Among polyestrous breeders, the basal level of gonadotrophin secretion remains at a higher level than in the monestrous breeders throughout the year or during the breeding season, and frequent surges (i.e., at intervals of about 2–4 weeks as a rule) of gonadotrophin secretion lead to frequent ovulations. Since LH has a luteolytic as well as a luteotrophic effect, one of the reasons for the difference between monestrous and polyestrous breeders in the length of the luteal phase is postulated to be the relative absence of LH in the monestrous breeders after each ovulation.

(Mead and Swannock, 1978). The lack of any difference in the duration of the luteal phase between the pregnant and nonpregnant dog (Smith and MacDonald, 1974; Concannon *et al.*, 1975), wolf (Seal *et al.*, 1979), mink (Canivenc *et al.*, 1966; Møller, 1973a), ferret (Carlson and Rust, 1969; Heap and Hammond, 1974; Blatchley and Donovan, 1976), roe deer (Hoffmann *et al.*, 1978), and only a small one between pregnancy and pseudopregnancy in the cat (Verhage *et al.*, 1976) also suggest that the uterus does not affect the duration of progesterone secretion in monestrous breeders. Their failure to evolve a luteolytically active uterus thus also contributed to the evolution of the long-lived CL.

Thus, the capacity for autonomous progesterone secretion, protected by the action of prolactin, the reduction of the luteolytic potential of LH,

and the absence of a luteolytic uterus combined to produce a luteal phase long enough for most of intrauterine embryogenesis, and there was no selection pressure among the monestrous breeders, therefore, to evolve an endocrine placenta, or to become dependent on it for the maintenance of progesterone secretion during pregnancy. As in the marsupial, but for quite different reasons, the duration of pregnancy thus remained essentially the same as that of the luteal phase itself (Fig. 8).

## 2. *Polyestrous Breeding and the Evolution of the Short-Lived CL*

a. *The Pivotal Role of LH.* Species with short-lived CL and polyestrous breeding may have arisen directly from the polyestrous pantothenes but were more likely to have arisen from the early monestrous eutheria. The trophoblast-bearing placenta, as it slowly evolved among the latter species, almost certainly also evolved the ability to secrete hormones with luteotropic and/or antiluteolytic properties. If this occurred among species with a tendency toward polyestrous ovulation cycles, and if they also evolved a CL which could respond to the effects of LH, the combination of these three things might have made it possible for them to return to polyestrous breeding and still retain a eutherian form of viviparity. The reasons for this are in the following discussion.

The essence of the difference between monestrous and polyestrous ovulation cycles is in the CNS control of gonadotrophin secretion. Polyestrous ovulation cycles are the result of a CNS control that induces a higher basal rate of LH and FSH secretion throughout the year than occurs in species with monestrous ovulation cycles. In the latter, the basal level of gonadotrophin secretion becomes very low soon after ovulation, and does not rise again to the equivalent of the polyestrous breeders' basal level until the next breeding period (Fig. 7). The cyclic character of gonadotrophin secretion among polyestrous breeders follows from interactions between the ovaries, pituitary, and CNS (see Karsch *et al.*, 1979, 1980, for example). The general importance of polyestry for CL function, therefore, is this: if the CL can respond to LH, its luteolytic effect (Table VIII) and the relatively high basal level of its secretion would tend to shorten the duration of the luteal phase. LH's luteolytic effect, I suspect, is probably much older in phylogeny than its luteotropic effect. Its primary role in vertebrate reproduction may have been the stimulation of intrafollicular PG production, and among the reptiles and mammals it also released inhibition over the granulosa cells' ability to make progesterone. The former effect thus caused ovulation itself, and the latter the formation of the CL. It is also able to stimulate PG production in luteinized granulosa cells, however (Table VIII), and because of this, it became an important cause of luteolysis and therefore an important element in the evolution of

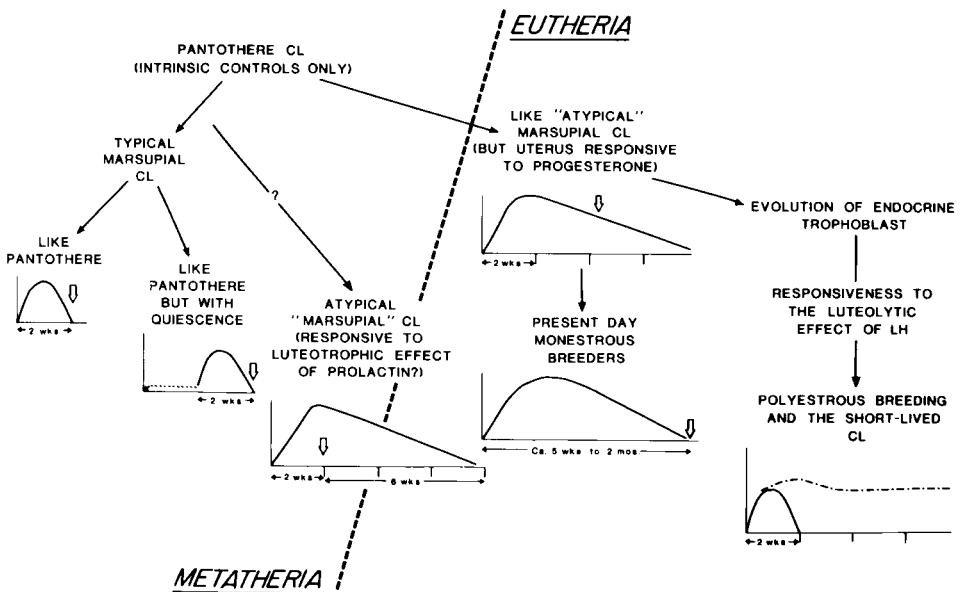


FIG. 8. The evolution of the mammalian CL in relation to the pattern of viviparity. The information in this figure continues from where Fig. 6 left off, and is intended to make the transition from metatherian (marsupial) to eutherian viviparity and CL regulation more understandable. The broken line represents the dichotomy between marsupial and eutherian viviparity. Luteal phase patterns are shown by the curves with time on the abscissa and progesterone secretion on the ordinate. The open arrows represent the time of parturition. The fine lines ending in arrows represent probable lines of descent and/or progression of change. Among the marsupials, intrauterine gestation remained very short, probably not much different from that in the pantothere ancestor; the young are always born in an extremely altricial (immature) state, even among the bandicoots (third curve from the top on the metatherian side), which have long-lived CL (Gemmell, 1981). The exact path of evolution of the bandicoot CL is unknown. The first eutherian CL (upper curve to right of the broken line) is presumed to have been like the bandicoots, and may have arisen from a pantothere ancestor (as shown), or from a bandicoot-like marsupial ancestor (not shown). Its longer life span is believed to have been the result of responsiveness to the luteotrophic effect of prolactin. As the uterus evolved responsiveness to the effect of the prolonged secretion of progesterone, the time of parturition was postponed and the duration of embryonic development increased; this led eventually to the pattern of viviparity seen in modern monestrous breeders. This progression also gave rise to the fully developed trophoblastic placenta, and with it, both the protection of the embryo against immune rejection by the mother, and the secretion of placental luteotrophic/antiluteolytic hormones. With the evolution of the endocrine placenta, therefore, polyestrous breeding, which was otherwise incompatible with a prolonged intrauterine gestation (because of the shortness of the luteal phase) became possible, since the placental hormones were secreted into the maternal bloodstream after implantation; their action on the CL prevented regression and prolonged the secretion of progesterone (lowermost curve on the right). Because of the prolonging effect of prolactin on CL activity, the return to the short luteal phase of the polyestrous ovulation cycle is believed to have occurred through the evolution of responsiveness to the luteolytic effect of LH. The appearance of responsiveness to the luteotrophic effect of LH also helped to make the short-lived CL compatible with eutherian viviparity; eventually this effect of LH predominated over the CL's capacity for autonomous progesterone secretion.

TABLE VIII  
*Evidence Suggesting or Showing the Luteolytic Effect of LH (or Related Hormones,  
such as PMSG, hCG)<sup>a</sup>*

Species	Remarks	Reference
Stimulation of intraluteal PG production		
Rat	LH increased PGF production by CL in organ culture	Demers <i>et al.</i> (1973)
	PMSG-induced luteolysis <i>in vivo</i> inhibited by indomethacin	Basu and Chatterton (1978)
Rabbit	LH doubled PGF production by luteinized GC in tissue culture	Erickson <i>et al.</i> (1977)
Pig	LH increased PGF production by CL <i>in vitro</i>	Guthrie <i>et al.</i> (1979)
Cow	LH increased PGF production <i>in vitro</i> by late luteal ph. CL	Shemesh and Hansel (1975c)
Rat, others	LH frees arachidonic acid from cholesterol esters by activating cholesterol esterases	Kuehl (1974)
Desensitization and related effects		
Rabbit	LH (or hCG) on day 9 psp. causes luteolysis	Spies <i>et al.</i> (1967b); Kelly and Stormshak (1969); Flint <i>et al.</i> (1974); Hunzicker-Dunn and Birnbaumer (1976)
Rat	LH (or hCG) after day 6 causes luteolysis in PMSG-hCG treated immat.	Catt <i>et al.</i> (1979)
Other effects		
Monkey	In presence of LH, PGs had only a luteolytic effect on luteinized GC in tissue culture	Channing (1972)
Rat	LH treatment of hypophysectomized pituitary transplanted or PRL-injected rat reduced duration of P secretion	Rothchild (1965); Macdonald <i>et al.</i> (1970)
	LH treatment [like PGF (Lamprecht <i>et al.</i> , 1975)] increased 20 $\alpha$ -OHSDH activity in CL	Hashimoto and Wiest (1969); Rodway and Kuhn (1975)
	LH decreased 3 $\beta$ -OHSDH activity in CL	Lawrence <i>et al.</i> (1978)
Sheep	LH treatment on day 12 o.c. increased estrogen production <sup>b</sup>	Baird <i>et al.</i> (1976)
Hamster	LH causes rapid luteolysis of o.c. CL	Choudhary and Greenwald (1968)
Dog	Inverse relation between plasma LH levels and duration of P secretion	Jones <i>et al.</i> (1973b)
Rabbit	Estrogens prolong CL activity after hypophysectomy but not in intact; LH prevents prolonging effect of estrogens in hypophysectomized rabbits	Spies <i>et al.</i> (1968a)

<sup>a</sup> See also Rothchild (1965, p. 277).

<sup>b</sup> Estrogens are luteolytic in sheep during late luteal phase (Horton and Poyser, 1976; Kann and Denamur, 1974).

eutherian polyestry and the short-lived CL. The selective advantage of the luteolytic effect of LH was that in cycles in which ovulation was not followed by conception, it helped to keep the luteal phase short, and since progesterone inhibits ovulation (Rothchild, 1965), this kept the interval to the next ovulation no longer than the length of such a luteal phase plus whatever time was needed for the completion of follicular growth.

This speculation does not mean that the luteolytic action of LH in modern polyestrous eutheria is the *only* factor responsible for initiating regression of the CL. During the course of evolution, other factors, in addition to or in place of LH, became incorporated into the luteolytic component of the basis for polyestrous breeding among some of the modern eutheria, but I think that in the *early* eutheria, prolactin had blunted the effectiveness of the intrinsic process, and responsiveness to the luteolytic effect of LH, therefore, was probably the change that had the greatest chance of succeeding as a counterbalance to its effect; it thus served as the first step in the evolution of other luteolytic mechanisms.

Polyestrous breeding, because it was inseparable from the short-lived CL, was also incompatible with eutherian viviparity except under the condition that the embryo's trophoblast hormones could prevent at least the luteolytic effect of LH and thus delay the onset of regression of the CL. My speculations to this point suggest that the sequence of evolutionary changes that allowed this to happen was as follows. The responsiveness of the pantothere CL to prolactin led to the evolution of the long-lived CL. This, among other things, made the evolution of the trophoblast placenta possible, and eventually, also, the evolution of luteotrophic/antiluteolytic hormone secretion by the trophoblast. When responsiveness to the luteolytic effect of LH then appeared in the CL of these eutheria, it made *both* the return to polyestry and eutherian viviparity possible, because the secretion of hormones by the trophoblast had evolved *before* responsiveness of the CL to the luteolytic effect of LH.

Successful reproduction in the early polyestrous eutheria thus came to depend crucially on the hormones secreted by the trophoblast, but the efficacy of these hormones must obviously also have hinged on the CL's ability to remain responsive to them until they were secreted into the bloodstream. This interval would have been determined by the time it took for the zygote to reach the uterus and implant there, and, in general, it could not have been much less than 1 week from ovulation; more than this would probably have been required for the amount of trophoblast hormone in the circulation to reach a level of dependable effectiveness. The early polyestrous eutheria, therefore, must have also possessed mechanisms that could prolong the plateau phase of the CL's life cycle at least enough for the CL to remain responsive to the trophoblast hormones when these finally reached it.

The blunting action of prolactin on both the luteolytic effect of LH and the intrinsic luteolytic process must have been one of these mechanisms; it was probably not until much later, as other control systems evolved, that the CL of some species lost their original dependency on it. Another change which eventually became a prominent element of the luteotrophic process among the polyestrous eutheria, occurred, I suspect, around the time these changes were taking place. This was the appearance of responsiveness to the *luteotrophic* effect of LH.<sup>4</sup>

The many ways by which LH facilitates progesterone secretion by the CL (see Rothchild, 1965; Armstrong, 1968; Savard, 1973; Dorrington, 1977; Channing and Tsafiriri, 1977; Birnbaumer *et al.*, 1979, for reviews) make it fairly clear that LH acts quite differently from prolactin. Even though it seems to act permissively in some conditions (Table II), its action *in vitro* on the CL of so many species is so clearly dose-related, while that of prolactin, as already noted, is not, that it is best characterized, as far as its general luteotrophic effects are concerned, as a *stimulatory luteotrophin*. It probably works simply by raising the rate of progesterone production (see, e.g., Armstrong, 1968), while prolactin, by contrast, presumably has no direct effect on the rate, but simply protects the process of production from interference, and so allows it to continue. Neither, according to the theory, changes the basic nature of the process itself.

The two effects, in fact, probably account for most of the examples of synergism between prolactin and LH shown in Table VI. The action of LH is not synonymous or even compatible with a prolonged luteotrophic effect, but even a short one, if it increased the rate of progesterone secretion, would be valuable in postponing the onset of regression, because of the suppressing effect of progesterone on intraluteal PG production. This effect, therefore, helped to ensure survival of the CL during the interval before the trophoblast hormones reached it.

Thus, the first polyestrous eutheria started out with a CL, which, in addition to the intrinsic controls inherited from the pantothere ancestor, had become responsive to three things that could affect those controls: prolactin's protective action on, and the stimulating effect of LH on the rate of progesterone secretion; and the facilitating action of LH on intra-

<sup>4</sup> It is worth noting at this point, that, if the dog and ferret are typical, the long-lived CL of monestrous breeders never became crucially dependent on LH as a luteotrophin, although the CL may respond to it (Concannon, 1980); in the dog, treatment with LHAS even late in the luteal phase does not stop progesterone secretion (P. W. Concannon, personal communication), and in the ferret, prolactin alone will maintain the CL after hypophysectomy (Murphy, 1979) (see also the discussion of the relation between the need for LH and the luteolytic effect of LH, in the rat, p. 256).

luteal PG production. Combined, these effects resulted in a luteal phase of about 2 weeks, long enough for the zygote to reach the uterus, implant, and secrete its hormones into the mother's bloodstream, yet short enough for the animal to enjoy the selective advantage of polyestrous breeding.

