NORMAL AND INDUCED DELAYED PARTURITION IN SWINE

by

RICHARD WILLIAM DANIELS

AN ABSTRACT

Submitted to the College of Agriculture, Michigan State
University of Agriculture and Applied Science
in partial fulfillment of the requirements
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Approved by John E Nellor

ABSTRACT

Parturition was delayed in swine by the oral administration of 6-methyl-17-acetoxyprogesterone (MAP). Dosages of 0.7-1.5 mg. MAP/lb. B.W. daily were utilized. The dosage level of 1.0 mg. MAP/lb B.W. daily was found to be the lowest sufficient level to maintain uterine quiesence and hence, delay parturition. The dosage of 1.0 mg. MAP/lb. B.W. daily was administered alone or in combination with levels of 0.0186-0.056 mg. of Diethylstilbestrol (DES)/lb. B.W. daily. The gilts utilized in these studies were subjected to manual or mechanical mammary evacuation during prolonged gestation subsequent to milk let-down. The day of milk let-down (i.e., the copious, spontaneous let-down of milk into the mammary ducts and cisterns) was considered to be the physiological due date. The characteristics of labor following withdrawl of MAP treatment were loss of placental integrity and an abnormally long parturitional sequence. The administration of MAP and DES concommitantly resulted in the maintenance of placental integrity but failed to shorten the parturitional sequence to a normal duration.

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Approved by John E. Miller

to the memory of

John Raymond Leeds

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TABLE OF CONTENTS

LITERATURE REVIEW

The hormones controlling gestation and its termination are of major importance to a study of the physiology of pregnancy. There appears little doubt that the physiological roles of progesterone, estrogen and oxytocin are of paramount importance.

Corner and Allen (1929), working with rabbits, found progesterone to be the stabilizer of pregnancy, and to cause the uterus to remain quiescent and tolerate the fetal parasite. Following ovariectomy, pregnancy was noted to be terminated. It was further observed that extracts of luteal tissue maintained pregnancy. The creation of a quiescent state of the uterus by progesterone has been substantaited by further studies (Nelson et al., 1930; Robson, 1936; Allen and Heckel, 1939; Csapo, 1956c; Kuriyama and Csapo, 1959; Marshall and Csapo, 1959; Csapo and Lloyd-Jacob, 1961). An increase in the myometrial resting membrane potential and minimal excitability threshold have been postulated as the mode of action by which progesterone promotes uterine quiescence in rabbits (Csapo, 1956c; Goto and Csapo, 1959) and women (Barnes and Kumar, 1961; Kumar and Barnes, 1961).

Estrogen, in contrast, increased the amount of contractile protein and the spontaneous contractibility of the uterus of rabbits (Reynolds, 1935; Csapo, 1948; Csapo, 1950; Csapo and Corner, 1953; Schoefield, 1962) guinea pigs, (Bozler 1938), rats, (Marshall 1959) and women (Cretius, 1957; Hirano, 1961). The contractility of the uterus due to estrogen stimulation is enhanced and coordinated by oxytocin in the rabbit (Csapo, 1954; Cross, 1958 a and b; Kuriyama and Csapo, 1959; Marshall and Csapo, 1959) cattle (Fitzpatrick and Walmsley, 1962), swine (Staub and Johannes, 1959), women (Caldeyro-Barcia, 1959; Jung, 1961), and in the rat and cat (Jung, 1961).

Estrogen has been reported to decrease membrane potential, and the excite-ability threshold, and to increase spontaneous activity of the myometrium in rabbits (Coutinho and Csapo, 1959; Goto and Csapo, 1959), rats (Marshall, 1959) and women (Hirano, 1961). Oxytocin has been reported to enhance the estrogenic effects by decreasing further the uterine myometrial threshold of excitation of the rabbit (Csapo, 1954; Csapo, 1956b), rat (Fielitz et al., 1960; Jung, 1961) and of the cat and human (Jung, 1961).

