

THE ENDOCRINE FACTORS
RESPONSIBLE FOR THE MAINTENANCE OF PREGNANCY.

Studies in the Interruption and Prolongation
of Pregnancy in the Rat and Mouse.

by
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Being a Thesis submitted for the Degree of Doctor of
Philosophy in the University of Edinburgh,
December, 1931.

Ph. D., 1931.



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I. HISTORICAL SURVEY.

The foundation of our knowledge of the sexual cycle was laid by Walter Heape (1900); he divided Mammalia into two groups: Monoestrous and Polyoestrous, in accordance with the frequency with which mating was possible during the breeding season. Animals in which oestrus (or "heat") does not recur during the breeding season Heape called Monoestrous; those in which there is a recurrence of the dioestrous cycle during a single season have been designated Polyoestrous animals. Frequently animals which are monoestrous in a state of nature become polyoestrous under the favourable conditions of domesticity. Possibly the sheep in its wild state belonged to the first group: it is now in the second. The bitch, cow, mare, sheep, rabbit, rat, etc., are polyoestrous.

A further division of animals into animals having diphasic and monophasic cycles is made with reference to their sexual activity. In the higher mammals, e.g., the monkey and man, there is a reproductive phase only, the sexual phase being continuous; in most mammals both the sexual and reproductive phases are present and separate. Each phase is characterised by structural changes in the genitalia, but the whole cycle, though it consists of/

of different functions and though different factors appear, is a biological entity. The ovaries play a fundamental part in the phenomena associated with the sexual cycle in that the sexual and reproductive phases, though having different manifestations, are equally dependent on ovarian hormones.

Brown-Séguarđ (1889), first gave any knowledge of the internal secretion of the sex-glands. Since his work on the ovarian and testicular hormones, great advance has been made in the study of this complicated subject, and the active principles secreted by endocrine glands have been isolated. Biedl (1913) strongly criticised all experiments with sexual hormones; he drew attention to the fact that substances are frequently introduced into the body (in the extracts) which are never secreted; that only some of the substances normally secreted by the gland are given to the experimental animal, whereas in the living state the effect is possibly due to an interplay of various factors; and, further, that the quantitative and time relations differ from those in the body - that it is not possible to imitate the rhythm of the normal function. It is probable that his contentions are not entirely unsound and the conflicting results that are sometimes obtained with extracts of the same organ/

organ may in part be due to some of the factors which he enumerates.

Alpha (or Oestrin).

The sexual phase (which is associated in animals with "heat" or Oestrus) is characterised by maturation of the follicles of the ovary and by the secretion of alpha, and is accompanied by changes in the uterus and vagina which are taken as typical of the condition. The follicular hormone has been designated by various authors, oestrin, menformon; folliculin and alpha; the latter term is employed throughout this paper. As early as 1906 Marshall and Jolly demonstrated that the ovary produces a hormone which causes the phenomena of oestrus. Adler (1911), Fellner (1913), Iscovesco (1914), Hermann (1915), and others extended and amplified this observation. Fellner worked with extracts of both placenta and ovaries, and obtained hyperaemia and thickening of the muscle layer of the uterus. Hermann induced great enlargement in the immature uterus, and "extraordinary changes" in the vagina. These were accompanied by follicular growth in the ovaries; both Fellner and Hermann observed increased growth in the mammae. Mammary development has been found in association with prolonged injection of alpha by E. Allen (1927), Laqueur, et al (1928). Iscovesco/

Isovesco found that oestrus could be produced in castrated females with an extract of follicular fluid. An observation of the first importance was made by Stockard and Papanicolaou (1917), who were the first to discover that mucosal transformations in the generative tract of the guinea-pig are accompanied by dehiscence of epithelial cells so that the vagina has a characteristic cell content. This has been applied to other animals and in this way it is possible to determine with ease in the living animal the occurrence and progress of the oestrous cycles; the vaginal smear method is used in all such cases. Allen and Doisy (1923) developed an accurate method of biological assay of alpha, and used as their unit the amount which induced cornification of the vagina in the castrated animal.

As alpha was first prepared from follicular fluid, and follicular maturation is accompanied by oestrous phenomena in the uterus and vagina, it was thought that the follicles were the sole source of the hormone. However, Parkes (1926) prevented follicular maturation by the application of X-rays to the ovary and found that oestrous periodicity was uninterrupted. This demonstrated the production of alpha elsewhere than in the follicles. It has been found in large amounts/

amounts in placenta, in amniotic fluid, in cystic corpora lutea, and in the blood and urine of pregnancy, and in ovarian substance. (Wiesner (1925), Zondek and Aschheim (1928), Allen et al (1930).). Allen, Pratt, Newell and Bland (1930) implanted amounts of human ovarian tissue into rats in order to ascertain its hormonal content; corpora lutea, follicles and the ovarian cortex were used. A high yield of hormone was obtained from corpora lutea from the 13th-17th days of the menstrual cycle and in the first third of pregnancy, after which there was a considerable decrease. Full term pregnancy corpora gave negative results, from which it would appear that the corpus luteum of the end of pregnancy is not responsible for the large amounts of alpha to be found in the placenta, blood and urine at that time. The follicle walls contained little alpha and the cortical stroma gave negative results.

If alpha is injected into the immature animal oestrous changes take place in the uterus and vagina but the ovary is not stimulated and ovulation does not occur. Precocious oestrus after injection of alpha was described by Zondek and Aschheim (1926), Mahnert and Siegmund (1927), Goormaghtigh (1929) using mice, Meyer, Leonard and Hisaw (1930) using rats, Loeb and/

and Kountz (1928) working with guinea-pigs, and Lipschutz (1930) with cats. Slonaker (1927), and Steinach and Wiesner (1925) induced oestrous changes in the senile animal following injection of alpha and Steinach, Kun and Hollweg (1928) are said to have caused ovulation in senile mice by similar treatment.

In the mature animal continued injection of alpha causes irregularities in the cycle tending to its prolongation but not to its obliteration; this is in contrast to the persistent vaginal cornification which occurs in the immature or in the castrated animal, Goormaghtigh (1929), Smith (1926a) and Mahnert and Siegmund (1930). Loeb and Kountz (1928) found that prolonged injection of "follicular substances" inhibited ovulation in the adult guinea-pig. Injected during the first half of pseudo-pregnancy, alpha caused cornification and copulation in mice, and there were marked changes in the corpus luteum; in some instances ovulation occurred in spite of the presence of functional corpora lutea (Deanesly, 1931).

Oestrus does not occur during lactation provided a normal sized litter is suckled (Crew and Mirskaia 1930); it can be evoked by the injection of alpha, the dose required varying with the size of the litter (Parkes and Bellerby, 1927). Oestrus very rarely occurs during pregnancy: Long and Evans (1922) attributed/

attributed its inhibition to the effect of foetal tissue. Swezy and Evans more recently (1930) have found that "the cycle of ovogenesis in the rat is not interrupted by pregnancy, but continues throughout with the usual 4 or 5 day periods. At the end of each period ripe follicles are present. With the beginning of the new cycle at the 5th, 10th, 14th and 18th days, or thereabouts, newly formed corpora lutea are found, some of which contain a segmenting ovum". The presence of large follicles did not cause oestrus.

If alpha is injected during the first part of pregnancy it causes abortion (Wiesner (1925), Smith (1926b), Parkes and Bellerby (1926), Simonnet (1927).) Very large amounts are required to produce this effect in the later stages (Courrier (1928a), Smith (1926) and Parkes (1930).). The latter found that a given amount of alpha was far more effective when given in a series of injections over 24-48 hours than when given all at once. Courrier (1928b) is of opinion that the abortion caused by injection of alpha is due to an inhibition of the corpus luteum by that hormone. If alpha is injected into pregnant rabbits during the last week of pregnancy the decidual reaction cannot be produced after birth (Courrier, 1930a).

The/

The extraction of alpha from placenta or urine has been carried out mainly with ether after mild acidification (Zondek and Aschheim (1928a)). Ether was found to be ineffective in extracting alpha from neutral or nearly neutral solutions (Marrian (1929)), and strong acidification is necessary. According to Zondek and Aschheim, as reported by Odescalchi (1928), alpha in human pregnancy urine in the latter half of pregnancy reaches a concentration of between 5,000 and 7,000 mouse units per litre. It is readily soluble in fat solvents; more recently water-soluble alpha has been prepared by Marrian (1929), Parkes (1930), Collip (1930) and Butenandt (1931).

The Corpus Luteum.

The theory has been held in some quarters that the follicular hormone is the specific female hormone which suffices to produce all the morphological phenomena and functions in the genital tract; Allen, Mahnert, Siegmund, Zondek, Brouha and Simonnet are of this number. The view most commonly held is that the follicular and lutein hormones are two distinct and different hormones: Fraenkel, Ancel and Bouin, Loeb, Marshall, Hammond, Parkes and Bellerby are all of this opinion.

Whereas the sexual phase is associated with the secretion/

secretion of alpha and certain typical morphological changes in the ovary, uterus and vagina, the reproductive phase is associated with the development and function of the corpus luteum. The two phases are sometimes spoken of as the first and second phases of ovarian activity respectively. The exact origin of the corpus luteum is still in dispute: different schools support its origin from the theca interna of the follicle (Von Baer, (1827)) from the granulosa (Bischoff, (1842) and Sobotta (1895), and from both together, (R. Meyer (1911) and Loeb (1906)). Asdell (1928), Andersen (1926), Hill and Gatenby (1926), and Deanesly (1930 a and b) have studied the structure of the corpus luteum, while Corner and Allen (1928, 1929, 1930) have supplied invaluable information as to its physiological behaviour.

Corpora lutea are formed as the result of ovulation or of the rupture of follicles; such corpora are functional in most animals, but not in the rat and mouse, which require the stimulus of copulation (Long and Evans (1922) and Parkes (1929a)). About a third of all ovulations exhibit one or more instances of retention of the ovum within the corpus luteum; such corpora are atretic. Long and Evans found that in 182 ovulations studied, an average of five corpora per/

per ovary occurred. Lipschutz (1928) has formulated a theory - "the law of follicular constancy" - based on the assumption that some factor exists limiting the number of ova ripening at one time. The morphological changes occurring in the corpus luteum have been examined by Deanesly (1930b) who states that the corpus luteum of ovulation in the mouse reaches its full size at 2-3 days after ovulation, and in the rabbit at $4\frac{1}{2}$ days; no further enlargement of the lutein cells takes place. According to Long and Evans (1922) the corpus luteum of pregnancy is of the same size as that of ovulation until the 10th day when it enlarges to the maximum which it retains to the end of pregnancy.

The corpus luteum of pregnancy is more vascularised than that of ovulation and the cells forming the fibrous reticulum are more numerous (Deanesly, 1930b). About the time of parturition, or at the end of the life of the cyclic corpus luteum, the size of the gland, which had been decreasing but slightly, drops suddenly. This change is associated with degeneration, the lutein cells showing marked fatty degeneration. Persistence of morphological structure does not necessarily indicate continuance of function, e.g., in the rat, the corpora lutea of several ovulations may be present without interfering with the ovulation of/

of the immediate cycle; the lutein cells of such corpora are filled with large lipoid globules (Asdell, 1928). Asdell is in agreement with Long and Evans (1922) in ascribing the increased life of the corpus luteum during pregnancy to the "presence of the products of conception", but at the same time admits that the facts are puzzling in view of its persistence in pseudo-pregnancy and after hysterectomy. Loeb (1923₂) found after removal of the uterus of a guinea-pig, that the corpus luteum persisted for 60 days instead of the usual 14-16 days. Asdell (1928) removed the uterus of a rabbit before puberty, and when ovulation occurred, the corpus persisted for 24-29 days (instead of the normal 16 days of pseudo-pregnancy).

Various functions are ascribed to the corpus luteum. The idea that the corpus luteum exercises an inhibitory influence on follicular growth and ovulation was first formulated by Beard (1897) and by Prénant (1898) and was verified subsequently by Loeb (1910) and by numerous authors who found an absence of ovulation during the lutein phase (pregnancy or lactation), after implantation of lutein tissue or injection of extracts of corpus luteum. According to Loeb (1923₂) in the guinea-pig the follicles may mature during the functional life of the corpus luteum/

luteum but ovulation itself is inhibited. Removal of corpora lutea from the ovaries of the pregnant goat or guinea-pig causes ovulation and, in the case of the former, abortion (Loeb, 1917, (guinea-pig), Drummond Robinson and Asdell, (1926), goat).

Extracts of corpus luteum have been prepared which inhibit ovulation and oestrus. Corner and Hurni (1918), however, obtained no effect with an aqueous extract of sow's corpora lutea. Papanicolaou (1926) postponed ovulation indefinitely in the guinea-pig by injections of corpus luteum extracts at weekly intervals; oestrus and ovulation were restored a few days after cessation of the injections. Parkes and Bellerby (1927_b), Hisaw et al (1928), Brouha and Simonnet (1929) and Patel (1930) inhibited ovulation and oestrus, the latter for as long as forty days in some cases (mice).

An illustration of the inhibiting effect of the corpus luteum hormone (beta) upon alpha is afforded by an experiment performed by Courrier (1930_b) upon the newly littered guinea-pig. It was found that in such circumstances, a much larger amount of alpha was required to override the proliferative action of beta than was needed to cause cornification in the castrate. It has, however, been demonstrated by experiments/

experiments by Brouha and Simonnet (1928 and 1929) and Deanesly (1931) with alpha, and by Zondek and Aschheim (1928 a and b) with pituitary implants and pregnancy urine, that alpha can act in spite of "parenchyme lutéinique" in the ovary.

One of the main functions of the corpus luteum is to effect such changes in the uterus as will prepare it for the embedding of the ovum; it is essentially responsible for the phenomena of the reproductive phase of the sexual cycle. Fraenkel (1903) showed that the corpus luteum is essential for the implantation and nourishment of the embryo. Bouin and Ancel (1909) described the normal relationship of the corpus luteum to the changes which take place in the endometrium of the rabbit. A detailed, histological study of the rat's endometrium throughout pseudo-pregnancy and pregnancy has recently been carried out by Allen (1931) and illustrates in a striking manner the nature of the uterine activity at the different stages of pregnancy under the influence of ovarian activity.

The proliferation of the endometrium of the fourth to sixth days of pregnancy and pseudopregnancy permits of the embedding of the ovum and the development of the placenta. This structural change does not take place in the absence of the corpus luteum, and the effectiveness of corpus luteum extracts has accordingly/

accordingly been tested by their ability to produce these changes, and to cause the uterus to react to irritation with the formation of decidual tissue. That the uterus does not retain this sensitivity after the sixth day-(Allen (1931)) had a few cases of the seventh day)- was demonstrated by Loeb (1909) in the guinea-pig, Courrier (1929_b) and Courrier and Kehl (1930_a) in the rabbit, Corner and Warren (1919) and Long and Evans (1922) in the rat, and Parkes (1929_c) in the mouse. The test was carried out by means of inserting a thread through the uterus of a pseudo-pregnant animal (i.e. after sterile mating to a vasectomised male) or of an animal injected with corpus luteum extract. About the sixth day after the operation deciduomata were formed at the sites of trauma. Experiments carried out by Corner and Allen (1929 and 1930), in which proliferation characteristic of early pregnancy was induced in newly ovariectomised rabbits by means of extracts of corpus luteum, proved conclusively that the corpus luteum was responsible for effecting this sensitivity and causing the progestational proliferation. It was shown to be possible with such extracts to maintain pregnancy to term in rabbits ovariectomised eighteen hours after mating. Extracts causing proliferation of the uterine epithelium of the rabbit have been prepared by/

by Corner and Allen (loc. cit.), W.M. Allen (1930), Hisaw and Leonard (1930) and Clauberg (1930); in the guinea-pig, by Goldstein and Tatelbaum (1929) and Weichert (1928) Loeb and Kountz (1928); in the rat, by Nelson and Pfiffner (1930); and premenstrual changes in the monkey by Hisaw, Meyer and Fevold (1930).

Weichert (1928) and Hisaw (1929) took as their test of the effectiveness of their extracts, the relaxation of the symphysis pubis of the guinea-pig as at the end of pregnancy. Allen (1930) assayed his corpus luteum extracts on the spayed adult female rabbit; a rabbit unit is the amount which produces proliferation equal to the eighth day of pregnancy. He calls the substance isolated "progestin": Wiesner gives the name "beta" to the same hormone.

Experiments performed by Hisaw and Leonard (1930) on castrated rabbits show that no gestational proliferation is obtained in the uterus of such animals after injection of corpus luteum extract, if castration is performed a considerable time prior to injection. From this experiment and others in which they ovariectomised rabbits soon after mating and endeavoured without success to maintain the proliferation of normal pregnancy, they conclude that the progestational proliferation of the rabbit's uterus is the/

the result of the combined effect of the corpus luteum and follicular hormones. They showed, furthermore, that the latter brings the uterus into the proper physiological condition to respond to the corpus luteum hormone.

Until Ancel and Bouin (1910-11) pointed out the significance of the mammary development which is observed during pseudo-pregnancy in the rabbit, mammary development was ascribed to the foetus or placenta. A similar phenomenon was described by O'Donoghue (1911) in *Dasyurus* and by Heape (1906) in the bitch. Loeb (1923^a) found that hysterectomy does not prevent mammary development. Loeb and Hesselberg (1917) removed the corpora lutea from guinea-pigs during early pregnancy and noted that mammary development had ceased when they examined their animals at about the 24th day after conception. Ancel and Bouin removed the corpus luteum after ovulation and interrupted the growth of the mammary glands. Though the corpus luteum may be responsible for the stimulus required for mammary growth, extracts of this body have failed to induce in ovariectomised animals a development comparable with that in a normal animal in the luteal phase. The character of mammary growth in pregnancy changes at mid-period in the pregnant rabbit, and the amount of mammary tissue is considerably increased/

increased (Asdell (1928)). Experiments point to two facts: that the increased growth of the mammae during pregnancy is due to the persistence of the corpus luteum for a longer period than pseudo-pregnancy, and that their development is not dependent on foetal tissue. The experiments reported by Corner (1930) are of great significance. He caused mammary growth equal to that at the end of pregnancy, together with milk secretion, by injecting alkaline extract of pituitary into virgin rabbits from which the ovaries and uterus had been removed. The evidence forthcoming up to the present does not eliminate the possibility of other organs, beside the hypophysis, being concerned in mammary development.

A further function has been attributed to the corpus luteum of some animals; an extract of corpus luteum prepared by Knaus (1930), when injected in the non-pregnant rabbit inhibits the reaction of the uterus to pituitrin; the action is similar to that which occurs during pregnancy. Robson and Illingworth (1931) have recently confirmed the statement of Knaus and describe experiments which suggest that the proliferative and inhibitory actions of the corpus luteum extract may be due to different factors.

The Removal of the Ovaries. Castration in the female animal/

animal leads to atrophy of the genital organs; the results are more pronounced when castration takes place before puberty. Gonadectomy in mammals and birds leads to the development of an intermediate or neutral type; the effects upon the secondary characters, upon basal metabolism, and on other ductless glands have been examined (1) by Goodale (1916), Pézard (1918), Boring and Pearl (1918), etc. in fowls, and by Steinach (rats and guinea-pigs) 1912, Tandler and Keller (1910) (cow); (2) by Lewy and Richter (1899) and Blair Bell (1920); and (3) by Hammond (1913) and Blair Bell. The latter found that ovariectomy causes hyperactivity of the pituitary, thyroid and adrenal. Ovariectomy causes an increase in body weight due to accumulation of adipose tissue (Hatai, (1913) on rats). He states that spaying does not modify the weight of the hypophysis. This is contrary to the findings of Fichera (1905) in guinea-pigs and rabbits but supports those of Marrassini and Luciani (1911). After castration in male rats the hypophysis becomes much heavier, but obesity does not occur (Hatai, (loc.cit)). Unilateral ovariectomy in rats causes no increase in body weight and the sex ratio of litters born to such animals is not affected. (H.D. King, (1911), Crew (1927)). The size of the litter is/

is slightly reduced (King, Asdell (1924) Crew, etc), but unilateral ovariectomy does not decrease the number of litters a female can produce.

Compensatory hypertrophy of the remaining ovary has been observed by several workers; Bond (1906), Carmichael and Marshall (1908), Hatai (1913), Arai (1920), Slonaker (1927) in rats and mice; Hartman (1925) in the opossum; and Lipschutz and Voss (1925-6) in cats. Hatai (1913 loc.cit.) found that the surviving ovary attains almost twice the weight of the normal single ovary. This confirmed the conclusion of Carmichael and Marshall (1908), and Bond (1906), etc. The latter thought hypertrophy involved mainly the stroma but Hatai and Arai (1920) demonstrated conclusively that this was due to the formation of additional corpora lutea.

The effect of unilateral ovariectomy upon the oestrous cycle was examined by Papanicolaou (1920 guinea-pig) and by Emery (1931, rat), both of whom noted a tendency to an increase in the period of cornification. Engle (1928) showed that the anterior lobe of the pituitary is the determining factor in compensatory hypertrophy. It has also been observed that implantation of ovaries increases the number of oestrous cycles (Friedman (1930)).

Removal/

Removal of the ovaries during pregnancy was performed on the rabbit by Fraenkel in 1910 at Born's suggestion. A parallel experiment was carried out in which all corpora lutea were removed and it was found that the results were the same in both instances: pregnancy was terminated in all cases where the operation was performed before the 20th day of pregnancy and in many cases subsequent to that date. The effect of removal upon the course of pregnancy appears to be determined by the duration of the activity of the corpus luteum: opinions vary regarding this, some believing its activity to be confined to the first part of gestation (Fraenkel (1910)(rabbit), Loeb (1917 and 1923), Herrick (1928) (guinea-pig)). Recent work tends to show that the corpus luteum is necessary throughout pregnancy; Marshall, Hammond, Asdell, Parkes etc. are of this opinion. Hammond (1917) found that bilateral ovariectomy in the rabbit between the 16th and 27th days of gestation invariably causes abortion. This was confirmed by Courrier and Kehl (1929b) who observed that lutein activity, as manifested in the uterine endometrium of that animal, was continued till the 26th day.

Fraenkel was of the opinion that the corpus luteum was not essential in the later stages of pregnancy/

pregnancy but more recently it has been shown that the removal of the ovaries during pregnancy in the cow, goat, rabbit, mouse and opossum is followed by resorption or abortion. In the guinea-pig this takes place more slowly than in other animals and, according to some authors, does not occur after the middle of pregnancy (Herrick (1928), Courrier, Kehl and Raynaud (1929)). Marshall and Jolly (1906) carried out on dogs a similar series of experiments to that performed by Fraenkel; abortion occurred in all cases where the ovaries were completely removed. In rats, they found that pregnancy was invariably interrupted where the operation was performed during the first six days, but some normal litters were obtained after ovariectomy in the later stages. (Daels (1908) found that bilateral castration in guinea-pigs and rats always interrupted the course of pregnancy if performed in the first half of the gestational period. Removal of the ovaries from a pregnant woman need not interfere with pregnancy after the eighth week. Edgar Allen (1928) records the appearance of menstruation in monkeys following on ovariectomy; also experimental menstruation in spayed monkeys followed the cessation of injections of ovarian and placental extracts.

The Anterior Pituitary Body.

The cyclic activity of the ovary is under the control/

control of the anterior pituitary lobe. The experiments of Smith (1926a) of Smith and Engle (1927) and of Zondek and Aschheim (1927) demonstrated this fact conclusively. Precocious sexual maturity and ovulation were induced in immature female mice after the implantation of pituitary tissue; marked hypertrophy of the ovary and follicular maturation were accompanied by the typical oestrous phenomena in the genital tract; removal of the hypophysis prevented the occurrence of these phenomena, and implantation of the tissue in hypophysectomised animals caused a renewal of ovarian function. It was shown that implantation into the pregnant animal invariably caused abortion or resorption except in the latest stages (Engle and Mermod (1928)); small amounts of tissue were implanted intramuscularly daily. Parkes and Bellerby (1926) and Smith (1926b) confirmed these results, and recently Philipp (1931) caused abortion in rabbits by implanting human hypophysis which had been found "to contain Prolan B" (the name by which he designates "the luteinizing principle" of the pituitary). Zondek and Aschheim (1928b) caused ovulation during pregnancy by pituitary implants. Implants in ovariectomised females had no effect upon the uterus or vagina (Smith and Engle, (loc. cit)).

Zondek/

Zondek and Aschheim (1928a) discovered a hormone in the urine of pregnant women which, when injected into female mice, produced effects closely resembling those caused by pituitary implants, and have based their pregnancy diagnosis test on this fact. This test has been repeated by several workers and has proved accurate and reliable (Philipp (1929), Brühl (1929), Odescalchi (1928), Allan and Dickens (1930), Crew (1930)). Zondek and Aschheim (1928a) demonstrated that ovulation could be induced in immature mice by implanting 0.1 gm. of placenta; blood serum from pregnant women, had the same effect. Extracts of human placenta have been prepared by Wiesner (1930) and by Collip (1930) which give similar results; the latter claims effectiveness for oral administration also. Pregnancy urine has been extensively used with a view to isolating the pituitary hormone: the different results obtained according to the amount and concentration of the extracts have led to the theory that two pituitary gonadotropic hormones exist (Rho factors (Wiesner(1930)); Prolan A and B (Zondek (1930, 1931) and Philipp (1931)).

Amongst those who have prepared urine extracts giving follicular maturation with small amounts and luteinisation with large and/or the combined effect are: Wiesner and Marshall (1931), Hill and Parkes (1930/

(1930-1 a, b, c,) rabbit and ferret); Zondek and Aschheim (1928a), Biedl, Botschkareff, Brouha, Ehrhardt (all 1929)). The four last worked with Prolan as prepared by Zondek; human pregnancy urine was acidified with acetic acid, concentrated, shaken with ether (to remove oestrin) and dialyzed. P.E. Claus (1931) claims to have separated the two factors in crystalline form. Cyclic variations in the hormone content of the anterior lobe are indicated by an experiment by Wolfe (1931), in which sow's pituitaries were implanted into rabbits. Zondek (1931) has found a similar variation in those of women. He has recently estimated the number of mouse units of the two factors contained in the anterior lobe of both men and women and finds that whereas the hormone content is relatively high in the non-pregnant woman, the anterior lobe of the pregnant woman contains only a very slight amount of hormone, but the placenta on the average 2000-3000 units. Philipp (1931), on the other hand, found no luteinising effect in rabbits after implantation of full-term placenta.

By alkaline extraction of the anterior lobe Evans (1924) prepared an extract which had a strong luteinising effect in all dilutions; NaOH was used in weak concentration. Follicles became luteinised without/

without rupturing, and oestrus and ovulation were inhibited during injection and for some time afterwards. Teel (1926) Wiesner (1930) and Brouha and Simonnet (1928) confirmed these results. Courrier and Kehl (1930b) caused fresh corpora lutea to form in a pseudopregnant rabbit after injection of a NaOH extract of pituitary but though uterine activity was prolonged if injections started on the 4th day the normal atrophy was not long postponed. This is contrary to the results obtained by Teel (1926 - rat) and recently by Robson (1931 - rabbit). Teel (1926) prolonged pregnancy in rats for 2-6 days by causing functional corpora to form after administration of Evans' alkaline extract. Whereas in the rat, mouse and rabbit the alkaline extract has a strong kyogenic or luteinising effect, in the cat it causes follicular maturation if administered in small quantities and luteinisation in large amounts — (Courrier and Kehl (1929a)). Bellerby (1929) has caused luteinisation in the rabbit with an acid-alcohol extract of pituitary.

