THE MAMMALIAN FETUS: PHYSIOLOGICAL ASPECTS OF DEVELOPMENT

COLD SPRING HARBOR SYMPOSIA ON QUANTITATIVE BIOLOGY

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The Mammalian Fetus: Physiological Aspects of Development

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FOREWORD

During the eight days from June 7 to June 14, 1954, more than a hundred and twenty-five American and European scientists in the fields of anatomy, physiology, genetics, and clinical medicine met at the Biological Laboratory to attend the nineteenth Cold Spring Harbor Symposium on Quantitative Biology. This year's meeting was on the development and physiology of the mammalian fetus, a topic that has not been taken up before in our Symposia. According to the plans prepared by the chairman of the program committee, the primary aim of this conference was to evaluate the present knowledge about the morphological, physiological, and biochemical factors that affect the development of the embryo, in particular the human embryo. The meetings began with a consideration of early embryonic development, then took up a variety of integrated physiological activities necessary for the development of the fetus, and finally discussed the metabolic characteristics of the fetus as a whole and of its different parts.

Following the custom established during the past few years, this volume contains the lectures presented by invited participants, in the order in which they were given, and also the discussions which followed them, whenever these were submitted in manuscript form to the editor of the volume.

Because Europe is the research center for the problems discussed at the meeting, it was important to have a considerable number of European scientists present. This was made possible by special grants made to the Laboratory by the National Science Foundation and the Association for the Aid of Crippled Children, in addition to the long-term grant received for the expenses of the Symposia from the Carnegie Corporation of New York. We were able to invite twenty scientists from abroad. I wish to acknowledge this support and express our gratitude to the organizations who gave it.

I want also to convey grateful appreciation to the members of the program committee, Donald H. Barron, Louis B. Flexner, A. St. G. Huggett, J. S. Nicholas, and Clement A. Smith, and particularly to its chairman, S. R. M. Reynolds, for their extremely valuable and effective cooperation in organizing the meeting.

M. DEMEREC

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COLD SPRING HARBOR SYMPOSIA

ON QUANTITATIVE BIOLOGY

Founded in 1933

by

REGINALD G. HARRIS

Director of the Biological Laboratory

1924 to 1936

The Symposia were organized and managed by Dr. Harris until his death. Their continued usefulness is a tribute to the soundness of his vision.

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DEVELOPMENTAL CHANGES AND FUTURE REQUIREMENTS

S. R. M. REYNOLDS

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We are now assembled for the opening of the nineteenth Cold Spring Harbor Symposium. Some have come from great distances, and not a few have come from other countries. We are a varied group, with respect to origins and training, as well as to areas of interest. We have come, however, to discuss and consider one common problem, namely, that of the functional development of the mammalian organism. This common interest has many sides. When your Committee began to consider participants for this Symposium, it was clear at once that so many and so varied were the topics that *might* be considered that it would be necessary to formulate a statement of what *ought* to be considered. We agreed upon the following statement:

Objective of the Symposium

The primary function of this Symposium is to bring into view and to evaluate the principal morphological, physiological and biochemical factors that are essential for the immediate well-being, and so the future development, of the mammalian organism. This involves the relation of hereditary and other factors to fetal development and mother-fetus relationships. It involves understanding of the development and function of the fetal membranes. In short, attention should be brought to bear upon the homeostatic factors which contribute to normal somatic and visceral development of the embryos to meet the requirements for living before, during and just after birth.

Various conferences in recent years have dealt with factors in embryonic development. The approach is usually that of experimental pathology, biochemistry and physics in relation to abnormal development. Seldom is an attempt made to fit together these broken pieces in order to understand the normal mechanisms which may have been altered to give rise to one or another abnormal condition. It is usually considered sufficient to describe and classify the end results. But normal processes are not usually to be understood by enumerating abnormal ones. Therefore, the limited experimental approaches that lead to different kinds of fetal malformation are to be avoided in this Symposium.