In order to elucidate the hormonal actions reported above, studies have been designed to demonstrate changes in ionic concentration as the biochemical result of hormone action. A cellular shift of calcium ions has been reported as being significant in muscle contraction (Niedergerke, 1956; Bianchi and Shanes, 1959). Experiments conducted on rabbit uterine tissue suggest that calcium is labile when the uterus is estrogen dominated and strongly bound under progesterone domination (Csapo, 1956c: Coutinho and Csapo, 1959; Goto and Csapo, 1959). In addition it has been proposed that changes of the ionic profile of sodium and potassium are involved in the actions of the hormones. Increased intracellular potassium or extra-cellular sodium have been reported to increase the resting membrane potential and the reverse of either to cause increased myometrial activity (Csapo, 1956 a and c; Coutinho and Csapo, 1959; Barnes and Kumar, 1961; Kumar and Barnes, 1961). Atpresent the ion or ions involved in the actions of the hormones are not known and further work is necessary before actual mechanisms are elucidated.

FACTORS AFFECTING THE CORPUS LUTEUM

The lifespan of the corpus luteum appears to vary according to the species of animal studied (Zuckerman, 1962) but the effects of hormonal factors influencing this gland appear to be relatively consistant.

It was first reported by Selye et al. (1935) that estrogen administration prolonged the diestrous phase of the estrous cycle in rats. He concluded that estrogen had a luteal maintaining ability. The ability of estrogen to maintain functional corpora lutea was further confirmed in the rat (Merckel and Nelson, 1940) and in the rabbit (Allen and Heckel, 1936; Robson, 1937; Heckel and Allen, 1939; Robson, 1939; Robson, 1940; Greep, 1941; Hammond, 1952; Hammond, 1956). In swine luteal maintenance effect was reported by Kidder et al. (1955). He theorized that corpora lutea had been maintained by luteinizing hormone release evoked by the estrogen administration. Gardner et al. (1963) and Rigor et al. (1963) found that the corpora lutea of estrogen treated swine decreased in size but that their concentration of progesterone increased. It was concluded that corpora lutea function had been maintained with estrogen treatment.

In cattle contradictory results have been reported. Hammond and Day (1944) implanted cycling heifers with diethylstilbestrol and reported that corpora luteal life had been prolonged. Greenstein et al., (1958) reported that estrogen caused early regression of corpora lutea in the dairy heifer and Loy et al., (1960) stated that estrogen does not prolong luteal life in the heifer. In addition, it was hypothesized that the maintenance of luteal tissue in cattle is dependent upon an optimal ratio ofprogesterone and estrogen.

Although estrogen is capable of maintaining luteal tissue by a direct action upon the ovary (Greep, 1941), it has not been resolved whether corpus luteum maintenance is a normal physiological action of estrogen during gestation. There is very little evidence of a luteal maintenance function for naturally secreted estrogen. Evidence which appears to obsolve this possibility seems quite strong. First, estrogen administration at levels reported to prolong luteal life, result in fetal death;

second, post parturient estrus indicates high levels of estrogen to be present in late pregnancy; and third, estrogen has not been located in the placenta of rodents, a structure which when removed hastens corpus luteum degeneration (Greep, 1941; Zuckerman, 1962).

Before ruling out the possible role of estrogen upon corpus luteum maintenance, the effects of hysterectomy must be considered. Persistence of corpora lutea for the length of normal gestation has been reported following hysterectomy in the rat (Murphy, 1934; Bradbury, 1937; Hechter et al., 1940; Malvern and Hansel, 1962) and guinea pig (Loeb, 1923; Loeb, 1927; Desclin, 1932; Rowlands and Short, 1959). Hysterectomy of the rabbit results in the maintenance of luteal tissue approximately 26 days post ovulation which is 5 days less than the period of gestation but 10 days longer than normal pseudopregnancy (Asdell and Hammond, 1933; Siegmund, 1934; Loeb and Smith, 1936; Micale, 1940; Chu et al., 1946). Hysterectomy of the cycling heifer extended corpora luteal life to 154 days while the same procedure in ewes resulted in luteal maintenance for 100 days (Wiltbank and Casida, 1956). Reports upon the effects of hysterectomy in cycling and pregnant gilts have shown corpora lutea to persist for periods of normal gestation and in some experiments 5 days beyond (duMesmil et al., 1959; Spies et al., 1960; Duncan et al., 1961). On the other hand, hysterectomy has been reported to have no effect upon luteal maintenance in the opossum (Hartman, 1925), monkey (Burford and Biddle, 1936), and ferret (Deanesly and Parkes, 1933). The significance of estrogenic maintenance of luteal tissue is important in that the ability of the uterus to convert estrone to estriol was proposed by Pincus (1937) and further that where estrone was not metabolized to estriol it was reported to cause luteal persistence (Bradbury, 1937; Hechter et al., 1940; Heckel, 1942).