Through a long evolutionary digression,<sup>5</sup> the polyestrous eutheria thus ended up with a CL which had a life span, determined by the balance between intrinsic and extrinsic controls, not much different from that of their pantothere ancestors or marsupial cousins, whose CL had a life span determined almost entirely by intrinsic controls. The digression, however, had permitted the evolution of the endocrine placenta. The short luteal phase, therefore, no longer put a limit, as it did in the marsupials, on the ways in which intrauterine viviparity could be practiced.

Responsiveness to the luteotrophic effect of LH, I would guess, was probably of the greatest importance in the balance between the forces promoting progesterone secretion and those promoting PG production within the CL, and eutherian viviparity among polyestrous breeders owed a significant part of its success as a way of reproduction to this effect. It thus became fixed in the genome of the polyestrous eutheria. LH seems to have the characteristics of a universal luteotrophin, not because it really is one, but because we have been more aware of sheep, pigs, cows, guinea pigs, people, than we have been of kangaroos and brush-tailed possums, or of minks, skunks, ferrets, dogs, and other such mammals, in which it is probably not an essential luteotrophin.

*b. How the CL of Modern Polyestrous Eutheria Came to Differ in the Extrinsic Factors That Regulate Their Activity.* Most of these differences probably arose as the result of the spreading apart of certain traits, once common to all, through the process of selection. The most important of these probably were: the relation between the critical intraluteal progesterone concentration and the switch-on of the increase in intraluteal PG production; the responsivity to the luteolytic effect of LH; and the balance between autonomous progesterone secretion and a dependency on the luteotrophic actions of LH and prolactin. Selection could have led, eventually, to species which differed enough from each other in each of

<sup>5</sup> It is, of course, theoretically possible that the short-lived CL of the polyestrous eutheria could have arisen directly from the pantothere CL. However, since the latter was presumably entirely autonomous to start with, such a transition could have been associated with eutherian viviparity only if it occurred simultaneously with the appearance of responsiveness of the CL to pituitary and trophoblastic luteotrophins, of hormone secretion by the trophoblast, of the ability of trophoblast to protect the embryo against immune rejection by the mother, and of responsiveness of the uterus to the progesterone secreted by the CL. It is more probable, therefore, that these changes occurred sequentially, as I have suggested above, than simultaneously.

these traits to account for most if not all of the diversity we see today in the regulation of the CL. For example, at one extreme might be species like the rat, in which responsiveness to the luteolytic effect of LH increased so far above the norm, while the level at which the intraluteal progesterone concentration switched on the increase in PG production decreased so far below the norm, that these changes become responsible for the evolution of the ultra-short luteal phase (discussed in detail in the next section).

At the other extreme, selection could have led to species with CL so insensitive to the luteolytic effect of LH that the switch-on of intraluteal PG production would again have come to depend entirely on the intrinsic controlling factors, as in their pantothere ancestors. In these species, however, prolactin was now presumably also controlling progesterone secretion, and its action would have reduced the effectiveness of the intrinsic controls, and so would have prolonged the regression phase of the CL life cycle. Such species (perhaps the roe deer is an example) might, therefore, have returned to a long luteal phase, similar to that of the monestrous breeders. Most, however, probably remained polyestrous because of the evolution of a connection between the venous drainage of the uterus and the arterial circulation to the ovary (Ginther, 1974). In these species, the uterine PGs acted in place of LH as the agent that switched on intraluteal PG production or may even have supplanted the latter, in the process that kept the CL short-lived. The view that species evolved intraluteal PG production as a compensatory trait, arising from the lack of such a uterus-related mechanism of luteolysis induction, is probably the exact opposite of what actually happened, an interpretation already hinted at by Patek and Watson (1976).

Among all the eutheria, at least two, possibly three things, in addition to the intrinsic controls themselves (as in the theory), seem to have evolved, that lead to the switch-on of intraluteal PGs, and that affect the rate of the regression phase. These were LH, the uterine PGs, and possibly estrogens (either intraluteal or follicular). Examples of the relative importance of the intrinsic controls, LH, and the uterine PGs can be seen by comparing their effects in the rat, and by comparing the rat with the guinea pig and the dog (Figs. 9 and 10). In the dog, intrinsic controls alone seem to be responsible for the switch-on of luteal PGs but since the CL's secretion of progesterone is maintained by an extrinsic luteotrophic stimulus (probably prolactin), regression is a slow, long drawn-out process (Figs. 9 and 10A). In the guinea pig (as a representative of species like the sheep, pig, cow) the action of the uterine PGs, either alone or with the switched-on intrinsic PGs, induces the rapid rate of regression typical of the short-lived CL (Fig. 9); in their absence, as after hysterectomy, the rate of regression

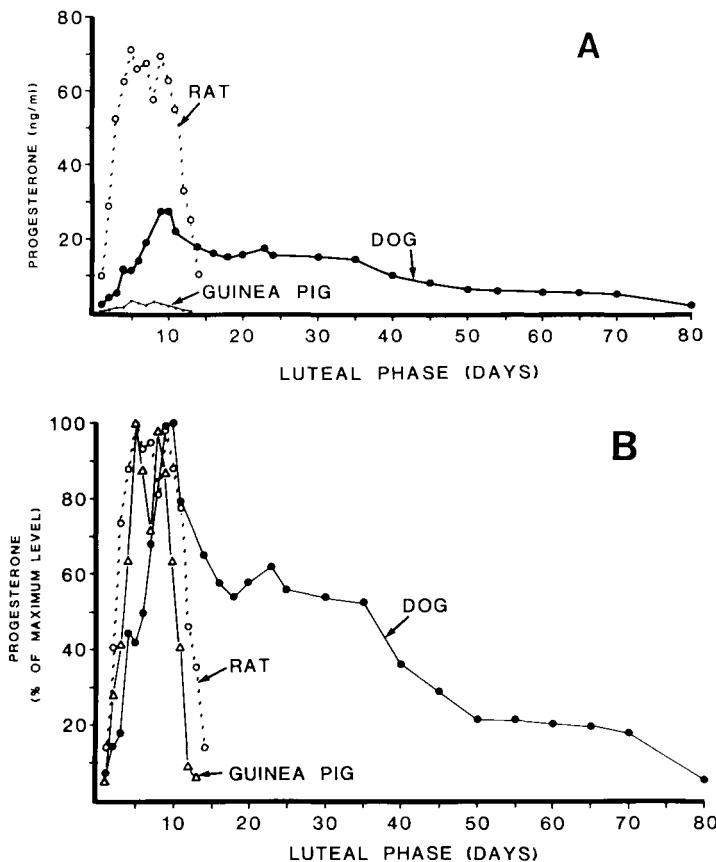


FIG. 9. A comparison of the pattern of progesterone secretion in three mammals. (A) The short luteal phase of the guinea pig and of the pseudopregnant rat, and the long luteal phase of the nonpregnant dog, are compared, in terms of actual levels of progesterone in peripheral serum or plasma. (B) The same values are expressed as the percentage of the maximum value. (Rat data are from Pepe and Rothchild, 1974; guinea pig data are from Blatchley *et al.*, 1976; dog data are from Smith and MacDonald, 1974.)

would be determined only by the intrinsic process, and, as in the dog, therefore, regression is a very slow, long drawn-out process (Fig. 10A). In the rat, LH, the uterine PGs, and the intrinsic controls seem to be involved (Figs. 9 and 10B). In the human, estrogens and intrinsic controls may have come to have the same importance that LH, the uterine PGs, and the intrinsic controls have in the rat.

In the evolution of changes in the balance between the capacity for autonomous progesterone secretion and a dependency on the luteotrophic

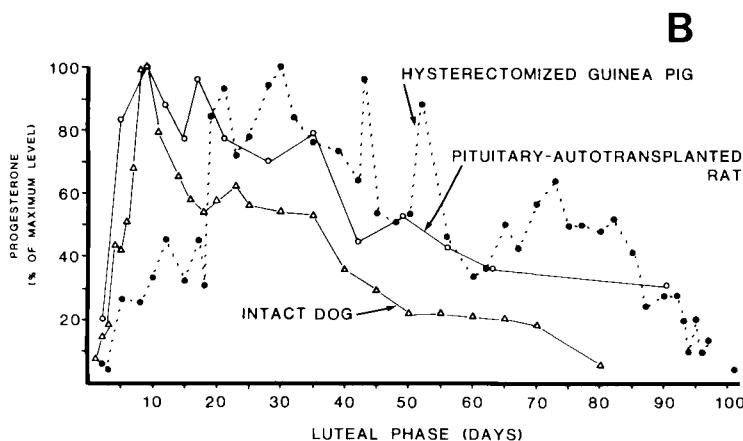
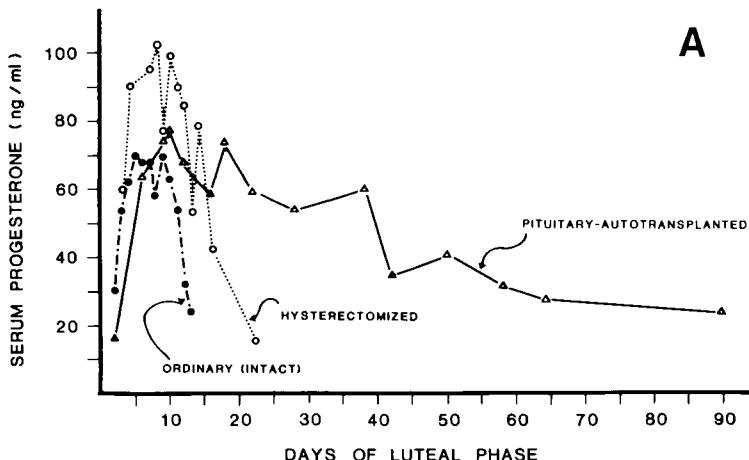


FIG. 10. The factors affecting the duration of the CL's life span in the nonpregnant animal. (A) The ordinary (intact) pseudopregnant rat, compared with the hysterectomized pseudopregnant rat, and with the hypophysectomized pituitary-autotransplanted rat. (B) The hysterectomized guinea pig compared with the pituitary-autotransplanted rat and the intact dog (continuation of Fig. 9, with the values expressed as percentage of maximum). The two figures illustrate the principle that regression is inevitable because of the action of intrinsically produced PGs, but that the rate of regression can depend on how rapidly the CL produces PGs, and on the presence of an extrinsic source of PGs. In the intact rat (A), three sources of PGs affect the CL: those produced intrinsically, those produced in response to LH, and those produced in the uterus. Regression is thus rapid. In the hysterectomized rat the rate of regression is reduced because the uterine PGs are absent. In the pituitary-autotransplanted rat almost no LH is secreted, and the uterus, subjected only to the action of progesterone, produces almost no PGs; thus regression is very slow under the influence of only the intrinsically produced PGs. In the intact dog (B), neither the uterus nor LH seems to be involved in the regression of the CL, and a similar slow regression occurs. In the hysterectomized guinea pig (B), the absence of the uterine PGs presumably also leaves only the intrinsically produced PGs as the cause of regression. Hysterectomized guinea pig data from Horton and Poyser (1976); pituitary-autotransplanted rat data from Ochiai *et al.* (1981).

effects of prolactin and LH, at least one general trend seems to be discernible. This is a reduction in autonomy, and an increase in dependency on LH; this change seems also to have been accompanied by a reduction in or loss of dependency on the luteotrophic effect of prolactin.

Among some species, as, e.g., the pig or sheep, the capacity for autonomous progesterone secretion has remained almost as high as the CL's maximum capacity for progesterone secretion, and one sees little difference in the pattern of progesterone secretion, therefore, between an intact animal and one hypophysectomized shortly after ovulation. In others, as, e.g., the human, the capacity for autonomous progesterone secretion probably accounts for only a very small fraction of the CL's maximum capacity for progesterone secretion, but the latter can be realized through the luteotrophic action of LH. In the rat (discussed in detail in the next section) the capacity for autonomous progesterone secretion may be intermediate between these extremes, but can be expressed only through the permissive effects of prolactin; maximum capacity for progesterone secretion differs from the capacity for autonomous secretion only after about day 9 of the luteal phase, and so depends on the luteotrophic effects of LH to be realized after this time.

### C. THE BASIC PRINCIPLE UNDERLYING DIVERSITY OF CL REGULATION

This is probably no different from that underlying diversity in the control of many other physiological processes. For example, the maintenance of a constant body temperature among warm-blooded vertebrates came about through a primary change in the hypothalamus which allowed it to recognize deviations from a given core temperature and to correct them by whatever means were available. The fact that all species do not use the same body processes in this regulation does not confuse us at all, because we recognize that the basic and common element in the regulation of body temperature is the organization of the hypothalamus and not the particular body process through which body heat is lost, conserved, or increased. An exactly parallel situation in the case of the CL, i.e., the use of either prolactin, or LH, or estrogens, or no extrinsic control at all, in the stimulation of progesterone secretion, by different species, confuses us because we have not understood what the basic controlling system was. The basic principle behind the diversity in CL regulation is the evolution of processes that could work through the basic system in a way that was compatible with mammalian viviparity, and particularly, eutherian viviparity. The theory describing this basic system may come close to what it actually is, and may help, perhaps, to reduce some of the confusion in our

understanding of the CL. Although not *all* of the peculiarities of the CL described in the Introduction are immediately understandable with the help of this theory, one example of its usefulness is the interpretation of the ultra-short luteal phase and its peculiar relation to the development of LH dependency. This is discussed in the next section.

#### IV. The Ultra-Short-Lived CL and LH Dependency

The ultra-short-lived CL is the third variety of eutherian CL (Fig. 2). Its characteristics have been most thoroughly documented in the rat; I will refer to its main features and the peculiarities of its dependency on LH, using the rat as the type example. From work done on the need for LH as well as for prolactin in the hamster and mouse (Greenwald and Rothchild, 1968; Munshi *et al.*, 1973; Ford and Yoshinaga 1975d; Mukku and Moudgal, 1975; Terranova and Greenwald, 1978, 1979a,b) and on the need for prolactin in the vole (Breed and Clarke, 1970; Milligan, 1975; Charlton *et al.*, 1978) and gerbil (Rich, 1968), it is clear that the rat is fairly representative of the group.

The ovulation cycle is 4 or 5 days long; if the day of ovulation is taken as day 1, the CL is fully organized (although not yet fully grown) by day 2 (Long and Evans, 1922; Mossman and Duke, 1973; Terranova *et al.*, 1980) and the peak of progesterone secretion occurs during the night between days 2 and 3 of a 4-day cycle (Uchida *et al.*, 1970b; Butcher *et al.*, 1974; Hiroyoshi and Suzuki, 1974; Smith *et al.*, 1975; van der Schoot and de Greef, 1976; de Greef and van der Schoot, 1979; Nequin *et al.*, 1979). Between day 3 and day 4 (proestrous of a 4-day cycle) the CL stops secreting progesterone and begins a slow regression in size.

Prolactin transforms this CL into one equivalent to the short-lived CL of the other polyestrous eutheria (Rothchild, 1965; Greenwald and Rothchild, 1968; Hilliard, 1973) but responsiveness to this effect does not appear until the morning of day 3 (Döhler and Wuttke, 1974; Smith *et al.*, 1975; Day *et al.*, 1980); the CL then remain dependent on prolactin to the end of the luteal phase ("pseudopregnancy"). Not enough prolactin is secreted during the interval between ovulations to have this effect, but copulation, or an equivalent stimulus (Long and Evans, 1922), induces a large enough increase in its secretion, which occurs in the form of two large daily surges (see Table II, under "rat") to transform the CL into the short-lived type.

When the CL are 8 or 9 days old, LH dependency becomes as important as the dependency on prolactin; the need for LH persists to the end of the luteal phase in pseudopregnant rats but only until day 12 in pregnant ones (Raj and Moudgal, 1970; Morishige and Rothchild, 1974; Akaka *et al.*, 1977). The peculiar appearance of LH dependency only after the first

week of the CL's life cycle may be an integral part of what accounts for the ultra-short life of the CL itself. To show this connection I will first enlarge on the main characteristics of LH dependency, so far only briefly described in the Introduction. Since a good part of the information comes from work done in our own laboratory, a description of methods and related material would be in order.