It is both wise and necessary to make some selection for emphasis among the various physiological processes whose normal functioning might be discussed. Thus, digestion and absorption of foods are not factors of crucial importance during the period of adjustment from intrauterine to extra-uterine life. On the other hand, circulation, tissue anabolism, respiration, the complex of kidney-endocrine-body water-electrolyte functions are critical. Similarly, comparative evaluation of some mechanisms such as temperature regulation, for example, sheds light on comparative fetal maturity at birth. What, we may ask, are the beginnings of these and similar mechanisms in development? How are they interrelated? Exactly what mechanisms are essential? How may morphological organ system interrelations be best defined? And how are these related to intracellular mechanisms?

The underlying design of this Symposium should be clear, therefore. We should avoid frittering away time unnecessarily in discussions which "lead one down the garden path." Our limited positive objective is to attempt to assess current knowledge concerning the requirements for normal living before and just after birth. It should then be possible to evaluate current practices of management of newborn and premature infants, and to understand those conditions of early embryonic development which provide the basis for normal somatic, visceral and mental development in later life.

Our avowed purpose, then, is to assess those aspects of development which are clearly essential at all times for normal living before and just after birth. This objective requires a degree of elaboration, for the embryo and fetus present a quite different organism from the post-natal animal, with which most investigators are familiar. There, we are wont to think of basic survival in terms which Claude Bernard defined when he conceived of the *milieu intérieur* of the body. We are wont to think, too, of an organism, as described by Cannon, which responds to changes in its external environment by a wide range of compensatory internal physiological adjustments which he called *homeostasis*. Or again, we think, as does the physiologist Rein, that metabolic need in a tissue is supplied by the mechanism of reactive hyperemia *after* the demand for it is created by activity.

Each of these concepts finds its own special application in embryonic and fetal development. None of them, however, defines the truly *cardinal* characteristics of functional development before birth. I refer to a fact which each of us knows, to which each one pays a certain lip service, but which we have not genuinely recognized. I refer to a very special aspect of embryonic development, which finds exemplification not only in the embryo and fetus. We see it, too, in the maternal reproductive tract, from the ovaries throughout the length of the birth canal. Let me give you some examples of what I mean.

The embryonic development of the chick heart, as visualized by Alexander Barry, reveals a succession of stages each one of which suits its present needs but would be insufficient for future needs and development. In the first instance, the heart begins as a small tube. It possesses a small lumen, a thick gelatinous layer called "cardiac jelly", and outside this, a very thin contractile syncytial layer of embryonic cardiac muscle containing coarsely striated myofibrilli. There are no auricles, ventricles or valves. The heart consists only of an unpartitioned ventriculo-truncus.

Blood is propelled in such tubes because a muscle twitch begins at the caudal end of the tube and moves toward the other end. The muscle twitch reduces the lumen of the tube locally by deformation of the cardiac jelly. This closed portion is swept ahead of the twitch. This is, therefore, a kind of "functional" valve that propels the blood ahead. Contractile energy deforms the viscous jelly and propels blood. Upon release of tension when the muscle relaxes, the deformed cardiac jelly springs back into its original shape. This, then, aids in the refilling of the embryonic heart supplementing the effect of the venous return pressure—a primitive venous return mechanism, if you will.

This arrangement suits the embryo very well for a brief time. The organism grows both larger and more complex, however. The cardiac tube grows in proportion. The cardiac jelly occupies proportionately less space in the cross section as the lumen becomes larger, so that greater muscular effort is necessary to occlude the lumen. This is possible to a degree by an increase in the quantity of cardiac muscle. As the force of the heart beat becomes stronger, so does the velocity of the stream of blood within it. This creates forces which are not only suited to the *immediate* circulatory requirements of the embryo, but also have a beneficial result for the *future* of the embryo! The viscous cardiac jelly is swept along the walls of the growing, bending tube by the force of strong contractions on swift, momentary currents of blood. The jelly is forced into positions which are predetermined by the shape of the heart, the distribution of the jelly, and the magnitude and direction of the forces which set on it and the length of time over which these forces act. These local "accumulations" of cardiac jelly are the precursors of the valves of the heart and of the chordae tendinae which become organized out of the cardiac jelly masses. *We see, therefore, that the character of a present structure and its function simultaneously contribute in a very precise and definite manner to the immediate support of the embryo and to conditions that affect the future welfare and development of the fetus.* If the transition did not occur *before* the need arises that need could not be fulfilled, and the whole course of development would be thwarted.