It has been reported that progesterone administered to cycling animals causes the regression of corpora lutea in pregnant swine (Sammelwitz and Nalbandov, 1958; Spies et al., 1958; Alfred et al., 1959; Nellor, 1963), cattle (Loy et al., 1960) and the guinea pig (Alfred et al., 1959). Studies in rats (Alfred et al., 1959; Rothchild, 1960) suggested that progesterone did not affect the ovaries. Samelwitz and Nalbandov (1958) reported that although progesterone had a luteolytic effect on formed corpora lutea, no effect was seen on corpora lutea during their formative period. Considering the naturally short luteal life of the rat, it is possible that a luteolytic action of administered progesterone might not be apparent in this species.

There is conflicting evidence on the influence of oxytocin administration upon the corpus luteum. Oxytocin was reported to decrease corpus luteum progesterone content in cattle (Armstrong and Hansel, 1959; Hansel and Wagner, 1960; Staples and Hansel, 1961; Staples et al., 1961). In contrast, Mares and Casida (1963) reported that oxytocin treatment significantly increased corpus luteum progesterone content in cattle when studied <u>in vivo</u> and decreased progesterone in <u>in vitro</u> experiments. In their experiments the ovaries were removed within a 24 hour period following systemic oxytocin treatment. If oxytocin has a luteolytic effect as reported, destruction of luteal tissue could cause an immediate increase in progesterone content due to destruction of tissues which might normally have bound progesterone. It is possible that had the ovaries been removed at a later time, a decrease in size and progesterone content would have resulted. As to the mode of action of oxytocin upon the corpus luteum, it appears to be an indirect effect mediated through the anterior pituitary gonadotrophin release rather than a direct effect upon the ovary itself.

Experiments performed with rats (Evans et al., 1941 a and b; Desclin, 1949; Everett, 1954; Everett, 1956; Meites and Shelesmyak, 1957; Nikitovitch-Winer and Everett, 1957; Nikitovitch-Winer and Everett, 1958) and mice (Browning et al., 1962) demonstrated that luteo-trophic hormone (LTH) was capable of maintaining corpora lutea for periods longer than their normal life span. However, Duncan (1961) reported luteal tissue of swine in vitro to be unaffected by LTH administration.

Autotransplantation of the pars distalis from its normal relationship with the hypothalamus to the left kidney in rats resulted in cessation of follicular growth. The transplant was further observed to maintain corpora lutea for periods 4-5 times the length of gestation (Desclin, 1953; Everett, 1954; Everett, 1956; Nikitovitch-Winer and Everett, 1957; Nikitovitch-Winer and Everett, 1958). This suggested the secretion of a neural or neuro-humoral agent from the hypothalamus which inhibits the release of luteo-trophin by the pars distalis (Everett, 1954; Everett, 1956). It was reported that the corpora lutea of oxytocin treated heifers failed to reach normal size and the size and number of luteal cells were reduced concommitantly with an increase in connective tissue (Armstrong and Hansel, 1959); these observations corroborated the previous findings. Oxytocin was suggested by these workers as the neurohumoral agent responsible for inhibition of luteotrophin release.

The removal of the pituitary gland was reported to have varying effects upon gestation length and on corpus luteum survival. Termination of pregnancy concommitant with corpus luteum regression invariably has been found to follow hypophysectomy in the rabbit (Deanesly et al. 1930; Smith and White, 1931; Firor, 1933; Robson, 1937) and in the dog (Aschner, 1912; Houssay, 1935). Although corpora lutea have been

reported to regress, extirpation of the pituitary gland during the second half of pregnancy did not result in abortion in the guinea pig (Pencharz and Lyons, 1934; Nelson, 1935) cat (Allan and Wiles, 1932; McPhail, 1935) and mouse (Selye et al., 1933; Newton and Beek, 1939). Although Gardner and Allen (1942) were in agreement with the findings that termination of pregnancy did not occur in the mouse due to hypophysectomy, they reported that luteal tissue appeared similar to that found in the intact animal. Hypophysectomy during the second half of pregnancy did not result in abortion in the monkey (Smith, 1954) and human (Little et al., 1958). Removal of the pituitary gland during the second half of pregnancy did not cause termination of pregnancy in the rat (Pencharz and Long, 1931; Pencharz and Long, 1933). Luteal tissue has been reported to persist for as long as 14.5 months following hypophysectomy in this species (Smith, 1930). This suggested an extra hypophyseal source of luteotrophic hormone to be present.