#### A. METHODS

The induction of luteolysis by a single injection of 0.5 ml of a horse antiserum to beef LH (LHAS) was taken as evidence for LH dependency. The antiserum is specific for LH and the dose is more than enough to neutralize the available LH in the circulation (Morishige and Rothchild, 1974). If this treatment did not induce luteolysis, we assumed that the CL were not dependent on LH. The primary indication of luteolysis was a fall in the rate of progesterone secretion [determined by the change in the peripheral serum level, since the latter reflects the change in secretion rate (Pepe and Rothchild, 1973)] within 72 hours of the LHAS injection (Fig. 11) to one too low to prevent ovulation or to maintain pregnancy or

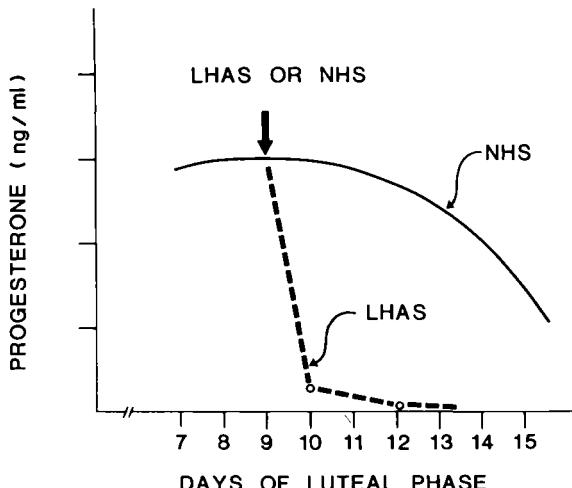


FIG. 11. The test for LH dependency in the rat. On the chosen day (e.g., day 9 in the figure), the rat is injected with either an antiserum to LH (LHAS) or with normal horse serum (NHS), and the serum progesterone level is determined from samples collected just before the injection, and 24 and 72 hours afterward. If the progesterone level falls, as shown, and is accompanied by a shortening of the vaginal diestrus, abortion, or regression of decidual tissue in the uterus (depending, of course, on the kind of rat the test is done on), the CL are judged to be LH-dependent. If the progesterone level in response to the LHAS injection is not different from that of the rats that received NHS, and if no change occurs in the length of the vaginal diestrus, etc., the CL are judged to be LH-independent.

decidual tissue (DT) growth in the uterus (Morishige and Rothchild, 1974; Rothchild *et al.*, 1974).

We also used the transplanted rat pituitary's ability to secrete prolactin (Everett, 1956; Meites *et al.*, 1972) to study prolactin's effect on LH dependency. The peripheral serum level of prolactin in a rat bearing a single pituitary transplant beneath the kidney capsule was about the same as the peak level of the two daily surges secreted by the *in situ* pituitary of the pregnant or pseudopregnant rat (Lam and Rothchild, 1977), and was fairly constant throughout the day (de Greef and Zeilmaker, 1978). In a rat bearing a pituitary transplant, therefore, the level of prolactin secretion was higher than in a pregnant or pseudopregnant rat secreting prolactin only by its *in situ* pituitary (Garris and Rothchild, 1980; Nanes *et al.*, 1980). The amount of prolactin secreted was also almost directly proportional to the number of pituitaries transplanted (Lam and Rothchild, 1977).

The role of LH in the induction of LH dependency was studied in two general ways. One was by the effects of hypophysectomy and pituitary auto- or homotransplantation. This reduces the secretion of LH markedly (e.g., see Lam and Rothchild, 1977) at the same time that it increases the secretion of prolactin. The other was by treating rats daily with LHAS for various periods of time, starting at different times in the CL's life cycle (Lam and Rothchild, 1977; Nanes and Rothchild, 1981). The effects of treatment with LH in the pituitary transplanted rat also helped to define some aspects of the action of LH.

Since almost all the information about LH dependency comes from experiments done on pseudopregnant or pregnant rats, the following background material may also be helpful. An *ordinary* pseudopregnancy lasts for about 13 days, and pregnancy for 23 days. Hysterectomy at any time on or before day 10 of pseudopregnancy prolongs pseudopregnancy to a mean duration of about 20 days; DT induction by scratching the uterus on day 5 has the same effect (Rothchild, 1965, p. 283). The pseudopregnancy of lactation also lasts for about 3 weeks, if the litter is a large one, but for about 16 days if the litter is less than 3 pups (Rothchild, 1960). Pregnancy in lactating rats lasts longer than in nonlactating rats, by an interval (about 1 week) equivalent to the delay in implantation induced by lactation (e.g., Yoge and Terkel, 1978).

## B. THE MAIN CHARACTERISTICS OF LH DEPENDENCY

LH dependency probably develops primarily because of an effect of LH exerted directly on the CL (Lam and Rothchild, 1977; Nanes and Rothchild, 1981). As a result of this effect, LH dependency appears by the morning of day 8 in pregnant rats (Morishige and Rothchild, 1974) and by

the next day in pseudopregnant ones (Rothchild *et al.*, 1974; Garris *et al.*, 1980). In the presence of the inducing action of LH from day 1 onward, an excess secretion of prolactin (e.g., by one or more pituitary transplants) can postpone LH dependency (Lam and Rothchild, 1977; Garris and Rothchild, 1980) but not to beyond day 12 (Nanes *et al.*, 1980), and the progestational uterus (Garris and Rothchild, 1980), especially if it contains implanting blastocysts (Morishige and Rothchild, 1974), can advance the time of appearance of LH dependency, but not to before day 8 (Fig. 12). If the inducing action of LH is delayed, LH dependency is delayed (Veomett and Daniel, 1971, 1975a,b,c; Rothchild *et al.*, 1974; Ford and Yoshinaga, 1975a,b; Lam and Rothchild, 1977; Nanes and Rothchild, 1981), and if it is permanently prevented, LH dependency is also prevented (Lam and Rothchild, 1977; Ochiai *et al.*, 1981). The inducing action of LH on LH dependency is over a threshold effect by day 5; i.e., the CL will become LH-dependent by day 9 even if they are no longer exposed to LH after day 5, if they have been exposed to LH until day 5 (Lam and Rothchild, 1977; Nanes and Rothchild, 1981).

The time of appearance of LH dependency corresponds closely to the transition from the plateau to the regression phase of the CL's life cycle. This is obvious in the case of ordinary pseudopregnant rats, in which regression clearly begins about day 9 (Pepe and Rothchild, 1974); it is less

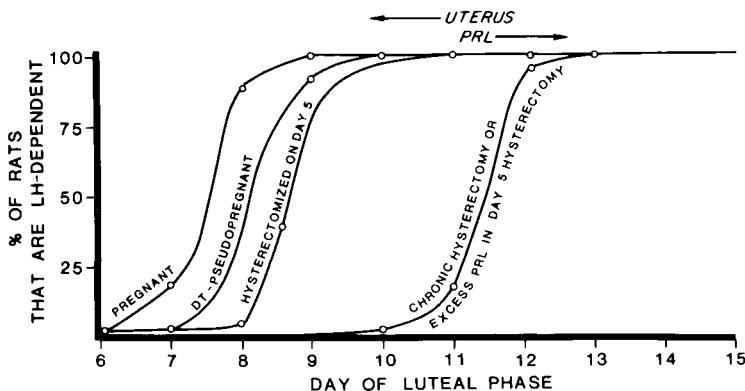


FIG. 12. The pattern of appearance of LH dependency in various types of luteal phase conditions in the rat. The values are the incidence of rats with LH-dependent CL, in relation to the days of the luteal phase. Among pregnant rats, all will have LH-dependent CL by days 8 to 9. DT-bearing pseudopregnant rats will all have LH-dependent CL by days 9 to 10, and rats hysterectomized on day 5 will all become LH-dependent about a day later. Hysterectomy before day 5, or treatment with an excess of prolactin, postpones the appearance of LH dependency to about day 12. The figure illustrates the principle that the uterus and prolactin do not affect the intensity of the need for LH but only *when* the full intensity is reached (Nanes *et al.*, 1980).

clear in the case of DT-bearing or hysterectomized rats because their regression phase lasts longer than in ordinary pseudopregnant rats (Pepe and Rothchild, 1974; de Greef *et al.*, 1976) and the transition from plateau to regression is less easy to see. LH dependency appears in pregnant rats' CL when regression *would* have started if they were not pregnant; the hormonal influence of the placenta prevents regression but does not prevent LH dependency until after day 12 (Raj and Moudgal, 1970; Morishige and Rothchild, 1974).

The mutually opposing actions of the pregestational uterus and prolactin on LH dependency (Lam and Rothchild, 1977) are related to one another quantitatively (Garris and Rothchild, 1980). In the absence of an excess of prolactin, the advancing action of the uterus on LH dependency is over a threshold effect by day 5, and in the presence of an excess of prolactin, by day 8 (Garris and Rothchild, 1980) (Fig. 13). DT, or the change in the uterus associated with the DT-inducing stimulus (e.g., needle scratch of the endometrium), advances the time of appearance of LH dependency more than does the pregestational uterus of the ordinary pseudopregnant rat (Rothchild *et al.*, 1974); this is a specific effect of DT,

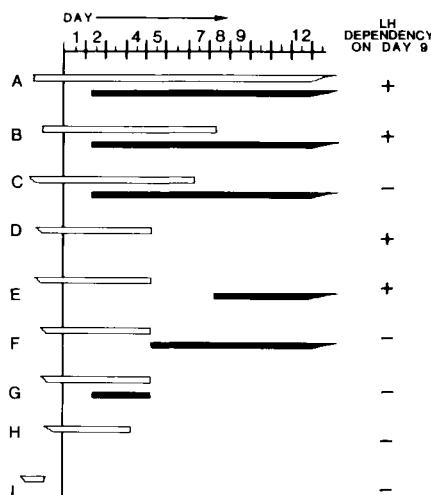


FIG. 13. The effects of prolactin and the uterus on the appearance of LH dependency by day 9 of pseudopregnancy in the rat. Solid bar: an excess of prolactin, secreted by a single pituitary homotransplant; open bar: the presence of the uterus. The figure illustrates the principle that the action of the uterus which leads to the appearance of LH dependency by day 9 is over a threshold effect earlier in the absence (D) than in the presence of an excess of prolactin (A, B, C). It also illustrates the principle that the earlier the uterus is removed, the less prolactin is needed to have a delaying effect on LH dependency (D-I). The figure is another way of looking at the same principles illustrated in Fig. 12 (from Garris and Rothchild, 1980).

and it includes a shortening of the delay in appearance of LH dependency caused by preventing the inducing effect of LH early in the CL's life cycle (Nanes and Rothchild, 1981). The actions of the uterus and prolactin do not affect the intensity of the need for LH, but only when the full intensity of this need appears (Nanes *et al.*, 1980) (Fig. 12). The action of the uterus does not seem to be essential for the appearance of LH dependency (Ford and Yoshinaga, 1975c; Garris and Rothchild, 1980; Nanes *et al.*, 1980), if the inducing effect of LH is present.

The life span of LH dependent CL is considerably shorter than that of CL which are not LH dependent (Lam and Rothchild, 1977; Takahashi *et al.*, 1978; Ochiai *et al.*, 1981), probably because of the process that induces LH dependency, rather than LH dependency itself. In spite of the crucial need for the luteotrophic action of LH, the CL cannot secrete progesterone in response to LH in the absence of prolactin or an equivalent hormone (e.g., placental hormone(s) secreted between the time of implantation and day 12) (Morishige and Rothchild, 1974). The luteotrophic effect of LH may be at least partially substituted for, during the period of LH dependency, by estrogens, either as such (Gibori *et al.*, 1977b; Gibori and Richards, 1978; Garris *et al.*, 1981), or in the form of androgens that can be converted to estrogens within the CL (Gibori *et al.*, 1978). In pseudopregnant rats the effective amount of estrogen is much less early than late in the period of LH dependency and is also much less in the absence than in the presence of the pregestational uterus (Garris *et al.*, 1981).

The amount of LH bound to CL cell membranes and the affinity of LH for these membranes is the same in LH -dependent and LH-independent CL (Garris *et al.*, 1980). The signs of LH dependency, that can be brought out by treatment with LHAS, will also appear if LH secretion is prevented by hypophysectomy (Alloiteau and Bouhours, 1965; Ahmad *et al.*, 1969; Gibori and Richards, 1978), by treatment of intact rats with an antiserum to GnRH (Nishi *et al.*, 1976), or if LH secretion is reduced by increasing the intensity of the suckling stimulus [which depresses LH secretion (Hammons *et al.*, 1973; Ford and Melampy, 1973)] in lactating pregnant rats (Veomett and Daniel 1971, 1975a,b,c; Ford and Yoshinaga, 1975a,b).

### C. THE RELATION BETWEEN LH DEPENDENCY AND THE ULTRA-SHORT-LIVED CL

#### 1. *The Physiology of the Ultra-Short Luteal Phase*

I have previously summarized (p. 233) many of the peculiarities of prolactin's luteotrophic effect and suggested that it is best characterized as a permissive luteotrophin, its action being one that protects the CL's

ability to secrete progesterone. This implies that the ultra-short-lived CL relies on prolactin, not to initiate progesterone secretion, but to maintain its ability to secrete progesterone autonomously. This in turn implies that there is an important difference between these CL and the short-lived CL of other polyestrous eutheria, as, e.g., the monkey's or the human's, which seem to depend on LH from very early on in their life cycle. Because LH acts luteotrophically by raising the rate of progesterone secretion (p. 242), the need for LH in species with short-lived CL means that the CL have lost most of their capacity for autonomous secretion of progesterone, and have come to depend on LH to secrete enough of it to satisfy the needs of reproduction. The absence of any need for the luteotrophic effect of LH in the rat during the first 8 days of the CL's life cycle, therefore, implies that, during this period at least, the CL can secrete enough progesterone autonomously for the needs of reproduction, as long as this ability is protected by prolactin. The luteal phase of these CL is ultra-short in the absence of prolactin, in other words, not because the CL cannot secrete progesterone for a long time, but because something keeps them from doing so, and their peculiar need for prolactin arises from its ability to reduce the effect of this interfering process.<sup>6</sup>

I think the interfering process has two parts: the ultra-short-lived CL is hypersensitive to the luteolytic effect of LH; and, it switches on PG production in response to an abnormally low critical intraluteal progesterone concentration. These changes probably evolved as intensifications of the normal traits that were part of the inheritance of the early polyestrous eutherian CL. The species survived as successful reproducers through whatever accident it was that coupled these changes with the one that suppressed prolactin secretion throughout the ovulation cycle, except after copulation [or during proestrus (Butcher *et al.*, 1974), when the CL can no longer secrete progesterone (Krey and Everett, 1973; Krey *et al.*, 1973)]. The whole complex of changes led to a highly efficient form of viviparous reproduction, among other reasons, because the ultra-short-life of the CL meant that, in the absence of a chance to copulate, only 4 or 5 days were lost, instead of about 2 weeks, before another chance for conception presented itself.

Whether the luteolytic effect of LH and the switch-on of intraluteal PGs through the critical intraluteal progesterone concentration are any different from those of the short-lived eutherian CL, except in sensitivity to these factors, is unknown. It is also unknown (as I hinted above, p. 233) whether prolactin acts on the CL to do more than suppress PG production

<sup>6</sup> There is a tantalizing implication of a similar idea about how prolactin acts in the rat, in an abstract by Chatterton and Greep (1965).

or the response to PGs. In any case, a few findings fit with this interpretation of the physiologic basis for the ultra-short-lived CL, even if they do not establish its validity.