There are numerous other instances of this principle of present developmental changes which anticipate future requirements. Thirty years of intensive research in physiology of reproduction have turned up many examples. Thus, we know of the necessity of estrogenic stimulation of the uterus as a prerequisite for progesterone action. Sensitization of the uterus for implantation necessarily precedes the onset of the implantation process. We are familiar with the fact that uterine hyperplasia precedes the stimulus for and fact of uterine hypertrophy, and we now recognize that uterine growth is virtually complete *prior* to the time when the daily increment of fetal growth is so great that it stretches the uterus very considerably.

Before gestation,—indeed even before fertilization,—a very great vascular proliferation takes place in order to support a possible future pregnancy. Menstruation in some species is evidence of this fact.

At the end of gestation, we know that the myometrum progressively acquires a biochemical make-up and character which favor the uterine contractions of labor. These changes involve a gradient of activity with respect to coordination of contractility and power.

Returning to the conceptus, we know that the placenta attains its maximum size before the fetus is of any appreciable size. It serves, at this time, as lungs, kidneys, intestines and liver, and possibly as an endocrine system, as well. All of these potentialities are developed in anticipation of a future need in the development of the fetus.

This list could be enlarged very much. I conceive it to be a function of this Symposium to lay down the ground rules for this game of—what shall we call it? We need a term which is an analogue to that of *milieu intérieur* and *homeostasis*.

I discussed this point several years ago with Prof. R. G. Harrison, of Liverpool. He has proposed (through the agency of Prof. A. H. Armstrong, Professor of Greek at Liverpool) that we coin a word. He suggests

PROPHTHASIS

PRO means to favor; PHTHASIS from the word *phthanō*, means to arrive first, or to anticipate. Prophthasis, therefore, would mean the occurrence of developmental changes in the growing organism and its environment which provide critically for the future survival of the organism. Prophthasis could refer to a wide range of morphological and functional changes taking place in an embryo or fetus during a limited stage of development which critically anticipate future morphological and functional requirements for the survival and welfare of the organism. This could be projected, no doubt, from womb to tomb, and could relate, I do not doubt, to *mens sana et corpus sanus.*

SOME ASPECTS OF THE ENDOCRINE ENVIRONMENT OF THE FETUS

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Thirty years ago our ideas in England about the endocrine background of the sexual cycle, derived largely from the writing of F. H. A. Marshall, were comparatively simple. Graafian follicles developed in the ovary, and in doing so secreted a hormone which brought the animal into oestrus. Ovulation then took place, with or without the stimulation of mating, and a corpus luteum developed from the shell of the ruptured follicle and produced another hormone which prepared the uterus for the reception of the fertilized egg. If implantation took place, the corpus luteum persisted, maintained pregnancy, caused development of the mammary gland and then degenerated at some stage before parturition. If conception did not follow ovulation the corpus luteum maintained a pseudopregnancy which according to the species might be of trifling duration or as long as true pregnancy, and in which the reproductive tract underwent varying degrees of development. Somewhere in the background lurked a "generative ferment", the fluctuating supply of which, combined with events in the reproductive tract, regulated the cyclic changes in the ovary. This simple outline was vindicated in its bare essentials by the preparation of oestrus-producing extracts of ovary, by the preparation of progesterone from the corpus luteum and particularly by the tracking down of the "generative ferment" to the gonadotrophins of the anterior pituitary body. In all but essentials, however, it has been subjected to one shock after another, so that present views, while they may be more comprehensive, are certainly no longer simple. One of the shocks was administered by me, in showing that cyclic activity in the reproductive tract might persist in the absence of the cyclic structures of the ovary. Most of the shocks, however, have related to the endocrinology of pregnancy, and here I can plead not guilty. Allen and Doisy's early oestrus-producing preparations were obtained from *liquor folliculi*, or whole ovary, as was right and proper, but similar activity was very soon found in human and other placentae, as might have been expected from the earlier work of Herrmann and others using tests less specific than vaginal cornification. This presence of large amounts of oestrus-producing substances in the placenta was difficult to reconcile with the idea of pregnancy simply as an extended luteal phase, and with the observed fact that in some species, notably the rabbit, pregnancy could be terminated up to perhaps half-term by the administration of comparatively small amounts of oestrogen. I remember well the perplexity caused to Marshall by this apparent anomaly, and the indignation with which I wrote late in 1926 that for the placenta to elaborate "oestrin" would be to attempt suicide. However, the difficulty remained, and, at best, one could only fall back on the idea that the oestrogen found in the placenta was not elaborated there. Shortly afterwards, the association of oestrogen with pregnancy was confirmed by the discovery of large amounts in the urine of pregnant women and of mares. In rather the same way, the discovery of the presence of gonadotrophins in the anterior pituitary body was followed very soon by the discovery of large amounts of similar substances in the urine of women and the blood of mares during early pregnancy. In both cases there is good reason to believe that the extra-hypophyseal gonadotrophins found during pregnancy are elaborated, if not by the placenta, at least by products of conception. Of the three main types of hormones associated with the sexual cycle, therefore, only the hormone of the corpus luteum, originally thought to be characteristic of that phase, does not appear in large amounts during pregnancy. This curious paradox is perhaps made less striking by the fact that our knowledge of oestrogens is largely derived from active excretion products, whereas progesterone is excreted as the inactive pregnanediol. It is doubtful, however, whether progesterone in relation to its physiological activity, ever appears during pregnancy in anything like the amounts in which the other two types of hormone are found.