EXPERIMENTAL PROLONGATION OF PREGNANCY

Gestation was first experimentally prolonged by Teel (1926) with bovine pituitary extracts. The extract was injected daily into rats beginning day 1 of gestation. Pregnancy was found to be prolonged 2-6 days by this procedure and all fetuses were stillborn. In this study it was noticed that the fetuses had attained a size greater than that found in normal gestation. Fetuses were alive at the time of normal parturition. Corpora lutea persisted for abnormally long periods with this method of treatment. The conclusion as to the cause of fetal mortality was that delayed implantation had occurred, although it was recognized that the persistence of corpora lutea might possibly be involved in the failure of the birth mechanism.

Nelson, et al., (1930) prolonged pregnancy in rats with extract meticulously collected from the ovaries of sows. He noted that a delay of partuition for over 70 hours resulted in the death of fetuses or of the mother. A delay of more than 60 hours resulted in an illness of the pregnant female. It was concluded that there was a limited time in which fetuses might remain viable in utero past normal term. Fetal size was again found to be greater than that during normal gestation. It was also stated that there was no relationship between the number of fetuses and the length of time parturition was delayed. No impairment of mammary gland development was noted due to treatment.

Extracts of human pregnancy urine were utilized by Snyder (1934) to prolong gestation in the rabbit. Parturition was delayed an average of 15 days following injection. This coincides with the length of pseudopregnancy in the rabbit. It was hypothesized that the treatment had caused ovulation and formation of fresh corpora lutea which maintained pregnancy for their normal life span. Pituitrin was ineffective in inducing parturition in treated animals when doses 1000 times greater than normally effective were utilized. Fetuses were found to be viable until day 35 of pregnancy. The placenta appeared healthy and functional on day 41 of pregnancy. All fetuses were dead by day 41 but the placental attachment appeared excellent. Abortions which took place occurred 2-3 days post injection. It was concluded from this work that the termination of pregnancy appeared to coincide with the termination of corpora luteal life.

Hoopes (1934) also utilized pregnancy urine extract to prolong pregnancy in rats. Injections were administered 19 days post coitus. Parturition was successfully delayed in 16 of 19 treated animals. All fetuses were dead by day 26 of gestation but a graded death was noted beginning

23 days post coitus. Laparotomy performed on day 40 of gestation showed a striking distension of the uterus by fetuses.

In a preliminary experiment, Koff and Davis (1937) prolonged pregnancy in the rabbit with progesterone. Labor was prolonged and difficult; this was attributed to increased fetal size. In a subsequent experiment 5 mg. of progesterone were injected daily beginning day 28 of gestation. When treatment was terminated, a delay of 24-48 hours occurred prior to labor. It was concluded that a change in the uteroplacental environment, rather than in the fetus itself, was the cause of fetal mortality and that the uterine environment was not created for continuing the life of the fetus far beyond normal term. Furthermore, it was stated that when progesterone or gonadotrophin were utilized to prolong pregnancy, a delay of parturition to less than 36 days from conception resulted in fetal viability whereas any delay greater than 36 days resulted in fetal death.

The first use of estrogen to prolong luteal life and thereby prolong gestation in rabbits was reported by Heckel and Allen (1939). A minimal dose of 175 I.U. of estrogen daily was necessary to delay parturition. A dosage of greater than 500 I.U. daily resulted in one normal litter on day 33 of gestation, but in all other cases the fetuses were dead. In all cases in which parturition was significantly delayed by estrogen treatment, all fetuses were found to be dead. It was concluded from these experiments that estrogen given one day prior to parturition would maintain functional corpora lutea and that if the high estrogen levels of pregnancy were to decline prior to parturition the corpora lutea could decline, allowing birth to occur.