Evans (1924) and Teel (1926) claim that the alkaline extract causes growth, in addition to inhibition of oestrus and of ovulation, but have not attempted to isolate the factor responsible. The same/

same extract also furthers mammary development (Corner (1930)).

Hartman et al (1930) claim to have found yet another hormone in the pituitary - responsible for menstruation. They induced menstruation in one-year old and spayed monkeys with Allen and Doisy's ovarian hormone and Squibb's alkaline extract prepared from pregnancy urine.

That the pituitary is not inactive in the immature animal has been demonstrated by Engle (1931) who made a study of the prepubertal growth of the follicles in the ovary. Meyer et al (1930) report that continuous injection of alpha decreases the gonad-stimulating power of the pituitaries of immature rats when implanted into other immature animals.

Pregnancy. Only two aspects of this phase need be considered in view of the particulars given under the heading of corpus luteum, viz. the embedding of the ovum and parturition. J.F. Daniels (1910) H.D. King (1913) and Mirskaia and Crew (1930a) observed prolonged gestation in mice suckling a large litter. Kirkham (1916) ascertained that the ova which are normally implanted at the end of the 6th day are, in such cases, free in the lumen of the uterus from the 6th to the end of the 14th day post-partum.

The phenomena associated with parturition appear to/

to be intimately connected with ovarian activity (Marshall (1927)) and with a definite stage in the ovarian cycle.

Blair (1922), Keye (1923), Corner (1923) and others noted the occurrence of cyclical variations in the contractility of the uterus and observed that, coincident with the greatest development of the corpus luteum the contractions are slight. Reference has been made under the heading: corpus luteum, to the recent experiments of Knaus (1930) and Robson and Allingworth (1931) which demonstrate the existence in the corpus luteum of the cow, of a substance which inhibits the reaction of the non-pregnant uterus of the rabbit to pituitrin. The part played in parturition by the oxytocic principle of the posterior pituitary was first suggested by Dixon and Marshall (1924), who observed an intimate connection between the activity of that organ and the degeneration of the corpus luteum. They found, moreover, that ovarian tissue from sows in oestrus induced secretion of pituitrin when implanted into pregnant animals, whereas those from animals in dioestrus were ineffective. Bourne and Burn (1928) reported that alpha increased the response of the isolated uterus of the virgin guinea-pig to pituitrin. In the pregnant mouse the sensitivity/

sensitivity of the uterus to pituitrin was found to be increased after repeated injection of alpha (Parkes 1930)), and it has been thought that the same factors were involved in both cases.

SCOPE OF THE THESIS.

The series of experiments to be described, was undertaken to determine the factors underlying the maintenance of pregnancy, and with a view to a general study of ovarian secretion in relation to the second phase of the ovarian cycle. In view of the relationship known to exist between the hypophysis and the gonads, an important part of the investigation was the examination of the effects upon pregnancy of the administration of pituitary factors. For this purpose the following substances were tested upon pregnant rats in varying amounts and at different stages of pregnancy:

- (i) an extract of human pregnancy urine (as prepared in the Macaulay Laboratory, Edinburgh)
- (ii) an alkaline extract of anterior lobe of pituitary (after D.H. Evans and H.M. Teel).
- (iii) implantation of anterior pituitary tissue.

Observations were made of additional experimental data regarding the effects of a corpus luteum extract/

extract administered from early to mid-pregnancy; the removal of ovaries at different stages of pregnancy in mice; growth of the mother and of the foetus during pregnancy, haemorrhage, and the cell content of the vagina under administration of the various treatments employed.

II. METHODS.

1. Experimental Animals.

In the whole of this series of experiments Albino rats inbred by brother-sister mating, from stock originally received from the Wistar Institute, were employed. The colony has arisen from two pairs originally supplied by the Wistar Institute and by the system of inbreeding adopted, a homozygous stock has been obtained. Actually the animals used in these experiments were surplus to the immediate need and brother-sister matings were not maintained throughout. In all, 178 nulliparous animals, between 150 and 180 grams weight and from 3-5 months old, were used. They were kept in a well-ventilated hut, the heating of which was thermostatically controlled. The cages are constructed in units of twelve and their component parts are readily detached for cleaning. Straw or hay was used as bedding and not more than three animals were housed in one cage. The diet/

diet was varied as much as possible and on a sound vitamin basis. Wheat and oats (both boiled and uncooked) and flaked maize were the principal items of diet and to these were added cod liver oil (once a week), milk powder, cocoanut, bran, or fish oil. Skimmed milk was given daily in dishes machine-washed in boiling water, the strictest attention being paid to cleanliness; lettuce and bread were given twice a week.

For the major portion of the experiment the female was removed from the male immediately after mating and was placed with other pregnant females until the 15th day when each was placed in a separate cage. At no time were more than three females housed in one compartment.

In the experiments in which the effects of ovariectomy were examined 187 mice were employed; these were of mixed breeds and colours, divided between stock of our own breeding and stock supplied by Messrs. Tuck, The Mousery, Rayleigh, Essex. They were kept in groups of five with a male until mating occurred, whereupon they were placed with other females that mated on the same day, and a different male was attached to them. This course was followed, as it was found that the mice did not become pregnant/

pregnant so readily as the rats and, in the event of the first mating proving infertile, a second mating would occur four or eight days later. The mice were placed in individual boxes about the 15th day of pregnancy, the same procedure being followed as in the case of the rats. Throughout the experiment they were fed on oats, flaked maize and broken biscuits, and were given lettuce at least three times a week.

2. Extracts used and their preparation.

The extracts, after preparation, were kept in the Frigidaire between injections. Injection was carried out in the morning, prior to smearing and weighing. In the experiments in which pituitary tissue was implanted, the operation was performed within eight hours of receipt of the glands, that there might be less risk of their proving ineffective through standing. The technique used in the various preparations is as follows:-

(i) Extract of Human Pregnancy Urine: This extract was prepared in the Macaulay Laboratory (Edinburgh) by Mr. P.G. Marshall, from urine obtained from the Royal Simpson Memorial Maternity Hospital. The technique used may be briefly stated here as follows:-

After preliminary precipitation of proteins with dilute/

dilute sulphosalicylic acid, the urine was concentrated to vol/10, and ground in a mortar with barium hydroxide; the barium was thereafter removed by the gradual addition of sulphuric acid. After filtration, the ammonia resulting in the process was expelled under reduced pressure and the filtrate adjusted to neutrality with acetic acid. The active principle was then precipitated with 95% alcohol, and after centrifuging, was again washed with alcohol for dehydration. Subsequent stages deal with the final precipitation of proteins with sulphosalicylic acid and the removal of this acid together with precipitation of the active substance, by alcohol. The precipitate is boiled with ether and the ether discarded. A pale fawn powder results which is water soluble. This was used in the present series of experiments in the dilution of 10 cc. of water to the yield of approximately 2 litres of urine. It was observed that the yield was not always constant nor was the amount an invariable indication of the strength of the active principle obtained. Thus 0.1 gm. from one consignment amounting to 2 litres might be stronger than 0.2 gm. from another consignment of the same volume. In addition, to the barium-alcohol precipitation method of extraction, a shorter method was employed in which the precipitating medium used/

used was phosphotungstic acid. The details of both methods are fully described in a paper by Wiesner and Marshall (1931).

That there might be a reasonable prospect of securing a potent extract for the purpose of the experiment, the yield from a number of consignments of urine was combined. This procedure was adopted only in the later stages of the experiment as it was necessary to ascertain, prior to such a course, whether the extract deteriorated with age. Material 6-14 weeks old was combined, and, on injection, was found to retain its potency. This was tested by means of injection in five unmated mature female rats (G1-5). Its effectiveness was judged by a phenomenon which appears to be a characteristic of the extract, at least in mature animals, viz., the presence in the vaginal smears of large epithelial cells with a darkly-staining nucleus, which persist for a number of days either alone or in the presence of a few leucocytes. This feature is examined in fuller detail later.

(ii) Alkaline Extract of Anterior Pituitary:
Bovine pituitary glands were received in a frozen state/

state from the Glasgow Slaughterhouse a few hours after killing and were either immediately prepared for extraction or kept frozen until used. After the covering layer of connective tissue had been removed, and the pars posterior and pars media had been discarded, the anterior lobes were weighed and then partially sterilized by being placed for ten minutes in 40% alcohol. They were then ground with a little sterile sand in a sterile mortar and extracted overnight in the Frigidaire with an equal volume of N/20 NaOH. Neutralisation to Phenol Red was effected the next morning by means of N/10 or N/20 Acetic Acid, after which the extract was centrifuged at high speed for fifteen minutes and the supernatant fluid decanted. The yield was approximately volume for volume. The method of extraction described is that employed by Teel (1926).

(iii) Implantation of Anterior Lobe Tissue:

The glands were prepared in the same way as those used for extraction. After partial sterilisation in 40% alcohol, the glands were cut up very finely and portions taken from several glands were mixed before implantation, so as to make reasonably sure of effective material being employed. The minced glands were kept under cover on sterile watch-glasses during the operation of implanting, and sterile instruments were used/

used. Intraperitoneal implantation was performed dorsally through an incision made near the ovaries. With the exception of one group of animals, a single implant was given, and its weight was calculated on the basis of one ox-pituitary weighing 1.5 grams (the average of ten weighings).

(iv) Corpus Luteum Extract:

The standard corpus luteum extract as prepared by Mr. R. Illingworth in the Macaulay Laboratory was employed in both sections of the experiment. The method of preparation was as follows:

The solid corpora lutea, dissected out of cows' ovaries a few hours after slaughtering, were minced and extracted with two volumes of ethyl alcohol (95%) in the cold for 24 hours, and thereafter with ether in a Soxhlet. The alcohol extract was evaporated under reduced pressure and the residue extracted with ether. The ether solutions were combined and the ether distilled off; the residue was extracted with ethyl acetate; a thick brownish liquid formed on evaporation of the ethyl acetate soluble fraction. The yield is about 25 cc. per kilo of corpora lutea; the pure extract was taken up in its own volume of maize oil and each animal was injected with a total of not less than 1.5 cc. of the pure extract or the equivalent/

equivalent of 60 grams of corpus luteum tissue. This was estimated to be beyond the minimal effective requirements on the basis of 1 cc. producing progestational proliferation in the rabbit (Corner and Allen (1929); Robson and Illingworth (1931)). The extracts were actually tested on rabbits as a control, and, with one exception which will be alluded to later, were found to contain no alpha, and to give the typical proliferative reaction associated with the reproductive (or second) phase of the ovarian cycle, and in particular with that stage of pregnancy which is concerned with the embedding of the ovum and the early development of the embryo, and which is characterised by the formation of decidua and hypertrophy of the uterine endometrium.

(v) Removal of Ovaries - Mice:

The mice were anaesthetised (with ether) and the fur plucked from the back over each ovary for a space of about 10 mm. square. A dorsal incision was made, and the ovary raised with forceps. Thread was passed over the top of the ovary, and tied through the base of the oviduct but leaving the Fallopian tube free. Section was made immediately above the ligature, and, thereafter, the inner and outer layers of skin were stitched separately.

3. Histological Technique.

Material for histological study was preserved in 10% Formol saline for 24 hours and thereafter transferred to 5% Formol saline. Paraffin sections were made and haematoxylin and eosin were used for staining.

4. Additional details of procedure.

Pregnancy in both rats and mice was dated from the appearance of vaginal plug. This obstruction of the vagina and cervix, first described by Lataste (1887), is associated with the mating act in some rodents and is derived from a secretion of the seminal vesicles coagulated by Gley's fluid. According to Long and Evans (1922) it will "remain in the vagina about twelve hours, but occasionally as long as twenty-four hours". Frequently however, in rats, it is expelled two or three hours after mating, a fact which makes the collection of animals of known date of mating an arduous undertaking. Allusion is made later to cases where plug was visible for a period considerably in excess of Long and Evans' maximum. Daily examinations for vaginal plug were made both of rats and mice every morning. For this purpose and for the taking of vaginal smears, a small glass rod was used to which a strong resistant wire had been fixed. Smears were taken each morning on glass slides and were/

were fixed in methyl alcohol and stained with Giemsa's stain. In the experiments in which pseudopregnant animals were observed for bleeding, smears were taken morning and evening from the eighth day onwards.

The gestation period in both rats and mice, is estimated as from the appearance of vaginal plug, though there can be a deviation of some hours owing to the stage in oestrus at which mating occurred. In rats, the gestation period averages 21.5 days (Long and Evans (1922)) i.e. parturition takes place on the 22nd day (calculated by adding 21 to the day of mating); as instances occurred among the control animals where pregnancy lasted till the 23rd day such a slight deviation from the normal in the experimental animals has not been attributed to the effect of treatment. In mice, the gestation period is normally 19 days, calculated in the same manner, i.e. parturition occurs on the 20th day. From the day that normal term was reached in the rats, their cages were examined three or four times daily for litters, the last examination being made about 10 p.m. After ovariectomy in the mice, the same procedure was followed.

Placental Sign - The occurrence and duration of this phenomenon were recorded in both rats and mice. In the former it was observed that the extracts etc. administered had an effect upon its occurrence and persistence/.

persistence. In rats this feature normally occurs from about the 13th to the 17th days (Long and Evans (1922)) and in mice about the 11th and 12th days of pregnancy.

Weighings. With the exception of the rats injected with the corpus luteum extract, and those employed on the early stages of the urine extract experiment, all animals (both rats and mice) were weighed daily. The animals used on the corpus luteum experiment - both injected and controls - were killed on the fifteenth day of pregnancy and the fetuses and placentae weighed to the nearest milligram, and corrected to 10 mgs. In all the other experiments, with the exception of a single group, pregnancy was allowed to run its course.

III. EXPERIMENTAL PROCEDURE.

I. Extract of Human Pregnancy Urine.

In all, 45 animals were employed in this experiment which falls into four groups:

- Group I. 12 animals receiving small daily doses over a long period.
- " II. 2 animals injected from the appearance of placental sign;
- " III. 8 animals with the same amount of extract at various stages of pregnancy.
- " IV. 23 animals injected with the same extract in different amounts at parallel stages in pregnancy.

The procedure and results are set forth in Table I.

TABLE I.

Effects of injection of varying amounts
of Extract of Human Pregnancy Urine at different stages
of pregnancy - Albino Rat.

Concentration = 2 litres urine: 10 cc. H₂O.

Daily Dose	Days after vag. plug.	No. of injections.	No. of Rats.	Effect on Pregnancy
Group I.				
A. 0.1 cc.	2	13	4	Pregnancy interrupted
B. 0.15 cc. to 0.2 cc.	2	13	3	" "
C. 0.2 cc. * to 0.3 cc.	6	9	5	" "
Group II.				
* 0.2 cc.	from appearance of placental sign.	9	2	Prolongation of pregnancy, followed by resorption.

TABLE I. (Contd.).

Daily Dose	Days after vag. plug.	No. of injections	No. of Rats	Effect on Pregnancy
Group III.				
0.2 cc.	2 - 3	7	2	1. Resorption after bleeding 1 Prolongation 4 days
0.2 cc.	5	4	1	Resorption after bleeding
	6	"	1	Prolongation 4 days
	8	"	1	Prolongation 4-5 days
0.2 cc.	10	5	3	Prolongation 4-5 days
Group IV. N.B. One and the same extract was used for all subsequent animals:-				
0.4 cc.	6	5	4	Resorption after bleeding
0.4 cc.	10	5	4	Prolongation 4-5 days
0.4 cc.	2	5	3	(Normal Pregnancy (2)); live litters.
0.4 cc.	15	5	3	(Resorption after bleeding (1) Prolongation 5-7+ days
0.1 cc.	6	5	3	Resorption after bleeding
0.1 cc.	10	5	3	(Resorption after bleeding (2). Prolongation 4-5 days; dead litter (1).
0.1 cc.	15	5	3	Prolongation 6-7 days.

* Same extract employed.
 † A double injection was given on the first day.

Group I.

<u>No. of Animals</u>	<u>Dose</u>	<u>No. of days of Injection</u>	<u>From</u>	<u>Gen. Results</u>
(A) 4	0.1 cc.daily	13	3rd day of pregnancy	Pregnancy interrupted.

Details:

<u>Animal</u>	<u>Pregnancy bleeding.</u>	<u>Findings at necropsy etc.</u>
Rat 769	8 - 15 days	Killed 15th day. <u>Uterus and vagina full of blood and distended</u> ; ovaries a mass of corpora atretica ("Mulberry" appearance - Evans); no follicles
" 803/2	13th - 15th days	See photo of 803/2 in which 25 atretic corpora were counted in one ovary.
" 806	12th - 15th "	
" 810/9	11th - 18th days and 27th day	Injected for 17 days. During this time she mated four times: on the 10th, 15th, and 17th days after the first plug. After the last plug daily smears were taken and showed leucocytes only or with a few epithelial cells. On the 27th day she again mated. A vaginal smear taken revealed leucocytes and no cornified cells. Necropsy the same day showed both uterine horns (and vagina) to be full of blood; uterus was distended and contracted readily as in oestrus; two blood points were seen in one ovary; both ovaries contained many atretic corpora though fewer than in 769, 803/2 and 806.

Histological/

Histological examination: Rat 803/2. uterus: full of blood and epithelial cells; large lacunae under the outer layer of high columnar cells, the latter resembling intestinal cells; lumen distended - pro-oestral. (See Plates: uterus, ovary, vagina P.188).
Ovary: Strong luteinisation; 2 primary follicles and one from which ovum had recently been shed.
Vagina: high mucous stratum; typical pregnant vagina (cf. Barrington (1916)).

(B) The three rats in this group received a total of 2.3 cc. of extract in 13 injections. If pregnancy existed, it was interrupted before the ova were embedded, as the rats mated repeatedly during the experiment. Necropsy on the 15th day showed the ovaries to contain several large follicles in addition to many corpora atretica.

(C) This group consisted of five animals on which laparotomy was performed six days after vaginal plug in order to ascertain if they were pregnant before injection was commenced. At this stage the ova are free in the lumen of the uterus and in the blastula stage; embedding is about to take place (Kirkham (1916); Huber (1915)). The animals were deemed to be pregnant from the general appearance of the ovary and uterus; the ovary contained a number of bright red corpora lutea and the uterus was hyperaemic and flaccid/

flaccid.

They received nine injections of 0.25 cc. daily from the seventh day of pregnancy. Pregnancy was interrupted in all cases. In one rat four implantation sites were visible in the uterus at necropsy, and in Rat 814/3 nine implantation sites were counted. The uterus of this rat was distended and full of blood; the stroma showed great glandular activity. The ovaries, as in all the animals of this group, were a mass of corpora lutea. Those of Rat 814/3 measured 9 and 8 mm. in diameter respectively; in the left ovary was one large follicle measuring $3\frac{1}{2}$ mm. and a blood follicle 3 mm. in diameter. The right ovary contained 3 blood follicles (See Plate XII).

Group II.

This group consisted of two animals only. It was thought worth while to ascertain the effect of the extract when given at an even later stage of pregnancy, and accordingly two animals were used in which the pregnancy sign of bleeding had been observed for the first time; the date of vaginal plug was not known. The same extract was employed on both for a period of 9 days, 0.2 cc. - 0.3 cc. daily, and it is noteworthy that this identical material was given to the animals in Group IC (i.e., those deemed pregnant at sixth day laparotomy/

laparotomy). Their weights were taken daily and showed a daily increase for as much as 14 days after the first appearance of the placental sign, the increase in that period being 55 and 50 grams respectively. A drop of 15 grams was noted two days after the maximum was reached and it was thought advisable to make a ventral incision and examine the condition of the uterus.

An examination under ether gave the following picture:

Rat 802/5. Right horn of the uterus contained one small foetus near the ovary, and at least one fully developed foetus lower down. There was much dark blood in the uterus and apparently inside the amniotic membrane.

The left horn contained several fully developed foetuses and much dark blood. There were what appeared to be functioning corpora lutea in both ovaries and also some newly forming follicles.

Rat 813. Ventral laparotomy was performed the same day as on Rat 802/5, i.e., the 16th day after the appearance of placental sign. Large foetuses were observed in both horns (none atrophic) and they seemed larger than normal. There was much dark blood inside the amniotic membrane/

membrane and uterus. Unfortunately in an effort to ascertain the number of foetuses, the uterus was accidentally punctured with the forceps, as the result of which the rat died the following day.

Comments.

Long and Evans (1920) found that the placental sign normally occurs in rats from the 13th to the 16th days of pregnancy. From the details given in a later portion of this paper it will be seen that, while such figures no doubt apply to the stock which their examination covered, they are not of general application. On the basis of the first appearance of bleeding being on the 10th day of pregnancy, the gestation period in these two rats lasted for as much as 26 days; if it be taken as the 13th day, pregnancy lasted for 29 days an extension of 5 or (possibly) 8 days. The average length of the gestation period in the rat is, according to Long and Evans, 21.5 days, and coincides with the average for our own stock.

Group III.

Eight animals were now set aside with the object of prolonging pregnancy. The extracts were administered over a shorter period and their effect at various stages of pregnancy was examined.

Details/

Details:

A. 2 animals (Injected with 0.2 cc. daily for 7 days from 3rd and 4th days of pregnancy respectively.

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect of pregnancy.</u>
1804/3	15th - 19th days	<u>Resorption:-</u> Maximum weight reached on 14th day - only 15 gms. above initial weight.

1804/8	11th - 24th days	<u>Prolongation. 4 days;</u> litter of three born on the 26th day - partly eaten but not decomposed. Maximum weight of rat attained on 25th day. Increase in weight 10 gms. between normal term and parturition. Laparotomy on the 14th day of pregnancy revealed hyperaemic ovary with active corpora lutea and small foetus in the uterus (W.B. at that time weight was only 7 gms. above initial weight).
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B. 3 animals: injected with 0.2 cc. daily for 4 days.

A 4	10th - 23rd day, 25th days	<u>Resorption after bleeding:</u> Injected from 6th day of pregnancy. Maximum weight reached on 22nd day, 31 grams above initial weight.
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A 1	14th, 16th-27th days	<u>Prolongation 4 days:</u> Injected from 7th day of pregnancy. Littering at 11 a.m. on 26th day of pregnancy. At noon 5 foetuses (all dead) and 4 placentae removed from cage. Weights of foetuses: $6\frac{1}{4}$, $6\frac{1}{2}$, $6\frac{1}{8}$, $6\frac{1}{2}$, 6 grams. i.e. larger than normal/
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<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect of Pregnancy.</u>
		normal which is 5.8 gms. Placentae: weights, 0.400, 0.310, 0.293, 0.310 grams. which is smaller than normal, viz., .428. N.B. Maximum weight was reached on the 25th day of pregnancy; Between normal term and actual parturition the increase in weight was 6 grams.

F 4	13th - 26th days	<u>Prolongation 4-5 days.</u> Injected from 9th day of pregnancy. No litter was found in the cage but a drop in weight of almost 20 grams occurred between the 25th and 27th days. Other signs pointed to the rat having littered. Maximum weight was reached on the 25th day of pregnancy.
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C. 3 animals: Injected with 0.2 cc. daily for 5 days from 11th day of pregnancy.
The same extract was used throughout this group.

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect of Pregnancy.</u>
C1	15th-18th, 20th, 22nd-28th days.	<u>Prolongation 4-5 days:</u> Dead foetus was in the vagina at 6 p.m. on the 26th day of pregnancy. This was probably not the first born as there had been a drop of 12 grams in weight overnight. On the 27th day four partly eaten foetuses with placentae attached were found in the cage. Weights; about 5 grams each. Maximum weight was attained on 23rd day, being 7 grams above that at term. N.B. Protracted parturition due to upset of birth mechanism.

Animal Pregnancy bleeding.

Effect of Pregnancy.

F6 14th - 26th days

Prolongation 4 days:
Four dead fetuses found on 26th day, two partly eaten; the other two weighed $5\frac{1}{2}$ and 6 grams, respectively. Vaginal "bridge" persisted till normal term. Maximum weight was attained on 24th day and was four grams above that at term.

E5 14th-17th, 20th-22nd
 24th-27th.

Prolongation 5 days:
Eight dead fetuses were found on the 27th day of pregnancy, their weights being: $7\frac{1}{2}$, $6\frac{1}{2}$, 7, 6, $5\frac{1}{2}$, $5\frac{1}{2}$, 6 grams (the 8th was partly eaten). Five were clearly above the average. The maximum weight of the mother was 50 grams above that of the 14th day. This was attained on the 25th day and was 6 grams above that of term.

Comments.

The following facts are noteworthy:

1. That in all cases where pregnancy was prolonged the litters were still-born.
2. The high incidence of bleeding.
3. The average weight of the foetus (in successful pregnancies) was higher than the normal average. That this is probably due to growth having continued beyond term is supported by the continued increase in weight of the mother after term is reached.

Group/

Group IV.

It was decided to examine the effects of the same extract in both large and small amounts when given at the same stages of pregnancy; in this way strictly comparable results were obtained. That the extract would not lose its potency when kept for more than a month had been first ascertained. For Group IV the yield of 8-10 litres of urine, consisting of several different consignments, was combined, giving approximately 40 cc. of extract, i.e., the equivalent of 0.8 gm. active principle in powder form.

In addition to the barium-alcohol precipitation method of extraction a shorter method was employed in which the precipitating medium adopted was phosphotungstic acid. The details of both methods are set forth in the paper by Wiesner and Marshall (1931). The material was combined and injections spread over a month; 26 animals were used in the group, which falls into two sections:

Section I covers those cases - 14 - in which a total of 2.0 cc. of extract was given to each animal, i.e. 0.4 cc. daily for five days. Injections were commenced on the 3rd, 7th, 11th and 16th days of pregnancy, four animals falling within each of the first three groups and three in the last. In the details which follow, the actual order in which the experiments were carried out is observed, for reasons which will be obvious later.

A 4 animals: Injected from the 7th day of pregnancy.

<u>Animal</u>	<u>Pregnancy bleeding.</u>	<u>Effect on pregnancy.</u>
A8	15th; much blood 16th-23rd; 24th-26th, blood.	<u>Resorption:</u> Maximum weight reached on 23rd day was 27 grams above initial weight and 2.5 grams above that at term. Rat killed on 26th day: small placenta in cervix and another in left horn; blood in both horns and 3 or 4 implantation sites; uterus hyperaemic Ovaries contained many small atretic corpora and one small follicle in each.
A13	14th; much blood 15th-19th; little blood 20th	<u>Resorption:</u> Maximum weight reached on 19th day was 27 grams above initial weight. In full oestrus on 23rd day.
D1	little blood 13th; much blood 14th-18th; little blood 19th, 20th; blood 21st, 22nd; much blood 23rd; little blood 24, 25th.	<u>Resorption:</u> Maximum weight reached on 18th day was 43.5 grams above initial weight. Weight remained stationary for 6 days.
D6	blood 14th; much blood 15th- 19th; blood 20th-25th	<u>Resorption:</u> Maximum weight reached on 24th day was 50 grams above initial weight and 2.5 above that at term.