When rat granulosa cells luteinize spontaneously in tissue culture, the duration of their progesterone secretion, in the absence of prolactin or other hormones added to the medium (even though the amount secreted is not very impressive), is about 4 to 6 days, instead of the 2 to 3 days of the estrous cycle *in vivo* (Crisp, 1977). If the 4-day cyclic rat is given a single injection of LHAS before the day 2 peak of progesterone secretion, this secretion, which presumably comes from the CL (de Greef and van der Schoot, 1979), continues for about another 24 hours (Sánchez-Criado and Rothchild, 1981). Although this resembles the effect of *LH* treatment on day 2 (Alloiteau and Acker, 1969; Buffler and Roser, 1971, 1974; Roser and Buffler, 1972; Boehm *et al.*, 1980) it is not the same, since the latter effect is almost certainly the result of partial luteinization of follicles (Chateau, 1969; Chateau and Aron, 1970; Buffler and Roser, 1972). Indomethacin treatment during the same part of the estrous cycle also tends to prolong progesterone secretion by about a day (Sánchez-Criado and Rothchild, 1981).

The explanation for the physiologic basis of the ultra-short-lived CL may make clear a hitherto unexplained finding of Ferin *et al.* (1969); after a single injection of an antiserum to estradiol into cyclic rats on day 2, about 50% of the rats went into a diestrus that lasted as long as an ordinary pseudopregnancy. The luteolytic effect of LH could be exerted through its role in stimulating estrogen production, since estrogens can stimulate PG release (p. 210). The inhibition of the estrogen effect by the antiserum, thus might have allowed progesterone secretion to continue long enough for it to initiate the surges of prolactin secretion, since progesterone has this effect in the rat (Alloiteau and Vignal, 1958; Everett, 1963; Rothchild and Schubert, 1963; Murakami *et al.*, 1980; see also p. 187). It is very interesting that in both Everett's (1963) study and that of Ferin *et al.* (1969), the maintenance of the long diestrus depended on isolating the rats and not taking daily vaginal smears.

The explanation also fits with prolactin's ability to prevent the luteolytic effect of the preovulatory surge of LH secretion (see Krey and Everett, 1973; Krey *et al.*, 1973; and Day and Birnbaumer, 1980, in Table VII). It also may help to clarify the finding that LH-stimulable adenyl cyclase activity in the rat's CL parallels the pattern of progesterone secretion during the ovulation cycle (Birnbaumer *et al.*, 1979), and accompanies the rise in progesterone secretion that occurs in response to prolactin during the transition to pseudopregnancy (Day *et al.*, 1980), even though the need for the *luteotropic* action of LH will not appear until about a week later. If

the luteolytic effect of LH, like its luteotrophic one, works through the adenyl cyclase-cAMP system, the ability of LH to stimulate adenyl cyclase activity at this time in the life cycle of the CL is probably an expression of its *luteolytic*, not its luteotrophic effect.

## 2. The Physiologic Basis of LH Dependency

a. *The Essentiality of LH.* The critical question in defining the cause of LH dependency is: *What action of LH induces the dependency in LH?* If my explanation of the basis for the ultra-short-lived CL is correct, it becomes immediately obvious that it must be the luteolytic effect of LH which induces the latter crucial need for its luteotrophic effect. I think this comes about in the following way.

The luteolytic effect of LH probably works in all of the polyestrous eutheria by facilitating luteal PG production. In these CL, the intrinsic capacity to produce PGs has not been lost, but has come to depend on extrinsic factors such as LH and/or the uterine PGs. Essentially the same relationship between extrinsic and intrinsic control of luteal PG production probably exists in the species with ultra-short-lived CL as in the other polyestrous eutheria, except that the *normal* level of sensitivity to the effect of LH and the *normal* threshold of response to the critical intraluteal progesterone concentration depend crucially on prolactin. PG production thus begins as the luteal phase of pseudopregnancy or pregnancy begins, but at a very slow rate.

I will also suggest that, thus protected by prolactin, the rat CL's capacity for autonomous progesterone secretion is the same as its maximum capacity for progesterone secretion during all of the rising phase. LH, therefore, has no luteotrophic effect during this period because the CL are already secreting at maximum capacity; depriving the CL of LH also has no effect, since progesterone secretion is autonomous. Because of the self-stimulating quality of PG production and because progesterone potentiates this production as it suppresses it, an increase in intraluteal PG concentration is inevitable, although the rate of increase, especially during the rising phase, is slow. This is probably because progesterone suppresses PG production best only when the CL secretes it at maximum capacity.

Because PG production does increase, however, and because of the effect of the critical intraluteal progesterone concentration, a point will eventually be reached at which the PG concentration is just high enough to induce a slight decrease in the CL's capacity for autonomous progesterone secretion. At this point the plateau phase begins. It progresses because of the interplay between progesterone secretion and PG production, as described in the theory, and as it does so, the gap between the

CL's maximum capacity for progesterone secretion and its capacity for autonomous progesterone secretion widens asymptotically. The regression phase is about to begin when this gap has reached a critical value (Fig. 14).

Since LH is postulated to act luteotrophically by raising the rate of progesterone secretion, the CL probably responds to this effect as soon as the gap between maximum capacity and capacity for autonomous progesterone secretion appears, but because the gap is very small at first, the effect of LH is not noticeable until the gap has reached a critical size. At this point the CL's response to LH is a large one. It is also an essential one, for without it the CL will undergo immediate luteolysis, because the capacity for autonomous progesterone secretion has now fallen too far

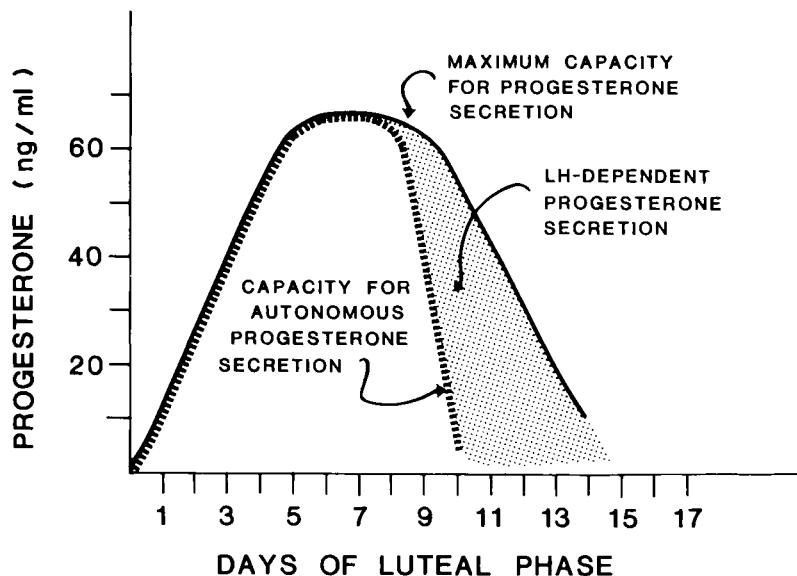


FIG. 14. A schematic representation of the hypothetical cause of LH dependency in the rat. During the rising phase, the lack of difference between the CL's capacity for autonomous progesterone secretion and its maximum capacity for progesterone secretion explains why neither LH treatment nor LH withdrawal affects progesterone secretion. Because LH is presumed to facilitate PG production by the CL, the PGs eventually lead to a fall in the capacity for autonomous progesterone secretion and, therefore, to a gap between this capacity and the CL's maximum capacity for progesterone secretion. When the gap reaches a critical value, the need for LH becomes very easy to see, since its ability to raise the rate of progesterone secretion restores progesterone secretion to the CL's maximum capacity, and in its absence, progesterone secretion falls precipitously. The luteolytic effect of LH thus induces the need for its luteotrophic effect. The latter, however, does not prevent regression, since this comes from the already switched-on PG production, but it slows down the rate of regression.

below the maximum capacity, for progesterone secretion to maintain itself. Progesterone secretion would stop almost immediately, therefore, if LH was not available to maintain it at the CL's maximum capacity (Fig. 14). The CL thus appears to have suddenly become crucially dependent on LH.

The ability to respond to the luteotrophic effect of LH, however, does not prevent the CL from regressing, but it reduces the rate of regression. The response to LH reduces this rate least of all in the ordinary pseudopregnant rat, because it is opposed by the effects of both the uterine and luteal PGs. It reduces regression more effectively in hysterectomized or DT-bearing pseudopregnant rats, because it is opposed only by the effect of the luteal PGs. In the pregnant rat, regression is even halted by the response to LH, because the response is supported by the placental hormones, and the uterine PGs do not oppose it.

Regression occurs in spite of the effect of LH, presumably, because the CL's maximum capacity for progesterone secretion has been under constant attrition from the effect of PGs, and at the point where LH dependency appears, PG production itself has progressed to where its continued increase cannot be prevented, but can only be kept from becoming a rapid increase by the combined effects of progesterone, and of prolactin. The PGs thus progressively reduce the CL's maximum capacity for progesterone secretion, until this secretion stops altogether. Preliminary findings in our laboratory fit with this, since the luteolytic effect of LHAS treatment of hysterectomized pseudopregnant rats, on day 10, can be prevented by indomethacin treatment (Sánchez-Criado and Rothchild, 1981).

Some of the other aspects of the effects of LH, in relation to LH dependency, become clearer with the help of this explanation. Thus, in the virtual absence of LH, as in the rat hypophysectomized and pituitary transplanted on day 2, the luteolytic effect of LH is also absent, and the need for its luteotrophic effect never appears (Lam and Rothchild, 1977; Macdonald, 1978; Ochiai *et al.*, 1981). If LH secretion is reduced, as in lactation (Ford and Melampy, 1973; Hammons *et al.*, 1973), or postponed, as in rats treated with LHAS daily from days 2 to 5 (Lam and Rothchild, 1977; Nanes and Rothchild, 1981) or longer (Lam and Rothchild, 1977), the dependency on LH also appears later than it would otherwise. Similarly, the shorter life span of LH-dependent than LH-independent CL can be seen to be the result of the differences between them, most probably in the rate of PG production rather than in the need for LH. Luteal PG production probably goes on in the LH-independent CL also, but at such a slow rate that the CL's capacity for progesterone secretion never falls

below its maximum capacity, and the regression phase, therefore, is greatly prolonged (Fig. 10).

It is also probable that when rat CL, which *in vivo* do not respond to LH and do not stop secreting progesterone when deprived of LH, are placed *in vitro*, the *in vitro* conditions themselves induce a difference between their maximum and their actual capacity for progesterone production; LH, therefore, by raising progesterone secretion to the cell's maximum capacity, now appears to have a dose-related luteotrophic effect (e.g., Shiota and Wiest, 1979; Rodway and Rothchild, 1977; Strauss and Flickinger, 1977).

This explanation for the role of LH in LH dependency, incidentally, also helps to resolve the puzzle of why LH treatment, in spite of its luteolytic effect in hypophysectomized, pituitary transplanted, or equivalent rats (Rothchild, 1965; Macdonald *et al.*, 1970; Takahashi *et al.*, 1978; Ochiai *et al.*, 1981), cannot shorten the duration of the luteal phase to less than that of an ordinary pseudopregnancy. It is because the balance between the CL's capacities for progesterone secretion and for PG production is such that the change from high progesterone/low PG to high PG/low progesterone, which leads to regression, never takes less than this amount of time, even in the presence of an optimal amount of LH. This, in turn, is because LH has two effects. Treatment with a luteolytic PG like PGF<sub>2α</sub>, however, by throwing the balance toward high PG/low progesterone after the plateau phase has begun, can shorten the duration of the luteal phase (Behrman, 1979). LH treatment may prevent this effect of PGs (Behrman, 1979) possibly in the same way that estrogens (Gibori *et al.*, 1977b, 1978), even in very small amounts (Garris *et al.*, 1981), prevent the luteolytic effect of LHAS, i.e., by causing a temporary increase in progesterone secretion, which in turn prevents a full scale switch-on of intraluteal PG production.

*b. The Delaying Effect of Prolactin.* According to this explanation, the balance between the processes promoting progesterone secretion and those promoting PG production will determine when LH dependency appears. Prolactin's effects array it on the side of progesterone secretion and against PG production, and this tends to postpone the appearance of LH dependency. This delaying effect is probably the reason for the continued need for prolactin after LH dependency appears, since it is only the combined effects of prolactin and the progesterone secretion stimulated by LH that hold the increase in PG production to a moderate one.

The action of prolactin, however, must be such that in the presence of the inducing effect of LH and even in the absence of the uterus, which works with this effect of LH (see next subsection), it can put off the

change in PG production that leads to LH dependency only until about day 12 (Fig. 12). In the absence of the inducing effect of LH, on the other hand, and probably in cooperation with the suppressing effect of progesterone itself on PG production, prolactin can postpone LH dependency indefinitely and thus greatly prolong the CL's ability to secrete progesterone. This, presumably, describes the conditions of the hypophysectomized, pituitary transplanted rat (Fig. 10).

c. *The Advancing Effect of the Uterus.* The effect of the uterus in the events leading to LH dependency seems to be part of those which (like that of LH) promote luteal PG production, but this effect is different from that on the CL's life span. The latter is without question the result of the production and delivery to the CL of luteolytic PGs (Horton and Poyser, 1976), and these, together with those produced by the CL itself, determine the rate of regression of the CL, for example, in the ordinary pseudopregnant rat. There are at least two reasons to postulate that its effect of advancing the appearance of LH dependency is different from this. One is that the effect on LH dependency is over a threshold by day 5 (Garris and Rothchild, 1980) (Fig. 13), while the life-shortening effect can be prevented by hysterectomy even as late as day 10 (Silbiger and Rothchild, 1963; Melampy *et al.*, 1964). The other is that both the DT-bearing uterus and the uterus of early pregnancy advance the appearance of LH dependency at least as much as (or even more than) the ordinary pseudopregnant rat's uterus (Morishige and Rothchild, 1974; Rothchild *et al.*, 1974; Nanes and Rothchild, 1981), yet they prolong the CL's life span as much as does hysterectomy (Rothchild, 1965, p. 283).

The uterus hastens LH dependency, therefore, through the action of a different substance from that which shortens the CL's life span (Nanes and Rothchild, 1981). The hypothetical substance, like LH, may facilitate the production of intraluteal PGs, and thus oppose prolactin's delaying effect. Some of its characteristics have an uncanny resemblance to those of LH, and it is interesting that both Morishige, in a pilot study (see Rothchild *et al.*, 1974) and de Greef *et al.* (1976) in a definitive one, found significantly lower peripheral serum levels of LH in chronically hysterectomized than in ordinary pseudopregnant rats. In the presence of LH, the uterine substance is not essential for the induction of LH dependency, since chronically hysterectomized rats do become LH dependent by day 12–15 (Ford and Yoshinaga, 1975c; Nanes *et al.*, 1980), but whether it would induce LH dependency in the absence of LH has never been tested definitively. It seems at least to cooperate with LH, in inducing LH dependency, and both this effect of LH and that of the uterus are over a threshold effect by day 5.

The uterine substance must also be made by the DT-bearing uterus,

which differs from that of the ordinary pseudopregnant rat's uterus only after day 5 [the day on which an endometrial scratch or other stimulus most effectively induces DT formation (De Feo, 1967)], and until day 8, only in the extent of the small amount of DT which has formed by then (De Feo, 1967). By day 8, the ordinary pseudopregnant rat's uterus is over a threshold effect for preventing the delaying effect of prolactin on LH dependency (Garris and Rothchild, 1980). There is thus little or no essential difference between the DT-bearing uterus and the ordinary progestational uterus in their action of advancing the appearance of LH dependency, and the slightly greater effectiveness of DT may be due perhaps only to an enhanced release of the uterine substance at the time of endometrial scratching. Why the implanting blastocyst also advances the appearance of LH dependency, however, remains a mystery.