An extensive study on the effects of progesterone, estrogen and hypophysectomy on prolonged pregnancy was reported by Robson (1940). In

pregnant hypophysectomized rabbits, pregnancy was prolonged with progesterone or with different estrogenic substances. Progesterone administration had a delaying effect upon parturition, but by day 35 of gestation, fetuses were dead and macerated. Furthermore, it was found that death had occurred between day 33-35 of pregnancy. When progesterone was administered corpus luteum degeneration was noted. Estrone, estradiol and estradiol benzoate were all capable of delaying parturition. Estradiol benzoate was found to be the most effective. In all cases, however, death of fetuses following estrogen injection was quite rapid. The estrogenic substances were reported to maintain corpora lutea. Larger amounts of estrogen were necessary to prolong pregnancy in the intact than in the hypophysectomized female. The explanation of this finding was that hypophysectomy removed oxytocin and thereby diminished the factors stimulating the uterus to contract. It was also suggested that although the corpus luteum is maintained by estrogen, the ultimate control is by the anterior pituitary.

The ability of prolactin (LTH) to prolong pregnancy in the rat was studies by Meites and Shelesmyak (1957). It was reported that 4 mg. of prolactine per day was the most suitable level for delaying parturition in the intact animal. When ovariectomy was performed, prolactin had no delaying effect on pregnancy. It was therefore advanced that prolactin prolongs luteal life and thereby gestation. The observation was made that at the time of normal parturition the mammary gland contained a milk like secretion, indicating that the treatment utilized did not interfere with the galactopoetic abilities of the gland. Furthermore, it was noted that if parturition was delayed more than two days, the fetuses were stillborn.

Progestin in the forms of an oil suspension of progesterone and an aqueous suspension of 6 methyl-17-hydroxyprogesterone acetate (6 MAP) was utilized to artificially prolong gestation in sheep (Bengtusson and Schoefield, 1963). The most successful treatment consisted of the daily intramuscular injection of 40 mg. 6 MAP. This dosage was able to prolong gestation in seven of eight attempts. In no case in which prologation of pregnancy occurred did delivery of live lambs result. In most cases when treatment with 6 MAP was removed a continuing inactivity of the uterine myometrium resulted and caesarean section was therefore performed. Surgery revealed a flacid uterus lacking contractile tone. The administration of estradiol and of stilbestrol was reported to increase myometrial tone but insufficiently to affect spontaneous delivery. A graded death of fetuses in cases of twins and triplets was noted but the actual cause of fetal mortality was left unresolved although the possibility of a progesterone toxicity effect upon fetuses was suggested. It was concluded that the normally secreted placental progestin could not be successfully replaced by a systemically administered progestin.

Progestationally induced delayed parturition was reported in swine and cattle (Nellor, 1963a). The levels of 6-methyl-17-acetoxyprogesterone (MAP) utilized (0.5-1 mg./lb. bodyweight daily, by the oral route), although not capable of supressing follicular growth for long durations in the cycling gilt, blocked parturition for up to 23 days following the "physiological due date." These levels did not increase fetal mortality or markedly incluence fetal welfare if administered throughout normal gestation and did not interfere with normal parturition if treatment was discontinued at the "physiological due date," that is on the day of the spontaneous copious letdown of milk. There was no attempt to estimate

the mammary secretory capacity during delayed parturition. Fetal death occurred during delayed parturition approximately 12 days from the "physiological due date" and was concommitant with increased follicular growth in the ovaries at this time. The apparent cause of fetal death was placental insufficiency, a phenomenon not involving all fetuses *in utero* at one time. It was apparent that the normal mechanism of parturition was abolished subsequent to discontinuation of progestational treatment during delayed parturition at any time after 2 days of parturition block. Increased uterine and marked abdominal contractions were noted approximately 18 hours following the end of treatment, without dilation of the cervix. Fetal death occurred at this time and the dead young were expelled from the uterus with complete cervical dilation 24 hours to 5 days later.

METHODS AND PROCEDURE

Progestin in the form of 6-methyl-17-acetoxyprogesterone (MAP) was utilized in this study to artificially induce delayed parturition in gilts. Diethylstilbestrol (DES) was the source of estrogen. The hormones were mixed with a standard swine ration so that four pounds of feed would contain the amounts of hormone required daily (table 1). The gilts were fed twice daily receiving two pounds of feed at each feeding.

Fifteen Hampshire, Yorkshire or crossbred gilts, averaging 380 pounds were utilized for this study. The average gestation length was accomplished either naturally or by artificial insemination and the expected due dates were calculated as 114 days following the breeding date.

Of primary concern in this study was the influence of continuous milking on fetal survival and upon the labor sequence which followed the artificially prolonged gestational period.