Comments.

In all cases with the exception of D1 large epithelial cells were a feature of the vaginal smear up to the time that severe haemorrhage occurred. The significance of this phenomenon is discussed later.

B. 4 animals: injected from 11th day of pregnancy.

Details:-

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect on pregnancy.</u>
A5	much blood 15th-16th; blood 17th; <u>little</u> blood 18th- 25th; much blood 26th, 27th. little blood 28th.	<u>Prolongation 5 days:</u> Maximum weight reached on the 26th day was 4 grams more than that at term. Litter on 27th day: 3 (dead) of which two were partly eaten; whole one weighed $5\frac{1}{4}$ grams.
D5	little blood 13th, 14th; much blood 15th-17th; blood 18th, 19th; <u>little</u> blood 20th, 22nd; much blood 24th, 25th.	<u>Prolongation 4-5 days:</u> Maximum weight reached on the 25th day was 13 grams more than that at term. Nest was made at 10.30 a.m. on the 26th day, and litter- ing took place at 1.15 p.m.: 4 (dead) of which 2 were partly eaten. Weights: $7\frac{1}{4}$, $6\frac{1}{2}$ grams. Still pregnant weight fell 16 grams overnight other foetuses eaten.
F3	blood 14th, 15th; much blood 16th; <u>little</u> blood 17th- 19th; 21st, 22nd; much blood 24th- 26th; blood 27th.	<u>Prolongation $4\frac{1}{2}$ days:</u> Maximum weight reached on 24th day was $6\frac{1}{2}$ grams more than that at term. Litter on 26th day at 6.30 p.m.: 6 (dead). Weights: $6\frac{1}{2}$, 7, $6\frac{1}{2}$ grams - other three half eaten; placentae normal size.
F7	Blood 14th, 15th much blood 16th; blood 17th; <u>little</u> blood 18th, 19th 21st; blood 22nd, 23rd.	<u>Prolongation $4\frac{1}{2}$ days:</u> Maximum weight reached on 22nd day (term). Nest was made at 10.30 a.m. on 26th day - litter at 6.30 p.m.: 9 (dead). Weights: $4\frac{1}{2}$, $4\frac{1}{4}$, 4, 5, $4\frac{1}{4}$, $5\frac{1}{2}$, $4\frac{3}{4}$, $4\frac{1}{2}$, 5 grams.

Comments/



Comments.

It will be observed that in all cases bleeding was severe on the 15th or 16th day and again just before littering. In those cases where the maximum weight was reached after normal term the continued growth of the foetuses is reflected in their weights. These may be compared with the weights of F7's foetuses which reached their maximum at term.

C. On the basis of the results so far obtained, it was apparent that injection of the urine extract in the early stages of pregnancy was attended with greater risk than when that condition was further advanced. It was accordingly decided that in those animals which were to receive the maximum dose, i.e., 0.4 cc. daily from the 3rd day of pregnancy, two would be given a double injection on the first day, thus spreading the injection over four days instead of five. In one animal (♂M4) the injections were carried out in the usual way, i.e., 0.4 cc. daily for five days; it will be noted that this was the only case in which resorption occurred.

3 animals: Injected from the 3rd day of pregnancy.

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect on pregnancy.</u>
M3	blood 13th-16th; little blood 17th, 19th; blood 20th, 21st.	<u>Normal pregnancy:</u> Live litter. Maximum weight reached at term. Litter on 23rd day: 9. Average 4.8 grams.

<u>Animal</u>	<u>Pregnancy bleeding.</u>	<u>Effect on pregnancy.</u>
M5	blood 14th; little blood 16th- 21st	<u>Normal Pregnancy:</u> live litter. Maximum weight reached at term. Litter on 23rd day: 6. Average weight 4.7 grams.
M4	<u>little</u> blood 10th; much blood 11th; blood 12th, 13th; much blood 14th; little blood 15th.	<u>Resorption:</u> Maximum weight reached on 17th day was 28 grams above initial weight. Weight remained almost stationary from 15th to 19th days after which it slowly fell. Cornifica- tion occurred on the 18th day.

Comments.

With the exception of M4, bleeding was notably scanty in this group as compared with that observed in the animals injected at a later stage of pregnancy.

D. 3 animals: injected from 16th day of pregnancy.

Details:-

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect on Pregnancy.</u>
B5	blood 14th-19th; little blood 20th- 23rd. blood 24th- 29th.	<u>Prolongation 7+ days:</u> foetuses dead. Maximum weight, attained on 23rd day, was 8 grams more than that at normal term: weight remained fairly stationary for 3 days then fell. On 29th day 10 large dead foetuses were removed by Caesarian cision. The ovaries were hyperaemic and contained several corpora lutea. Weights of foetuses; 7, 7 $\frac{1}{4}$, 7 $\frac{1}{2}$, 6, 6 $\frac{1}{4}$, 6, 7, 7 $\frac{1}{2}$, 6, 5 $\frac{1}{2}$ grams. Weights of placentae: 10 weighed 2.476 grams. Average: 0.248 gram (i.e. smaller than normal).

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect on pregnancy.</u>
K7	Little blood 12th; blood 13th-16th; <u>little blood 17th-</u> 22nd; blood 23rd- 25th.	<u>Prolongation 5+ days:</u> foetuses dead. Maximum weight attained on 25th day, was $3\frac{1}{2}$ grams above that at term. A nest was made at normal term. On 27th day, 9 large dead foetuses were removed from the uterus by means of Caesarian cision. The amniotic membranes of one horn were septic and there was venous blood in the lumen. Ovaries were large and a mass of atretic corpora (See Plate XI) Right ovary was removed: measured 8 mm. x 5 mm. at the hilum, weight: 0.096 grams. Left ovary contained two small follicles. Weights of Foetuses: 7, 7, $6\frac{1}{2}$, $6\frac{1}{2}$, $6\frac{1}{4}$, 7, 7, 7, $7\frac{1}{4}$ grams. Weights of placentae: 9 weighed 3.325 grams. Average: 0.370 grams. The placentae had to be detached from the uterine wall.
K5	blood 15th; little blood 16th; blood 17th-19th; little blood 22nd; blood 24th, 25th.	<u>Prolongation 3+ days:</u> foetuses removed alive. Maximum weight attained on 24th day, was 2 grams more than that at term. At 5.30 p.m. on 25th day 6 living foetuses were removed from uterus by Caesarian cision. They were foster- mothered but died. Weights of foetuses: $6\frac{1}{4}$ grams average. Weights of placentae: 0.500, 0.600, 0.650 grams (only three were weighed) - consider- ably above the average size.

Comments/

Comments.

The most noteworthy fact in this group is that the foetuses retained in utero for 3½ days beyond normal term were still alive, even though not viable.

SECTION II.

Section II comprises 9 animals which received only 0.5 cc. of the same extract as was used in Section I, i.e., 0.1 cc. daily for 5 days. Injections were commenced at the same stages in pregnancy as in Section I but the earliest stage - the third day - was omitted in view of previous experiments.

A.3 animals: injected from the 7th day of pregnancy.

Details:-

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect on pregnancy.</u>
D7	blood 14th; much dark blood 15th-17th; blood 18th-23rd; much dark blood 24th, 25th; <u>little</u> blood 26th.	<u>Resorption:</u> Maximum weight, attained on 23rd day, was 43 grams above initial weight and 1 gram above that at term; gradual drop in weight after 23rd day.
E6	much dark blood 15th-17th; blood 18th-20th; <u>little</u> blood 22nd, 23rd.	<u>Resorption:</u> Maximum weight, attained on 23rd day was 36 grams above initial weight and 3 grams above that at term; gradual drop thereafter.
F5	Blood 14th; much blood 15th- 17th; blood 18th- 22nd; <u>little</u> blood 23rd-25th; much blood 26th- 30th.	<u>Resorption:</u> Maximum weight, attained on 23rd day, was 49 grams above initial weight and 1 gram above that at term. Stationary for three days then a gradual drop. Nest was made on 24th day and rat was fierce but would not carry an 8-day old rat.

Comments.

The excessive bleeding from the 15th to the 17th day is noteworthy, and comparable with that of the animals in Section I, Group A. injected at the same stage in pregnancy but with a larger amount. Reference is made to this phenomenon in the section entitled Haemorrhage during Pregnancy.

B. Laparotomy was performed on the 11th day of pregnancy on each of the three animals in this group, in order to make sure that pregnancy existed as an interruption was anticipated.

3 animals: injected from 11th day of pregnancy.

Details:-

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect on pregnancy.</u>
K3	Blood 13th; much blood 15th, 16th; little blood 17th; blood 18th, 19th, 20th; much blood 21st; <u>little</u> blood 22nd.	<u>Resorption:</u> Maximum weight attained, on 21st day was 28 grams above initial weight.
B2	much blood 14th-16th; blood 17th-22nd; little blood 23rd; much dark blood 24th- 27th; blood 28th.	<u>Resorption:</u> Maximum weight attained at term was 34 grams above initial weight.
K4	much dark blood 14th and 15th; <u>little</u> blood 16th- 18th; blood 20th; little blood 22nd- 24th; much dark blood 26th and 27th.	<u>Prolongation 4-5 days:</u> Maximum weight attained on 20th day was 2 grams above that at term. It remained almost stationary for 6 days at which point the rat made a nest (25th day) and seemed about to litter. At noon on the 26th day a decomposed foetus was removed/

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect of pregnancy.</u>
		removed from the vagina, and the "bridge" across the opening, which had persisted throughout pregnancy, was cut to free the foetus. At 10.30 p.m. the same day, another half-eaten foetus was removed, and on the 27th day two decomposed foetuses were removed. The weight of one was $5\frac{1}{2}$ grams.

Comments.

It would seem that for this stage in pregnancy and for this particular extract the effective unit was somewhere in the neighbourhood of 0.5-0.6 cc. While prolongation resulted in one case, the dose was not sufficient to secure this result in the other two animals.

C. 3 animals: injected from the 16th day of pregnancy.

Details:-

<u>Animals</u>	<u>Pregnancy bleeding</u>	<u>Effect on pregnancy.</u>
K1	little blood 16th-21st, 24th; blood 25th; much blood 26th-28th.	<u>Prolongation 6-7 days.</u> Maximum weight attained at normal term. It remained almost stationary for 3 days and then fell gradually. Between the 27th and 28th days there was a drop of 25 grams. The rat was very ill; a dead foetus was removed from the vagina at 9.20 a.m. on the 28th day; on the 29th day another dead foetus at 12.45 p.m. Weight 6 grams. The same day a Caesarian cision was made and 4 dead/

<u>Animal</u>	<u>Pregnancy bleeding</u>	<u>Effect of pregnancy.</u>
		dead fetuses were removed: weights 6, 6, 4 $\frac{1}{4}$, 5 $\frac{3}{4}$ grams. Placentae were decomposed and rather small. Follicles of moderate size were forming in the ovaries.
K8	little blood 17th-19th, 21st, 22nd; blood 23rd; much blood 25th-27th.	<u>Prolongation 6-7 days.</u> Maximum weight attained on 23rd day was 4 grams more than at normal term; it then fell slowly till the 27th day on which a drop of 20 grams occurred overnight. On the 28th day a dead foetus was removed from the vagina at 9.20 a.m.: weight 6.6 grams. On the 29th day the rat was dead: 6 dead fetuses were in utero: weights 7, 7.2, 6.75, 7.5, 6.4, 7 grams. placentae were normal, average weight 0.3 grams.
H4	little blood 14th, 17th, 18th; blood 20th; little blood 21st; much blood 24th-28th.	<u>Prolongation on 6 days:</u> Maximum weight, attained 24th day, was 6 $\frac{1}{2}$ grams above that at normal term. Between the 27th and 28th days a drop of 31 grams occurred during the night and parts of dead foetuses were found in the cage in the morning.

Comments.

Bleeding was notably scanty in this group, the haemorrhage after normal term being due to retention of foetuses.

The following facts emerge from an examination of the/

the results obtained in Group IV. As has been said, the results are strictly comparable as the same extract was employed throughout the group. (1) Administration of concentrated extract of human pregnancy urine in the early stages of pregnancy, i.e. before the 11th day, is followed by an interruption of that pregnancy at a later stage unless the initial stimulus is made so strong as to overcome the disruptive forces which otherwise supervene. The cases in Section IC demonstrate that this substance may be administered from the earliest stages of pregnancy without ill effects, provided that this point is observed. (2) The failure of the birth mechanism already observed in the earlier groups is a feature here also, and frequently involves protracted labour. (3) With the exception of the animals in Section IIB the results show singular consistency. A possible explanation of the variation in response in this particular group is offered in the discussion.

Two facts were noted in Group IV.

- (a) The frequency with which large epithelial cells containing a large, darkly-staining nucleus, appeared in the smears throughout pregnancy.
- (b) The presence of cornified and partly cornified cells in the smears from the 15th to the 18th day of pregnancy. An attempt has been made at a later stage to elucidate the part played by alpha at this stage of/

of pregnancy.

Seven ovariectomised mice were injected that it might be ascertained whether there was any direct effect of the extract upon the uterus. On five, an early extract was employed, two receiving 0.35 cc. in all, and three 0.8 cc. The extract used on Group IV was injected in two ovariectomised mice, each of which received 0.4 cc. for five days. They were killed on the seventh day and the genital tracts preserved. Histological examination showed that the effect of the extract is solely via the ovaries.

SUMMARY.

1. Injection of an extract of human pregnancy urine may prolong pregnancy from 2-7 days.
2. In the early stages of pregnancy it may cause interruption, but where the initial dose is a large one or is quickly followed by a second, this is less likely to occur.
3. An amount which causes an interruption of pregnancy in the early stages will prolong pregnancy at a later date.
4. Where pregnancy is prolonged for more than 3 days litters are still-born, and parturition is frequently protracted.

II. ALKALINE EXTRACT OF ANTERIOR PITUITARY.

The experiment by Teel (1926) formed the basis of that to be described, but owing to the excessive amount of protein given if Teel's course of injections were followed (viz., 1.0 cc. extract daily to term), an effort was made to find the minimum amount which would produce the same results. Thirty-six pregnant rats were employed in this series of experiment which falls into four sections: Section A deals with the injections of the extract in the various dosages stated. In Section B, age and filtration tests were made: an extract prepared from glands kept frozen for a week after killing, was injected when a month old; the effectiveness of the extract after this had been passed through a Seitz filter was also examined. In this Section, too has been placed one female to which the extract was given on the 20th day of pregnancy, and two mice which received the same extract. Section C deals with a feeding experiment in which the extract was mixed with the daily ration of food, that it might be seen if oral administration were as effective as injection. In section D the effect of various dilutions was examined.

TABLE/

TABLE II.

Effect upon pregnant rats of injection of alkaline extract of pituitary.

Section	No. of Animals	Dose	From	General Results
A (1)	4	1.0 cc. daily for 6 days	13th day of pregnancy	Pregnancy prolonged between 5 and 7 days; litters still-born
(2)	2	") ") ")	1st "	Pregnancy prolonged 2-2½ days; <u>live</u> litters
(3)	2	1.0 cc. daily for 4 days		
(4)	1	0.3 cc. daily for 6 days	6th "	Pregnancy prolonged 2 days; <u>live</u> litter
	6	"	11th, 12th, 13th days of pregnancy	Pregnancy prolonged 2, 4, 5, 6 days; litters still-born
B Age Test (Month old Extract)	3	1.0 cc. daily for 5 days	3rd day of pregnancy	Probable prolongation of pregnancy in two cases

TABLE II. (Contd.)

Section	No. of Animals	Dose	From	General Results.
Filtration Test	3	0.8 cc. daily for 4 days 0.4 cc. "	3rd day of pregnancy) 2nd ")	Probable prolongation in all cases
Unit Test	3	0.4 cc. daily for 5 days	1st "	? any effect
	1	0.2 cc. "	" "	No effect
Late pregnancy (or abortion test)	1	3.0 cc. in 24 hours	20th "	Pregnancy prolonged 4-5 days: litter still-born
	2 (mice)	0.4 cc. (one injection)	14th "	Pregnancy prolonged 3+ days
C Feeding Experiment	3	Tot: 8.0 cc.	2nd, 7th, 11th days of preg.	No effect
	2	Tot: 12.0 cc.	15th day of preg.	" "
	3	Tot: 0.4 cc.	4th day "	" "
		" 0.2 cc.	3rd " "	" "
		" 0.1 cc.	12th " "	" "
D Very dilute extract	<u>36 +</u> <u>2 mice</u>			

Section A: 15 Rats.

(1) In the four animals (C4, F9, B3, C5) which received a total of 6.0 cc. of extract from the 13th day of pregnancy, pregnancy was prolonged between 5 and 7 days. In three cases growth continued after normal term, 11, 12, and 14½ grams being added. In only one case (C5) did the maximum weight coincide with the normal term; it remained stationary for five days. The foetuses were above the normal average and were specially large in C4's case, where the average of seven was 6.75 grams.* Those of C5 which attained her maximum at term were, however, within the average, viz. 5 grams. In this rat parturition was protracted, two dead foetuses being born on the afternoon of the 28th day of pregnancy and six more the following morning. All the animals in this group received injections of pituitrin (Parke, Davis & Co) on the 26th or 27th days. Bleeding occurred from the 15th to the 26th days, with a break on the 17th day, and was severe during the later stages.

(2) Where the alkaline extract of anterior lobe is injected/

*The placentae were correspondingly large: 1.097, 1.202, 0.510, 0.519, 0.532, 0.533, 0.520, 0.495 grams. One was attached to the uterine wall by fibrous tissue. The ovaries were large and a mass of corpora atretica; their weights were 0.091 and 0.079 grams. (See Plate IX)

injected in the early stages of pregnancy a slight prolongation results but live litters are obtained, a result which Teel also secured.

Pregnancy was prolonged for $2-2\frac{1}{2}$ days in each of the four rats (C3, F1, A6, B1) receiving between 4 and 6 cc. of extract commencing the first day of pregnancy. Growth continued after term in three cases, 4-5 grams being added, but this did not affect the weight of the foetuses to any marked extent, the average of 15 being 5.6 grams.

In no case did littering occupy more than $4\frac{1}{2}$ hours. Bleeding took place between the 14th and 17th days in those animals which were injected to the sixth day but was later in appearing in those which received the smaller amount of extract, e.g., in A6 scanty bleeding occurred on the 17th, 21st and 22nd days; in B1 bleeding was rather more severe and occurred between the 16th and 22nd days with slight traces from the 25th to the 28th. In this case the continued appearance of blood in the vaginal tract after parturition would seem to indicate resorption of unexpelled foetuses. As the litter numbered only two and the maximum increase in weight was 73 grams over the initial weight there is little doubt that this condition existed.

(3) In subsequent experiments a decreasing amount of extract/

extract was employed in order to ascertain if such a course would affect the result. After the initial test on one animal - Rat 1804/6 - had proved successful, tests were made at varying stages in pregnancy with the smaller amount.

In 1804/6 which received a total of 1.8 cc. of extract from the 6th day of pregnancy over a period of six days, a live litter was born two days after term. Growth had continued beyond term, $10\frac{1}{2}$ grams having been added. The average weight of the fetuses was 5.45 grams. Bleeding which was scanty occurred on the 15th, 16th and 21st days.

(4) Six animals (B6, D2, F8, C2, C6, F7) were injected with 0.3 cc. of alkaline extract daily for six days from the 11th, 12th or 13th days of pregnancy with effect on pregnancy in every way comparable with those secured by Teel with twelve times as much material. In one case littering took place on the 24th day, i.e., a prolongation of two days; in all others, pregnancy was prolonged for 4, 5 and 6 days; the litters were still-born. Growth continued after term in only three cases, in one of which the difference between the maximum and normal term weight was merely 1 gram; in the other two the difference was 11 and 12 grams. In the latter case the fetuses were/

were considerably above the normal, the average weight of nine being 6.86 grams (one weighed 8 grams). In two animals the maximum weight was attained a day before term and remained stationary for 3 or 4 days. There were three instances of protracted parturition: B6 littered at 9.20 a.m. on the 26th day, and, still being pregnant, was given pituitrin. Another dead foetus was born at 3.30 p.m. At 6.30 p.m. hysterectomy was performed and 5 dead foetuses were removed. There was much venous blood inside the amniotic sac. D2 littered at 10 a.m. (Six dead) and again at 10 p.m. C6 littered during the night and again at 1 p.m.

Bleeding in this group occurred at two stages: between the 14th and 20th days (approximately) and again before littering - between the 25th and 27th days.

Section B: 13 Rats, 2 Mice.

(1) Age Test. In this group and those which immediately follow, the lengthening of the gestation period is so slight owing to the early stage at which injections were commenced, that each case has to be examined on its merits. This is rendered necessary in view of the fact that instances were found in the normal controls in which pregnancy lasted till the afternoon/

afternoon of the 23rd day.

Three animals (P3, P1, K6) were injected from the third day of pregnancy for five days with 1.0 cc. daily of the extract already employed with success on the animals in Section A (2). The same extract was also used on the late pregnancy test to be described (Rat F2). It had been kept frozen for a month and was found to have retained its potency. Pregnancy was prolonged from $1\frac{1}{2}$ to 2 days and live litters were obtained. In one case the maximum weight was attained after term, the difference being 13 grams; in the other two the weight remained stationary for 30 and 40 hours respectively. The average weight of 15 foetuses was 5.6 grams. Haemorrhage was of a scanty nature in K6, and in P1 and P3 was confined to the 15th-18th days with slight traces before and after. There was no instance of protracted labour.

(2) Filtration Test. Five animals (P4, P7, P8, K14, R7) were employed on this test, of which three were injected with a total of 3.2 cc. and three with only 1.6 cc. of extract. In both groups the injections were spread over only four days, commencing on the third and second days of pregnancy respectively. Litters were born on days 23, $23\frac{1}{2}$, $23\frac{1}{2}$, 24 and 24. It is noteworthy that the two animals (R7, K14) which received/

received only 1.6 cc. of extract were those in which gestation persisted for two days beyond normal term. With one exception (K14)^{*} the litters were alive, but the average weights were on the whole considerably lower than normal, e.g. 3.8 grams (P4), 4 grams (P7) 5½ grams (P8), 5.04 grams (R7), 4 grams (K14). The low average weights may have been largely due to the fact that the maximum weight was generally reached before term and remained stationary for a considerable time before birth e.g. P4: 36 hours, P7: 72 hours (litter 10, of which 7 were dead), P8: 30 hours, R7: 65 hours. The extract may have delayed development of the embryo at the outset but have been incapable of making this good at a later stage. (It is interesting to note that the placentae of K14 were abnormally large, thick and vascular: their weights were: 0.7, 0.8, 0.9, 0.5 grams. The horn containing the three first was twisted on itself and there was marked venous congestion. The foetuses in this horn were between 3.5 and 4.5 grams in weight).

Bleeding was scanty and occurred about the 15th day preceded and followed by slight traces. In K14 it occurred earlier and was probably associated with the abnormal condition of the uterus.

(3)/

* K14 died on the 24th day of pregnancy. The condition alluded to below may have been due to a fall about the tenth day.

(3) Unit Test. Four animals were employed on this section (M1, M6, R4, R5); the first three were injected with a total of 2.0 cc. and the fourth with 1.0 cc. of extract commencing the first day of pregnancy, injections being spread over five days.

In the three animals receiving the larger total: 2.0 cc., live litters were obtained on days 23 and 23½. The maximum weight was attained at term in two cases and on the day following in one. In M1 the difference between the initial and maximum weight was 98 grams. The average weights of the fetuses in the three cases were: 5, 5.2, 6 grams. The animal receiving a total of 1.0 cc. littered at the normal time, 21.5 days; the fetuses were alive and averaged 5.25 grams.

As no phenomena indicative of prolonged gestation occurred in any of the members of this group it is doubtful whether an effect was obtained. It is concluded therefore that the effective unit is 2.0+ cc. when given in five daily injections at an early stage of pregnancy.

Bleeding occurred, on an average, between the 14th and 16th days and was exceedingly scanty. This feature was associated with the appearance of cornified cells in the vaginal smears, the significance of which is dealt with later.

(4)/

(4) Late Pregnancy Test: one animal.

Rat F2 was given a total of 3.0 cc. of alkaline extract as follows:

1.0 cc. at 8 p.m. on the 20th day,

" " 10 a.m. " " 21st "

" " 6 p.m. " " " "

Results.

23rd day: a foetus was born alive at 10 a.m.; the cord was cut to secure its release; it was eaten at 12.30 p.m. the same day. No more foetuses were born till

26th day: one dead foetus was in the cage at noon; weight, 5 grams.

27th day: rat was killed under ether - 3 more foetuses were in the uterus which was septic; weights, 5, 7, and 5 grams. The ovaries contained several recently formed corpora lutea.

It is remarkable that the main effect secured by Teel by the injection of 20+ cc. of extract over a period of 21+ days (viz. prolongation of pregnancy) can be secured by the injection of a seventh of that amount (probably much less) in only 60 hours.

It is generally assumed, in the experimental injection of extracts in the mouse, that the reproductive/

reproductive system requires 84-96 hours for a definite reaction to be manifest macroscopically in the living animal. Our figures are based on experiments with both placental and pituitary extracts, the figures given by many workers, and on the procedure adopted in the Zondek and Aschheim pregnancy test where the animals are killed on the fourth day after the first injection. It is admitted that there is an ovarian response much sooner than the maximum period fixed by the test but this is of an incomplete nature.

A.S. Parkes (1930) mentions 84 hours as being required for the maximum vaginal reaction to alpha, i.e. 48 hours after a 36 hours period of injection. Smith and Engle (1927) however, induced sexual maturity in the immature female mouse in 36 hours; usually the reaction required three days, and in the rat longer.

This instance would seem to demonstrate conclusively that the prolongation of gestation induced by the extract is the result of some failure of the birth mechanism and not due, in this instance, to size of the foetus interfering with its delivery, nor to a late maturation. At this stage of pregnancy, the growth of the foetus must have been already complete or nearly so - in only one case is the weight above normal. In those cases where injection was started sufficiently/

sufficiently early to produce enlargement of the foetus, this was undoubtedly a contributory factor to protracted parturition. It is not, in our opinion, the main factor.

Two minor experiments might be mentioned here. In our mouse colony an animal (C2) was encountered which had a history of abortion about the 14th or 15th day of pregnancy, at which time a large amount of blood was to be seen in the vagina. In the third pregnancy in which this occurred, an injection of 0.4 cc. of the alkaline pituitary extract was given in one dose, on the 14th day, which was the occasion of the first appearance of bleeding. Haemorrhage persisted till the 17th day and was scanty on the 18th and 19th; the weight of the animal remained stationary during that time but rose 5 grams between the 18th and 22nd days (to 43 grams). On the evening of the 25th day she was killed when a partly resorbed foetus and large placenta were found in the uterus; there were three blood points in the ovary.

In the above case the prolongation of gestation can definitely be ascribed to the injection of the extract but it is impossible to determine whether it was equally responsible for the continuance of pregnancy.