We have here, therefore, to recognize the rather odd situation that oestrogens and gonadotrophins are the most prominent hormones found during pregnancy in certain species, and may appear then in enormous quantities. The pregnant female and perhaps her fetus are thus subjected to concentrations of oestrogens and gonadotrophins far greater than those encountered at any other period of life. We must ask, therefore, whether any effects appear in either which can be ascribed to the presence of these vast amounts of hormones, irrespective of whether or not the effects can be regarded as playing any essential part in the physiology of the pregnant female or the development of the fetus. This question is particularly relevant in the case of the oestrogens, which were long ago shown by Courrier to cross the placenta and affect the fetus when injected to the mother in late pregnancy.

In considering the possible effects of endogenous oestrogens on the fetus it is necessary to delve back into history, because at least one phenomenon has been known for centuries which can be ascribed to the hormones circulating in pregnancy — the "witches' milk" sometimes secreted by newborn human infants. The fact that many mammals are normally born in a more advanced state of sexual development than their own endocrine make-up can support, and that the affected organs regress after birth, has now become well recognized. The condition long ago received the apt description of "crise genitale du nouveau-né" from Courrier, who described hyperplasia of the vaginal epithelium in the newborn guinea pig, and, because it was not maintained in the post-natal guinea pig, ascribed it to the action of maternal oestrogens.

It is my intention in this paper to discuss briefly some aspects of this fascinating subject, giving particular attention to lesser known examples and to effects produced on the maternal ovaries by the oestrogens and gonadotrophins of pregnancy. It is not my intention to deal with other hormones, though it must be remembered that these are very much involved in the fetal life, and that the adrenals of the newborn human infant suffer a crisis as severe as anything seen in the reproductive organs.

MAN

The facts are well known and need be mentioned only for comparison with those relating to other animals[1]. Oestrogen and gonadotrophin both begin to appear in appreciable quantities in the urine of the pregnant woman during the sixth week after the last menstruation. The oestrogen increases, slowly up to about 100 days and then much more rapidly, to give amounts at full-term varying between 10 and 40 mg per 24 hrs, and a total excretion during pregnancy which must sometimes be of the order of several grams. Pregnanediol excretion follows a similar course, but it is difficult to know how far this indicates progesterone activity. The gonadotrophin excretion, by contrast, rushes up to a peak at about 60 days, then falls equally rapidly up to about 80 days, and almost disappears by 120 days (Browne and Venning, 1936; Venning, 1948). The circulating gonadotrophin follows a very similar curve, except that the peak concentration seems to last rather longer in the blood than in the urine, and the active substance appears qualitatively to be of the same nature as the urinary gonadotrophin (Boycott and Rowlands, 1938). The rise in gonadotrophin content seems to correspond with the later stages of the corpus luteum of pregnancy and with the transfer of its function to the placenta, but it has no obvious direct effect on the ovaries of the mother. The oestrogen in pregnancy is no doubt intimately concerned with the development of the reproductive tract and mammary gland, but it is a remarkable fact that a woman becomes more or less immune to exogenous oestrogen during pregnancy and will tolerate doses which would otherwise cause grave disturbances. Both oestrogens and gonadotrophin are present in the placenta in large amounts throughout pregnancy and are apparently elaborated there, since they appear respectively after removal of the ovaries (Waldstein, 1929) and in cultures of placenta tissue (Jones, Gey and Gey, 1942).