By contrast, there is a very big difference between the ordinary pseudopregnant rat's uterus and the DT-bearing uterus in their contribution to the pool of PGs which determines how rapidly progesterone secretion falls during the regression phase. Whatever the exact cause for it the DT-bearing uterus lacks the ability—which the ordinary pseudopregnant rat's uterus has—to contribute to this pool. As a result, the regression phase of the CL's life cycle, in rats bearing DT, is like that of a hysterectomized rat (see, e.g., Pepe and Rothchild, 1974).

### 3. Pregnancy and LH Dependency

Pregnancy and its effect on the CL is a subject altogether too large to be included in this article except to say that the prolongation of progesterone secretion in the rat, for example, by pregnancy (Fig. 15) is typical of the evolution of the CL-trophoblast relationship among the polyestrous eutheria. Even the change in LH dependency in the rat's CL caused by pregnancy is a subject in itself, and I will not do more at this point, therefore, than mention some of the more important and interesting of its aspects.

There is only suggestive evidence (Haour *et al.*, 1976; Blank *et al.*, 1979) that the rat placenta secretes a chorionic gonadotrophin (rCG) (i.e., a hormone homologous with hCG), in addition to the placental luteotrophin (rPL) (Kelly *et al.*, 1976). There is good evidence that estrogens stimulate progesterone secretion after day 12 of pregnancy (Takayama and Greenwald, 1973; Gibori *et al.*, 1977a, 1978; Kato *et al.*, 1979; Ochiai and Rothchild, 1980; Rodway *et al.*, 1981), but estrogens are apparently not essential, since progesterone secretion can be maintained at the level reached by day 12, until at least day 20, by treatment with only the serum of day 12 pregnant rats, after hypophysectomy and hysterectomy on day 12 (Gibori *et al.*, 1977a). We have also found only equivocal evidences so

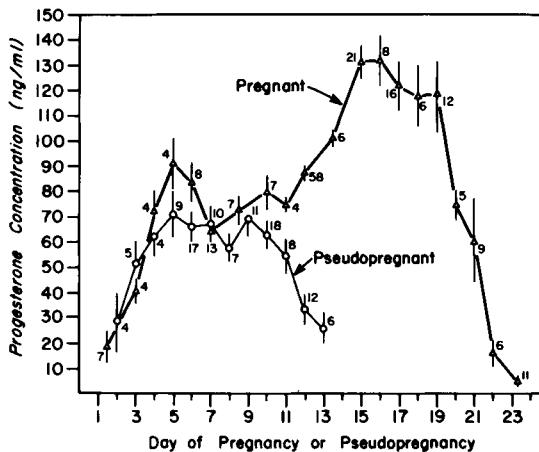


FIG. 15. The pattern of progesterone secretion during pseudopregnancy and pregnancy in the rat. The rat has an ultra-short-lived CL, whose life span is increased ("pseudopregnant" curve) to that of a short-lived CL (Fig. 2) by the prolactin secreted in response to sterile mating or an equivalent stimulus, such as mechanical stimulation of the cervix. The prolongation of progesterone secretion by pregnancy in the rat is an example of the fact that pregnancy has this effect in all polyestrous eutheria (from Pepe and Rothchild, 1974).

far that antiestrogen treatment during the period after day 12 interferes with progesterone secretion (Rodway and Rothchild, unpublished findings).

Although the evidence is still far too sketchy to be certain that any interpretation of the loss of LH dependency after day 12 is correct, the most likely one may be that the placental hormones change the progesterone/PG relations within the CL in such a way that support of progesterone secretion through a mechanism like the luteotrophic action of LH may not be necessary for at least the first several days after day 12; if it is necessary beyond that point, it is through a hormone other than LH itself. The pattern of progesterone secretion after day 12 (Fig. 15), the fact that the CL secrete progesterone autonomously for about 3 days after day 12 (Rothchild, 1973; Rothchild *et al.*, 1973), and that neither prolactin (Rodway *et al.*, 1981) nor rPL (in the form of day 12 pregnancy serum) (Gibori *et al.*, 1977a) will raise the rate of this secretion form the basis for this interpretation. In fact, it is almost strikingly obvious, from a comparison of the progesterone curves for pregnancy and ordinary pseudopregnancy (Fig. 15), that the hormonal conditions of pregnancy have induced a new life cycle in the CL, almost identical in pattern to that of pseudopregnancy, but superimposed on the pseudopregnancy cycle at the point where regression would have begun. On a time scale about 20 times longer than in rat pregnancy, the same thing seems to happen in the roe deer

(Fig. 16). The placental hormones, thus, not only prevent the CL from regressing, but in some way reactivate the capacity for autonomous progesterone secretion to such an extent that by day 12, the CL are no longer LH dependent, and they can even secrete progesterone in the absence of both pituitary and placentas for 3 days (Rothchild *et al.*, 1973).

How this comes about and why estrogens at this point assume the importance they have in inducing the rise in progesterone that occurs between days 12 and 15 will have to be the subject of another article.

### V. Summary

The CL is a peculiar endocrine gland, characterized by a bewildering diversity of patterns of progesterone secretion, of regulation of this secretion, and, even among the mammals, in the relationship between CL activity and viviparity. Its unique and universal characteristic, however, is ephemerality, which is expressed in a typical nonoscillating pattern of

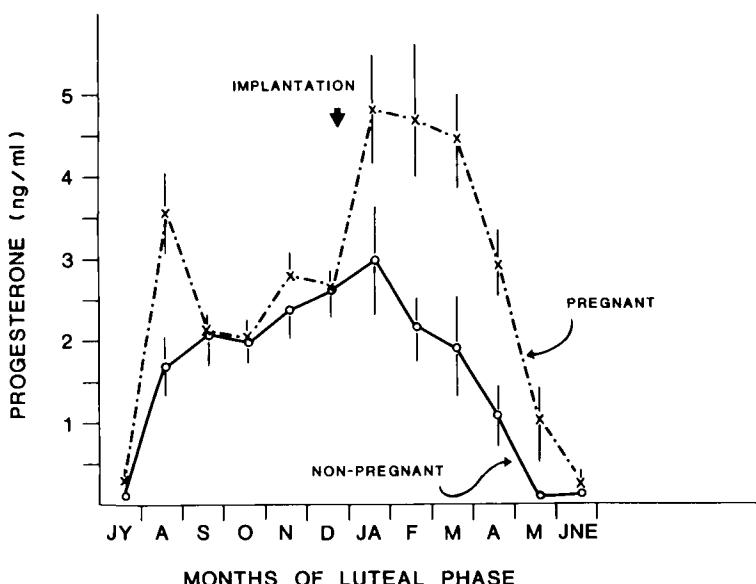


FIG. 16. The difference in pattern of the luteal phase between the pregnant and nonpregnant roe deer, a monestrous breeder. (Adapted from data of Hoffman *et al.*, 1978.) The difference is not in duration of the luteal phase, but in the amount of progesterone secreted after implantation, and is almost exactly like that between the pseudopregnant and pregnant rat, in which the whole cycle of pregnancy occupies a period of only about 3 weeks (Fig. 15). The armadillo (Peppler and Stone, 1980) and the prairie dog (Foreman, 1962), which are also monestrous breeders, probably also have long-lived CL with a duration of activity little different from that during pregnancy.

progesterone secretion and change in size, in which a rising phase, a plateau phase, and a regression phase are easy to see. A group of characteristics of the mammalian CL—although they do not seem to be universal ones—appear to be crucial clues to the cause of this ephemerality. These are: the ability of the CL of several species to secrete progesterone autonomously, i.e., in the absence of any known extrinsic stimulus; the luteolytic effect of prostaglandins (PGs); the ability of the CL to make PGs; the self-stimulating quality of PG production; and, the ability of progesterone to suppress PG production, and at the same time to stimulate the potential for PG production. The implications of autonomy are that progesterone itself may be the primary stimulus of its own secretion, a possibility for which suggestive evidence can be found.

From a consideration of how these characteristics are related to one another, I have theorized that ephemerality arises from the nature of the basic and common system of regulation of all CL; this system can be described by the following five postulates:

1. The CL secretes progesterone autonomously.
2. Progesterone is the primary stimulus of its own secretion.
3. The CL also makes PGs, but progesterone suppresses their production, at the same time that it increases the CL's potential ability to make them.
4. The luteal PGs are also made autonomously, are self-stimulating, and they prevent progesterone secretion without increasing the CL's potential for progesterone secretion.
5. As progesterone secretion rises, so does the intraluteal progesterone concentration; when this reaches a critical level, it reduces the rate of further increase in progesterone secretion and switches on an increase in PG production. The self-stimulating quality of progesterone secretion and PG production and their mutually opposing effects lead inevitably to regression of progesterone secretion and eventual disappearance of the CL.

The essence of the CL's ephemerality is thus that both progesterone secretion and PG production are regulated by positive feedback and are mutually opposing. Extrinsic factors that stimulate (luteotrophins) or inhibit (luteolysins) progesterone secretion presumably have their effects by acting on each of these intrinsic processes.

The pathway to diversity in the regulation of the CL (as well as in its other attributes) began with the evolution of the CL itself. The principal effect of luteinization of the postovulatory follicle (POF), when it first occurred in the primitive reptile ancestors of modern reptiles and mam-

mals, is postulated to have been the postponement of the POF's production of PGs. This postponed the production of PGs in the female reproductive tract and thus also the expulsion of the egg. From this *first* step, through a series of intermediate stages, such as the ovoviviparity of the monotremes, until the limited form of viviparity of the modern marsupials, the principal effect of the progesterone secreted by the CL probably remained essentially unchanged; the intrinsic regulating system of the CL probably also remained essentially unaffected by outside factors.

The eutherian CL evolved because of the appearance within it of responsiveness to extrinsic factors. The first of these must have been the pituitary hormones, and later ones included placental hormones, estrogens, uterine PGs, etc. I have speculated that the eutherian CL and eutherian viviparity evolved according to the following sequence.

The first change was responsiveness to the luteotrophic effects of prolactin, which are postulated to work mainly through depression of either or both the effects or production of intraluteal PGs. Prolactin, therefore, increased the duration of progesterone secretion well beyond the approximately 2-week period of the marsupial and earlier CL. Among modern marsupials, the bandicoot's CL has apparently evolved to this stage. The second change was responsiveness of the uterus to the gestation-prolonging effect of the CL's progesterone. The bandicoots have not yet evolved to this stage, their young still being born only 12 days after ovulation, in spite of a prolonged secretion of progesterone. The first eutherians were probably monestrous breeders, in which prolongation of progesterone secretion through the effect of prolactin, and of intrauterine gestation through the effect of progesterone, provided the *minimum* conditions for the transition from the marsupial to the eutherian form of viviparity. Eventually this led to the pattern of viviparity seen in modern eutherian monestrous breeders, in which the luteal phase in the absence of pregnancy is as long as it is during pregnancy (e.g., in the dog, about 2 months).

Another change probably occurred almost simultaneously. This was the evolution of the trophoblastic placenta, which, together with the effects of progesterone on the uterus, helped to prolong intrauterine gestation by protecting the embryo from immune rejection by the mother. During the course of its evolution, the trophoblast acquired the ability to secrete hormones with luteotrophic/antiluteolytic properties. Although these may have affected the amount of progesterone secreted by the monestrous breeders' CL, they did not affect the duration of CL activity, and were probably not essential for the maintenance of pregnancy. Their appearance, however, made it possible for polyestrous breeding to be compatible with eutherian viviparity.

The next change was the appearance of responsiveness to the *luteolytic* effect of LH. This effect, probably a facilitation of intraluteal PG production, together with an increase in the basal rate of secretion of LH and FSH, made polyestrous ovulation cycles possible, since it shortened the life span of the CL to about 2 weeks, and thus also the interval to the next ovulation. Polyestrous cycles were not compatible with eutherian viviparity, however, until they appeared in the descendants of the monestrous breeders in which an endocrine placenta had already evolved. The latter prevented the luteolytic effect of LH and thus prolonged the CL's life span enough to make eutherian viviparity possible.

Such CL were already presumably responsive to the luteotrophic action of prolactin, and this helped preserve their responsiveness to the placental hormones until these were secreted into the blood stream. The next or almost simultaneous change was the appearance of responsiveness to the *luteotrophic* effect of LH, one believed to act by raising the rate of progesterone secretion. This also helped to preserve CL activity and responsiveness to the placental hormones, and eventually became a major element in the luteotrophic process of most, if not all, polyestrous eutheria.

The first polyestrous eutheria were thus species with CL responsive to the luteotrophic effect of LH and prolactin, and to the luteolytic effect of LH, and with a placenta which secreted hormones that prevented the latter. The later paths of evolution of CL regulation involved changes primarily in these three attributes, and in the balance between them and the CL's capacity for autonomous progesterone secretion; changes in the factors contributing to the intraluteal control of PG production, and in the source of PGs responsible for the CL's regression also occurred. Examples of these are described.

One example of the usefulness of the theory is in the interpretation of the rat's ultra-short luteal phase of the estrous cycle, and its relation to the rat's peculiar need for the luteotrophic effects of both prolactin and LH. The ultra-short luteal phase is postulated to be the result of evolution of a supernormal sensitivity to the luteolytic effect of LH and of a subnormal threshold for the switch-on of an increase in intraluteal PG production. Both changes limit the life of the CL to less than about 3 days, but prolactin, by reducing both effects, allows the CL to express its capacity for autonomous progesterone secretion. This is enough to maintain progesterone secretion for at least 8 or 9 days after ovulation.

The luteolytic effect of LH causes the development of the crucial need for the luteotrophic effect of LH; this need appears by day 8 or 9. Although the luteolytic action of LH is reduced by prolactin, it is not altogether prevented, and it leads eventually to a gap between the CL's capacity for autonomous progesterone secretion and its maximum capac-

ity for progesterone secretion. By raising the rate of progesterone secretion, LH thus maintains the CL's maximum capacity, but it does not prevent regression. This occurs because of the cumulative effect of the PGs, which eventually reduce the maximum capacity for progesterone secretion to zero.

Thus, *the essence of a CL*, in a word, is that it is a vertebrate postovulatory follicle whose inevitable regression, itself the result of its intrinsic ability to make PGs, is postponed by its ability to make progesterone. The duration of this postponement is determined primarily by the duration of progesterone secretion.