Mouse NN1, also, was injected with the alkaline extract/

extract. This animal was unilaterally ovariectomised on the sixth day of pregnancy; from the tenth day she received daily injections of 0.4 cc. for three days. The placental sign appeared on the 12th day and was observed in diminishing amount for four days. The maximum weight was reached at term and thereafter fell slowly till the animal died on the 22nd day. One foetus was found in the cage; two others were in the uterus and weighed slightly over 1 gram each. The placentae were larger than normal: 0.183, 0.159, 0.221 grams.

As in the case of mouse C2, it is here, also, impossible to state whether the extract had any effect in stemming abortive tendencies. That it caused a slight extension of the gestation period is likely as also increased placental growth beyond the normal.

Section C.

Feeding alkaline extract of pituitary. Five pregnant animals were set aside for this experiment; each rat occupied a cage to herself and as much maize was placed on a dish as was likely to be consumed in the 24 hours. With this the extract was mixed each day for four days and any food left over was placed on the top of the next day's ration, thus making reasonably sure that each animal received the same amount of extract/

extract. The treatment was commenced: from the 2nd, 7th, 11th, 15th and 15th days of pregnancy respectively; a total of 8.0 cc. was given to the first three animals and 12.0 cc. to the last two, but without effect. Live litters were born on the 22nd and 23rd days in all cases.

Section D.

In view of the experiments carried out by Courrier and Kehl (1929)^a with this extract upon cats, it was decided to test the effect of various dilutions on three rats. Each animal was given four injections; one received a total of 0.4 cc. one 0.2 cc. and one 0.1 cc. Pregnancy was uninterrupted and normal litters were born at term.

SUMMARY.

1. Pregnancy is prolonged after administration of an alkaline extract of pituitary.
2. Where injection covers mid-pregnancy, the gestation period is prolonged from 2-7 days but litters are invariably still-born and parturition is frequently protracted.
3. Where injection ceases before mid-pregnancy, the gestation period is prolonged for 2-2½ days but litters are born alive.
4. If the extract is administered to a rat two days before term, parturition is delayed for 3-4 days.
5. The extract has no effect upon pregnancy when administered orally.
6. In dilute form it does not interrupt pregnancy.

III. IMPLANTATION OF ANTERIOR LOBE (PITUITARY)
SUBSTANCE.

By means of implantation of small amounts of anterior lobe tissue in pregnant rats and mice, Engle and Mermod (1928) caused pregnancy to be interrupted. In none of the animals receiving implants beginning the first day after insemination were pregnancies obtained, and interruption occurred in all cases where implantation commenced between the 4th and 6th days, and was associated with severe haemorrhage at a later stage. Where implantation started from the 12th to the 15th days, pregnancy was interrupted in only 40% of the cases, but 8 out of 12 rats receiving implants between the 15th and 17th days aborted. The heavy discharge of blood from the vagina was a very noticeable feature in both rats and mice. Large follicles had formed in the ovaries and advanced oestrous changes existed in the uterus.

It has been found that an extract containing pituitary hormone (Rho factors) caused follicular maturation when given in small amounts but luteinisation of the follicles when larger quantities are administered. On this assumption, it was thought that large implants of anterior lobe tissue would cause fresh luteal tissue to form and so prolong pregnancy as in the case of the alkaline extract experiment/

experiment described.

There was no means of reckoning what amount of tissue was likely to produce kyogenic results i.e. to cause development of luteal tissue in the ovary, so this was made the matter of experiment. The first group of animals comprised four pregnant rats: two had reached the seventh day of pregnancy, one the sixth, and one the fifth day. The total amount of tissue (which was implanted in two operations) was equal to half an ox-pituitary; in order to ensure a definite reaction, portions were taken from four different glands which were finely minced, and, after mixing divided into four portions. No ill-effects were experienced, but in order to give the animals time to recover entirely from the effects of the first operation, 48 hours were allowed to elapse between the first and second implantations. In all those cases where pregnancy was permitted to proceed to term gestation was slightly prolonged; in no case was there an interruption of pregnancy. The small amount of bleeding is noteworthy, more especially in view of Engle and Mermod's results.

Group I/

TABLE
Effect of Implantation of Large amounts of
Anterior Lobe Tissue in Pregnant
Rats.

Tot. Amount of Tissue	No. of Animals	Time of Implantation	Effect upon Pregnancy
Group I Half ox- pituitary	4	7th and 9th days " " " " 6th and 8th " " 5th and 7th " " of pregnancy	Prolongation of Pregnancy 1-2 days; two live litters; protracted parturition in one case.
Group II. Whole ox- pituitary	5	13th, 13th, 11th, 9th, 4th days of pregnancy	Prolongation of Pregnancy 4+ and 6 days in 3 cases; Resorption in 2 animals.
Group III. Small piece ox-pituitary	2	4th day of pregnancy	? any effect.

Group I;

Details:

<u>Animal</u>	<u>Implants</u>	<u>Bleeding</u>	<u>Effect on Pregnancy</u>
1804/7	7th and 9th days	14th,16th, 17th days.	<u>Prolongation 1½ to 2 days with protracted parturition.</u> Three dead foetuses were born at 6 p.m. on the 23rd day; weights 5.6, 5.2, 6.0 grams. At 10 a.m. on the 24th day 2 males were born <u>alive</u> and a female dead. The maximum weight was reached on the 23rd day and was 8 grams above that of normal term. The weight fell 57 grams on littering.
1804/1	7th and 9th days	12th day	Killed on 12th day in order to ascertain the degree of development of the embryo as it was anticipated that this might be retarded. Embryo and membranes measured 6 mm.
1804/2	6th and 8th days	15th,16th days "little bleeding" on 18th and 21st.	<u>Prolongation one day.</u> <u>Nine foetuses born alive</u> on the 23rd day; average weight was 5.5 grams. Maximum weight reached 21st day (day before term) and remained stationary for 30+ hours.
1804/5	5th and 7th days	14th-16th days <u>little bleeding</u> on 17th,18th and 22nd.	<u>Prolongation one day.</u> <u>Two newly born foetuses</u> were partly eaten at 10 a.m. on the 23rd day. Maximum weight was reached on the 20th day i.e. 2 days before term and fell 30 grams on littering.

Comments.

The results set forth bear a strong resemblance to those obtained by injection of the alkaline pituitary extract in the early stages of pregnancy: pregnancy was slightly prolonged and live litters obtained. While the extension of the gestation period over the normal is only a slight one, the uniformity of the results appears to have some significance and to point to a definite effect. This is especially the case when the facts are correlated with the protracted gestation in one case and the incidence of maximum weight in all.

Group II. consisted of five pregnant rats, in each of which anterior lobe tissue equal to one whole ox-pituitary was implanted intraperitoneally at one operation. Tissue actually from five anterior lobes was implanted in each animal, though the amount received by each was equivalent to one in weight. The animals were in a state of partial collapse the day following the implantation and it was difficult to rouse them from their lethargy and to induce them to take food. Presumably this was due to the excessive amount of protein administered. They were kept as warm as possible, and stimulant was added/

added to hot milk which was freshly prepared twice daily; they refused all their ordinary food except lettuce. This state of semi-prostration lasted for about four days, diminishing towards the end of that time except in the case of E2 which required to be hand-fed daily throughout pregnancy.

Pregnancy was prolonged in three cases and resorption occurred after bleeding in two.

Details:

<u>Animal</u>	<u>Transplants</u>	<u>Bleeding</u>	<u>Effect on Pregnancy.</u>
A3	13th day	11th, 13th 18th; "little bleeding" 19th-22nd; blood 23, 25, 26.	<u>Resorption.</u> Weight was only slightly above the initial weight throughout pregnancy.
B4	13th day	19th-24th	<u>Prolongation 4+ days.</u> Killed under ether on 26th day; 5 fetuses removed (septic); uterus septic; total weight of fetuses 30 grams. Cold abscess formed where tissue implanted. Maximum weight reached 21st day - 20 grams above initial weight, remained stationary for three days, then fell slightly.
E2	11th day	?	<u>Prolongation 6 days.</u> Weight not taken. Litter born 28th day; 8 fetuses (dead and somewhat decomposed); placentae had been eaten/

<u>Animal</u>	<u>Transplants</u>	<u>Bleeding</u>	<u>Effect on Pregnancy.</u>
			eaten. Weights of fetuses: $4\frac{1}{2}$, 4, 4, 3, $3\frac{1}{4}$, $4\frac{3}{4}$, 3 grams, and one partly eaten.
A7	9th day	15th, 17th- 19th, 21st- 22nd, 24th- 28th (Severe)	<u>Prolongation 6 days.</u> Four dead fetuses (of which 2 half-eaten) on 28th day - decomposed; two weighed $7\frac{1}{2}$ grams each. Maximum weight reached 24th day, remained stationary for 4 days; 15 grams above weight at normal term.
E7	4th day	14th, 16th, 18th-23rd.	<u>Resorption after bleeding.</u> Maximum weight reached on 20th day, 25 grams above initial weight.

Group III.

In view of the extensive experiment carried out by Engle and Mermod with the repeated implantation of small amounts of anterior lobe tissue it seemed unnecessary to devote time and animals to a renewal of this experiment. However two were set aside for a single implant. That a single implant is without effect was demonstrated by Zondek and Aschheim (1927 and 1928), but it was deemed advisable to use this method of control for the larger amounts.

The material for implantation was administered at one operation on the 4th day of pregnancy. The tissue, (consisting of portions from six glands) measured approximately 3-4 mm. square x 1 mm. thick.

Details:

<u>Animal</u>	<u>Bleeding</u>	<u>Effect on Pregnancy.</u>
J3	little bleeding 15th, 16th, 18th days.	<u>Probably no Effect.</u> Ten living foetuses were born at 5.45 p.m. on the 23rd day, the average weight of which was 5 grams. The maximum weight was reached on the 23rd day, this was 5 grams above that of normal term, and $76\frac{1}{2}$ above the initial weight.
J4	14th-17th; 19th, 20th (<u>little</u> bleeding)	<u>Probably no Effect.</u> Ten foetuses were born on the 23rd day, of which 8 were alive; their average weight was 5 grams. The maximum weight was reached on the 23rd day; this was 7 grams above that of normal term and 83 above the initial weight.

As there were no indications to a prolongation of pregnancy, it is assumed that an implant of this size has no effect upon the pregnant animal.

SUMMARY.

1. Implantation in the rat of a very large amount of bovine anterior lobe tissue at one operation may cause pregnancy to be prolonged for 4-6 days; litters are still-born.
2. Implantation of half this amount of tissue causes a shorter prolongation and litters are born alive.
3. A single small implant has no effect upon pregnancy.

IV. Additional Observations.

Data were collected both amplifying and arising out of the experiments already made. The effects of the removal of one or both ovaries in mice at various stages of pregnancy, and of injection of a corpus luteum extract into rats until mid-pregnancy, were investigated. The daily smearing, weighing etc., afforded material for such studies as: haemorrhage in pregnancy, the growth of the mother and of the foetus in pregnancy, and the cell content of the vagina under the various treatments administered. These data are given herewith:

A. Corpus Luteum Extract - Rats.

It was thought possible that injection during pregnancy of an extract containing that principle which promotes decidual growth in the uterus, might so affect the metabolic processes of reproduction as to cause enlargement of the placenta and/or of the foetus at a relatively early stage in pregnancy. The group of experiments to be described was carried out with a view to examining this point; twenty pregnant rats were injected daily from the 4th, 5th, 6th or 7th day of pregnancy to the 15th day, with 0.3 cc. of the prepared corpus luteum extract as described at the outset of this paper. In this way each animal received/

received not less than 1.5 cc. of the pure extract which is in excess of the amount required to cause progestational proliferation in the rabbit. On the fifteenth day of pregnancy, the rats were killed and the fetuses and placentae were weighed and compared with the weights of those of nine control pregnant rats. In one of the injected animals pregnancy had been interrupted at an early date; the placental sign had not occurred but implantation sites were visible in the uterus. The results of the experiments are set forth in the following tables and histograms.

TABLE IV.

Minimum and Maximum weights of fetuses and placentae
(corpus luteum extract)

	foetus	placenta
minimum control	160 mgm.	130 mgm.
" injected	160 "	110 "
maximum control	380 "	260 "
" injected	530 "	280 "

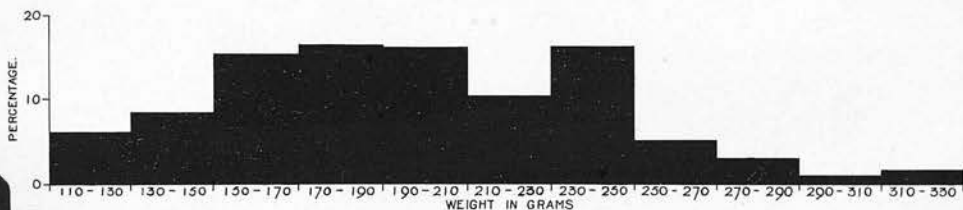
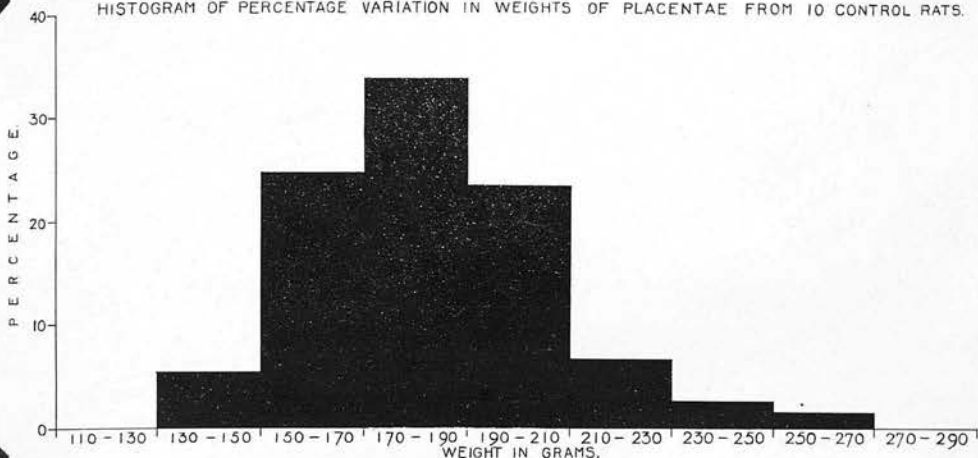
The average weights as shown in Table V present a truer and fuller picture for purposes of comparison; these are based on the average weights of the fetuses and placentae in each experimental animal from which total averages are derived.

TABLE V.

Average Weights of Foetuses and Placentae in Control
and Injected Rats. (Corpus Luteum)

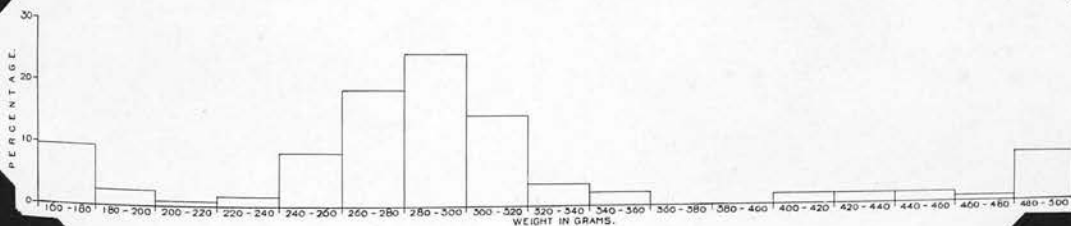
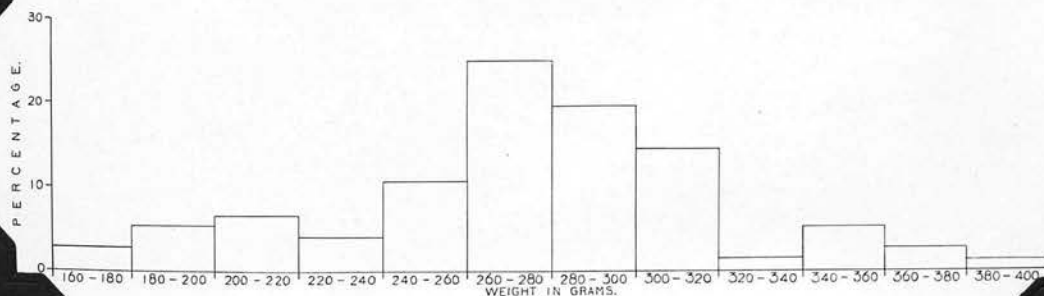
Controls		Injected	
Foetus (average)	Placenta (average)	Foetus (average)	Placenta (average)
226 mgm.	154 mgm.	223 mgm.	152 mgm.
254	167	440	130
271	171	294	215
288	176	291	162
330	193	273	184
272	185	330	277
298	187	294	164
238	238	263	177
261	170	284	207
		250	198
		284	237
		166	148
		155	171
		310	213
		300	177
		449	216
		170	143
		272	242
		493	254
Tot. 2438 mgm.	1641 mgm.	5541 mgm.	3667 mgm.
Av. 271 mgm.	182 mgm.	292 mgm.	193 mgm.

HISTOGRAM OF PERCENTAGE VARIATION IN WEIGHTS OF PLACENTAE FROM 10 CONTROL RATS.



% Variation in weight of 142 Placentae from 20 Rats Injected with Corpus Luteum Extract.

HISTOGRAM OF PERCENTAGE VARIATION IN WEIGHTS OF 75 FOETUSES FROM 10 CONTROL RATS.



% Variation in weight of 142 Foetuses from 20 Rats Injected with Corpus Luteum Extract.

It will be seen from the histograms that there is a slight shifting to the right in the curve for the foetuses of the injected animals, and a flattening of the curve for the placentae of these animals. If this was attributable to the extract and not merely a fortuitous circumstance, it is possible that the foetus benefited from an acceleration of the metabolic and assimilative processes taking place in the placenta owing to an excess of hormone. The curves are so nearly similar, however, that one cannot assign any appreciable effect to the extract, and one must conclude that probably the excess of secretion over that absorbed in the genital tract, was expelled through the ordinary channels.

Histological findings.

Control) Killed 15th day of pregnancy.
Rat 119) Ovary contained large active corpora lutea - the luteal tissue was vascularized and the central cavity filled in with connective tissue; also two primary follicles, one of which was moderately large; the ovum in this was detached from the base of the follicle and the cells of the discus proligerus were loosening from one another and from the rest of the granulosa.
(See Plate IA, II).

Rat 453: Injected from 4th to 15th day of pregnancy; killed on 15th day. Ovary contained corpora lutea similar in structure to those of the control, also three small primary follicles.
(See Plate IB).

In/

In addition to the pregnant controls employed, four unmated females received the equivalent of 1.5 cc. pure extract of corpus luteum over a period of twelve days. This amount did not inhibit oestrus nor render sterile a mating which took place during the injection. It should be noted, however, that this particular batch of extract when tested on rabbits for its power to cause gestational proliferation of the uterus, and inhibition of its reaction to pituitrin (Robson and Illingworth (1931)) proved inactive. This was the only consignment (out of four employed) of which this can be said. Its injection into ovariectomised mice revealed the presence of alpha when the equivalent of the daily dose given to a rat was administered, viz., 0.3 cc. of the prepared extract. The difficulty of eliminating alpha in the preparation of corpus luteum extracts has been experienced by Corner and others, and for this reason great care was taken to discard all cystic corpora at the outset, as the fluid in such cysts has been found to contain alpha. The glands used in the extract which caused cornification in ovariectomised mice, had already undergone the initial stages of preparation before reaching the Macaulay Laboratory, and this precaution had not been observed. It can be said, however, that one unit of alpha administered daily to a rat is insufficient to interrupt/

interrupt pregnancy. In the rabbit, the presence of alpha in fairly considerable quantities, up to 1,000 mouse units, does not override the progestational reaction of the uterus to beta or the corpus luteum hormone (Robson and Illingworth (1931)). According to the results reported by Patel (1930) this cannot be said of the mouse.

Furthermore, it is noted that extracts which cause progestational proliferation in the rabbit do not, however, cause a significant increase in the growth of the foetus or placenta. From our own and Teel's experiments, it would appear that such growth is capable of modification only after the first half of pregnancy and not in the initial stages. This conclusion is probably not of general application, but may refer only to such animals as the rat, mouse, etc. in which the major portion of the growth of the foetus takes place in the last third of pregnancy. The injection of corpus luteum extracts in the rabbit may produce different results owing to the fact that in this animal intra-uterine growth is most rapid between the 9th and 15 days, when 704% of its weight is added daily as against only 212% daily from the 15th to the 20th day. (Minot (1907)).

A comparison of the effects produced by injection of corpus luteum and pituitary extracts, leads one to the conclusion that to effect any change of consequence in/

in the ovarian cycle, and more especially in the reproductive phase of that cycle, the method of approach should be through the ovary via the pituitary, and not directly upon the uterus.

SUMMARY.

The injection of pregnant rats with a corpus luteum extract which contains the factor responsible for progestational proliferation of the uterus of the rabbit, does not have any appreciable effect on the size of the placenta or of the foetus up to the 15th day of pregnancy.

B. Removal of Ovaries - Mice.

The experiments to be described were carried out with a view to ascertaining (a) at what stage ovarian secretion ceases to be necessary to pregnancy, and (b) the extent to which the remaining ovary (after unilateral ovariectomy) is able to support a pregnancy which was initiated in the presence of two ovaries. It has been established (Long and Evans (1922)) that there is a definite relation between the number of corpora lutea of pregnancy and the number of foetuses; to what extent this relationship may be divorced in pregnancy without danger to the life of the foetus, appeared worthy of investigation. The readjustment to/

to the new conditions imposed upon the surviving ovary in the non-pregnant animal (as manifested in the oestrous cycles) was also examined.

The experiments to be described fall into two sections: bilateral ovariectomy and unilateral ovariectomy.

For bilateral ovariectomy 90 mice, mainly multiparous were employed; these were arranged in groups for operation on the following days:

on the 7th day after vaginal plug - 18 mice;

on the 10th " " " " - 11 "

on the 13th " " " " - 12 "

on the 16th " " " " - 8 "

on the 19th " " " " - 3 "

Pregnancy was terminated in all cases except the last: one mouse bilaterally ovariectomised early on the 19th and two late the same day had live litters at term (H_6 , D_3 , F_8). In the earlier groups large numbers were employed as one was not certain that pregnancy existed. In the three last groups the placental sign had been observed.

As the element of shock could not be wholly ignored, the second ovary was removed in 19 animals in a pregnancy subsequent to that in which the first was removed. Only the late stages of pregnancy were examined/

examined in order that the threshold of safety might be ascertained. At the same time attention was paid to the degree of activity of the ovary as a whole, and the stage at which degenerative changes in the corpora lutea were most manifest.

The effects on pregnancy were as follows:-

16th day: removal of the second ovary on the 16th day was followed by resorption.

17th day: (5 animals) abortion took place the following day in four cases; the foetuses were dead and weighed from 0.5 to 0.6 gram each. One animal littered at term, but laparotomy showed that some ovarian tissue had been left when the first ovary was removed in the previous pregnancy and this had hypertrophied. This occurred also in one animal from which the second ovary was removed on the 18th day; a litter was born a day before term of which four were living and three dead.

18th day: out of six animals allocated to this group dead litters were born to five on the 19th day. The single case (C₄) in which a live litter was born on that day, had had her previous litter prematurely. In two cases (H₅, F₉) the second ovary was removed between 5 and 9.30 p.m. on the 18th day. One aborted the following morning; one foetus was alive and/

and one dead; at the time the ovary was removed the bursa ovarica was distended with blood - a possible indication of the approaching collapse of the vascular supply. The other mouse had a live litter at term (the 20th day). It is noteworthy in this case that, at the time that the ovary was removed it was anaemic, and histological examination showed considerable degeneration of the corpora lutea. Two mice submitted to laparotomy and manipulation (D_1 , K_4) had live litters, one at term and the other a day later.

19th day: Of five animals from which the second ovary was removed on the 19th day, four had live litters and one still-born.

Histological examination of ovaries:

- 6th day ovary: Corpora lutea contained no degenerated cells; numerous small Graafian follicles were present.
(TT_4)
- $17\frac{1}{2}$ day ovary: Mature corpora lutea as defined by Sobotta; thecal ingrowth marked; some blood in centre of corpora.
(K_2)
- 18th day ovary: Full of active corpora lutea - mature; theca at the centre; a few degenerated luteal cells.
(K_3)
- $18\frac{1}{2}$ day ovary: A few degenerated luteal cells in corpora lutea: theca cells still apparent.
(F_9)
- 19th day ovary: (1) degenerating corpora lutea, definitely passés.
(G_2) (noon)
- (H_2) (2) corpora lutea definitely degenerated.

(H₆) (3) lutein cells large and soft, prior to degeneration; numerous large follicles present.

With the exception of TT₄ (6th day ovary) and H₆ (19th day), the ovaries examined were removed from a pregnancy subsequent to that in which the first ovary was removed. In the case of H₆ both ovaries were removed at one time. It would appear that only in those cases in which degeneration of the luteal tissue of the ovary has already commenced, can the ovaries be dispensed with without danger to the life of the foetus. This degeneration probably commences late on the 18th day of pregnancy; by the middle of the 19th day degeneration is far advanced and little danger attends removal of both ovaries at this stage. The mechanism of parturition appears to involve a number of factors and is discussed elsewhere in this paper.

Control experiments were carried out in which manipulation of various degrees was employed: 19 animals were used. Two such cases have already been described. The oviducts of both ovaries were ligatured by passing the thread under the oviduct instead of over the ovary and section was made immediately above the ligature without interfering with the blood supply to the ovary; normal pregnancies ensued.

In framing the second series of experiments, i.e. that dealing with the effects of unilateral ovariectomy it/

it was assumed that provided ovarian secretion was present, pregnancy would not be interrupted, or if at all, only in the early stages. For this reason the 13th day was the latest stage at which the operation was performed. The results showed from what point one ovary is able to fulfil the function of two in a pregnancy initiated in the presence of two. The strain on the remaining ovary would naturally be greater at a time when the pregnancy changes are not fully established than in the later stages when the placenta is approaching its maximum development. 97 nulliparous mice were used on this experiment and were fed and housed under the same conditions as those used for bilateral ovariectomy. The vagina was examined every day and the mice weighed daily throughout pregnancy.

13th day: On the 13th day after vaginal plug 7 mice were unilaterally ovariectomised, and bore litters between the 19th and 21st days.

10th day: On the 10th day after plug 14 mice had one ovary removed; of these -

9 bore litters between the 19th and 20th days;
2 " " on the 17th day;) still-born
1 " " " 18th day;)
2 aborted on the 16th day.

The average number per litter in the live litters was/

was seven. The partial withdrawal of ovarian secretion at this stage appears to have had some adverse influence upon pregnancy in that there are only nine normal litters out of fourteen. It was thought that such an effect (if operative) would be more strongly felt on that side of the uterus from which the ovary was removed, but it would appear that where abortive forces are initiated, these generally affect the whole uterine system simultaneously. On the 19th day of pregnancy, two mice were opened and fully developed foetuses were found to be present in both horns of the uterus.