Initially, milking was accomplished by the use of a milking machine, similar to that of Hartman and Pond (1960), which was developed for use in this experiment. A complete description of the apparatus is reported in figures 1-4.*

Due to the inhibitory effects upon milk ejection of adrenal in which is released in response to stress (Cross, 1955) a period of conditioning of the gilts to the experimenter and to the milking apparatus was initiated two weeks prior to the expected due date. The conditioning process of gilts mechanically stimulated included an initial week of twice daily feeding in the milking parlor following which the teat cups were applied for short periods of time, not exceeding five minutes. At the end of a week, frequency of feedings was increased gradually to six times daily and the milker applied following each feeding. Gilts were induced to assume a lying position by manual mammary stimulation. Although the milking machine created intense mammary stimulation it failed to completely evacuate the udder. A further disadvantage of the milking machine occurred as a result of confinement of gilts on the concrete floors of the barn for 2 weeks prior to parturition. This confinement created considerable lameness which was further irritated by the climbing of the ramp into the milking parlor. For these reasons manual stimulation and partial evacuation of the udder was most satisfactory.

Conditioning to manual milking required approximately one week. During this period of time the experimenter manually stimulated the mammary glands and induced gilts to assume a lying position. This process

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*The milking machine was developed by Dr.E.R. Miller, Department of Animal Husbandry, M.S.U.

was accomplished approximately four times daily until milk let down occurred. Following milk let down, frequency of milking was increased to ten times daily. This procedure was continued until parturition ensued following the cessation of MAP administration.

One experimental gilt received two intramuscular injections each consisting of 30 mg. DES dissolved in sesame oil. The injections were given thirty hours and fourteen hours prior to expected time of parturition. The further administration of 80 I.U. of oxytocin injected intramuscularly every 45 minutes was initiated twelve hours after the second DES injection and continued for the duration of labor.

The estrogenic treatment was further modified in gilts 6-10 and 6-11 where the initial dosage of 0.028 mg. of DES daily was increased to 0.056 mg. daily, four and three days respectively from the day of milk let-down.

In all other gilts, treatment was discontinued 24 hours prior to the time desired for labor and a record of anamalies either prior to or during labor was kept. In addition, fetal weight, sex and condition at parturition were recorded.

In all cases in which a caesarean section was performed*, ovarian and uterine condition were noted concommitant to the fetal data above.

*Caesarean sections performed by Dr. A. Shaikh, D.V.M. Department of Animal Husbandry, Michigan State University.

Table 1. SUMMARY OF TREATMENT

Gilt No.	Gilt Wt. lbs.	Treatment[1] mg/lb. B. W. Daily MAP	DES	Duration Treatment[2] Pre Let-down MAP	DES	Duration Treatment[3] Post Let-down MAP	DES	Expected[4] Date Due	Physiological[5] Date Due
68-1	298	0.7	----	7	---	---	---	4-26	4-27
63-7	340	0.8	----	65	---	4	---	6-20	6-19
66-1	354	0.9	----	15	---	7	---	6-28	6-30
80-2	290	1.0	----	74	---	4	---	6-15	6-16
9-5	370	1.0	----	13	---	4	---	7-7	7-8
60-3	366	1.3	----	10	---	5	---	6-23	6-24
66-4	362	1.5	----	13	---	4	---	6-27	6-30
63-4	457	1.0	0.0266	16	7	4	4	7-30	8-2
10-1	390	1.0	0.0186	8	6	4	4	7-24	7-25
64-3	402	1.0	0.031	10	2	11	11	7-24	7-27
13-3	345	1.0	0.028	8	0	4	4	7-26	7-25
27-1	462	1.0	0.028	6	0	5	5	11-1	11-1
27-2	418	1.0	0.028	8	0	4	5	11-2	11-4
60-3	433	1.0	0.028	37	0	12	13	11-2	11-2
6-10	390	1.0	0.028	8	0	4	4	4-5	4-3
			0.056				+3		
6-11	370	1.0	0.028	8	0	15	3	4-8	4-4
			0.056				+12		
24-1	418	---	0.028	---	1	---	0	11-8	11-7

[1]Amount of 6-methyl-17-acetoxyprogesterone (MAP) and diethylstilbestrol (DES)/lb. B. W. Daily.
[2]The number of days during which treatment has been administered prior to milk let-down and approximated from the expected due date.
[3]The number of days following milk let-down during which treatment was continued.
[4]Calculated as 114 days from the breeding date.
[5]The day of the spontaneous copious let-down of milk in mammary gland ducts and cisterns.