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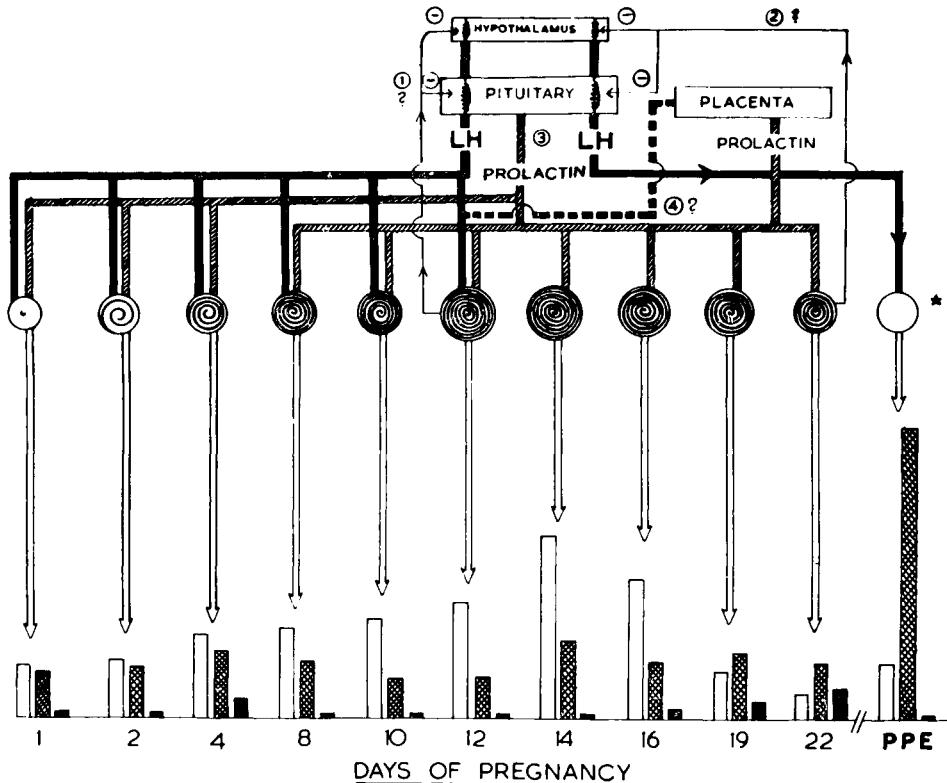


FIG. A. Proposed model for hormonal control of pregnancy in the intact rat. (From Raj and Moudgal, *Endocrinology* 86, 874-889, 1970.) This model was proposed from the then available data on hormone levels and our work using LH antiserum. (a) Feedback effect of high progesterone and low estrogen titers on pituitary and hypothalamus to cut off LH. (2) Feedback effect of low progesterone and high estrogen titers on the pituitary and hypothalamus to reinitiate LH release. (3) Prolactin from the pituitary and hypothalamus to reinitiate LH release. (4) Prolactin from the pituitary and placenta facilitating conservation of progesterone. (4) Nature of luteotropin from twelfth day placenta had not been clearly established at the time this model was proposed, hence a question mark. It was proposed to be LH like. Open squares, progesterone; solid squares, estrogen; hatched squares, 20 $\alpha$ -OH-P. Synthesis under tropic stimulation of LH. Secretion of 20 $\alpha$ -OH-P, however, is inversely related to prolactin stimulus due to the ability of PRL to initiate 20 $\alpha$ -hydroxysteroid dehydrogenase and conserve progesterone. PPE, Postpartum estrus. Asterisk indicates effect of LH on the corpora lutea of PPE and pregnancy. Pattern of steroid output would once again change with the initiation of suckling-induced prolactin stimulus.

#### DISCUSSION

**H. G. M. Raj:** I have one figure which rather refreshes our memory in the evolution of ideas on the control of corpus luteum and the luteotrophic hormone. This (Fig. A) shows a working hypothesis that we proposed exactly 10 years ago and was published in *Endocrinology* (86, 814, 1970) at a time when prolactin was being considered to be the sole luteotrophic

hormone. While parts of this model were based on our observations, many aspects were hypothesis that served to promote further work in the field. However, our work using LH antiserum in the pregnant rat clearly showed that LH is required for normal maintenance of pregnancy and progesterone synthesis, during pregnancy. I wouldn't take the time to explain all the features of the model, but we clearly demonstrated the need for LH to maintain pregnancy. In the preimplantation period, especially on day 4, LH is the tropic stimulus that induces estrogen production necessary for implantation. However, administration of LH antiserum between day 8 and 12 induces abortion and this was not reversed by various doses of estrogen or prolactin. Only progesterone could reverse the abortifacient effect. This certainly implicates LH as a luteotropic hormone necessary for progesterone production. This work was extended later by Morishige and Rothchild who demonstrated that LH dependency sets in between day 6 and 8, as mentioned in the talk. While LH is not the sole luteotrophic hormone, it certainly is the only hormone capable of stimulating side-chain cleavage and conversion of cholesterol to pregnenolone. From this viewpoint, and using the then available data from other investigators, we proposed that LH is the only tropic influence that stimulates steroidogenesis (i.e., progesterone synthesis per se). Further, we proposed that prolactin modulates and conserves the progesterone from being catabolized. As you presented, your group, as well as Drs. Keyes and Gibori, have, in recent years, elegantly demonstrated that LH acts via intraluteal estrogen formation. However, I am concerned that it has lead to the "nomination" of intraluteal estrogen as a luteotropic hormone, to the exclusion of LH. Clasically the term luteotropic hormone has been used to denote pituitary and placental hormones that impinge on the ovary from outside to exert this tropic action. If we are going to say that intraluteal estrogen is to be called a luteotropic hormone and not LH, this quite leaves the door open for considering other metabolites like LH-induced testosterone as luteotropic hormone and LH-induced cAMP as luteotropic. By the same reasoning, estrogen-induced mRNA (through which estrogen itself exerts its action) also will have to be considered as luteotropic.

**I. Rothchild:** As far as the luteotropic effect of LH is concerned, it is incorrect, I think, to talk about LH as the *primary* luteotrophin in the rat or any other species. For example, if the rat's corpus luteum is not exposed to LH after it has been formed, it does *not* depend on LH at all to maintain its progesterone secretion. The pattern of progesterone secretion in the pituitary-autotransplanted rat (test Figs. 9 and 10) shows this very nicely, and other findings, such as those of Takahashi *et al.* and of Macdonald (see References) fit with this conclusion. This and other evidence I've discussed indicate that it is LH itself which induces the need for its luteotropic effect. I've discussed LH dependency in the rat in detail in the article, and there is not enough time to do so here, but very briefly, my point is this. The need for the luteotropic action of LH arises in the rat (and probably in other polyestrous eutheria also) as a result of the action of LH on the prostaglandin (PG)-producing part of the corpus luteum, but because LH also probably acts as a luteotrophin because it can raise the rate of progesterone secretion, it is the *luteolytic* action which later makes the CL depend on the luteotropic action to continue secreting progesterone. In the absence of the luteolytic effect, the need for the luteotropic one doesn't arise.

As far as defining a luteotrophin is concerned, I think this can be reduced to the very simple statement that anything can be a luteotrophin if it stimulates progesterone secretion. I've included a set of definitions in the article, not just for luteotrophin but luteolysis, etc. An essential part of my theory is that progesterone stimulates its own secretion; anything, therefore, which facilitates this, acts luteotrophically and anything which hinders it acts luteolytically. The basic underlying factor within the CL that accounts for its typical short life span is the dichotomy between how progesterone secretion is regulated and how PG production is regulated. Any substance or process, therefore, that can act on these can

regulate the corpus luteum. We've identified only a few—LH, prolactin, estrogens, placental hormones, uterine PGs—but there may be others still unidentified, perhaps because we haven't thought of looking for them.

**K. Savard:** The point I would like to make is that there is no question of the episodic existence of the corpus luteum and that this is unique in endocrinology—at least among the steroid-secreting tissues. Nevertheless the corpus luteum cannot be set apart from the other steroid-producing tissues, insofar as its intrinsic biochemical properties are concerned. All steroid-producing tissues share the capability of transforming cholesterol into progesterone and/or obligatorily, its immediate precursor pregnenolone. The enzymes of this critical step, and all other enzymes associated with it, are found only in this family of tissues, and this one fact reflects a read-out of unique genetic information which is not transcribed in other cells. Consequently we can say that all steroid-producing cells form progesterone (or at least its immediate precursor, pregnenolone) from cholesterol and therefore, the formation of progesterone is not necessarily unique.

However, in most cells this progesterone serves as a precursor for other transformations into corticosteroids (the adrenal cortex) or androgens or estrogens; very little if any progesterone is actually released or secreted as such (an exception to this is the adrenal of a certain strain of mouse, I've forgotten the reference to this observation published in *Nature* some years ago). What is unique to the corpus luteum of most species is that much of the steroidogenic process stops at progesterone and this is what is secreted. In certain species as we know, C<sub>19</sub> steroids and estrogens are also formed. We biochemists have yet to explain the basis for this selective accumulation and secretion of progesterone by such tissues as the human corpus luteum, which also has the capability of synthesizing estrogens.

I do not wish to detract from the thesis which you present, but I must call attention to the biochemical dogma which requires the corpus luteum to possess certain biochemical properties (i.e., enzymes etc.) in common with all other steroidogenic cells. I must admit that the luteolytic process which the corpus luteum undergoes is quite unique and cannot be explained as yet in biochemical terms.

When we consider the many factors which bear on corpus luteum formation, its function and lysis, on the variety of steroid products formed, among the species studied and reviewed, we are faced with the realization that the process of evolution has certainly impinged heavily upon this essential element of the reproductive-endocrine system. We can be thankful at least for the fact that in all the species, LH, LTH, progesterone, estrogen are discernible in their usual and accepted roles, and that we are not faced with a vast array of varied chemical factors having similar physiological roles. We can be grateful that all we have to unravel is their interplay.

**I. Rothchild:** One of the greatest difficulties in studying biology and especially reproductive biology is to grasp the fact that there is no purpose in a process which is obviously moving toward a specific goal. Doing without the idea of purpose is not easy, but it must be done if one is to try to understand how the process arose and changed. The story, as I've tried to tell it to you, is that each of the changes in CL function and regulation that took place in the progression from its earliest form in reptiles to the kinds of CL we see in modern mammals was the result of an accident that happened to be valuable for reproduction, or at least not incompatible with it, and so was retained. We don't see the ones that were incompatible with reproduction. I'm sure, for example, that the descendants of the first eutheria included some whose CL became responsive to the effects of LH, and whose CNS-pituitary system went through the changes associated with polyestrous breeding, before they had evolved an endocrine trophoblastic placenta. We don't see these today because they would have been unable to reproduce. We see only the ones in which the endocrine placenta evolved before the return to polyestry, and so it's easy for us to say that the endocrine

placenta evolved "in order to" prevent regression of the CL, etc., thus injecting the idea of purpose, however innocently and unconsciously it's done, into a process totally devoid of purpose.

What you say about the biochemical characteristics of all steroid-producing tissues helps of course to explain some of the vagaries of the CL, but they also help us lose sight of what a CL really is. I don't think it was "designed" as a progesterone secretor originally, but as a PG producer. PGs were nice and easy to make and they did a good job of getting rid of the postovulatory follicle, which, having nursed its egg and released it, was no longer valuable to the organism, and in fact was probably a nuisance especially in forms which either released millions of eggs, as in swimmers, or made very large eggs. When the accident of progesterone secretion appeared in the postovulatory follicle, it delayed the PG-induced regression and expulsion of the egg, and so it also began the long pathway toward viviparity and the mammalian corpus luteum as such.

**P. W. Concannon:** First, I would like to compliment you, Dr. Rothchild, on your presentation, particularly as regards the large number of diverse species you have considered in addressing luteal regulation from the aspects of intrinsic luteal autonomy and ephemerality. I would also like to take this opportunity to report some results from my laboratory on two of the species which you cited as examples of animals that experience "long-lived" luteal phases in the absence of pregnancy—namely, the dog and the mink.

In the dog, luteal function is dependent on the presence of the pituitary and LH administration will elevate plasma progesterone levels (Concannon, *J. Reprod. Fertil.* **58**, 407, 1980). We also have additional data demonstrating the luteotrophic function of LH in the dog. Around day 45 of the cycle pregnant or nonpregnant bitches were each given a single injection (10 ml) of an equine antiovine LH serum similar in potency to the antiserum used in your studies on rats. In each bitch serum progesterone levels declined abruptly for 24–48 hours after injection and then returned to control values within 4–6 days. More recently, however, we have obtained evidence that prolactin, also, functions as a luteotropin in the dog. Luteal-phase serum progesterone levels were measured in bitches administered the prolactin-suppressor ergocryptine (CB-154) or control vehicle, daily, for 6 days (Fig. B). The doses of CB-154 were 0.1 mg/kg/day. As shown in the middle panel of Fig. B, the CB-154 treatment initiated on day 42 of the cycle in nonpregnant bitches caused an abrupt decline in progesterone levels to below 1 ng/ml. Progesterone levels remained below 1–3 ng/ml throughout the treatment. After termination of treatment levels increased transiently prior to a final decline and a foreshortening of the luteal phase. CB-154 injections initiated on day 42 in pregnant bitches induced similar rapid declines in progesterone and resulted in abortions within 3–4 days. Results for one such pregnancy are shown in the bottom right panel of Fig. B. When the CB-154 treatment was initiated on day 22 of the cycle the results suggested that the CL may be less dependent on prolactin (or perhaps more "autonomous") during the early luteal phase than during the late luteal phase. The ergocryptine-induced suppression of progesterone was not as severe or protracted as that observed in the bitches treated in the late luteal phase, as shown in the lower left panel of Fig. B, and was followed by recovery of progesterone levels to control values. This observation, in addition to the relative resistance of canine corpora lutea to the luteolytic effects of prostaglandin in the early luteal phase (Concannon and Hansel, *Prostaglandins* **13**, 533, 1977), suggests that the developing corpus luteum in the dog may have a certain degree of autonomy.

Our observations in the mink may be of greater interest to you in that they point to the evolution in this species of a dependence of luteal function on prolactin as a mechanism to regulate the time of implantation (and thus of parturition) in response to seasonal changes in the daily photoperiod. As indicated in Fig. C, in control pastel mink, following mating and induced ovulation in the first week of March, progesterone levels remain low until activation

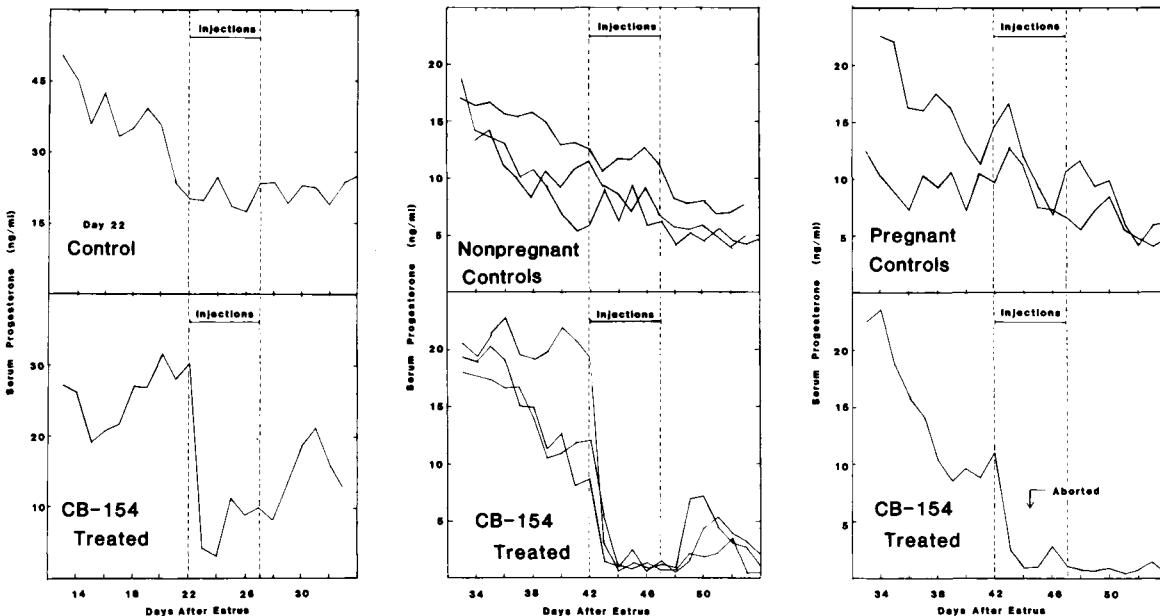


FIG. B. Serum progesterone levels in beagle bitches receiving im injections of ergocryptine (0.1 mg CB-154/kg/day) or control vehicle (20% ethanol, 0.1 ml/kg/day) daily for 6 days.