7th day: For unilateral ovariectomy on the 7th day of pregnancy 21 mice were employed; of these 10 had normal litters between the 19th and 21st days, and 2 had still-born litters on the 22nd day after seven days' bleeding. Abortions occurred in 9 cases:

1 on the day following the operation.

2 on the 14th day

4 " " 15th "

1 " " 16th "

1 " " 18th "

Cases of resorption and abortion are not uncommon in non-experimental animals; so high a percentage as 50, however, would seem to indicate some interference with the pregnancy mechanism. That this should/

should be so slow to manifest itself is difficult to explain; this point is touched upon in the discussion. There appeared little definite relation between the incidence of the placental sign and abortive tendencies; its duration did appear to reflect an abnormal condition in two cases: still-born litters were born on the 21st and 22nd days after 7 and 11 days' haemorrhage respectively.

6th day: W.B. Kirkham (1916) has shown that the ova only reach the uterus at the end of the fifth day after ovulation: it seemed desirable, therefore, to test the effects of unilateral ovariectomy at this early stage. In some cases the oviduct was not removed with the ovary, as three of the five days are occupied in the passage of the ova through the oviduct, and if mating occurred early in oestrus removal of the oviduct might entail their destruction before the uterus was reached.

Eleven mice were used; two bore litters, the average number per litter being seven. Resorption took place in one case, and abortions in eight on the following days: 7th, 10th, 11th, 11th, 12th, 14th, and 16th days.

One mouse (PP₉) which aborted crumbled fetuses on the 22nd day of pregnancy is disregarded as she mated during pregnancy: on the 9th day. Laparotomy in two cases/

cases showed that foetal development in that uterine horn from which the ovary had been removed was unaffected.

1st day: Unilateral ovariectomy was performed in 27 mice at the end of the day on which vaginal plug was observed, in order to ascertain if pregnancy could reach its normal termination after interference at such an early stage. In no case did pregnancy occur.

Ether only: As it was felt that the element of operative shock might play a part in the result obtained, 17 mice were given anaesthesia alone at the end of the day on which plug was observed. To make reasonably sure that a fair proportion of the animals were pregnant, several mice were employed that had mated a second time. There were only two live litters and two still-born; abortion and resorption occurred in two cases and in a doubtful third. This control experiment would seem to indicate that even relatively light narcosis at the earliest stage of pregnancy is attended with considerable danger.

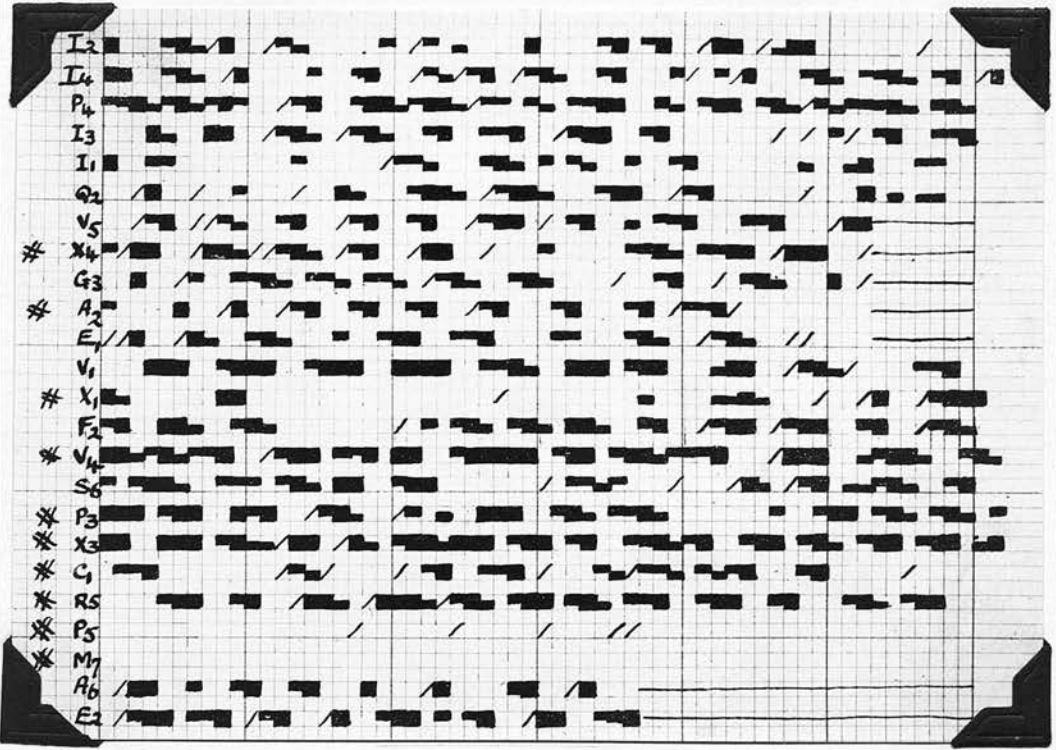
The results obtained in this experiment confirm the observations of Harris (1927-1928) in so far as bilateral ovariectomy is concerned, but not as regards unilateral ovariectomy. He found that, after bilateral ovariectomy, pregnancy was terminated in all cases where/

where the operation was performed in middle and early advanced pregnancy: subsequent to the 15th or 16th day of pregnancy, the operation was followed by abortion accompanied by a very high percentage of still births. Where ovariectomy was performed in the early stages, the animals were autopsied 2, 3, or 4 days after the operation; in a few cases about 10 days. The same procedure was adopted in the case of those animals which were unilaterally ovariectomised, and in the control animals where there was manipulation only. Harris used only six animals: three for unilateral ovariectomy and three, as controls, for exposure and manipulation of the ovary. In only two cases was pregnancy carried to term, and of these: (1) a dead litter was born (after unilateral ovariectomy at the 12th day) and (2) a live litter after the same operation on the 13th day. Of the other cases he states: "Neither unilateral ovariectomy nor exposure and manipulation of the ovaries during pregnancy terminate pregnancy". As the animals (with the two exceptions quoted above) were killed on the 12th, 6th, 12th, and 14th days of pregnancy, such a statement lacks foundation. It would appear from the particulars already given, that where unilateral ovariectomy is/

is performed prior to the sixth day of pregnancy, abortive tendencies (when these are at work) are manifested at an earlier date than in those animals operated upon after the sixth day. In the former abortion takes place about the 10th and 11th days; in the latter, it is postponed to the 15th day. In no case, therefore, had Harris even reached the stage at which he could, with any degree of accuracy, prognosticate the likely issue of pregnancy.

As a further control, the fertility and general fitness of the animals was tested by re-mating some of those that had aborted or resorbed, and examining the oestrous periods of others. Forty-four had live litters, the average per litter being 5.3 which compares not unfavourably with Hanson's average: 5.7 (1925). There were ten abortions.

Daily vaginal examination of twenty-one unilaterally ovariectomised mice was carried out for two months, and in two other cases for one month. Papanicolaou (1920) avers that "animals with only one ovary have a very regular oestrous cycle, the only peculiarity being that the oestrous cycle in such animals is about one day longer than in those possessing both ovaries". Emery (1931) on the other hand, remarks: "Irregular oestrous cycles, with a tendency to/
to/



- CC
- ▣ EC or E-C
- ▤ ECL
- / E
- L or EL
- No Smears

Smears of 21 unilaterally ovariectomised mice covering 2 months.

* Commencing 2 months after the operation; all others one month.

to an increase in the length of cornification, are very characteristic of semi-ovariectomised rats and are seen more frequently 4-8 months after the operation". He states elsewhere that the irregularity begins to appear about two months after the operation. He found that 16% were in oestrus for 10 consecutive days and 9% for 15. There were ten cases of oestrus for 25 consecutive days, two for 30 and two for 35 days. It is presumed that by "oestrus" is meant full cornification but this point is not clear. As cysts were found in the ovaries of 50% of the rats, this would account in no small measure for the extraordinary incidence of cornification.

In the graph produced on page 100a three features appear to be noteworthy:

- (1) The regularity of the oestrous cycles as a whole.
- (2) The relatively long period during which cornified cells appear in the smear (though frequently in the presence of others). This confirms the "tendency to increased cornification" as noted by Emery, and the "increase in the length of cornification" alluded to by Papanicolaou.
- (3) The occurrence of rest periods in fifteen out of twenty-one cases. The irregularity to which Emery refers may be of this nature. Had smearing been continued for two or three months longer it could have been/

been ascertained if such periods recur at regular intervals; they vary from 7-11 days and thus resemble a pseudopregnancy. It is probable that during such intervals a low level of alpha is secreted.

Mucification in the vagina of the mature ovariectomised mouse has been caused by repeated small doses of alpha prepared from follicular fluid (Robson, (1930)). It was at first thought that the irregularity observed might be peculiar to unilaterally ovariectomised animals, but it has been found also in normal mice (Mirskaia, and Robson - unpublished).

Certain features were noted in course of the experiment:-

(1) Pregnancy bleeding which normally occurs in the mouse on the 11th and 12th days of pregnancy, was occasionally found to exceed these limits though less frequently than in the rat. Such divergence was not necessarily attributable to the operation nor did it necessarily denote any interference with pregnancy. In four cases where there were 5 and 6 days' bleeding, and in one after 8 days', healthy litters were born. In many cases, however, where haemorrhage was protracted and severe, it signified that resorption was taking place or that the foetuses were dead. This was/

was applicable also to those cases where placental sign was delayed till the 15th day; ten such cases were observed.

(2) Sex ratio. In the cases covered by this experiment there would appear to be a slight, though not statistically significant, balance in favour of males. In 44 litters the ratio was 118.63:100 (on the basis of normal 106:100), or 54.28 ± 3.34 :

(3) A litter of ten was born from one uterine horn. This occurred in the pregnancy which immediately followed on that during which one ovary was removed. There had been no regeneration of the ovary and the ten implantation sites could be counted.

(4) Removal of one ovary, though lengthening oestrus slightly, does not apparently lengthen the course of pregnancy. Of 24 cases of successful mating after unilateral ovariectomy 20 had litters on the 20th and 21st days, and 3 on the 19th day.

(5) Two cases of possible superfoetation were observed. F7 and M12 were opened on the seventh day after mating for the removal of an ovary and in both cases a foetus was observed in¹the right horn of the uterus.

According to Kirkham (1916) the ova are merely in the blastula stage at this period of pregnancy; it appears likely, therefore, that these belonged to a mating which took place 5 days prior to the second. One other/

other case of mating during pregnancy has already been mentioned (page 87); there was one instance in which cornification was observed on the 4th and 5th days of pregnancy following which an abortion took place on the 19th day. Such a phenomenon appears to be rare: Long and Evans refer to two in the rat (1922) and W.O. Nelson (1929) to one; Crew and Mirskaia (1930)^b noted six cases in 100 mice during six months.

(6) "Regeneration of the ovary". Long and Evans allude to this possibility, and C.B. Davenport (1925) is stated to have found "regeneration" in nearly two-thirds of the mice bilaterally ovariectomised by him. Such a feature seems necessarily to involve a remnant of ovarian tissue from the operation; in our own experiment two such cases have been noted, in one of which the complete removal was queried at the time of operation.

(7) The average maximum number of ova shed at an ovulation by each ovary functioning synchronously is five (Engle, 1928), yet an instance occurred (page 103) in which ten ova were fertilised from one ovary, showing a doubling in power, if not in weight, of the remaining ovary.

A detailed study of compensatory hypertrophy followed/

following on unilateral ovariectomy has been performed by several workers on various animals (see Introduction); the question of the formation of definitive ova naturally arises from such an examination. Two theories are held on the subject: (1) that the number of ova is not added to post-natally or at least after the fourth day (Cowperthwaite, (1925); Crew, (1927)); (2) that new ova are formed up to one year of age (Arai, (1920) - rats), up to puberty (Kingery (1917)), at each oestrus (Allen (1923)), up to old age (Papanicolaou (1924) and Butcher (1927)). According to Professor Crew the number of ova is determined genetically and remains a constant factor. Where there is hypertrophy, a larger number of ova come to successful maturity owing to an increase in the nutritive material available. Engle (1928) carried out experiments in which he established that this hypertrophy is due to the additional pituitary secretion which is made available for the remaining ovary.

SUMMARY.

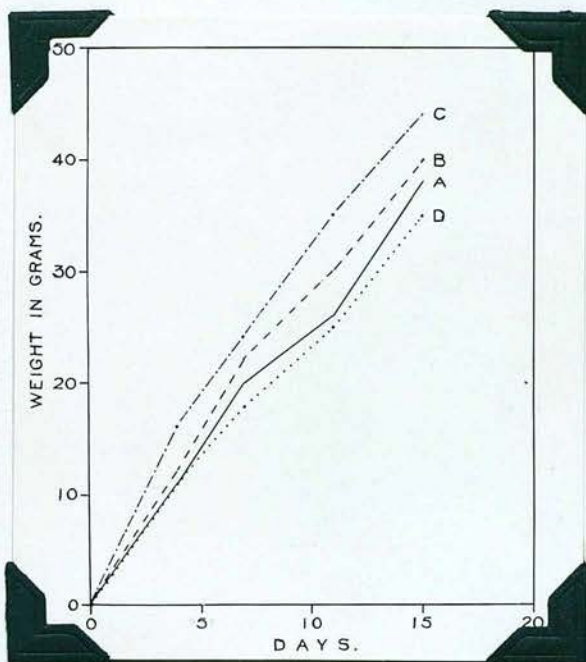
1. Removal of both ovaries in mice terminated pregnancy in all stages of the gestation period.
2. Where the second ovary is removed in a pregnancy subsequent to that in which the first was removed, the same result is obtained.
3. Removal of one ovary on or after the 13th day of pregnancy/

pregnancy has no ill-effects; on or before the 10th day there is danger of interruption of pregnancy; such interruption occurs at an earlier date in those mice in which the operation was performed before the 7th day.

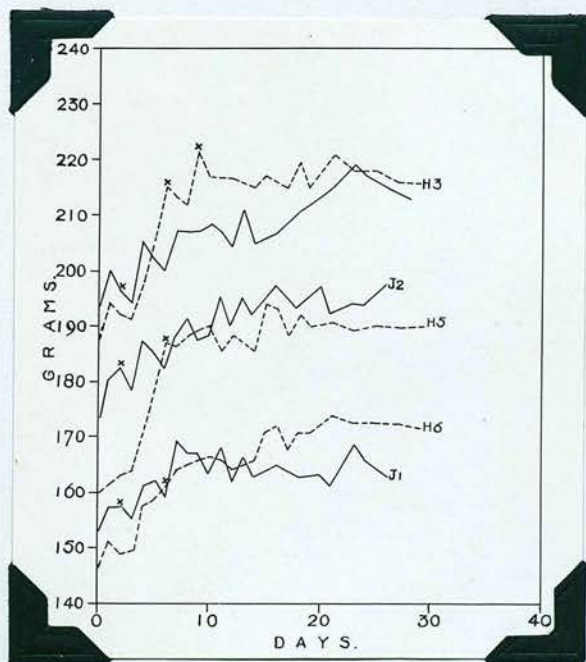
4. Where pregnancy is maintained, foetuses develop normally in both uterine horns.
5. No pregnancies occurred where one ovary was removed at the end of the first day.
6. Anaesthesia on the first day of pregnancy generally interrupts pregnancy.
7. Unilaterally ovariectomised mice tend to have longer periods of cornification.

C. Growth of the Mother and Foetus during pregnancy.

The purity of the alkaline extract of the pituitary has been called in question, owing to a noticeable increase in growth which has been found to accompany its administration. On this account, and also owing to the difficulty of diagnosing pregnancy before the appearance of placental sign, this point was investigated. In preparing the graph on page 106a twenty-six pregnant animals injected with the alkaline extract were arranged in five groups according to the total amount of extract given, and the average increase in weight of each group was taken from 1-4, 4-7, 7-11 and 11-15 days. From the 15th day of pregnancy the rapidly increasing weight of the foetuses and placentae (varying in number from animal to animal) renders useless/



Growth curve of pregnant animals injected with alkaline extract of pituitary.



Growth curve of unmated rats injected with alkaline extract of pituitary.

J = mature rats injected with total of 2.0 cc.

H = " " " " " " 8.0 cc.

less any further comparison.

Group A consisted of 4 animals receiving 6.0 cc. alkaline extract commencing the 13th day of pregnancy;

Group B consisted of 7 receiving 4-6 cc. commencing the 1st to 3rd days;

Group C consisted of 3 receiving 3.2 cc. commencing the 3rd day;

Group D consisted of 6 receiving 1-2 cc. commencing the first day of pregnancy.

For purposes of comparison, six mature unmated females were injected with the extract; three received a total of 8.0 cc. over a period of ten days, an interval of two days elapsing between the first four and the last four injections. Three others, of which vaginal smears had already been taken during two oestrous periods, were injected with a total of 2.0 cc. Vaginal smears and body weights were taken daily for four weeks. The same procedure was followed in the pregnant animals throughout their pregnancy.

The growth curves show an increase in weight of between 20 and 30 grams during the period of injection in those non-pregnant animals receiving 8.0 cc. of extract (H series). There is no further increase on cessation of the injections but the high level is maintained. The rapid increase during the first seven days is so uniform as to appear to be definitely related to some factor present in the extract/

extract. The animals receiving the smaller amount (J series) show only a slight upward tendency during the same stage, which cannot be ascribed, with any certainty, to the influence of the extract; the curve throughout is remarkably uniform.

The curves for the pregnant animals injected are of interest in that it is generally held that the only criterion of the pregnant condition in the rat prior to the 15th day is the placental sign, as the weight offers little indication of the condition.

In essence this is true; when one compares the weight on the 11th day with that on the 15th there is a difference of only 10 or 11 grams, whereas as much as 30-40 grams are added between the 15th day and term.

A somewhat rapid increase in weight is noticeable in all groups from the 1st to the 7th days, followed by a less marked increase till the 11th after which the rate is again slightly accelerated. The increase in the various stages is actually not so marked as would appear from the graph and it is only when the weight at, say, the 12th or 13th day is compared with the initial weight that one can hazard a guess as to the possibility of pregnancy existing. Normally between 25 and 30 grams are added to the weight during this time, and in view of the insignificant growth in the placenta/

placenta and embryo which accompanies this (at most equal to three grams) it becomes necessary to assign some reason for the phenomenon. It is known (Marshall (1922)) that there is excessive formation of nitrogenous compounds and retention of body-fluids during pregnancy, and there is little doubt that these factors play a part. If this is so, this increase is likely to be fostered by a substance so rich in protein as is the alkaline extract employed. Furthermore, the possible existence in the gonad of a non-specific metabolic stimulant cannot be ignored, in view of the experiments of various workers with transplants of ovarian and testicular tissue (Lipschütz (1924)). At a time when the gonad is at the height of its activity (i.e. in pregnancy) such stimulus is likely to be at its maximum.

The animals in Group A received the extract too late for any effect to be observable in the period covered; there are indications, however, of a sharpening of the upward trend of the curve at the end.

Groups B and C would seem to show some effect of the extract, more especially when compared with Group D which received the smallest total amount. The figures are, however, in no way comparable with those of Teel (1926) who obtained an average increase of
40/

40 grams in the first ten days as the result of daily injections of 1.0 cc. throughout the period. That this increase in weight was not attributable to the growth of the foetus and placenta, is demonstrated by the fact that the growth of the foetus and placenta had been arrested during this period as the result of the injections; necropsy at the eleventh day revealed embryonic growth equivalent only to a sixth day pregnancy.

Foetal growth under administration of pituitary hormone would appear to be greatly accelerated after the initial delay has been overcome. As the result of the early arrest of development, one would expect the maximum weight to be reached after normal term in all cases, or the foetuses to be underweight at birth; the tendency is certainly in favour of the former, the normal growth being attained at term. Where the urine extract was administered the maximum weight was reached before term in only one case other than those in which resorption ultimately took place. In fifteen cases, prolongation of pregnancy was associated with a continuation of foetal growth beyond term varying from one to four days; of these, one was for four days, five for three days, four for two, and five for one day.

The alkaline extract of pituitary gave the following/

following results: in three cases the maximum weight was attained the day before normal term, in two cases at term, in eleven cases after term. Correlation of these data with the amounts of extract given and the stage in pregnancy at which they were administered throws no light on the cause of these deviations. This would apply also to the implantation of anterior lobe tissue. In four cases the maximum weight was attained before term, and of these one was associated with resorption. Of the remaining five one attained full growth at term, two the day after, one two days and one three days after.

It will be evident therefore that growth is either extended beyond the average limits resulting in larger foetuses than normal, or there is a retardation of the normal development of the foetus at some stage or stages which necessitates a prolonged gestational period. With regard to the former, the weights of the foetuses are, with few exceptions, above the average, from which one would conclude that the normal processes of growth had been extended. Such an effect would be attributable to the continued activity of the corpus luteum, resulting in a persistence of the decidua and consequent increased blood supply, at a time when the latter should normally be coming under/

under the influence of those degenerative changes which culminate in parturition.

With reference to the second explanation, Teel has definitely shown that the prolonged gestational period was in large measure attributable to a delay in the implantation of the ova. This implantation normally takes place on day six, but was delayed to days 10-12. A substance is thought to exist which fosters either growth or reproduction. It is possible that for some reason (connected with the treatment administered) growth of the mother took place at the expense of growth of the foetus. Where implantation of the ova is delayed in animals that are suckling a large litter (Daniels (1910), King (1913), Kirkham (1918) Crew and Mirskaia (1930^a)), it is probable that this substance is expended upon mammary development and is passed on to the litter being suckled.

Many instances have occurred where the weight of the injected animal remained stationary for 2-4 days immediately before parturition. Ten uninjected pregnant rats were weighed throughout pregnancy and it was found that the increase in weight was continued up to the time of parturition. In those cases, therefore, where the weight remained unchanged for more/

more than 24 hours, it can be assumed that the gestation period probably was prolonged.

D. The Cell content of the vagina under administration of pituitary hormone.

Unmated mature rats and a number of pseudopregnant animals were used as controls for all of the groups experiments that have been described. Following the administration of the urine and pituitary extracts and implantation of anterior lobe tissue, vaginal smears were taken daily over a lengthy period. The cell content of the vagina was examined because of a phenomenon which appears to be directly connected with a certain phase of pituitary activity induced: a frequent picture is one of clusters or even masses of rather large epithelial cells containing a large darkly-staining nucleus. These are encountered in the pregnant animals injected but are much more numerous and persistent in those pregnant animals injected with the urine extract. In these, there is a tendency for the cells to lose their nucleus but they never become really cornified till about the 15th day. The reaction is obtained whether 0.1 cc. is injected daily or four times that amount; it is possible that if a still larger amount were injected this effect would not be got. Our statement is based on a tentative comparison of the strength of the urine extract/

extract with that of the alkaline extract of pituitary and of pituitary substance. The alkaline extract is volume for weight equal to pituitary substance; when a total of 1.0 cc. was injected large epithelial cells appeared in the vaginal smears only to the extent to which they are observable in an ordinary pregnancy. In larger amounts, i.e., where from 2-6 cc. were given, these cells were relatively absent, the normal small epithelial cell with leucocytes, or oftener leucocytes only being present.

With a view to ascertaining whether this effect was secured only in the presence of a pregnancy, non-mated and pseudopregnant rats were examined under the same treatment. Ten mature females were mated with vasectomised males and of these -

3 were injected with a total of 6.0 cc. of alkaline extract,

3 were injected with a total of 1.2 to 1.8 cc. of alkaline extract,

1 was injected with a total of 1.5 cc. of urine extract,

1 received an intraperitoneal implantation of anterior lobe tissue equal to one whole ox pituitary two days after the sterile mating.

Three others were kept as controls.

Nine unmated mature controls were used, of these -

3 were injected with a total of 2.0 cc. of alkaline extract,

3 were injected with a total of 8.0 cc. of alkaline extract,

3/

3 were injected with a urine extract in order to test its inhibition of oestrus.

Before examining the general results, reference may be made to the three unmated controls injected with urine extract (815 series). The extract did not inhibit oestrus, and on the second occurrence of this phase a male was placed in the cage. Mating occurred in two cases and a prolonged pseudopregnancy followed; it is possible that mating took place in the third animal without vaginal plug being observed. Rat 815/1 was pseudopregnant for 14 days according to the smears, but on the 15th day mating occurred; no cornified cells were observed 12 hours before mating or on the removal of plug approximately 12 hours after. In view of the numerous cases of oestrus and mating observed by Evans (1922), King (1913) and other workers, it is exceedingly unlikely that mating took place in the total absence of cornification, however partial this might be - it is well-known that the threshold of oestrus is lower in some animals than in others. It must be assumed therefore that pseudopregnancy terminated in Rat 815/1 on the 14th day. This case seems to demonstrate the advisability of keeping a male in the cage in all cases where tests are made for oestrus-inhibition, as it is apparent that/

that oestrus can last for only a few hours (as observed by Long and Evans) and its outward manifestation can readily be missed where vaginal examinations are made only once or at most twice in the 24 hours.

A study of the results obtained reveals a number of interesting facts: (1) that the large epithelial cells already described are not a feature of pseudopregnancy and, indeed, are noticeably absent from it; (2) that the appearance of these cells in the vaginal smear can be induced both in unmated and in pseudopregnant animals by injection of the alkaline or the urine extract. It is interesting to compare with this the reactions obtained by implantation of anterior lobe tissue. A whole gland, implanted at one operation, has the effect of depressing the epithelial cells so that leucocytes alone or with a few small epithelial cells are the characteristic picture. Half a gland, in two implantations, gives an almost similar picture, but a small piece of the gland implanted three days after mating causes a noticeable preponderance of the large cells. (3) The third noteworthy fact is the lengthening of pseudopregnancy by the alkaline extract of pituitary or the urine extract, and by the implantation of a large amount of anterior lobe tissue. The normal length/

length of pseudopregnancy in the rat is 11 days but 11-14 days are not uncommon. The alkaline extract prolonged pseudopregnancy to 16-19 days;

the urine extract	19-25	"
anterior lobe implantation (large amount)	16+	" *

In unmated animals 2 cc. of alkaline extract of pituitary caused inhibition of oestrus equal to a normal pseudopregnancy, viz., 12-14 days; 8 cc. of this extract caused oestrus inhibition for a period comparable with the actual pseudopregnancy prolonged by the same extract, viz., 17-22 days. A preponderance of large epithelial cells without any cornified cells for 6 and 7 days followed injection of the urine extract. As no male was placed with this group of females it cannot be said whether oestrus was inhibited or not. However, where the strength of the extract varies so considerably, it is useful to know that extracts causing this cell-picture in mature rats may be potent for the prolongation of pseudopregnancy and of pregnancy.

*This rat was unfortunately returned to stock before cornification occurred.

E. Haemorrhage during Pregnancy.

Owing to the relatively high incidence of bleeding in many of the pregnant animals which were the subject of experiment, a study was made of a number of normal pregnancies that more accurate data might be available for purposes of comparison.