It is not surprising that the fetus in this endocrine environment shows precocious changes in the reproductive organs. Hypertrophy of the mammary gland in newborn infants has already been referred to, and according to Fraenkel and Papanicolaou (1938) is the rule rather than the exception in both sexes. Other hypertrophic changes, which quickly disappear, are listed by Courrier (1945) and include more or less overt changes in the ovary and in the whole of the reproductive tract of the female. The paper by Fraenkel and Papanicolaou may be consulted for details. Analogous changes occur in the male. The peculiar histological characteristics of the human prostate gland at birth, which disappear within a few weeks, were ascribed by Halban as early as 1905 (Halban, 1905) to the action of maternal hormones. From our present viewpoint the important thing is that the peculiarities consist mainly of epithelial metaplasia, which is characteristic of the oestrogenized prostate, and they can be reproduced in the slightly older male child by the administration of oestradiol (Sharpey Schafer and Zuckerman, 1941). There is no indication of androgenic stimulation.

HORSE

The discovery of abundant gonadotrophin in the blood and of oestrogen in the urine during pregnancy led to the mare becoming the chief commercial source of these two hormones. As a result the periods of maximum concentration were worked out in some detail. The gonadotrophic phase lasts from about the 40th to the 120th day of pregnancy, and large amounts of the hormone are present in the circulating blood between the 45th and 80th day (Cole and Hart, 1930a). The peak concentration occurs at about 60 days, when as much as 200 I.U. per ml may be present, equivalent to about 3 million I.U. in the circulating blood (Day and Rowlands, 1940). This enormous quantity of gonadotrophin seems to originate in the endometrial cups opposed to the chorion which were described by Cole and Goss (1943) and Rowlands (1946). In spite of the high concentration in the blood stream little if any of the gonadotrophin is excreted in the urine. The effect of this circulating gonadotrophin on the maternal ovaries is dramatic. The corpus luteum of conception regresses by the end of the sixth week, but as Cole, Hart, Lyons and Catchpole (1933) originally recorded, the maternal ovaries undergo great development thereafter, that is, during the seventh week, and the growth of

[1] A few only of the relevant references are given in this section.

follicles leads to ovulation or to the formation of atretic corpora lutea. Rowlands (1949) records that the ovaries of 17 mares examined between the 46th and 74th day of pregnancy contained an average of five large follicles and three fully formed corpora lutea. One to three ova were obtained from the tubes of more than one half of the mares. Ovarian activity wanes as the amount of circulating gonadotrophin decreases, and by the 120th day no more follicles are being produced, though existing corpora lutea last for another month or so. Thereafter the maternal ovaries become completely quiescent. The large amounts of gonadotrophin circulating during the gonadotrophic phase appear to have no effect on the fetal gonads, which are rudimentary at this time, and it is probable that the placenta acts as an effective barrier to the hormone.