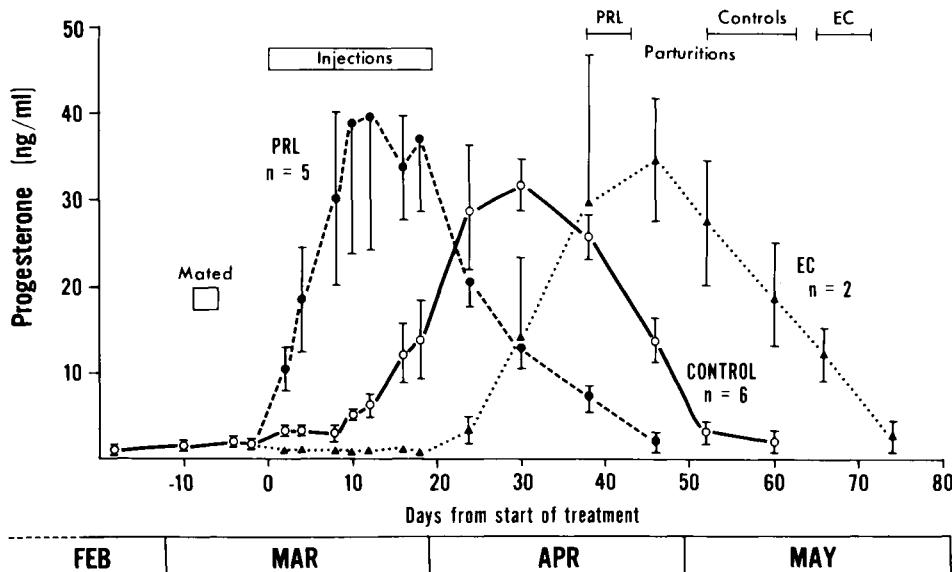


FIG. C. Mean ( $\pm$  SEM) serum progesterone levels in control (○), prolactin-treated (●), and ergocryptine-treated (▲) mink that whelped litters. The ranges of parturition dates are indicated for each group. (From Papke, Concannon, Travis, and Hansel, *J. Animal Sci.* 50, 1102, 1980.)

of luteal function during the fourth week of March, at or shortly after the vernal equinox. We have found that the rise in progesterone occurs at the same time in mink mated to either intact or vasectomized males in the last week of February, in the first week of March, or in the second or third week of March. An artificial increase in hours of light per day following early matings can advance the rise in progesterone and subsequent implantation (Allais and Martinet, *J. Reprod. Fertil.* 54, 133, 1978). I think this photoperiod-sensitive control mechanism may be mediated by prolactin. As you can see in Fig. C treatment with prolactin (0.5 mg NIH-B3/day) during embryonic diapause advanced the times of the rise in progesterone levels and of parturition. Conversely, administration of CB-154 inhibited luteal function and the subsequent delayed rise in progesterone resulted in delayed parturitions. However, prolactin may not be the only luteotrophic requirement in mink. Recently, in collaboration with Dr. Bruce Murphy, we have observed that prolactin NIH-S13 administered daily (0.5 mg/kg) to mink hypophysectomized during delayed implantation caused a rise in progesterone levels for 1-2 weeks and resulted in apparently normal implantations. However, continued prolactin treatment was not capable of maintaining progesterone secretion and implanted embryos underwent degeneration and partial resorption. Finally, I would like to report a related observation that is particularly interesting. In both of the studies involving prolactin administration to mink during delayed implantation we observed an immediate and dramatic shedding of the winter fur and initiation of the summer molt. Thus it seems that the fur molt and the activation of luteal progesterone secretion that normally occur in response to increasing daylight after the vernal equinox may both be initiated by an increased secretion of prolactin. This idea is supported by the recent finding of Martinet; she has measured an increase in circulating prolactin levels in mink at the time of luteal activation and has

evidence that the timing of this increase can be advanced by increasing the daily photoperiod. Our observations on the possible involvement of pituitary prolactin secretion in the initiation of the summer fur molt can be considered in agreement with the results of Rust, Shackelford, and Meyer (*J. Mammal.* **46**, 549, 1965) insofar as they found that hypophysectomy caused the retention of winter pelage and prevented the development of the summer pelage. However, as I recall, they suggested that ACTH might be the hormone involved. Although we have not tested the effects of ACTH and they did not consider the role of prolactin, perhaps the differences in results reflect differences in the purity of hormone preparations available then and now.

Dr. Rothchild, perhaps you could comment on these observations on factors regulating luteal progesterone secretion in the dog and mink in light of your present ideas on the regulation of the mammalian corpus luteum.

**I. Rothchild:** Most of my comments are probably in the article, but I'll try. The effect of prolactin on the pelage of the mink reminds me of what I think is somewhat similar effect on molting in birds, in relation to or associated with the transition from ovulation and lay of a clutch to brooding the eggs. This can be a behavior-induced change. In the mink it's obviously one connected with a seasonal change. In spite of the differences in the specific stimulus that sets off the change in prolactin secretion, both are again examples of the close connection between the secretion of prolactin and the postovulatory aspects of the reproduction cycle, a connection discussed in great detail by Riddle 17 years ago (see References), and one—though by no means the only one—of the reasons that I've postulated that responsiveness to prolactin was probably the first step in the transition from the metatherian to the eutherian corpus luteum.

Your evidence for the dog and mink and Murphy's for the ferret were some of the other things that persuaded me that the long luteal phase of the monestrous breeders is probably primarily dependent on prolactin. That doesn't mean that LH isn't involved at all; your evidence shows that it has a luteotropic effect in the dog, and the inability of prolactin by itself to maintain the mink's CL for more than a few weeks after hypophysectomy suggests that maybe LH may also be involved in controlling the mink's CL. But there isn't enough, at least in comparison with polyestrous breeders, after each season's ovulation in a monestrous breeder for LH to be more than a synergist with prolactin, and probably also not enough to induce a luteolytic effect, i.e., increase the CL's ability to make PGs equal to that induced in a polyestrous breeder. So, prolactin, through its anti-PG effect, can keep progesterone secretion going for a long time, very much as it does, perhaps, in the pituitary autotransplanted rat.

Your information about the mink adds another item to the list of peculiarities of the CL, since it shows us that even among the monestrous breeders things aren't uniform. The dog's CL may not need prolactin crucially until it is well past its third week of life, while the mink's CL seem to need prolactin even to get started! I wonder what the whole picture will be like when we've made a few more comparisons of this kind.

**S. Cohen:** I'm a little bit suspect of whether there is an involved stimulation and an inhibition occurring simultaneously, because to me that sounds too much like driving a car with one foot on the accelerator and the other on the brake, and pushing them both at the same time, and second, about autonomy, how can you be sure that if the corpus luteum can be formed only by LH hormone then how do you know that the so-called autonomous progesterone production may not be a residue of the LH stimulation?

**I. Rothchild:** The answer to your first question is one I've given a lot of thought to. One of the important points I'd like to make about the CL is that almost anything (probably everything) that can act on a CL can have two effects, a stimulatory one and an inhibitory one. That's true for LH, for prolactin, for estrogens, for progesterone, for PGs, for placental

hormones, etc. That's one of the reasons I postulated what I did, i.e., to fit this particular peculiarity of the corpus luteum with its ability to make both progesterone and PGs. So it isn't really a matter of stepping on the brake pedal and the accelerator at the same time. To mix metaphors a little, it depends on what else the CL is doing at any particular time.

For example, LH, through its activation of cholesterol esterases can increase the concentration of both free cholesterol and arachidonic acid within the corpus luteum, as Kuehl pointed out several years ago (see References). Whether this leads to more progesterone because there's more of its substrate available, or to more PGs because there's more of its substrate available will depend on the state of the corpus luteum at the moment when both these substrates become available in increased amounts. I can't, and in fact I don't want to try to specify what such conditions are right now because it isn't necessary to do so to grasp the idea that the diametrically opposite effects of a hormone like LH don't have to be exerted with equal force at all times. What happens within the CL, in other words, depends on which of these two effects is dominant at the time we look at it. In the end, because of the difference between the progesterone and PGs production system, as I pointed out, the PGs will always win.

Your second question I believe was about how one could be sure that what I called autonomy was not the residual effect of the LH that induced ovulation and luteinization. I suppose we will always argue that question but I think several findings indicate, to me, at least, that the ability to secrete progesterone in the absence of the pituitary is not due to any residual effect of the preovulatory LH. The best comes from the tamar wallaby, in which, even as late as 10 months after ovulation, the quiescent CL responds to hypophysectomy by becoming activated. All the evidence from the behavior of granulosa cells in tissue culture, cited in the article, fits with the interpretation that the preovulatory LH is not involved in progesterone secretion of these cells. In the rat's estrous cycle the short period of autonomous progesterone secretion is actually prolonged by treatment with LHAS, as the experiment by Sanchez-Criado in my lab has shown. As far as we know the turnover of hormones bound to a receptor is just too rapid to account for the prolonged duration of progesterone secretion, after withdrawal of pituitary hormones, on the basis of hormones like LH remaining within the CL. No, the findings, as I see them, are most reasonably interpreted as evidence of a capacity for autonomy.

**S. Cohen:** And yet in the human they sometimes speak of progesterone production beginning before the LH surge.

**I. Rothchild:** There is no question that an increased secretion of progesterone occurs in some species before ovulation, and in others, only after the CL has been formed. In the first, the increase comes from the preovulatory follicle, and it may progress without a dip into the pattern of secretion of the definitive CL, as, e.g., in the dog. In others, the preovulatory rise is followed by a dip, and this in turn by the rise of the definitive CL, as for example, in the rat and the human. In the cow, there is apparently no preovulatory rise. I don't yet know how to account for these variations.

**B. G. Steinetz:** This question has to do with the problem of luteolysis. If hamsters are treated with 1  $\mu$ g of PGF<sub>2 $\alpha$</sub> , on day 8 of pregnancy there will occur a marked decrease in plasma progesterone levels. If the corpus luteum is examined 24 hours later, it will show histological signs of involution. The animals will abort if nothing else is done. On the other hand, if at the time the PGH<sub>2 $\alpha$</sub>  is injected one also administers a small dose of progesterone, say 1 or 2 mg, there of course is no drop in plasma progesterone levels since you provided it, but there is also no change in the histology of the corpus luteum and the pregnancy continues uneventfully. How would your theories fit the results of this experiment?

**I. Rothchild:** Your findings are very similar to several others that were done on rats during the similar period of pregnancy except that luteolysis and the abortion that followed it were

induced either by hypophysectomy or by treatment with an LHAS. For example, Raj prevented luteolysis after LHAS treatment by giving the rats dydrogesterone a synthetic that acts very much like progesterone. Ahmad prevented luteolysis after hypophysectomy by treatment with progesterone together with a dose of estrogen that was probably too small to have a direct effect on the CL. Loewit and Zambelis prevented the LHAS-induced rise in intraluteal 20 $\alpha$ -OHSDH by treatment with progesterone, even in the absence of the pregnant uterus, suggesting very much that this was a direct effect of progesterone. All of these are in the article and I touched on them lightly in discussing the possibility that progesterone stimulates its own secretion. One of the interesting tie-ins with your experiment may be a preliminary experiment Sanchez-Criado has done in my lab in which this luteolytic effect of LHAS was prevented by indomethacin treatment. This probably means that there is little difference in the mechanism of luteolysis induced in your hamsters by PGF<sub>2</sub> for example, and in the rats by LHAS; this in turn suggests that your findings are another bit of evidence for a direct luteotrophic action of progesterone.

**J. Weisz:** I'd like to make a point in connection with Ken Savard's interesting statement that the peculiar aspect of the corpus luteum is its ability to secrete progesterone and prevent it from going further. Of course there are biochemical bases for it, but there may be proponents of the idea that the corpus luteum secretes progesterone and does a little bit of what we used to consider the prerogative of the protein secretory hormone secretors; they may be right and that would raise some interesting possibilities. Therefore it might be of interest to look at this question of packaging in different species and at different stages of the corpus luteum—what is your feeling about this?

**I. Rothchild:** Gemmel, the one who lent me the information about the bandicoot, has been much involved in the EM study of the CL and of packaging of progesterone within it. All I can say is that I don't know how to fit it with the rest of the secretion process, and that we'll have to wait to see how widespread this particular form of secretion packaging is. But to come back to the point that you and Ken Savard touched on: how come the CL is such a good progesterone producer? What is it that distinguishes it from other steroid-producing tissue in which the progesterone is used as a precursor for other steroids and is not the typical secretion product of the tissue. Why does this not happen in the CL? As I've already tried to show in my reply to Ken, the solution isn't in looking at the phenomenon teleologically, but in trying to define the characteristics of the CL. One way is by comparing the luteinized granulosa cell with the follicular granulosa cell, and the perhaps not so surprising thing about this is that the major difference between them is in how much progesterone they make, not in what they do with it after it is made. Neither cell can turn the progesterone into androgens very well, and the only reason the follicular granulosa cell makes lots of estrogens and the CL cell doesn't is because the former has a supply of androgens coming to them from the theca and the CL does not. Both cells can turn the androgens into estrogens very easily. But another even more important factor is the great increase in the cell's capacity to make progesterone that takes place when it luteinizes, and here is where the real mystery lies, i.e.: what is luteinization? I've tried to describe what I think happens after the CL is formed, and what was the path of evolution to the CL of modern mammals, but in case you haven't noticed, I avoided saying anything about luteinization, because I have so far only the barest glimmer of an idea about the nature of luteinization where the whole thing started.

**L. Birnbaumer:** It has been shown by Iqbal Khan in K. Ahren's laboratory, as well as by others, that pseudopregnant rats are refractory to the luteolytic action of PGE<sub>2</sub>, during the first 3 days. Full responsiveness to PGE<sub>2</sub>, was shown to be established by day 7 of pseudo-pregnancy.

Dr. Howard Kirchick and I wondered whether perhaps the luteolytic effect of LH in rabbits is mediated by prostaglandins formed *in situ* under the influence of LH. We therefore

injected 6-day pseudopregnant rabbits with hCG to induce luteolysis and tested whether simultaneous administration of indomethacin would interfere with the progesterone-decreasing effect of hCG as seen 18 hours later in the serum of the tested animals. The dose of indomethacin used was such to block the ovulatory effect of hCG known from work by Marsh and LeMaire to require prostaglandins. We found that indomethacin treatment, though blocking ovulation, did not alter progesterone production by the corpora lutea, both in control or in hCG-treated animals. As expected, the latter showed reduced serum levels of progesterone, indicative of initiation of luteolysis. Being aware of the fact that estradiol is essential for rabbit corpus luteum survival and taking into consideration the possibility that hCG treatment might result in a decreased estradiol secretion by neighboring follicles, we repeated the above described experiment in rabbits that had Silastic implants with estradiol of such a size as to increase basal estradiol levels about 3-fold. Again, indomethacin treatment did not interfere with the hCG-induced decrease in serum progesterone levels. It would appear, therefore, that prostaglandins are not the second messenger in the luteolytic effect of hCG. In addition, we also determined that indomethacin did not interfere with the desensitizing effect of hCG as seen in measurements of LH-stimulable adenylyl cyclase activity on corpus luteum homogenates.

I should like to ask you for your opinion as to what the mechanism might be by which LH is luteolytic in ultra-short cycle and in long cycle animals.

**I. Rothchild:** Wasn't this a desensitization effect?

**L. Birnbaumer:** The desensitization of the adenylyl cyclase system precedes the initiation of decreased progesterone production by several hours (up to 10 in the rabbit). However, I do not know whether there is a cause-effect relationship between hCG-induced desensitization and hCG-induced luteolysis. The two effects may also be parallel responses. Thus, receptor occupancy by hCG may result in both increased levels of cAMP and desensitization, i.e., interruption of the receptor-cyclase coupling process. If the luteolytic response is mediated by cAMP, then clearly desensitization and luteolysis, though correlated, are not dependent on each other.