Long and Evans (1920 and 1922) have described the bleeding which normally occurs about the 13th day of pregnancy as the "placental sign", and presumably the interpretation which should be placed on this term is that the bleeding is the outward sign of the presence of placenta in the uterus. The definition given by H.M. Evans (1928) would seem to indicate that he held the opinion that the haemorrhage actually emanated from the placenta. He defines it as "a leakage from the multiple new maternal vessels involved in placenta formation". Certain facts, however, would seem to point to some secretory factor as the source responsible for the phenomenon. Four circumstances might be cited:

(1) A mouse which was unilaterally ovariectomised during pregnancy mated again and during the ensuing pregnancy was killed on the first appearance of bleeding. The uterine horn of the side from which the ovary had been removed was found to be full of blood/

blood; the other horn contained four 10th day foetuses with blood between. There was no trace of ovarian tissue on the operated side, and all possibility of migration of ova from one horn to the other is precluded (Crew 1927, etc).

(2) In the pregnant animal, excessive haemorrhage follows upon the injection of certain extracts (from pregnancy urine etc.) containing anterior pituitary hormones; and in immature and ovariectomised monkeys a similar reaction, but of a milder nature was obtained by Hartman et al (1930) with extracts from the same source. It seems reasonable, therefore, to assume some hormonal basis for the phenomenon, and accordingly an effort has been made to correlate the incidence of bleeding with the amount and nature of the extract given, and to compare this with what normally occurs in pregnancy.

(3) Haemorrhage may occur also at the end of pseudo pregnancy (rat). Parkes (1929_a) and Long and Evans (1922) allude to such an occurrence, and three instances were observed in the course of this experiment, and are described later. As neither placenta nor foetus are present in the pseudopregnant animal, the haemorrhage cannot be derived from this source. While there are no grounds for assuming that the phenomena/

phenomena in the pregnant and pseudopregnant animal are identical, it is probable that the same causative factor underlies each; in which event the term "placental sign" would be a misnomer.

It is generally held that the haemorrhage is associated with a certain stage in the development of the foetus and that this is roughly equivalent to a growth of 12 days. (Teel 1926, Vol. 79). The rats in Group 1A receiving the urine extract, showed the placental sign about the normal time, yet no foetuses were found when the animals were killed three days later. Teel's statement referred to above, was supported by the fact that in the animals which he injected from the first day of pregnancy continuously to term, the appearance of the placental sign was delayed to the 18th-20th day of pregnancy, instead of appearing on the normal 13th or 14th days. This he attributed to interruption in the development of the embryo, due to the extract. Although Teel's experiment was not repeated by us, none of the animals injected either from the first or sixth day of pregnancy showed any significant departure from the normal in this respect.

A direct relation between the size of the foetus and the first appearance of the placental sign would imply/

imply the existence of a foetal stimulus for the release of the substance responsible for the haemorrhage, if such substance exist. A difficulty then arises, viz., to explain the nature of the haemorrhage associated with resorptions. Placentomata and deciduomata are not associated with bleeding; it would therefore be inaccurate to argue that the irritation set up by a foreign body was the responsible factor. On the whole it is unlikely that there is a different causative factor underlying each.

In view of the apparent connection between the administration of anterior pituitary substances and excessive haemorrhage it would seem reasonable to postulate that a certain stage in the growth of the corpus luteum is associated with this condition, and if this stage is artificially prolonged, bleeding will continue beyond the normal time. Those animals that were injected (or received implants) between the first and sixth days, manifested the normal condition; the corpus luteum degenerated at its accustomed time (or only a few hours later). When the injections* covered the period when bleeding normally occurs, bleeding was severe for two, or at the most, three days, and thereafter was of a scanty nature: (days 10-14: A5, F3, F7, D5, K4). Where the ovary came

under/

*Urine Extract.

under the influence of the extract between the 6th and 10th days (A8, A13, D6, D1, D7, E6, F5) haemorrhage was excessive, that phase of ovarian activity which is associated with haemorrhage being reinforced or stimulated immediately prior to its commencement.

An interesting circumstance has been observed in the course of vaginal examination of pregnant rats. In the pregnant control animals, leucocytes preponderate in the smears taken up to the 14th or 15th day. About the 4th, 8th and 12th days epithelial cells containing a darkly-staining nucleus are often found. It is a noteworthy fact that from about the 14th or 15th to the 17th days these epithelial cells are more numerous and are not rarely found in association with some cornified and partly cornified cells. The presence of both types of cells or of one only, indicates a measure of secretion of alpha. It was noted that where haemorrhage was of a severe nature both types of cell were absent, the smear being of leucocytes only; where both types of cell were present, haemorrhage was scanty. It is tentatively suggested that the part played by alpha/^{at}this stage of pregnancy is to prevent excessive haemorrhage, a kind of balance being set up between the factor responsible for this phenomenon (in the corpus luteum) and that concerned with its repression/

repression. Whereas the secretion of alpha at this stage of pregnancy may serve a useful purpose in normal pregnancy, it may have the opposite effect in one in which abortive tendencies were already at work at an earlier stage. If the secretion of alpha is induced or alpha itself is administered early in pregnancy, abortion or resorption frequently does not occur until mid-pregnancy is reached. What the initial secretion of alpha failed to accomplish, the subsequent reinforcement about the 14th day, achieves.

Where gestation is prolonged beyond term and more especially after the death of the foetus, other factors naturally play their part in the haemorrhage produced, e.g. loosening of the placental attachments and disturbance of the vascular connections. Such a haemorrhagic condition is likely to be reinforced as "the increase in blood supply to the generative organs is at its height towards the end of pregnancy".

(Marshall (1922)).

In order to ascertain if bleeding could occur in the absence of pregnancy, a series of experiments was undertaken in which 22 pseudopregnant rats were employed. Three mature males were vasectomised, as much as 10-12 mm. being cut out of each vas deferens in order to ensure no renewal of testicular activity. The first two matings proved fertile, as sperm existed in/

in the portion of the vas deferens between the section and the penis. Lipschütz (1924) and Miss Kropmann (See Lipschütz) noted that, in the absence of mating, spermatogenesis may continue for as much as seven weeks after the vas deferens is sectioned. Subsequent to the first matings, no pregnancies were obtained, the cervical stimulation initiating the pseudopregnant condition instead. This condition is marked by persistence of the corpus luteum, inhibition of oestrus, and a typical development of the uterine mucosa. The various stages in this development have been fully described and illustrated by Allen (1931). The vaginal smears associated with the condition show either leucocytes only or leucocytes in the presence of a few epithelial cells; the mucosa is moist and glistening and a variable amount of mucus is secreted.

As it was deemed possible that some irritation of the uterus might be a necessary prelude to the phenomenon, small portions of a uterine horn were cut in three animals on the sixth day after the sterile mating. The uterus was ligatured in two places, about $\frac{1}{4}$ inch apart and a portion equal to about one-eighth inch was cut out between. Well-developed deciduomata/

deciduomata formed at the point of section as was ascertained when the animals were killed. Vaginal smears were taken twice daily after the operation, but no haemorrhage was observed.

Fourteen pseudopregnant animals were then treated in the same manner as those in the group experiments already described, with a view to ascertaining if the condition could be induced in pseudopregnancy:-

7 were injected with a total of 1.6 cc. urine extract from the day of mating for 8-10 days;
5 were injected with alkaline pituitary extract (2 with a total of 1.8 cc. and 3 with 6.0 cc.);
2 received implants of anterior lobe tissue equivalent to one whole gland.

Haemorrhage occurred in one animal in each of the three groups: on the 15th day in the first group, on the 11th in the second, and on the 14th day in the third group. In the last-named, haemorrhage followed strong cornification on the previous day and was severe. The animal was not killed until some hours later, when no signs of the occurrence were visible; the rats belonging to the first two groups were killed immediately haemorrhage was observed, and the histological findings were as follows:-

Rat 770/6 (injected with urine Extract): haemorrhage
15th day.

Macroscopically: Left uterine horn contained much blood, distended; swellings as though atrophic fetuses present.

Ovaries: 3 recently degenerated corpora lutea,

6 large atretic corpora; 2 large follicles.

Right horn distended with fluid - hard swelling-? foetus.

Ovary: 8 atretic corpora; 2 new follicles.

Histologically: Uterus distended, passing from pseudopregnant to pro-oestral character: extravasation of blood within the uterine sub-mucosa; very strong glandular development; in uterine lumen large mass of old blood containing epithelial cells, polymorphs, and near the uterine wall, hydropic cells.

Rat 1812/1 (injected with alkaline extract of pituitary): haemorrhage 11th day.

Macroscopically: Ovaries contained several very red corpora lutea; blood in both horns, especially above the swelling in both; two swellings in right horn, one in left-?pregnant.

Histologically: Uterus full of blood and epithelial cells, distended - lumen the size of Stage I in Long and Evans (1922) endometrium not "hillocky" but typically pro-oestrous (Allen): epithelium columnar and high - with stratified muscular layer well-developed. (See Plate III)
Ovary: strongly luteinised; corpora degenerated, luteal cells loose and many fibroblasts; one newly-formed corpus containing cavity, showing ovulation had occurred; 4 large primary follicles in one of which ovulation was imminent (See Plate IV).

As it was thought that the haemorrhage might have been due to the extracts, five pseudopregnant rats, which were given no treatment, were observed throughout pseudopregnancy. From the eighth day vaginal smears were taken twice daily. Haemorrhage occurred on the 11th day in Rat 1816/1 which was immediately killed. Macroscopically the uterus had the appearance/

appearance of early pregnancy, as in the other two cases described.

Histological findings:

Uterus: lumen distended and full of blood and decidual tissue; glands and lacunae contained blood. It appeared as though decidua + blood had been shed; the margin between the uterine structure and the mass of decidua was not always clear. (See Plate VIII).

It has long been held that menstruation in woman is the end of a pseudopregnancy, and represents destruction of the prepared endometrium when no fertilisation has taken place. Heape (1900) held the view that menstruation was analogous with the pro-oestrous haemorrhage in dogs; but Marshall (1922) is of the opinion that it is a pseudopregnant degeneration and a pro-oestrous regeneration in one. The histological findings in the three cases given, support Marshall's view.

Duration of the Placental Sign. Long and Evans (1920) state that this occurs between the 13th and 16th days but "where animals are maintained on a low vitamin E diet the sign appears earlier, usually on the 10th or 11th day" (Evans (1928)). According to Teel (1926) "it occurs on days 13 and 14 and only in rare cases as late as day 15". The restriction of the appearance/

appearance of the placental sign to four days at most, is somewhat misleading; though these figures may be true of one stock, they are not of general application.

With the object of ascertaining the average bleeding incidence in the Wistar albino rats employed on this series of experiments, sixty-seven rats were examined daily from the appearance of vaginal plug until parturition, and the occasions on which bleeding occurred were recorded. From the particulars given in Table VI it will be seen that the placental sign covers a longer average period in the stock employed, than appears to have been the case in those used by Long and Evans.

Prolonged haemorrhage does not necessarily point to an unsuccessful termination to pregnancy; where such haemorrhage is excessive, however, it is generally an indication that abortive tendencies are at work.

One or two interesting features noted may be mentioned, in view of their general bearing on the experiments. In one case (R 774/9) vaginal plug occurred two days after parturition instead of within the customary 20 hours (Long and Evans). Only one other such case was recorded (R. 814/3). There would appear to have been two cases of protracted parturition (772/3/

PREGNANCY HAEMORRHAGE (or PLACENTAL SIGN) IN CONTROL RATS.

TABLE VI.

No. of days over which	(a) bleeding was spread.														
	(b) bleeding actually occurred														
(a)	15	14	13	12	11	10	9	8	7	6	5	4	3	2	1
No. of Cases	1	1	1	4	7	9	8	10	11	5	4	1	2	1	2
(b)	-	-	1	-	1	2	9	11	10	5	13	6	3	4	2

(772/3 and 776/3) though this is of relatively rare occurrence according to the literature; Miss King mentions two such instances: Rat 776/3 gave birth to two young on 19/6/30 and on 20/6/30 these were eaten by the male at 10 a.m.; at noon two more were born and met the same fate. An instance of difficult labour owing to excessively large foetuses was provided by rat 795/2. She appeared about to litter on the 22nd day but was unable to do so and was found dead the following morning with thirteen large foetuses in utero, their average weight being 7 grams.

In course of examination of these animals, it was observed that vaginal plug which is stated to persist for "about 12 hours, but occasionally as long as 24 hours" (Long and Evans), was visible for a much longer period. The maximum was sixteen days (K9) - it is to be noted that this female was removed from the male on the day that she mated; four instances of plug lasting three days were observed and in only one of these did pregnancy follow; the litter was still-born. In five cases out of nine where plug was visible for four days no pregnancy supervened. Three cases of a five day plug were associated with pregnancies, but two of seven days were followed by resorption and still-birth; in the latter the gestation period was prolonged/

prolonged (Rat 771/3) to the 24th day. On two occasions in rat 774/2 plug remained for eight days and was then removed; no pregnancy followed. Rat 32B, however, injected with corpus luteum extract, retained plug for the same period and had a normal pregnancy.

Where accurate information is required as to the length of pregnancy, it is important that the exact date of mating be known; the data given above, show that an error equal to 25% of the gestation period might occur if an unknown plug were estimated as at most 24 hours old.

DISCUSSION.

Certain facts are apparent from an examination of the foregoing experiments: In the mouse and rat, as in the majority of vertebrates, ovarian secretion is essential throughout pregnancy; a partial withdrawal of, or diminution in, this secretion in the latter half of pregnancy is generally attended by no ill effects, but in the early stages is liable to lead to an interruption of pregnancy, more especially if the interference occurs at a time when the ova are being - or are not yet - implanted in the uterine endometrium.

It has, moreover, been shown that the administration of corpus luteum hormone during the first two-thirds of pregnancy has no effect on the course of pregnancy during that period, and no appreciable effect on the growth of the foetus or of the placenta.

A large number of experiments on the effects on pregnancy of the administration of pituitary hormone have been carried out and both prolongation and interruption of pregnancy have been obtained under definite experimental conditions. Pregnancy is prolonged after implantation of relatively large amounts of bovine anterior lobe tissue, after injection of an alkaline extract of that organ and sometimes/

sometimes as the result of injection of an extract of human pregnancy urine. The results obtained with the first two methods are remarkably uniform, but more especially in the case of the alkaline extract injections. The urine extract shows considerable variability in its effect, even when the same material is employed throughout a series of experiments. As a rule, pregnancy is interrupted where this extract is administered in the early stages, unless the initial dose is speedily followed by a second dose or is itself a very concentrated one. However, an amount which causes an interruption of pregnancy in the early stages will, when given in the latter half of pregnancy prolong this condition.

The gestation period is lengthened, by any of the three means described, from $1\frac{1}{2}$ to 7 days; in other words, parturition is delayed until the 24th to 29th days. Where treatment commenced and ceased early in pregnancy, prolongation is of shorter duration than in those cases where treatment commenced later. A prolongation of pregnancy is sometimes attended by protracted parturition, a condition due to a failure in the birth mechanism. With the exception of those cases in which treatment commenced on or before the 6th day, foetuses are still-born and probably die in the/

the uterus some time before being expelled; fetuses were still alive in utero three days after normal term but were not viable: they are almost always above the average weight (5.8 grams) and range from 6 to $7\frac{1}{2}$ grams. This fact, together with the increase in weight of the mother for three and even four days after normal term was reached, points to the growth of the foetus being continued after its normal maximum weight is reached. Where the birth mechanism has failed, the placentae are sometimes found detached from the uterine endometrium; frequently they are much larger than normal (1.0^+ gram as against 0.400 gram) and occasionally, in such cases, are still attached to the uterine wall, showing that placental growth had continued beyond the normal term.

The persistence of lutein tissue formed as the result of administration of hypophyseal extract seems to be directly associated with the failure in the birth mechanism. The ovaries of the animals in each of the three groups receiving pituitary implants, alkaline extract and urine extract, are always large and present the "mulberry" appearance described by Evans (1924) due to excessive luteinisation. But it must be emphasised that an excessive formation of luteal tissue is not exclusively associated with cases of prolonged pregnancy and is frequently found in/
in/

in conjunction with resorption.

The main findings in connection with the combined series of experiments are:

- (1) That it is possible either to prolong or to interrupt pregnancy in the rat by modifying the secretory activity of the ovary.
- (2) That pregnancy is more readily interrupted in the early stages than in the later, and, further, that pregnancy is more readily prolonged by treatment administered in the later stages than in the early stages.

A number of previous observers have recorded data on this subject and their work may be described under three headings: (1) removal of the ovaries themselves; (2) implantation of anterior lobe tissue; (3) injection of alkaline extract of the pituitary. The effects of the injection of pregnancy urine upon pregnancy, however, have not hitherto been examined, but as a considerable amount of work has been carried out with this material on immature animals, the main findings are discussed in so far as they appear to be relevant.

Removal of the Ovaries:

Until recently it was held that in many animals the ovaries could be removed comparatively early in pregnancy/

pregnancy without ill effects upon the course of gestation, but later work has shown that ovarian activity is necessary until the latest stages, if not for the duration of pregnancy. Weymersch (1911) Blair-Bell and Hick (1909), McIlroy (1912), Asdell (1928) either removed both ovaries or extirpated all corpora lutea from rabbits at varying stages of pregnancy up to the 21st day and terminated pregnancy in every case. Marshall and Jolly (1905) obtained the same result in five bitches from which the ovaries were removed between three and four weeks after coitus. In the case of the guinea-pig the ovaries can be removed "in the last half of pregnancy" without danger (Courrier, Kehl and Raynaud (1929)). In this animal the placental development is not so marked as in other rodents and the early removal of the corpora lutea does not necessarily cause abortion (Loeb and Hesselberg (1917)). Mulon and Daels (both 1908), however, found that pregnancy was interrupted at whatever stage the ovaries were removed. In the human, the ovaries may be removed at the eighth week of pregnancy (cases of four weeks are known) without necessarily interrupting pregnancy (Asdell, (1928)). In all animals, removal of the ovaries or of all corpora lutea before the ova are implanted in the uterine mucosa is invariably attended by abortion. (Loeb/

(Loeb (1923) (guinea-pig), Fraenkel (1903 and 1910) (rabbit), Drummond-Robinson and Asdell (1926) (goat), Hartman (1925) (opossum), Hammond (1917 and 1925) (rabbit), Parkes (1928) (mouse). Even after removal of one ovary in the early stages, pregnancy is likely to be interrupted. Dick and Curtis (1912) found that in only one rabbit in five, was pregnancy continued after removal of one ovary before the 14th day.

That abortion following upon removal of the ovaries is directly attributable to a withdrawal of the corpus luteum secretion, was demonstrated by the parallel experiment carried out by Fraenkel (1910) in which he produced the same effect by removing all the corpora lutea in both ovaries. Hammond (1917) pointed out that in the rabbit, contrary to the report of previous observers, the corpora lutea persist throughout pregnancy. The part played by these bodies throughout pregnancy will be more fully examined later. That removal of one ovary should have any effect upon pregnancy at any stage is more difficult to explain, since Fraenkel found that if one corpus luteum was left it was sufficient to maintain pregnancy. It appears possible that the remaining ovary is unable to adapt itself to the demands/

demands which would be made upon it by embryos which had originally depended upon the secretion produced by two ovaries; in other words, the interruption of pregnancy in such cases is due to a lack of ovarian secretion. The possibility also exists that, owing to the additional amount of pituitary hormone made available for the remaining ovary, changes occur in the ovary which are antagonistic to the function of the corpus luteum, the primary concern of which is the preservation of the uterine mucosa in a state conducive to the implantation and nourishment of the ovum. Such changes would be more likely to have disastrous effects at an early stage in the development of the sensitive embryo than when growth is already advanced.

Implantation of Anterior Lobe Tissue.

Engle, and Smith and Engle, (1927) demonstrated that repeated implantation of small amounts of anterior pituitary tissue into the immature animal induced precocious sexual maturity. Excessive follicular development was usually followed by ovulation within 48 hours. Similar results were obtained by Zondek and Aschheim (1927) but there was no effect where ovariectomised animals were employed; that the effect was not purely ovarian was demonstrated/

demonstrated by the implantation of anterior lobe tissue into hypophysectomised animals; maturity changes took place in the reproductive organs of both immature and mature females in which the operation had caused a cessation of these functions.

By means of implantation of small amounts of anterior lobe tissue in pregnant rats and mice, Engle and Mermod (1928) caused pregnancy to be interrupted. This interruption was directly associated with a condition induced in the ovary and commonly connected with the secretion of alpha, and it was held that such secretion is antagonistic to the maintenance of that development of the uterine endometrium which is essential to the life of the ovum and embryo. If these phenomena are artificially imposed upon pregnancy an abortion is not likely to be long deferred at whatever stage the initial stimulus occurred; it is readily understood that it is more difficult to induce this condition in advanced pregnancy.

While it appears clear from these experiments on immature, mature and pregnant animals, that by means of implants of anterior lobe tissue, oestrous conditions were induced in the ovary and uterus, certain facts seem to indicate that the mechanism is less direct/

The material was taken from rats, mice, rabbits, etc.

direct and straightforward than appears on the surface. The specific effect of anterior lobe implants, according to Engle, is follicular maturation followed by ovulation. It may be asked, therefore, whether follicular maturation or ovulation was responsible for the results obtained in the pregnant animal. The following facts are to be considered: follicular formation occurs throughout pregnancy, and such follicles may attain a degree of development equal to maturity (see Plate of ovary of rat on 15th day of pregnancy). Further, the normal ovarian cycle is only modified during pregnancy and not wholly suspended, as is evidenced by the appearance of epithelial cells in the vaginal smears at approximately the usual cyclic intervals: the 4th, 8th, 12th, and 16th days, (Swezy and Evans (1930)) and by the fact that mating occasionally takes place during pregnancy (Long and Evans, (1922) Nelson, (1929) Watt (1931) Mirskaia and Crew (1930)). Apparently alpha is produced continuously; follicular formation (and consequently the secretion of alpha occurs in the pre-pubertal animal (Mirskaia and Wiesner (1930)) and Engle (1931). Engle has shown that at 15 days the ovary of the immature mouse contains follicles very nearly as large as those of the first oestrus. From/

From the histological examination of the vaginal mucosa carried out by Mirskaia and Wiesner (loc. cit) it would appear that a low secretion of alpha is associated with a definite structure in the vaginal epithelium strongly resembling the mucification of the pregnant animal. A similar effect was brought about in the mature mouse by small doses of alpha (Robson (1930)). Not only is alpha secreted during pregnancy and prepuberty but also during anoestrus, and it is apparent that a "threshold value" exists.

That the result which Engle and Mermod secured on pregnant animals after anterior lobe implants was not attributable to ovulation - which he found to be the specific effect on immature animals - is indicated by the fact that in no case did ovulation occur before abortion or the death of the foetuses. Moreover, Zondek (1928) induced ovulation by the same means, and with small amounts of pregnancy urine extract, without causing abortion.

The interruption of pregnancy was ascribed by Engle and Mermod to the induced secretion of additional follicular hormone which had the effect of directly inhibiting the action of the corpus luteum, as exhibited in the oestrous condition of the uterus. There is a possibility, however, of a direct action upon the uterus. The existence of variations in the sensitivity/

sensitivity of the uterus to the oestrus-producing hormone, and also the possibility of some mechanism inhibiting the effect of alpha, are discussed at a later stage.

Parkes and Bellerby (1926) and Smith (1926b) obtained similar results to Engle and Mermod, with injections of alpha but Allen (1924) and Brouha and Simonnet (1925) obtained negative results, which indicated that the luteal phase of ovarian activity directly associated with pregnancy need not necessarily be reversed by follicular hormone. More recently Robson (1931) and Philipp (1931) have obtained luteinisation in the rabbit with implants which is in direct confirmation of our own results. It is significant that the nature of the result obtained is determined by the amount of pituitary substance; this aspect of the question is discussed more fully later.

Alkaline Extract of Pituitary.

As the implantation of anterior lobe has always been associated with follicular growth, so, too has the injection of alkaline extracts of the pituitary been associated with luteinisation of the follicle (Smith and Engle (1928) Zondek and Aschheim (1927) Evans (1924), Teel (1926) Brouha and Simonnet (1927)). It has been shown that implants can induce both follicular formation and luteinisation, and it will later be shown that the alkaline extract has similar effects.

As has already been stated, Teel's experiment served as the basis of our own in the administration of Evans' alkaline extract of pituitary during pregnancy. While this was not a repetition of his work, it confirmed his results in the essentials. He found that the normal gestation period was prolonged between two and six days as the result of the injections, and that this prolongation was due to a delay in implantation of the ova. Foetuses were still-born if injections were continued beyond the sixth day; their death was due to a failure of the birth mechanism, and this was associated with a persistence of luteal tissue in the ovary due to the extract.

The delay in implantation of the ova and the persistence of luteal tissue were examined further since the phenomena were likely to be common to both experiments.

(a) A delay in the appearance of the placental ^{*}sign in those animals which were injected from the first day of pregnancy, induced Teel to examine the uteri of a number of injected animals on the ninth, tenth, and eleventh days of pregnancy. He found that the implantation of the ova had been delayed; six-day blastocysts/

* This did not appear till the eighteenth or nineteenth day, instead of the thirteenth day as is normal.

blastocysts (Huber 1915) were found free in the lumen of the uterus, without the slightest evidence of a placental reaction on the part of the uterus, and on the twelfth day implantation sites resembling seventh or eighth day development were observed.

That the ultimate death of the foetus was not due to injury in the early stages was proved by its subsequent implantation and development. The delay in implantation was thought to be due to a delay in the passage of the ova through the tubes as a consequence of the injections, but Teel found ova in the uterus between days one and six. Another suggestion was that the delay in implantation was due to a lack of sensitivity on the part of the uterine mucosa as a result of the injections. It was found, however, that placentomata were induced in ~~innmated~~, injected animals by the usual means: the animals were selected at random, injected with alkaline extract for five days when a thread was inserted through the uterus; injections were continued, and six days later placentomata were found. This showed that the delay in implantation above described, was not due to a lack of sensitivity on the part of the uterine mucosa on days five and six. It might be said that trauma such as has been described exerts a much stronger stimulus on/

on the mucosa than the delicate ovum, and that some factor, which is essential to its nourishment and development was lacking. A brief description of the development of the uterine endometrium during pregnancy and a possible modification which took place as the result of injection will make the point clear.

It is manifest from a histological study of the mucosa at various stages that the uterus does not maintain the same character through all stages of pregnancy. (W.M. Allen (1931)). Between the fourth and sixth days, during which time the ovum is normally implanted, the uterine epithelium has a height of twenty to thirty μ and the cells are columnar; there is hardly any mitosis. There is no definite basement membrane such as exists in pro-oestrus; mitosis is taking place in the stroma and the general appearance is one of secretion in the epithelial layers and of growth in the stroma. In the days that immediately follow (seven and eight), there is a transition to a low cuboidal type of epithelium, and the general appearance is that of growth rather than of secretion. It is not until the tenth day that the epithelium is thrown into crypts and folds and assumes the "hillocky" appearance which it retains till the end of pregnancy. With each of these stages is associated a certain stage of/
of/

of development of the embryo. The secretory activity of days four to six is evidently most favourable to the embedding of the ovum. Days seven and eight correspond to the period during which the developing embryo erodes the epithelium and becomes embedded in the endometrium. Possibly the rapid growth of the epithelium during this period aids in repairing the injury which occurs at the time of implantation. At about the tenth day the uterus enters on another stage which is associated with uterine haemorrhage. Whatever part this phenomenon plays, it is immediately followed by very rapid growth of the foetus and placenta as is shown by the following figures:

At fifteen days the average weight of the placenta	is 180 mgm.
" term " " " of the placenta	is 400 mgm.
" fifteen " " " of the foetus	is 270 mgm.
" term " " " of the foetus	is 5800 mgm.