The oestrogen of the pregnant mare follows a concentration curve reciprocal to that for gonadotrophin. It is present in only very small quantities in the urine during early pregnancy, and does not appear in large quantities until the gonadotrophic phase wanes. It increases to a peak in the seventh and eighth months of pregancy, when as much as 50 mg oestrone per liter may be present. The concentration then falls off to the end of pregnancy (Kober, 1938). This vast amount of oestrone is probably of placental origin (Hart and Cole, 1934). In any case, it can only be excreted via the circulating blood, although the concentration therein is very small (Cole and Hart, 1930b). As mentioned above, the maternal ovaries are atrophic during the oestrogenic phase and perhaps indicate the usual depressant effect of oestrogens on ovarian cortical tissue. By contrast, the fetal gonads undergo remarkable hyperplasia, which had frequently been recorded without arousing much interest in the half-century before the work of Cole and Hart brought its significance to light. Enlargement begins about the fourth month, and continues for three to four months, by which time the fetal gonads increase in weight from one g to 150 g, the latter being twice the size of the maternal gonads. They are five to seven cm long by about four cm in diameter, kidney shaped, dark red and smooth in outline. Dr. Rowlands tells me that they were known to Aristotle who regarded them as an extra pair of kidneys. This enormous development of the fetal gonad is due entirely to hyperplasia of the interstitial tissue; at all stages the embryonic tubules of the testis and the germinal epithelium and oocytes of the ovary are rudimentary and inconspicuous. After the eighth month the fetal gonads undergo regression by the involution of the interstitial tissue (Cole *et al.*, 1933), and at birth the ovary contains numerous follicles up to two mm in diameter, and a mass of degenerate interstitial tissue. Amoroso and Rowlands (1951) record that the genital tract of the female fetus shows effects typical of oestrogenization. Events in the male accessory organs do not seem to have been recorded. The coincidence of the rise and fall of oestrogen excretion with the hyper- and hypoplastic changes in the interstitial tissue of the gonad is so striking that it is difficult not to conclude that they are intimately related.

Seal

Highly suggestive observations on seals, reminiscent of those made earlier on women and mares and their newborn young, have recently been recorded by Amoroso, Harrison, Matthews and Rowlands (1951). As might be supposed, it has not yet been possible to determine the oestrogen and gonadotrophin content of the blood or urine of pregnant seals, but, as in the foal, remarkable conditions are found in the pre-natal and neo-natal seal pup. All the near-term female fetuses and newborn pups examined by Amoroso and his coworkers had ovaries greatly enlarged by hypertrophy of the interstitial tissue. As in the foal, the cortical tissue was insignificant. Associated with the ovarian hypertrophy there was enlargement and engorgement of the uterus, and growth of the vaginal epithelium. Histological examination showed that the uterine glands had developed in a way not normally found at oestrus in the seal and that the vaginal epithelium had undergone mucification. It may be inferred, therefore, that the reproductive tract of the fetus is acted upon by both oestrogen and progesterone, and that the female seal is born not merely showing signs of sexual maturity, but actually pseudopregnant. Retrogressive changes start within a few days of birth and during the second week the ovaries have shrunk to a quarter of their previous size and the uterus is in involution.

Amoroso and his colleagues have not yet published a full description of the existing material, and their preliminary account deals mainly with the female pup. The testes of the full-term and neonatal male, however, are also greatly enlarged by the massive development of interstitial cells, and the waxing and waning of this tissue seems to follow the same course as in the case of the ovary. In a private communication, Dr. Harrison tells me that the prostate gland is much enlarged in the newborn seal, but unlike the condition in the newborn boy, is suggestive of androgenic rather than of oestrogenic stimulation.

It has not yet been possible to obtain a complete set of material at different stages of pregnancy, so that the genesis of this remarkable condition is not known. The maternal ovaries at term, however, contain a single old corpus luteum—in addition to large follicles ripening for the postpartum oestrus—and there is no reason at present to suppose that ovulation takes place during pregnancy.

Elephant

A most intriguing addition to the story of the "crise genitale" has recently been provided by Perry (1953) in his work on the African Elephant. As with the seal, we do not yet have for the elephant any information about hormones circulating or excreted during pregnancy, but remarkable events take place in the maternal ovary and in the fetal gonads during the later stages and at the end of the 22 months' gestation period. The ovaries of the pregnant elephant are markedly different from those of the pregnant mare in that active corpora lutea are present throughout, and are contributed to by at least one supplementary ovulation. Perry enumerates as follows the problems raised by this state of affairs: "Several questions present themselves regarding the fate of the corpus luteum of conception, the stage at which accessory corpora lutea are first formed, and whether, once formed, they persist throughout gestation or are replaced once or several times." He concludes from his material that a number of accessory corpora lutea are formed early in pregnancy, probably at the same time as the corpus luteum of conception. The corpora lutea dating from the beginning of pregnancy last until about mid-term, when they are replaced by a similar set which last until term and then involute rapidly. On each occasion some of the corpora lutea are formed following true ovulation and some from follicles luteinizing without ovulation. The ovaries of one cow elephant, for instance, having a six kg embryo and estimated to be nine to ten months pregnant, had 26 newly-formed corpora lutea of which nine appeared to have resulted from ovulation. The first crop of corpora lutea remained only as small dark-colored bodies.