**I. Rothchild:** As I say, I'm not sure that desensitization, or rather, the luteolysis that goes with it, is an effect of PGs. It doesn't seem to be. We have some preliminary evidence that the luteolysis that follows withdrawal of prolactin in the rat also cannot be prevented by PG inhibitors, so it's conceivable that luteolysis could occur following the acute withdrawal of a luteotrophin because an action of the luteotrophin might be to facilitate a crucial step in the production of progesterone. The luteolytic effect of a large desensitizing dose of LH might in some way lead to such a condition, but the luteolytic effect of LH that I was referring to is one which I think happens only in response to a continuous low level rate of LH secretion, and is probably the result of the potentiation of luteal PG production by this level of LH. We're about to set up experiments with indomethacin treatment to test this idea.

**J. Nolin:** I must add my congratulations to the many others which have been offered you this evening. I also want to offer a bit of new information that we've come across recently. I think there can be no doubt that the ideal place to study hormonal regulatory activity would be in the target itself. Hormone in transit can at best tell us what might be available to a target and at worst, absolutely nothing about hormone-target interaction (J. M. Nolin and E. M. Bogdanov, *Biol. Reprod.* 22, 393-416, 1980). We think we have had some success over the last few years in actually looking at some of the peptidial hormones, particularly prolactin, in their targets and we invariably find positive correlations between response and the presence in individual target cells. Not long ago we were able to identify prolactin in the forming corpus luteum during the rat estrous cycle. I think this may not fit your hypothesis, if I understood it correctly.

**I. Rothchild:** The question really is whether the ability of the rats CL to secrete proges-

terone during the estrus cycle diestrus independently of the pituitary is because it has some prolactin within it as it is being formed. Two findings argue against a dependency on this prolactin at this time in the CL's life. One is an old experiment of Alloiteau and Acker (cited in the article), in which they hypophysectomized the rats early in proestrus, and induced ovulation with a single dose of LH. Their findings were the first to show that the CL formed under these conditions functioned autonomously. Uchida and his co-workers (see References) made similar findings. The other is from the studies of Smith and Neill and Day and Brinbaumer (see References) which show in the one case indirectly and in the other directly, that the rat's CL doesn't respond to prolactin until after its "autonomous" progesterone secretion has reached its peak value, i.e., between the second and third day after ovulation. But the main things about my idea of autonomy is that there is much more evidence for it than just the way progesterone is secreted during the rat's estrus cycle.

**J. Nolin:** Hypophysectomy, whether done before or after ovulation, involves removing only the source of prolactin. The question is: how long after hypophysectomy does any prolactin remain available to the ovary? We might also ask whether there might be any intercompartmental transfer of prolactin already present in the ovary.

As for the CB-154 approach, this drug produces a relatively precipitous decline in prolactin release from the pituitary gland but not obliteration of release.

Your third point, that treatment with exogenous prolactin doesn't change progesterone secretion until after day 2 of the luteal phase, at first glance, seems to be a strong argument for your hypothesis. Nevertheless, since it does not take into account the presence of endogenous prolactin already in the corpus luteum, it would seem to me that the question of prolactin involvement in corpus luteum function during the rat estrus cycle has not yet been altogether resolved.

**G. MacDonald:** My only response is that prolactin must be given to hypophysectomized rats quite soon after surgery to maintain progesterone secretion. This is independent of the day of the cycle that the animal is operated.

**L. Birnbaumer:** Dr. Sharon Day's experiments in my laboratory showed that the critical initial PRL surge, which rescues the CL of the cycle from regression allowing them to become CL of pseudopregnancy, is one that occurs on the morning of diestrus-2. These studies confirmed a similar conclusion made earlier by both M. S. Smith in Dr. J. Neill's laboratory and B. Fluchinger. It follows from this that the previous surges are there probably only because it is simpler to set the hypothalamic clock on the afternoon of proestrus (i.e., mating), to initiate surges immediately, as opposed to setting it in such a manner as to start 3 days later.

**G. MacDonald:** So that implies the data Dr. Rothchild quoted suggesting prolactin has to be applied within 24 hours of hypophysectomy are incorrect.

**I. Rothchild:** That's in the pseudopregnant rat. The general rule is that depriving rat CL which are actively secreting progesterone of prolactin for 24 hours or more leads to a luteolytic effect of the prolactin when it again acts on the CL. But if the CL have never been exposed to prolactin, as in the experiment of Alloiteau and Acker I just mentioned, they are not sensitive to this luteolytic action of prolactin for as much as 5 days after hypophysectomy. I think it may have to do with the ability to secrete progesterone autonomously. We also find the same resistance to the luteolytic effect of prolactin in the CL of pregnant rats during the period after day 12 (Lam and Rothchild, 1973), when the CL secrete progesterone in the absence of the pituitary and placentas for at least 3 days (Rothchild *et al.*, 1973).

**R. O. Greep:** How do you think your theory fits with or helps us understand the basis of the persistent corpus luteum in women?

**I. Rothchild:** I'm glad you asked that because I think it makes an interesting comparison with the others shown in Figs. 9 and 10. One can show the effect of changing the regression

process in the guinea pig and rat on the life span of the CL and its pattern of progesterone secretion because of the specific roles of the uterus and the pituitary in this process in these forms, but the uterus has no effect in primates. Perhaps phenothiazine treatments could reproduce in primates the conditions that prolong the life span in the pituitary-autotransplanted rat, but that can't be done ethically in women and nobody's tried it in monkeys. The persistent corpus luteum, however, seems to be the result of a change that resembles what has happened in the hysterectomized guinea pig and the pituitary-autotransplanted rat, and I'd be willing to bet that if one could measure progesterone levels in these women from the time the CL was formed until its eventual regression (usually about 3-4 months later) one would see a pattern essentially the same as those shown in Fig. 10B. It would also be interesting to see what effect treatment with either CB-154 or a PGF<sub>2</sub> analog would have; my guess is that either treatment would induce CL regression and a resumption of ovulation cycles.

**H. Adlercreutz:** I would like to discuss a new class of compounds in man and animals which is closely connected to gonadal function and especially to the corpus luteum phase of the menstrual cycle and early pregnancy. Others involved in these studies include K. D. R. Setchell, A. M. Lawson, F. L. Mitchell, D. N. Kirk, and M. Axelson.

During studies of steroid excretion by the vervet monkey, a number of phenolic compounds were consistently detected in polar steroid extracts during gas chromatography-mass spectrometric analysis. Although their mass spectra were atypical of steroids, the major compound was found to exhibit a cyclic pattern of excretion during the menstrual cycle in which a maximum and approximately 4-fold increase occurred in the luteal phase (Setchell, Bull, and Adlercreutz, *J. Steroid Biochem.* **12**, 375, 1979).

Previous observations of the presence of these compounds in human urine prompted an investigation of their behavior during the human menstrual cycle. Preliminary results from a limited number of subjects confirmed the initial observation of the cycling behavior and demonstrated a maximum and 4-fold increase during the early luteal phase of the menstrual cycle of human (Setchell and Adlercreutz, *J. Steroid Biochem.* **12**, xv, 1979). The excretion of the two major phenolic compounds (compounds 180/442 and 180/410) in one subject in relation to estrone-glucuronide and pregnanediol-3 $\alpha$ -glucuronide during a menstrual cycle can be seen in Fig. D.

Measurements of the major compound (180/442) in urine samples collected during pregnancy showed that the maximum excretion occurred between 14 and 22 weeks of gestation, thereafter levels declined. No significant difference in the urinary excretion in an anencephalic pregnancy was seen (Fig. E) and the extremely low levels in newborn infants indicate a maternal origin for these compounds during pregnancy. These preliminary findings were presented at the IXth International Study Group for Steroid Hormones, Rome 1979 (Setchell, Lawson, Axelson, and Adlercreutz, "Research on Steroids IX," 1980, in press).

An intensive investigation of the distribution of these compounds has led to their recognition as urinary constituents in human, baboons, and vervet monkeys and they have also been identified in human plasma and bile. Many of the general physicochemical and chromatographic characteristics in addition to the mass spectrum of the trimethylsilyl ether derivatives of the two principal phenolic compounds were reported recently (Setchell *et al.*, 1979).

Gas chromatography-mass spectrometry, nuclear magnetic resonance spectroscopy, infrared spectroscopy, and wet chemical techniques have shown that these compounds belong to a class known as lignans which contain a 2,3-dibenzylbutane skeleton as their basic structure. The two compounds were definitely identified, following the chemical synthesis of reference compounds: (1) 2,3-bis(3'-hydroxybenzyl)butyrolactone (previously referred to as compound 180/442), and (2) 2,3-bis(3'-hydroxybenzyl)butane-2,4-diol (previously referred to as compound 180/410). The chemical formulae of these two compounds are shown in Fig. F,

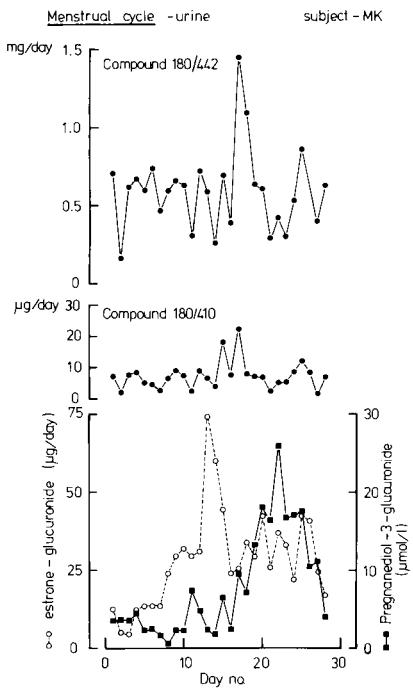


FIG. D.

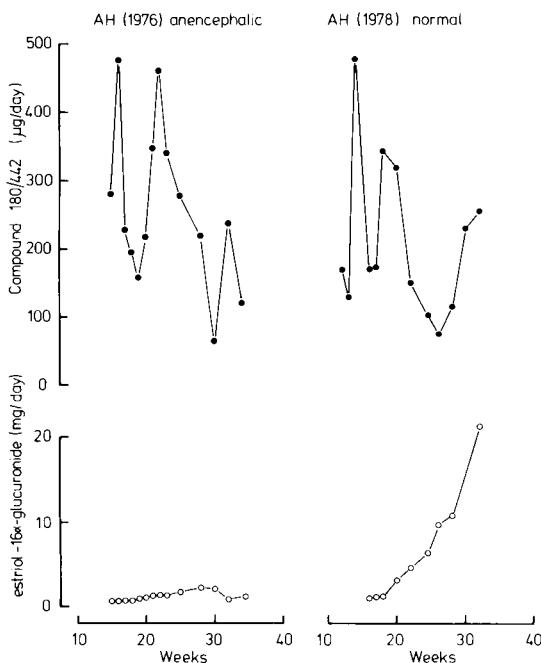


FIG. E.

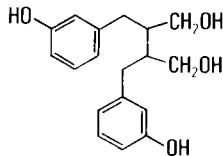
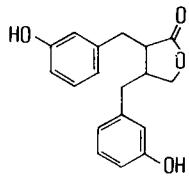


FIG. F.

and a comparison of the mass spectra of the trimethylsilyl ether derivatives of the natural (first and third panels) and authentic compounds are shown in Fig. G (Setchell, Lawson, Mitchell, Kirk, Adlercreutz, and Axelson, *Nature (London)* 1980, in press). These compounds have been found in urine conjugated to glucuronic acid.

The biosynthesis of these lignans in humans has not yet been determined but their highly aromatic nature might suggest a dietary origin or their formation by intestinal bacteria. Our preliminary results, however, indicate that they may be ovarian in origin or that their production is influenced by ovarian function. The cyclic behavior in the menstrual cycle, the much lower levels in newborn infants and children, and the reduced excretion in post-

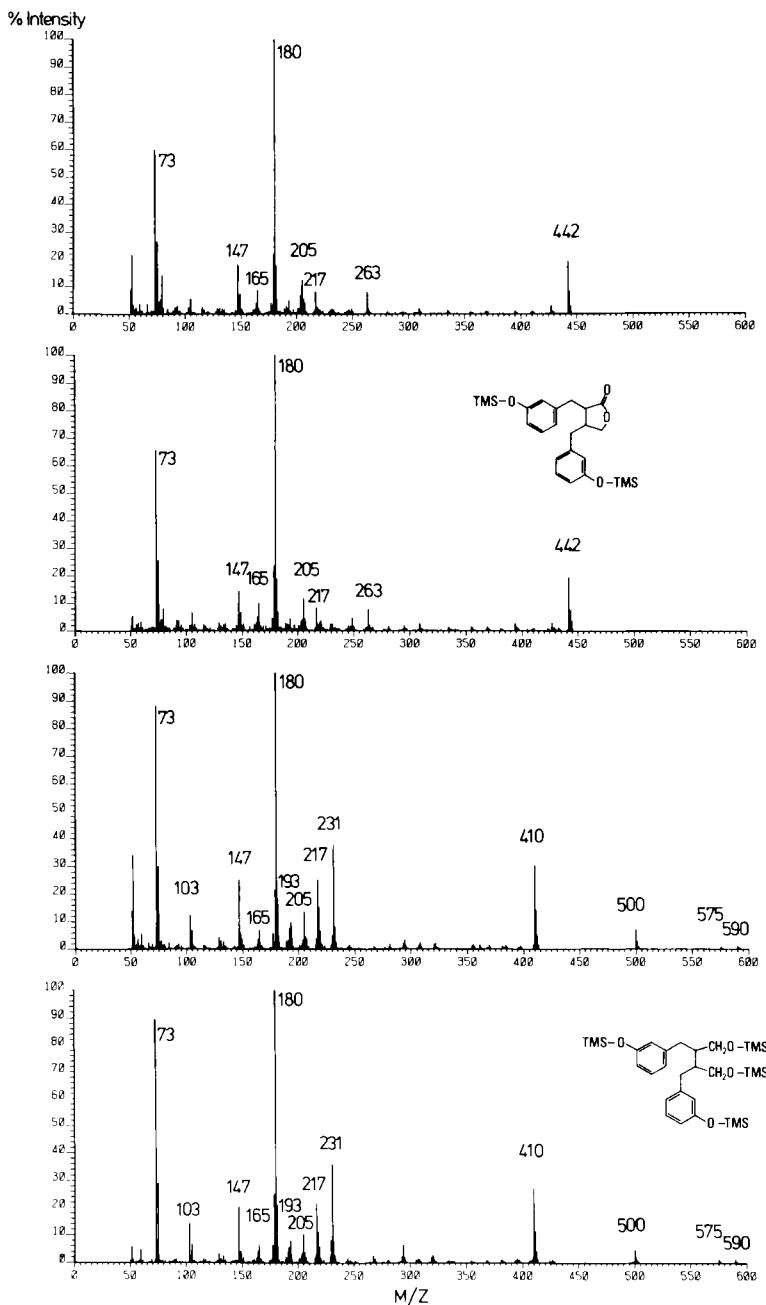


FIG. G.

menopausal and ovariectomized women would support this view. Both compounds have, however, been detected in adult males.

The occurrence of lignans in humans and our preliminary observations on their disposition pose questions as to their physiological and biological significance. Lignans are a group of uncommon plant constituents which have only been found in higher plants of the orders Gynerospermophytina and Angiospermophytina. They have been extensively investigated by the natural products chemists because of the known antimitotic activity of many plant and synthetic lignans and their potential anticancer activity. The structures of the lignans described here, however, have never been identified in plant species which presumably reflects differences in pathways of biosynthesis or metabolism. The diphenolic and highly aromatic nature of these compounds are characteristic of many substances which possess properties which are either highly estrogenic or antiestrogenic. Although it is yet to be determined if these lignans possess any biological activity, their cyclic excretion may suggest they play an important role in the regulation of the menstrual cycle.