On this basis the growth between the fifteenth day and term is:
placenta approximately 55 per cent of the total growth.
foetus " 95 per cent " " " "

With these different stages in the growth of the uterine epithelium, it is probable that differences in the/

the structure of the corpus luteum and, consequently, in the balance of ovarian secretion as a whole, are directly associated. R. Deanesly (1931) has shown that between two and three days after ovulation, the lutein cells have reached their maximum size. After this date, the corpus luteum enlarges by mitosis and at about the tenth day is completely formed and measures 2.7 mm. in diameter (Arai, (1920)). Long and Evans (1922) are of the opinion that the corpus luteum of pregnancy does not differ from that of ovulation until the tenth day and that at that time a change in size and in the number of lipoid granules takes place. If, as has been suggested, structural changes in the uterine endometrium are associated with a balance between follicular and luteal hormone in the ovary, it may readily be imagined that the formation of abnormal lutein tissue is likely to have as far-reaching effects on pregnancy as the excessive secretion of follicular hormone. Allen (1931) has shown that the effect of alpha on the uterus is anabolic, and only its withdrawal is accompanied by destructive changes. The pro-oestrous uterus bears a strong resemblance to the five days' pseudo-pregnant organ; in both cases there is marked secretory activity of the cells and the epithelium is high and columnar/

columnar (20-30 μ); but growth of the stroma is not characteristic of the pro-oestrous uterus. Both features are present in the uterus of early pregnancy, and it is suggested that the combined result is due to the interplay of the two ovarian hormones, alpha and beta, and that alpha is responsible for the secretory function and beta for the growth of the stroma. A formation of abnormal lutein tissue may cause an insufficient secretion of fluid by diminishing the amount of alpha. As there is probably always alpha present in the corpus luteum the result is not likely to be so serious as in the case of the reverse process effected by the anterior lobe implants, but appears to be sufficient to delay the implantation of the ovum.

A number of experiments have shown that it is impossible to prolong the luteal phase indefinitely and possibly, on this account, the ova were ultimately implanted. Mirskaia (1930) states that "the kyogenic reactions of the body cannot persist indefinitely," for it has been shown that, in mice, the lactation interval of three weeks is not prolonged though suckling is continued; Brouha (1928) showed that even when the life of the corpus luteum is extended by anterior lobe extracts, it is for a short time only that the sensitivity of the uterine endometrium is prolonged; and Courrier and Kehl (1930) found that the corpora lutea/

lutea which formed as the result of injection of alkaline extract were powerless to prevent the normal atrophy of the endometrium of the pregnant and pseudopregnant rabbit.

(b) The persistence of the additional luteal tissue formed as the result of the extract. Teel attributes the failure of the birth mechanism directly to this cause, and indirectly to an inhibition of ovulation consequent upon this persistence. He states "that ovulation and oestrus do not occur until the lutein tissue formed from injections disappears. This is a matter of twelve to twenty days after injections are stopped". This persistence of abnormal lutein tissue several days beyond normal term was a characteristic of our own experiments. A number of facts can be cited in support of Teel's contention that an inhibition of ovulation due to the persistence of lutein tissue accounts for the prolongation of pregnancy: it has been said that the injection of an extract of ovaries from other animals at oestrus will hasten parturition (Dixon and Marshall (1924)); and that the frequent injection of alpha (Parkes (1930)) and repeated implantation of small amounts of anterior lobe tissue will have the same effect (Engle and Mermod (1928)). If the presence of alpha is essential to parturition one may ask how parturition occurred eventually/

eventually. Ovaries removed from animals which had already expelled dead fetuses contained no follicles. (See plate XI). It has already been stated that follicles can mature and ovulation can take place in the presence of corpora lutea (Deanesly (1931), Zondek (1928), Swezy (1930), and our own experiments). Conversely, decay of the corpus luteum is not in itself sufficient stimulus to cause ovulation (Deanesly (1931)); yet ovulation always follows parturition. Moreover, alpha causes sexual maturity but does not cause ovulation, and injected in large amounts it fails to bring about parturition. It would seem, therefore, that some extra-ovarian stimulus plays a part in initiating the changes associated with parturition. This extra-ovarian factor may affect both the activity of the corpus luteum and the ovulation-and alpha-production mechanism of the ovary. This will be examined in more detail later.

While it would appear from the experiments of Wiesner (1930) and those described herein, that even in dilute form, the alkaline extract of pituitary gives only one reaction, viz. luteinisation, the results obtained by Courier and Kehl (1929a) demonstrate that the extract is capable of causing follicular formation, luteinisation, or the combined effect according/

according to the amount administered. Although Hill and Parkes (1930b) obtained oestrous changes in the vagina of the ferret with the same extract this is not necessarily significant, as apparently their preparation was toxic, for cystic follicles were formed. Courrier and Kehl (1929a) show clearly by their histological findings that the three effects were obtained: cats in dioestrus were given varying amounts of extract corresponding to from ten to twenty grams of fresh glands. "Avec les doses les plus faibles nous avons déclenché une phase folliculaire caractéristique: l'ovaire est volumineux, il renferme des follicules en maturité et parfaitement normaux;... la corne utérine a le plus souvent triplé de volume;... l'épithélium vaginal offre ici l'aspect caractéristique de la phase folliculaire normale. Chez une chatte ayant reçu des doses un peu plus fortes, le tractus génital est en phase folliculaire, et dans l'ovaire les follicules volumineux renferment leur ovocyte: mais quelques cellules de la granulosa se pycnosent tandis que les atres se transforment en éléments lutéiniques....On a l'impression que ces follicules, après avoir subi une maturation normale et déclenché dans tout le tractus genital les modifications du rut, sont en voie d'atrésie lutéinique.

Chez l'animal ayant reçu la plus forte dose

(20 gr. en 12 jours), on trouve un ovaire renfermant de nombreux corps jaunes;.....cette phase lutéinique a été précédée d'une phase folliculaire comme nous l'a montré l'étude quotidienne du frottis vaginal. La corne utérine nettement hypertrophiée n'a pas l'aspect typique de la phase lutéinique normale...il semble que l'action de ces corps jaunes soit moins intense que normalement".

The implantation of large amounts of anterior lobe tissue demonstrated that a substance which in small amounts causes follicular formation (or the first phase of the ovarian cycle) is capable, in greater concentration, of inducing the second phase, associated with luteinisation. Courrier and Kehl's experiments complete the chain of evidence in the other direction; they show that a luteinising extract can cause either the luteal or the follicular phase according to its concentration.

The Urine Extract.

It has been implied in the foregoing study that interruption of pregnancy is associated with induced follicular activity, and its prolongation with the persistence of luteal tissue, and that each of these results may be obtained by the administration of different concentrations of pituitary hormone. It has generally been observed that small amounts of pregnancy/

pregnancy urine preparations stimulate to sexual maturity in a short time, but if injected in large quantities for a longer period cause follicular luteinisation (Aschheim and Zondek (1930)) with Prolan, Biedl, Botschkareff, Brouha (all (1929) Wiesner (1931) etc.). The results were not so straightforward, however, as is implied, and it would seem that pregnancy urine stimulates the production of both the alpha and beta hormones of the ovary. The effect on pregnancy will depend on the stage of development of the uterine mucosa and not entirely on the dosage. The dual effect can be obtained in the same animal after injection of pregnancy urine: i.e. mature follicles, corpora lutea and corpora haemorrhagica, as has been shown by Wiesner and Marshall (1931), Hill and Parkes (1930-31), Zondek and Aschheim (1928a) Odescalchi (1928), etc.). This, indeed, is frequently a characteristic of pregnancy urine: if one presupposes that two pituitary factors are involved in these effects it would be necessary to assume that some follicles received one substance and other follicles another, in order to account for the different reactions. The following points are to be noted:

- (1) The urine extract employed is a highly purified substance and would be absorbed more rapidly than either/

either the untreated tissue or the less purified alkaline extract. The response on the part of the ovary would be likely to vary according to its secretory activity at the time. Undeveloped follicles would mature, mature follicles would undergo luteinisation or become haemorrhagic. If the first stimulus is rapidly followed by others, the development of luteal tissue will be accelerated. If the stimuli are spread over a relatively prolonged period the first effect is likely to be maintained. It follows, therefore, that the more prolonged the period of injection is in the pregnant animal, the more likely is resorption to take place. This was borne out by our results.

It was on these grounds that certain modifications were made in the latter half of the experiment with the urine extract on pregnant rats. Both Engle and Mermod's results with implants, and our own with urine extract, showed that pregnancy was more easily interrupted in the early stages than in the later; the embryo itself is likely to be more sensitive to changes in nutrition at an early period of its development. Also, if the corpora lutea of pregnancy are not fully formed till the tenth day (Arai, (1920), it is evident that the balance of/

of ovarian secretion will, during this period, be more readily weighted in favour of the follicular hormone than at a later stage. For this reason, members of a group receiving injections from the second day of pregnancy were given a second injection within ten hours of the first, three injections being given in the twenty-four hours. These had normal pregnancies; it is presumed that the effect was to accelerate luteinisation of the follicles in which the initial injection had induced development. In the animals which received the same total amount of extract over a longer period (and even from a later date viz. the seventh day) pregnancy was interrupted at a later stage. It would appear, therefore, that the strength of the initial stimulus largely determines the result to be obtained.

(2) An amount of extract which in the early stages of pregnancy is definitely disruptive (0.1 cc. daily) is able, when injected at a later date (eleventh and fifteenth days), to prolong pregnancy to the same extent as the most concentrated doses. If it is held that a certain substance exists in the small doses and another in the larger amounts, these respective quantities should always produce the same effect. Had pregnancy run its normal course one would have contended/

contended that as it is more difficult to interrupt the later stages of pregnancy, the extract had been ineffective. Since it prolonged pregnancy to the same extent as the larger dose, it seems evident that the effect was due to the same substance in both cases, and that the different response is one of degree and not of kind.

(3) That one hormone is responsible for follicular maturation and another for luteinisation is held by many authorities (Claus (1931) Hill and Parkes (1930) Wiesner (1930) Zondek and Aschheim (1930)); yet three points are noteworthy:

(a) Hill and Parkes (1930) found that luteinisation follows automatically on ovulation without further injection; and in the rabbit by similar treatment, corpora lutea are formed which are functionally equivalent, so far as their action on the uterus is concerned, with those of pseudo-pregnancy (Robson (1931)).

(b) Continued injection of an "oestrus-producing" hormone induces in the ovary and uterus, changes characteristic of the luteal phase (Wiesner (1931)).

(c) An "oestrus-producing" hormone can bring about oestrous changes in the uterus or vagina without follicular formation in the ovaries (Wiesner). Hill and Parkes mainly base their conclusions (that there are/

are two factors) on Engle and Mermod's experiments and state that the fact that pituitary tissue caused a "reversal" of the luteal phase instead of reinforcing it, is "the most conclusive experimental finding that two hormones are concerned". The results obtained from large implants, and from small amounts of urine extract in mid- and late pregnancy, when taken in conjunction with those of Courrier and Kehl (1929) with an alkaline extract of pituitary, seem to point to the existence of one gonadotropic hormone only.

Certain features are noteworthy in connection with the high incidence of resorption in animals injected with urine extract in the early stages of pregnancy.

(a) There was no outward manifestation of an interruption of pregnancy until a relatively late stage - the placental sign appeared at the normal time and it was only its excessive character that denoted an abnormal condition, and this was not an infallible sign. That abortion did not follow immediately or relatively soon after injection seems to point to the action being an indirect one.

(b) The most noteworthy feature, and one which requires to be emphasised, is, that in the rats in which resorption occurred, this condition was, almost without/

without exception, associated with marked luteal development in the ovaries. These were remarkable for the absence of follicles and invariably consisted of masses of corpora lutea; as many as 20-25 corpora lutea in one ovary were frequently found, and the ovaries were more than twice the normal size. The vagina of such animals showed none of the typical oestrous changes: and though the uterus was of pro-oestral type (Allen (1931)) and in some cases there were indications that ovulation had recently occurred, these had followed the interruption and were not its cause.

As the main results of the series of experiments undertaken fall into two headings: the interruption of pregnancy and its prolongation, it seems profitable to examine in fuller detail the factors underlying these phenomena.

Interruption of Pregnancy.

The features which were found in the animals which aborted in the experiment carried out by Engle and Mermod - follicular maturation etc. - are in marked contrast with those observed in the animals in which abortion or resorption occurred in the experiments which we have described. In the latter, the structure of the ovaries and vagina were typical of the luteal phase/

phase: luteinisation was excessive in the ovaries, and the mucous stratum of the vagina was high and vacuolated; these features, however, were found in conjunction with a condition in the uterus which is associated with oestrus (Allen, Long and Evans (loc. cit)). The difference between the picture presented by Engle and Mermod's animals and that which we have described is so marked that it would appear likely that the interruption of pregnancy was not due to the same cause in both experiments.

Several possibilities have to be considered:

(1) That the interruption of pregnancy was due to the secretion of alpha. "Follicles are not necessary for the continued secretion of the ovarian hormone which is responsible for oestrus" — (Allen, Pratt et al (1930)). Support of this fact has been provided in various forms. Parkes (1926) destroyed the ovarian follicles in a mouse by means of X-rays, but the oestrous rhythm was uninterrupted. Both Hill and Parkes (1930) and Wiesner (1931) found cornification in the vagina in the presence of atretic corpora lutea, and the former obtained the oestrous sign in the ferret (enlarged and swollen vulva) in the absence of any ovarian change. AS in both cases a preparation of pregnancy urine was employed, the findings are significant. The possible source/

source of alpha was not merely the ovarian stroma but the atretic corpora lutea themselves; Zondek and Aschheim found alpha to be present in such bodies.(1928a) The explanation does not appear to be altogether satisfactory, as histologically the corpora lutea formed after injection of urine extract appeared in no way different from those formed after injection of the alkaline extract of pituitary, which did not cause an interruption of pregnancy.

(2) That a follicular phase preceded the formation of corpora lutea, that luteinisation took place slowly under the influence of a weak luteinising extract, and that disruptive forces were initiated, (involution and regression of the uterus), which the formation of fresh corpora delayed for a little but was powerless to prevent.

(3) That the development of the corpus luteum was arrested and held in check. If a certain phase of development in the corpus luteum is associated with a definite structural phase in the uterus, (and this seems likely from the histological studies of Allen (1931)), it is reasonable to suppose that, by arresting further development of the corpus luteum, the character of the uterine endometrium may not change in accordance with the demands made upon it by the growing embryo. Such/

Such a circumstance would be attended with considerable danger to the foetus but need not cause abortion: that a particular stage of uterine development was "held" in this way, but without ill effects, was shown in the experiment with the alkaline extract, when the implantation of the ovum was delayed. Failure on the part of the corpus luteum of ovulation to enter upon that stage of growth which is associated with the corpus luteum of pregnancy, may account for the fact that resorption frequently takes place only after mid-pregnancy is passed.

Conversely, the development of the corpus luteum (and therefore of the uterine mucosa) might be so accelerated as to be ahead of the development of the ova. The placentomata test could have shown whether this were the case, but this was not performed.

(4) That some unknown factor in the pituitary was stimulated either directly, or through the ovary, or through some channel outside both. It is suggested that this factor may act directly on the uterus and not through the ovary: Courrier (1928) caused abortion in rabbits by injecting 'folliculin', but there were no structural changes in either the ovaries or the uterus; the decidual reaction was as intense as in the controls. He contends that the action was not through/

through the corpus luteum, as in this event the endometrium would have degenerated. Allusion has already been made to the action upon the pituitary of repeated stimuli (Engle and Mermod (1928), Zondek (1928_b) and Deanesly (1931)); the two former with pituitary implants and the latter with alpha induced ovulation. That the alkaline extract never has this effect may be in some way connected with its antagonism to ovulation (Evans (1924), Teel (1926) Brouha and Simonnet (1928)), for there would appear to be some connection between the mechanism which maintains the pregnant condition and that which suppresses ovulation.

Prolongation of Pregnancy.

The removal of corpora lutea brings pregnancy to an end (Fraenkel, etc.); their persistence prolongs pregnancy (Teel). This implies either

(a) that their period of functional activity is extended, or

(b) that their presence prevents the activity of some other factor which plays a part in the birth mechanism.

(a) That the corpus luteum does perform a definite function in the later stages of pregnancy, although perhaps a less vital one than at its commencement, is indicated/

indicated by the nature of the activity of the uterine endometrium. Allen (1931) states that from the tenth day the endometrium assumes the "hillocky" appearance which it retains till the end of pregnancy. "During this period the growth of the epithelium continues, but is slow, the stroma is oedematous, there is a definite basement membrane and the appearance is as of secretion". That there is marked growth both of the placenta and of the foetus at this time has already been shown; the fact that placental and foetal growth was so frequently continued after normal term in the injected animals, indicates that the functional life of the corpus luteum, judged by uterine activity, had been extended. An instance was found in which the placenta was actually embedded in the stroma which showed considerable glandular activity; all the placentae in this animal were 250-300 per cent larger than the average (See Plate).

(b) It must be evident that the activity of some other factor which is essential to the birth mechanism was inhibited. Various possibilities arise:

- (i) that this factor was ovarian
- (ii) " " " " uterine
- (iii) " " " " extra-ovarian and extra-uterine.

(i) That this factor was ovarian. The theory that an antagonism exists between the follicular and corpus luteum/

luteum hormones is of old standing and has perhaps been unduly stressed. It is based on two findings: the so-called oestrus-inhibiting properties of extracts of corpus luteum, and the interruption of pregnancy by alpha. The secretion of alpha during pregnancy when the corpus luteum is dominant, its existence in corpora lutea themselves (Allen, Pratt et al (1930) Zondek (1928a)), the necessity for its presence for the continued activity of the corpus luteum hormone in ovariectomised animals (Hisaw and Leonard (1930), Corner and Allen (1929)), and the lack of relation between its secretion and its outward manifestation in morphological changes in the genital tract, make it difficult to ascribe oestrus-inhibiting properties to corpus luteum extracts. The tendency is to consider this antagonism to be inherent in the hormones themselves, and their interrelation as a direct one, without reference to a possible intermediate factor, but there would seem to be indications in favour of the latter hypothesis. In any case, no experiments have reported oestrus-inhibiting properties with hormone content as determined by any well-defined test.

Since pregnancy can be interrupted by the administration of alpha (Parkes (1930) and Dixon (1924)), and as parturition is followed by oestrus and ovulation, there are indications that the secretion of alpha/

alpha plays a large part in the normal birth mechanism. So long as the corpus luteum is active, the full effects of alpha are inhibited. It would appear, therefore, that the persistence of lutein tissue had, by preventing the secretion of alpha, caused pregnancy to be prolonged.

This is not an adequate explanation of the failure in the birth mechanism, as is shown by the following: the uterine contraction which takes place at oestrus is slight, and insufficient to expel the foetus - (Bourne and Burn (1928)); also, the amount of alpha which, in the castrate, induces all the manifestations of oestrus is unable to cause abortion (Courrier et al (1929)). It was found by Bourne and Burn, and confirmed by Parkes (1930), that the oxytocic principle in the posterior pituitary which causes the uterine muscle to contract, acts more readily in the presence of alpha, and it was presumed that the degeneration of the corpus luteum permitted the secretion of alpha in amounts sufficient to sensitize the uterus to the activity of pituitrin. Two objections occur: Where parturition follows the removal of the ovaries (either after a short or long interval - as in man) the secretion of alpha cannot play a part in the birth mechanism. Moreover, Parkes (1930)/

(1930) affirms that alpha had to be administered in repeated injections spread over thirty-six hours, (mice), and that a more rapid or concentrated stimulus was without effect. This would involve its secretion in large amounts for a considerable period prior to parturition, but experiments indicate that the activity of the corpus luteum is continued until within a few hours of parturition. Pituitrin can act in the absence of alpha (Parkes (1930)) so that alpha cannot be described as playing an essential part. Siegert (1931) states that alpha can inhibit the reaction to pituitrin of the uterus (in vitro) of the virgin rat or guinea-pig. As the uterus of a pregnant rat in vitro responds to pituitrin, there would seem to be some mechanism inhibiting its activity throughout pregnancy (Siegmond, (1930)).

(ii) That the factor which failed to play its normal part in the birth mechanism was uterine, foetal or placental. It has been said that a foetal stimulus is active in changing the corpus luteum of ovulation into the corpus luteum of pregnancy (Long and Evans (1922)), just as by reaction of the pituitary to the stimulus of suckling (Crew and Mirskaia (1930a)), the corpus luteum of pregnancy is transformed into the corpus luteum of lactation. The reason that pseudo-pregnancy comes to an end, is that there is no uterine stimulus/

stimulus to extend the life of the corpus luteum. It may not be impossible, therefore, that parturition takes place not only "when a particular stage of the ovarian cycle is reached" (Parkes (1930)), but also when a certain stage in uterine development is reached.

This would presuppose that pregnancy comes to an end either because, owing to faulty conditions in the uterus, the foetus has not developed, and has therefore not stimulated the continued growth of the corpus luteum, or that foetal growth has reached a maximum, and that the stimulus which it exerts via the pituitary during its growth has consequently been withdrawn. The objection is that completion of foetal and placental growth is not sufficient to set en train the changes pertaining to parturition. It must also be borne in mind that "phenomena normally associated with parturition may occur when a similar ovarian stage is reached at the end of pseudopregnancy" for which no foetal stimulus can be held responsible. (Parkes (1929a) (Plate). Moreover, it would appear that certain uterine changes did take place in spite of the persistence of luteal tissue in the ovary, in that not only were the placentae in most cases detached from the endometrium but incomplete expulsion of the foetuses occurred. As parturition was frequently/

frequently protracted, it is evident that some factors which normally play a part in the birth mechanism were wanting; their absence was associated with the abnormal ovarian condition. That parturition did occur in the absence of those ovarian changes normally associated with it, may in some measure have been due to uterine contractions set up by increased abdominal pressure due to putrefactive gaseous distention.

(Routh (1898), quoted by Marshall (1922)).

(iii) That some factor, both extra-ovarian and extra-uterine, was prevented from playing its normal part in the birth mechanism. As neither alpha nor the completion of foetal growth appears to provide sufficient stimulus to cause parturition, it would appear likely that the prolongation of pregnancy was due to an inhibition placed upon some outside factor which is normally responsible for a renewal of pituitary activity. It is probable that the factor which is responsible for stimulating the pituitary at puberty, is also responsible for the ovulation which follows parturition, and that a high level of pituitary activity is as essential to pregnancy as is the existence of the corpus luteum.

Normal Pregnancy. From a study of the foregoing, certain facts emerge that may throw some light on the mechanism/

mechanism of normal pregnancy. It has been shown that the maintenance of pregnancy is due mainly to the existence of the corpus luteum secretion; its removal causes abortion in almost all animals except man; its function has been shown to consist chiefly in producing certain morphological changes in the uterus that are intimately connected with the implantation and subsequent nourishment of the ovum (a) Fraenkel (1910), Corner (1929 etc.) Hisaw (1929) Weichert (1928)), and that alpha is incapable of fulfilling these functions (Courrier (1928b)). Corpus luteum extracts injected into mature rabbits caused proliferation of the uterine mucosa but did not bring about this reaction in immature animals (Hermann (1915))). Such extracts have no effect on the ovariectomised animal, but if alpha is first administered, the usual response is obtained to the subsequent injection of corpus luteum hormone (Hisaw and Leonard (1930), Corner and Allen (1929)). This is not maintained, however, in spite of continued injections of corpus luteum extract (Hisaw and Leonard (1930)), and it would seem that beta requires alpha for its full activity.

A part has already been assigned to alpha in the structural changes which take place in the uterus during pregnancy and pseudopregnancy (Allen (1931)).

It/

It has been suggested that alpha is mainly responsible for the secretion of fluid throughout pregnancy, and for the development of the uterine musculature, while the activity of beta is chiefly concerned with the growth of the stroma. However, the secretion of alpha during pregnancy is apparently maintained at the low level of prepuberty and anoestrus (Engle (1931)); follicles form and reach a considerable degree of maturation but ovulation is rare.

It is generally held that the relation between beta and alpha is antagonistic as well as complementary. Inhibition of ovulation was regarded by Loeb (1923) as one of its chief functions, and only second to its action upon the uterine mucosa, as ovulation rarely occurs in the presence of a functional corpus luteum. As in most animals the termination of pregnancy is associated with the cessation of the functional activity of the corpus luteum, it follows that it is equally determined by the nature of the pituitary activity. Anything that diminishes the activity of the pituitary is likely to interrupt pregnancy, especially if the stimulus is a repeated one. The high level of pituitary activity may be maintained under stimulus from the corpus luteum, or through some other mechanism, since a low level of secretion of the pituitary can occur in the presence of strong luteal development/

development in the ovary.

It has been stated that the secretion of alpha performs a definite function in the pregnant animal, and although present in large amounts, e.g. in human placenta and urine (Zondek (1928), Wiesner (1930) and Collip (1930)) and to a less extent in mouse placenta (Mirskaia and Wiesner (1930)), it does not cause pregnancy to be interrupted. The amount of alpha which, in a castrated guinea-pig caused cornification, failed to cause an abortion in a castrated pregnant animal. While it may be said that the uterus varies periodically in sensitivity to the oestrus-producing hormone, it appears likely that some outside mechanism is responsible for the suppression of its full effects, and inhibits the reaction of the uterus. It is probable that oxytocin or some other factor acts upon the uterine muscle, causing it to contract and to expel the foetus, and that either its action is held in check or its secretion is inhibited during pregnancy (Bourne and Burn (1928), Parkes (1930), Robson and Illingworth (1931)). That the stimulus initiating the changes associated with parturition does not arise in the ovary, is indicated by the fact that in man and possibly in some animals (e.g. the guinea-pig), pregnancy can continue in the absence of both ovaries. That it may arise from the uterus either/

either directly or indirectly, would appear likely. It is possible that the degeneration of the corpus luteum towards the end of pregnancy is caused by a uterine stimulus, as hysterectomy causes a persistence of the corpus luteum (Loeb (1923a) Asdell (1928)). That it is not due to a foetal stimulus is shown by the fact that similar changes occur at the end of pseudopregnancy.

The conclusions are, briefly, as follows:

(a) For the normal course of pregnancy, ovarian secretion in the mouse and rat is essential throughout the gestation period.

(b) While the corpus luteum plays a dominant part throughout pregnancy, it requires the presence of alpha for its proper function.

(c) An "optimal quantitative relationship" exists between the follicular and corpus luteum hormones (Hisaw and Leonard (1930)), and ~~that~~ for pregnancy to run a normal course the secretion of alpha must be subordinated to that of beta.

(d) It follows that the degree of pituitary activity which is associated with the sexual phase of the ovarian cycle is subordinated to that which accompanies the reproductive phase.

(e) It is probable that some mechanism outside the ovaries/

ovaries is responsible for preventing this activity, and that this mechanism is connected directly or indirectly with the uterus.