The condition of the gonads in the pre-natal elephant fetus was very similar to that seen in the pre-natal foal. Hypertrophy was very marked in all fetuses of estimated age 16 months and older, the ovary becoming more than four times its neo-natal weight. In both sexes the enlargement is due to overgrowth of the interstitial tissue, which regresses after birth. According to Perry, in the case of the ovary the tissue looks remarkably luteal-like, a suggestive comment in view of the apparently pseudpregnant state of the newborn seal. Information about the state of the reproductive tract of the newborn elephant will be awaited with interest.

Other Species

Reference has already been made to the guinea pig. In this species the corpus luteum of conception is maintained throughout pregnancy. During much of the time large follicles are present, but no ovulation takes place, and nothing dramatic in the way of hormone circulating or excreted during pregnancy has been recorded. Moreover, the gonads of the newborn are not hypertrophic. The "crise genitale" in the guinea pig therefore seems to amount to hyperplasia of the vaginal epithelium, indicative of oestrogenization (Courrier, 1928), some glandular activity in the mammary gland (Florentin, 1936) and the copulatory reflex described in the newborn guinea pig of both sexes by Boling, Blandau, Wilson and Young (1939).

The cow is known to excrete appreciable amounts of oestrogen in pregnancy, and is similar to the mare in that the largest excretion takes place late in pregnancy (Turner, Frank, Lomas and Nibler, 1930). The amounts, however, are comparatively small, and Dr. Rowlands tells me that there is only slight evidence of oestrogenization in the newborn calf and no hypertrophy of the gonads.

The monkey is disappointing in that it might have been expected to provide an experimental approach to the endocrinological problems of the pregnant woman, but fails for the most part to do so. Some increase of oestrogen is found in the urine during pregnancy but the amounts are small, up to 200 I.U. per day (Dorfman and van Wagenen, 1941). Gonadotrophin is absent, and the endocrinological picture is quite different from that in women. In accord with this, no signs of oestrogenization have been reported in the newborn monkey.

In the cat, a "crise genitale" has been caused experimentally by the administration of oestrogen in late pregnancy, but does not appear to occur naturally.

Discussion

The most notable feature of the brief account given above is that no two species seem to be more than superficially similar in the behavior of the maternal ovaries and the fetal reproductive organs during pregnancy. We may be sure, therefore, that further work on the seal and the elephant, and the extension of the investigation to other little known animals, will much increase the harvest from this rich and varied field of reproductive endocrinology. The ordinary laboratory animals have made very little contribution to the subject, and we see again how the restriction of laboratory work to a handful of small rodents can limit ideas and cramp outlook. It is instructive to contemplate how our ideas of the reproductive endocrinology of pregnancy might have developed had they been based originally on work on the mare and elephant instead of on the rat and rabbit.

There is here a general principle to be seen. The animals described above are large and monotocous with the exception of the guinea pig, and all have a relatively long period of gestation at the end of which the young are born in an advanced state of development. As a class such animals are not very suitable as laboratory animals, and the characteristics which make them so may also necessitate the special endocrinological mechanisms of pregnancy which are now proving of such great interest. It is perhaps significant, for instance, that the elephant, with the longest known gestation period, also has the most developed system of relays of corpora lutea so far described. This situation calls loudly, but probably vainly, for a vast extension of the range of animals available for investigations by laboratory methods, and in particular for a long series of ovariectomy experiments on pregnant elephants.

Source and function of oestrogens and gonadotrophins of pregnancy. It is not my intention to discuss in detail the problem of the source of the oestrogens and gonadotrophins of pregnancy. The gonadotrophins of pregnant women and mares are almost certainly not of hypophyseal origin, and are elaborated by the maternal placenta or endometrium of the pregnant uterus. It is doubtful if they cross into the fetus or have any direct effect on it. They appear to be concerned essentially with the maintenance of pregnancy at a critical stage.