(f) Parturition is intimately connected with the decadence of the corpus luteum and with the mechanism concerned with ovulation.

The Part Played by the Anterior Lobe of the Pituitary during Pregnancy.

There are indications that pituitary secretion is maintained at a high level throughout pregnancy.

Though the growth and continuance of the corpus luteum may be furthered to some extent by foetal stimuli, it is unlikely that these play any considerable part in the early stages, and at no time directly upon the corpus luteum. Zondek and Aschheim (1928a) have demonstrated the presence of large quantities of pituitary hormone in the urine of pregnant women. Its production follows "almost like an explosion on the implantation of the ovum.....As early as the first month of pregnancy 'l'ormone ipofisaris si mobilizza' and rapidly reaches a concentration of 3-5000 units per litre of urine; it is maintained at this level until towards the end of pregnancy and disappears from the urine about the eighth day of puerperium" (Odescalchi, (1928)). The presence of pituitary hormone in urine was used by Zondek and Aschheim/

Aschheim in the diagnosis of pregnancy, in that it is almost specific for this condition. The presence of ovarian hormone could not be used for diagnostic purposes; it is not present in appreciable amount (300-600 units) during the first month and though it reaches at the fifth month a concentration of 5000-7000 units per litre and is maintained at this high level until term, it is frequently associated with conditions other than pregnancy, e.g. carcinoma, benign tumours, cystitis, amenorrhoea and the climacteric (Zondek (1928a), Odescalchi (1928)).

Experiments performed by Wolfe (1931) in which sows' pituitaries were implanted into rabbits, indicate that there are cyclic variations in the hormone content of the pituitary. The anterior lobes of the pregnant cow and sow, according to Zondek (1931) contain the same amount of hormone as those of the non-pregnant animals. This author also states that the pituitary of the pregnant woman contains only a small amount of hormone or none at all, but this does not necessarily indicate an absence of activity on the part of this gland.

An experiment performed by Haterius and Nelson (1929) supplies evidence of a high degree of activity on the part of the pituitary during pregnancy.

Ovaries/

Ovaries from virgin rats were implanted into pregnant females between the 9th and 13th days of gestation and the host's ovaries were removed four or five days later. Pregnancy was uninterrupted and normal young were born at term; histological examination of the ovarian grafts invariably showed the presence of lutein tissue. The immature ovaries were able to undergo adaptation and to develop masses of luteal tissue, so as to serve as functional substitutes for the original ovaries in the pregnant animal.

Mammary changes under the influence of the extract were not examined, but it seems to be fully established that the lutein phase of ovarian activity and mammary development are related and that the pituitary plays a part in this development (Ancel and Bouin (1911), Stricker and Grueter (1928), Corner (1930)). The presence of foetal or placental elements is not essential to mammary growth, as in those animals in which pseudopregnancy is marked e.g. the rabbit, ferret and dog, considerable growth occurs and even milk secretion during the pseudopregnant period (Parkes (1929a)). This activity is not, however, equivalent to full pregnancy development, nor can this be induced in unmated animals by means of corpus luteum extracts (Corner (1930)). For this reason/

reason it would appear likely that the placenta is largely responsible for the attainment of the maximum pregnancy development of the mammary glands. The influence of the posterior pituitary upon milk secretion was observed as early as 1913 (Hammond); that of the anterior pituitary has now been clearly demonstrated by Corner (1930). In experiments upon virgin rabbits, from which the ovaries and uterus had been removed, he induced abundant secretion of milk and full mammary development by means of an alkaline extract of the pituitary, showing that the effect of pituitary secretion may be a direct one.

From the data given therefore, it would appear highly probable that throughout pregnancy the anterior lobe of the pituitary maintains a high level of activity. Certain difficulties are presented by this conclusion: the part played by alpha during pregnancy, which is associated with the dominance of the corpus luteum; the existence of follicular activity in the presence of a high degree of luteinisation; and the mechanism responsible for maintaining pituitary secretion at this high level.

(1) The secretion of alpha in large quantities during pregnancy in the human being, is indicated by its presence in increasing quantities in the urine, and/

and in the placenta. Its secretion in the mouse and rat is surmised on the following grounds: its presence in mouse placenta (Mirskaia (1930)), and its outward manifestation in modifications of the cell content of the vagina associated with follicular activity in the ovary (Swezy and Evans (loc. cit)). It is an established fact, however, that alpha alone is incapable of either initiating or maintaining the pregnant condition.

(2) The corpus luteum hormone does not prevent the formation of follicles; it may inhibit their full maturation. Follicles form throughout pregnancy and reach a considerable degree of development (Plate II). It is unlikely that the high level of pituitary activity associated with the luteal phase could be maintained; the cyclic nature of its activity, indirectly reflected in the vaginal mucosa, is not wholly subordinated even during pregnancy. Were luteinisation to take place in the absence of follicular formation in the human, it is probable that the ova would suffer; the ovary itself would undergo atresia. It is possible that this is the factor underlying some forms of amenorrhoea. While the complaint in such cases would be attributable to hyperactivity of the pituitary, in many instances amenorrhoea is due to undersecretion of that organ: The level of activity remains of such a type as is associated/

associated with follicular formation in the ovary, and luteinisation does not occur. As the degenerative changes which occur at parturition are associated with the breakdown of the corpus luteum, so also, are those of menstruation, the latter being virtually a "pseudo-birth" (Wiesner (1929)). Amenorrhoea, therefore, may be due either to the persistence of the corpus luteum or to its failure to develop, i.e. to hyperactivity or to hypo-activity of the pituitary.

(3) It is to be assumed that some mechanism exists for determining the level of pituitary activity. From the fact that hysterectomy prolongs the life of the corpus luteum for a considerable period, (Loeb (1923a) and Asdell (1928)), it would appear likely that the uterus, either directly or indirectly, exerts some controlling influence on pituitary secretion. That maturity can be artificially induced in the immature animal but not maintained, may be due not merely to a lack of somatic development but to such an inhibitory factor as we have assumed.

A Consideration of the Existence of one or of two Gonadotropic Hormones in the Pituitary.

There would appear to be four degrees or forms of activity associated with pituitary secretion as exhibited in changes in the ovary: follicular formation without maturation, alpha secretion, ovulation/

ovulation and corpus luteum formation. Follicular maturation may occur without the changes in the reproductive system which are associated with oestrus; oestrus may occur without ovulation. The existence in the pituitary of two factors has been formulated in order to explain the different reactions on the part of the ovary, viz. follicular formation and corpus luteum formation. Extracts of anterior lobe, human placenta and pregnancy urine have been prepared which induce both follicular maturation and corpus luteum formation. The same preparation usually has the first effect when given in dilute amounts and the second when given in more concentrated form; moreover it appears that the first effect cannot be held indefinitely without the second supervening (Wiesner and Marshall (1931)). It has been assumed that two pituitary factors (Rho factors) are present in these extracts "in varying proportions and that both their relative concentration and their absolute quantity determine the effect" (Wiesner (1931)). Reference has already been made to the literature bearing on these results, and to the fact that frequently a combined effect is obtained in one animal, viz. follicles, corpora haemorrhagica and corpora lutea; in such cases one has to assume that the different follicles/

follicles received different substances or that the excitability of the different parts of the ovary varies.

Apparently it has not been possible to separate the two factors, though until recently it was believed that the kyogenic (i.e. the luteinising) hormone had been isolated in Evans' alkaline extract of the pituitary. While it would appear that initial treatment of the pituitary with alkali causes more efficient extraction with the saline, so that the active substance is present in greater concentration, Courrier and Kehl's experiments indicate that the kyogenic reaction is not obtained in all concentrations. (1929a) Recently P.E. Claus (1931) has claimed to have separated the two factors in human pregnancy urine. Acetic acid, ether and acid-alcohol were employed in the extraction, and crystals were formed which, dissolved in the equivalent of 1 cc.: 126 of urine, produced precocious sexual maturity in mice. It is perhaps inadvisable to make comparisons between the amount of urine and the effect secured as the concentration of the active substance varies so greatly in the materials used. It is interesting to note that the equivalent of 120 cc. of urine was the minimum required to produce the same effect in Wiesner's experiments (1931) and as much as 200 cc. in our own experiments failed to cause/

cause luteinisation in the rat. It is possible, therefore, that had Miss Claus concentrated her material still further, luteinisation would have occurred. In the preparation above described, a substance which was insoluble in alcohol was found to inhibit oestrus and ovulation, and to cause luteinisation. It would seem advisable for further tests to be made with these two preparations before the isolation of two factors can be claimed.

Whether it is assumed that the effect produced on the reproductive system is determined by one factor or by two, the existence of some mechanism which sets free at intervals a larger or smaller amount of hormone, seems to be implied. Whether such a factor exists in the pituitary itself, or is supplied by some other endocrine organ remains to be ascertained, as also the mechanism which governs its periodic activity.

With regard to the different response which animals make to the same extract or treatment, it is necessary to consider two circumstances:

(1) No two animals are at the same stage of ovarian activity at one time; only to a strong stimulus is the response likely to be uniform; to a relatively weak stimulus some may respond with follicular formation, some with luteinisation or atresia, while others may make no response at all. The last-named

is likely to occur in those animals in which the oestrous threshold is normally low, i.e. where the full oestrous signs are rarely or never present and cornification of the vagina is incomplete.

(2) Grounds exist for supposing that the reproductive system does not remain at the same level of sensitivity continuously. This variation in sensitivity is shown by the inability to extend unduly any phase of ovarian or uterine activity. Instances have already been given: artificially extended follicular activity passing into atresia (Wiesner (1931)), and the inability to extend for long, the functional period of the corpus luteum of pregnancy, pseudopregnancy or lactation (Courrier and Kehl), and Mirskaia).

Parturition and the part played by the Posterior Pituitary in Normal Pregnancy.

It has long been held (Tyler Smith (1849), Minot (1889) and Beard (1897) that there is a connection between parturition and menstruation, and that the two processes are homologous. According to Minot, "the menstrual and gravidal changes follow the same cycle of events, the pregnant uterus passing through a prolonged and intensified menstrual cycle". He thought it probable that there was a common cause for the termination of both. As in the rat and mouse/

mouse similar conditions are presented at the end of pseudopregnancy to those at menstruation and parturition, a careful study of this stage in the ovarian cycle of these animals might throw some light on both of these phenomena. It is exceedingly difficult to secure specimens of uteri at the stage when haemorrhage takes place, and it is likely that this is of very short duration. From the three cases which were examined, it would appear that the blood collects in the uterus, and, either owing to pressure, or to stimulation of some nervous mechanism, possibly situated near the cervix, is expelled, presumably as the result of a series of uterine contractions. The uterus in every case presented on the outside the lumpy appearance denoting early pregnancy, and it was at first thought that this condition actually existed. On sectioning, it was found that blood and decidual cells had collected in pockets, and it was this appearance which led to the surmise that a certain degree of pressure was being exerted. The uterus was distended and histological examination showed a development characteristic of pro-oestrus (see plate and text); the lutein tissue of the ovary was degenerated, and vascularisation was marked; in one case ovulation had occurred and in another was imminent. There seems little doubt that the phenomena associated/

associated with the dehiscence of decidual tissue and accompanying haemorrhage, were in some way attributable to the cessation of the functional activity of the corpus luteum, and that the inhibition which had been placed upon the secretion of the posterior pituitary or upon the effects of that secretion was withdrawn.

Extracts of the corpus luteum have in some cases given increased contraction of the uterine muscle, in others relaxation (Guggisberg (1913)). Sharpey Schafer (1917) states that the ovaries appear to produce two hormones, one inhibiting the contractility of plain muscle and the other increasing it.

Experiments carried out by Knaus (1930) and recently confirmed by Robson and Illingworth (1931) throw more light on this problem. It has been shown that the *in vitro* uterus of the non-pregnant rabbit which normally contracts on injection with pituitrin, does not contract during the dominance of corpus luteum secretion viz. during pseudopregnancy and the first 16 days of pregnancy (Knaus (1930)). Extracts of corpus luteum which caused progestational proliferation in the rabbit's uterus also inhibited the *in vitro* reaction to pituitrin, the action being, therefore, similar to that occurring during pregnancy (Knaus, and Robson and Illingworth). Experimental evidence/

evidence indicates that the proliferative and inhibitory actions of the corpus luteum extract may be due to different factors (Robson and Illingworth (1931)).

According to Bourne and Burn's experiments (1928) on the uterus of the guinea-pig, injections of alpha have a sensitizing effect upon the uterus to pituitrin (in vitro); Parkes (1930) working on the pregnant mouse (in vivo) noted a similar sensitizing effect, provided repeated injections were given. This is not in accordance with the findings of Robson and Illingworth (1931 - rabbit) and Siebert (1931) guinea pig), and it cannot be adduced that the secretion of alpha plays a part in the normal mechanism of parturition. It appears highly probable, however, that the reaction of the uterus to pituitrin is held in check throughout pregnancy by an inhibitory factor present in the corpus luteum. As extracts having a similar inhibitory effect upon the reaction of the uterus to pituitrin were prepared from human placenta (Robson and Illingworth (loc. cit), it cannot be said that the corpus luteum plays the sole part in this important function.

SUMMARY.

Experiments have been performed which examine some of the effects upon the pregnant animal of injections of an extract of human pregnancy urine, and of an alkaline extract of anterior pituitary, also of the implantation of anterior pituitary tissue. Pregnancy was interrupted in some cases, and in others prolonged.

Additional data were collected regarding the effects of a corpus luteum extract upon foetal development, and of partial and entire removal of the ovaries during pregnancy. The extract was without appreciable effect up to the 15th day of pregnancy. It was confirmed that ovarian secretion is essential to pregnancy, and that its partial removal in the early stages may cause pregnancy to be interrupted. A study was made of the growth of the mother and foetus during pregnancy, haemorrhage, and the cell content of the vagina under the influence of the various treatments administered.

Factors concerned in the interruption and prolongation of pregnancy have been examined. It is highly improbable that an interruption of pregnancy is due to an "oestrogenic effect". In the animals in which pregnancy was interrupted, the ovaries were found to/

to be full of corpora lutea; the possibilities of an interference with an extra-ovarian factor concerned in the pregnancy mechanism are discussed. The prolongation of pregnancy appears to be due to a continuation of the activity of the corpus luteum and to an inhibition thereby placed upon factors responsible for parturition.

Evidence in favour of the existence of Rho factors (pituitary) is examined, and it is concluded that the data, so far obtained, do not warrant making the conclusion that there are either one or two factors.

ACKNOWLEDGMENTS.

The suggestion to test the effects upon pregnancy of the urine extract emanated from Dr. B.P. Wiesner, to whom my gratitude is due. I also wish to express my deep indebtedness to Dr. J.M. Robson for his help and patient criticism, and to Professor Crew for his academic hospitality.

PLATES

PLATES.

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- B. Rat 453, ovary, 15th day of pregnancy, injected corpus luteum extract - x5 87.
- C. Rat 815, ovary pseudopregnant rat, injected Urine extract - x5 115.
- D. Rat 803/2, ovary 15th day of pregnancy, injected Urine extract - resorption - x5 41.

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III. Rat 1812, uterus, pseudopregnant haemorrhage, injected alkaline pituitary - x25 126.

IV. Rat 1812, ovary - x15 126.

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V. Rat 803/2, vagina (See Plate ID) - x70 41.

VI. Rat 803/2, ovary (See Plate ID) - x14 41.

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VIII. Rat 1816, uterus, pseudopregnant bleeding, uninjected - x70 127.

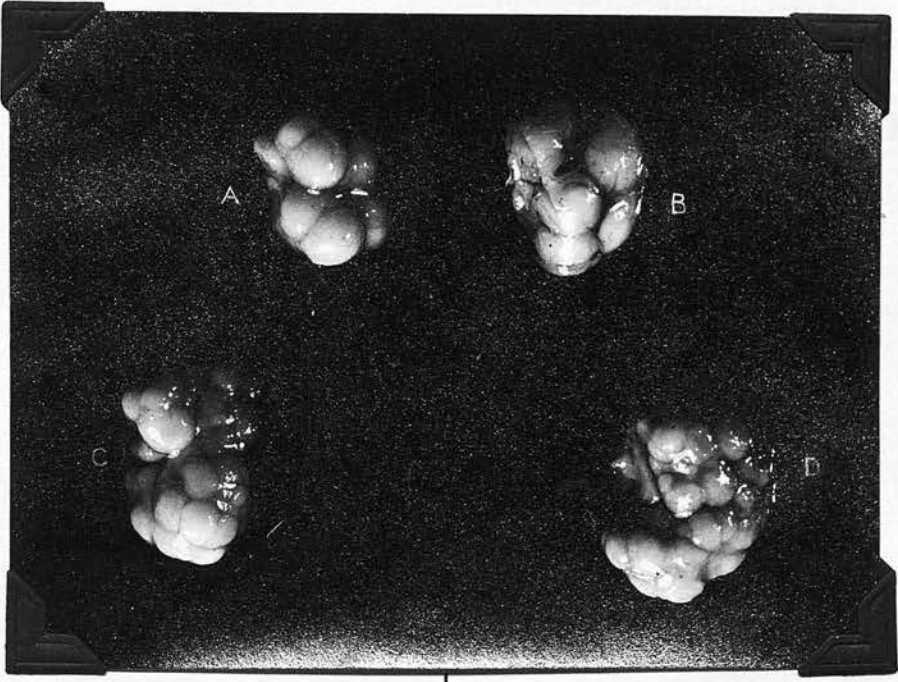
IX. Rat C4, placenta adherent to uterine wall; injected alkaline pituitary; removed 26th day of pregnancy - x70 63.

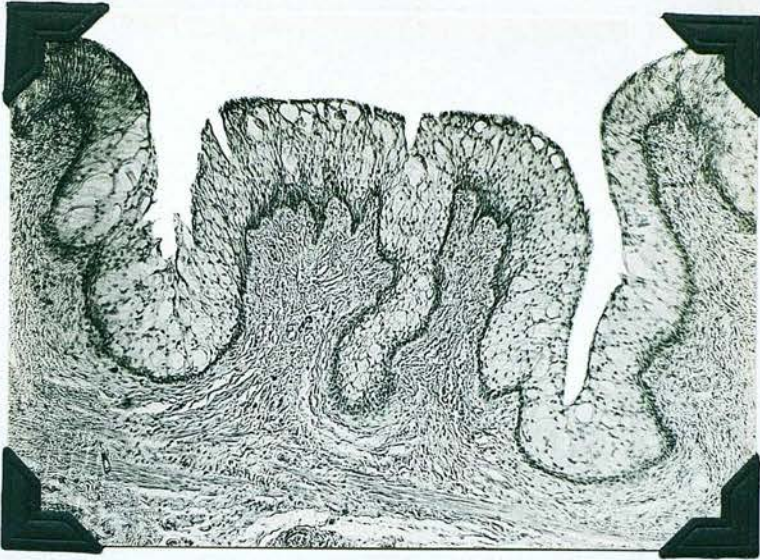
X. Placenta - normal and overgrown) - life size)

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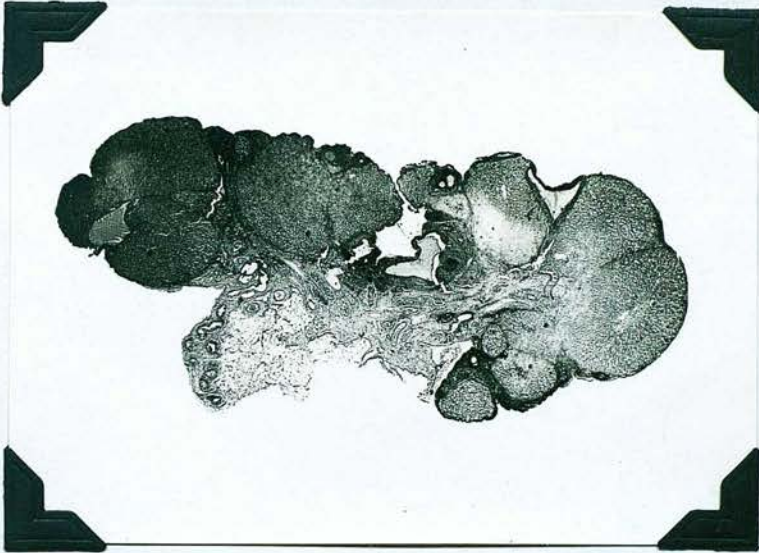
XI. Rat K7 - ovary removed 27th day of pregnancy - injected urine extract - x11 54.

XII. Rat 814/3 - ovary 15th day of pregnancy - injected urine extract - x14 43.

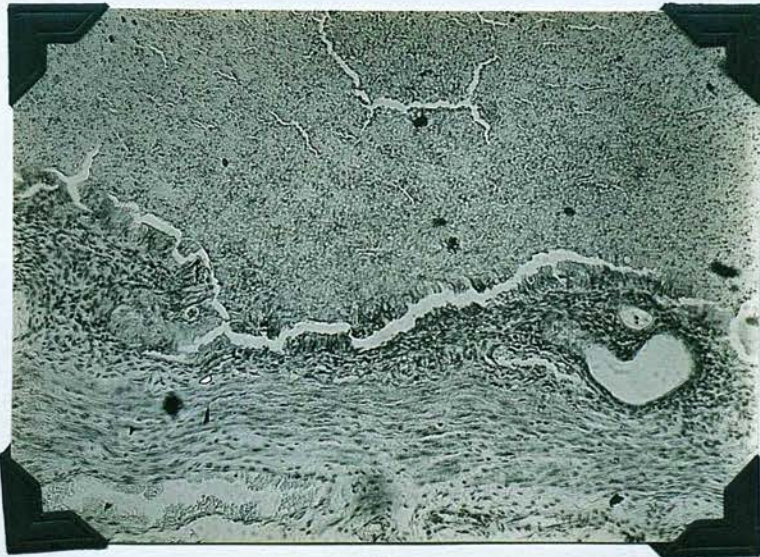




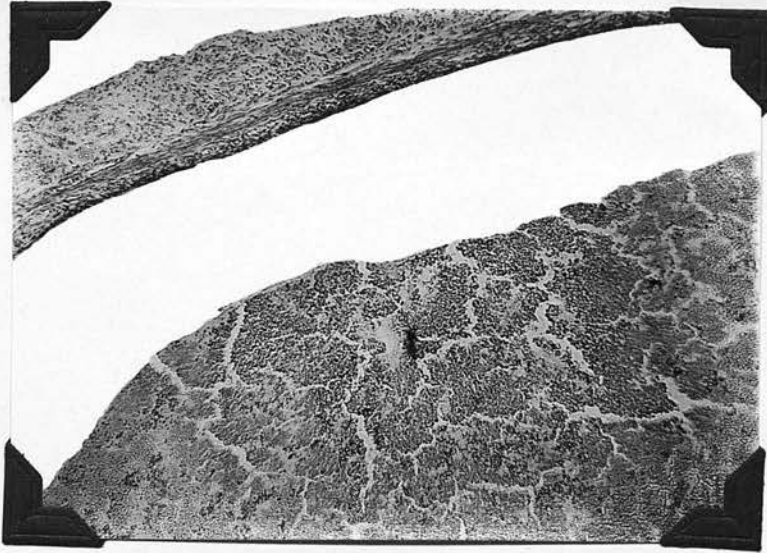
5



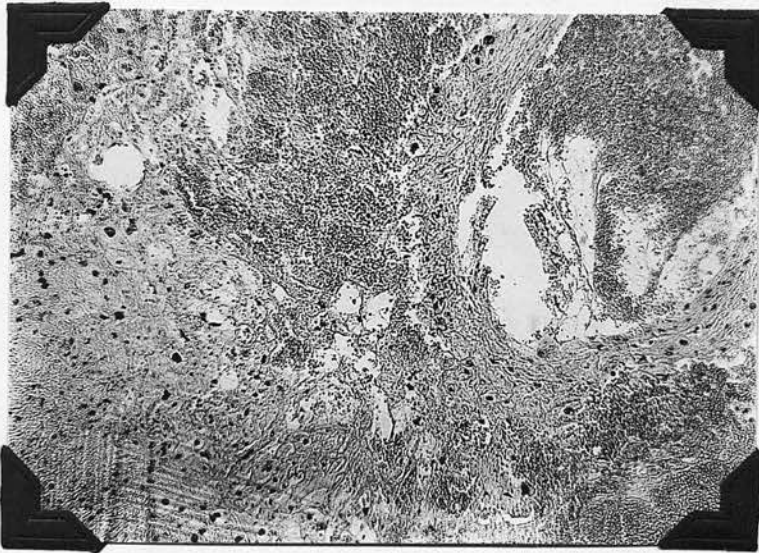
6



7



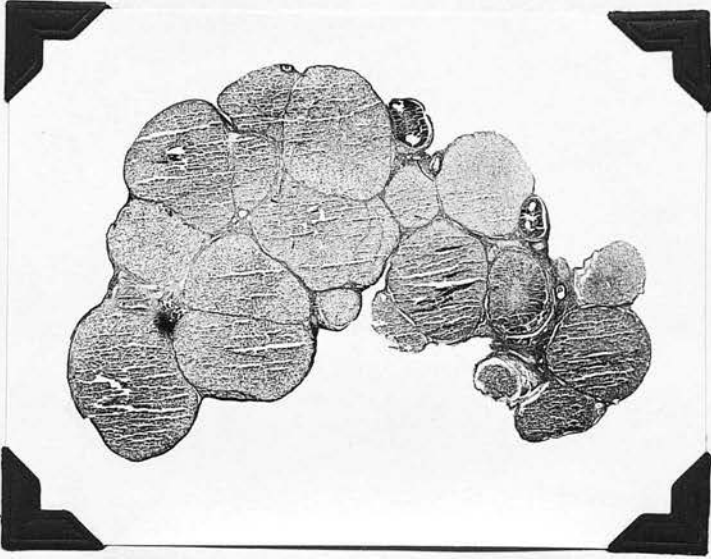
8



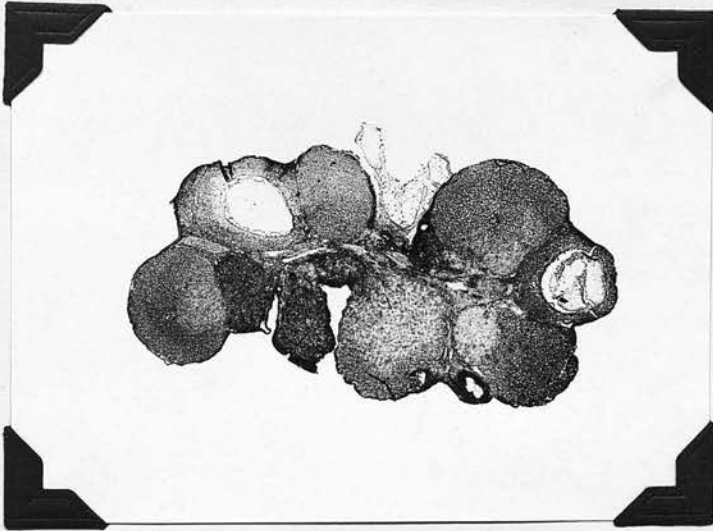
9



10



11



12

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