The origin and function of the additional oestrogens of pregnancy is a much more difficult question. Their source is uncertain, and investigation is hampered by the apparent ease with which they cross the placenta. Distribution studies cannot, of course, be conclusive, but they are compatible with placental origin in the pregnant mare (Catchpole and Cole, 1934). In women (Waldstein, 1929) and mares (Hart and Cole, 1934) ovariectomy during pregnancy does not affect appreciably the amount of placental and excreted oestrogen respectively. It is unlikely, therefore, that much contribution to the total is made by the maternal ovary, which is atrophic in the mare during the oestrogenic phase. Nor is a fetal source of origin very likely. The coincidence between the increase in fetal gonad size and the excretion of oestrogen in the mare, though most striking, does not in itself relate cause and effect. It may be that massive quantities of oestrogen are produced in the fetal foal by the hypertrophic gonad acting under some unknown stimulus, but it is, of course, quite possible, as happens with the ovary of the anoestrous mole, for masses of secretory tissue to be without apparent activity. The essential function, if any, of the vast amount of oestrogen found during pregnancy is also difficult to assess. No doubt it plays an important role in promoting muscular development of the uterus and vagina in the later stages of pregnancy and in preparation for parturition, but it is difficult to believe that the effects on the fetal reproductive organs have any essential place in the reproductive processes. It is possible that the fetal ovary is not so much a target organ for the oestrogen as one which accidentally gets in front of an endocrinological blunderbuss pointed in another direction and so receives a shower of unwanted hormone. This conclusion, however, would do nothing to explain by what mechanism exposure of the fetus to oestrogen produces such remarkable results on the gonads, and a wide and fascinating field of work is opened up.

Intersexuality. We shall hear from Dr. Jost later in this Symposium about the induction of intersexuality in laboratory animals by administration of exogenous androgens and oestrogens during pregnancy, and the story of the freemartin shows that similar effects can occur in larger animals. Why then is intersexuality not more common in species in which a large amount of oestrogen is circulating in pregnancy and is known to cross the placenta? The answer probably turns on the current stage of development. In the fetal foal the gonads differentiate at about eight weeks, during the gonadotrophic stage, and well before oestrogens reach high levels. By the time the oestrogenic phase is established the reproductive organs are fully differentiated and respond merely by qualitatively minor and reversible changes to heterosexual hormone stimulation.

Superfetation. I shall define superfetation as the condition in which two sets of fetuses of markedly different ages are present simultaneously in pregnancy. Many attempts have been made without apparent success to produce the condition by experimental means in laboratory rodents. Ovulation can readily be induced during pregnancy in an animal such as the rabbit, and one horn of the uterus can be kept free of embryos at the first implantation. The difficulties appear to be to effect fertilization of newly ovulated eggs during pregnancy and to provide suitable endometrial conditions for implantation at a much later stage than is usual. The relevance of this problem to my present theme is that *a priori* superfetation might be expected to occur occasionally in a monotocous animal having a bicornuate uterus and ovulating during pregnancy. No animal fulfilling these conditions is known at present, and even in the seal, which is monotocous and bicornuate, the sterile horn becomes at an early stage little more than an occluded rudiment. It is likely, however, that the endometrial changes taking place in the sterile horn in such a case would prove endocrinologically diagnostic, as they have in laboratory animals in which one horn has been rendered sterile artificially. A curious footnote to the superfetation story is provided by the fact that there is said to be some evidence of superfetation in the hare, an animal which produces a small litter of advanced young after a long period of gestation and which may therefore fufill some of the apparent qualifications for the spontaneous occurrence of ovulation during pregnancy.

Much more detailed knowledge on a far wider zoological basis will be required before firm general principles of the special reproductive endocrinology of pregnancy will emerge, but on the occasion of this Symposium it is pleasant to record that the foundations laid by H. H. Cole and his colleagues in California are being so fruitfully extended and built on by workers in England.

It is a pleasure to offer my grateful thanks to my friends and colleagues, Dr. R. J. Harrison, Dr. L. Harrison Matthews, Dr. J. S. Perry, Dr. I. W. Rowlands and Prof. S. Zuckerman, whose interest and