Upjohn Repromix was incorporated in a standard swine feed so that four pounds of feed daily fulfilled the requirement stated in Table I.

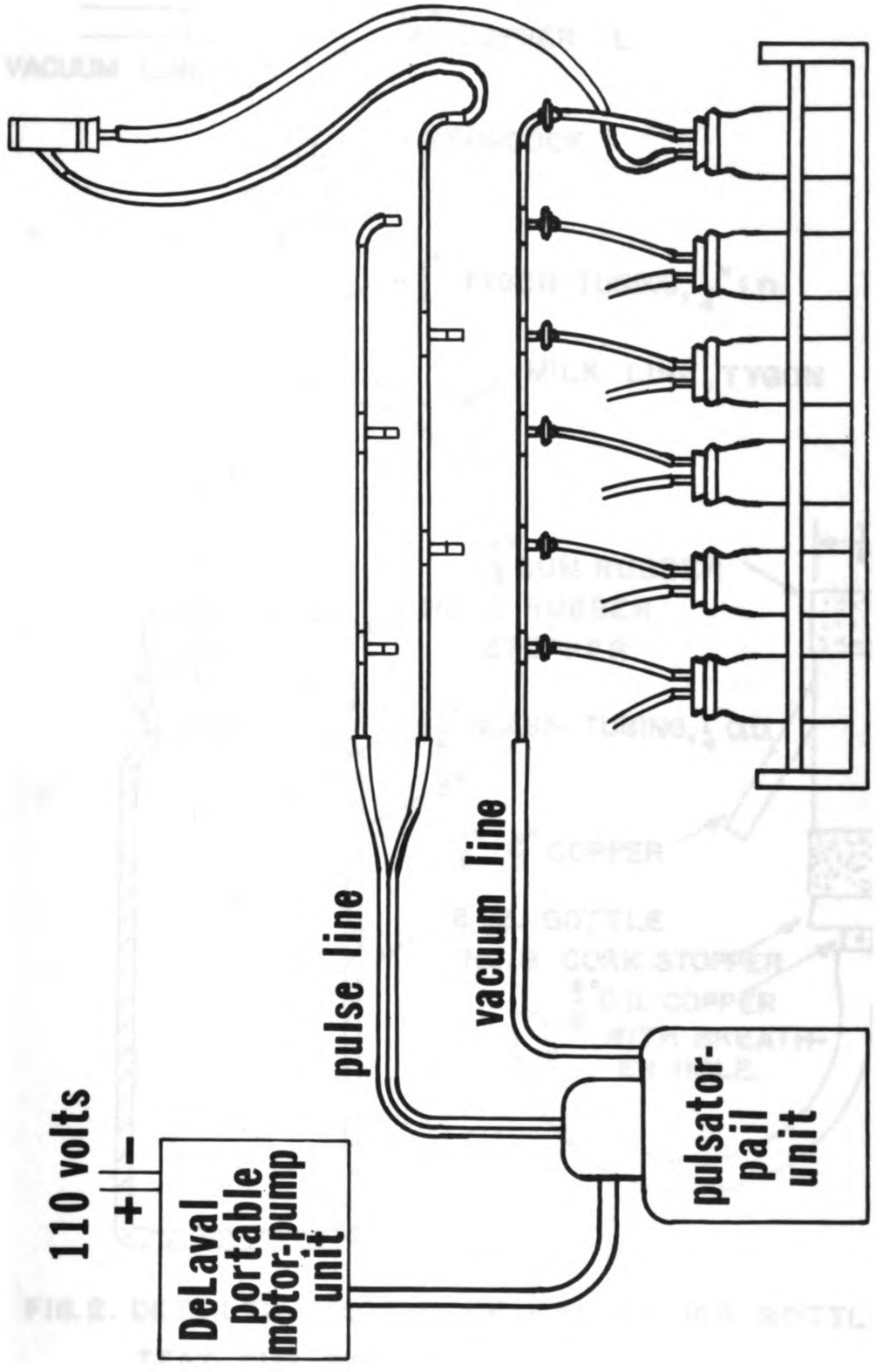

FIG. 1. DIAGRAM SHOWING ADAPTATION OF DELAVAL DAIRY MILKING MACHINE FOR USE IN SOW MILKING.

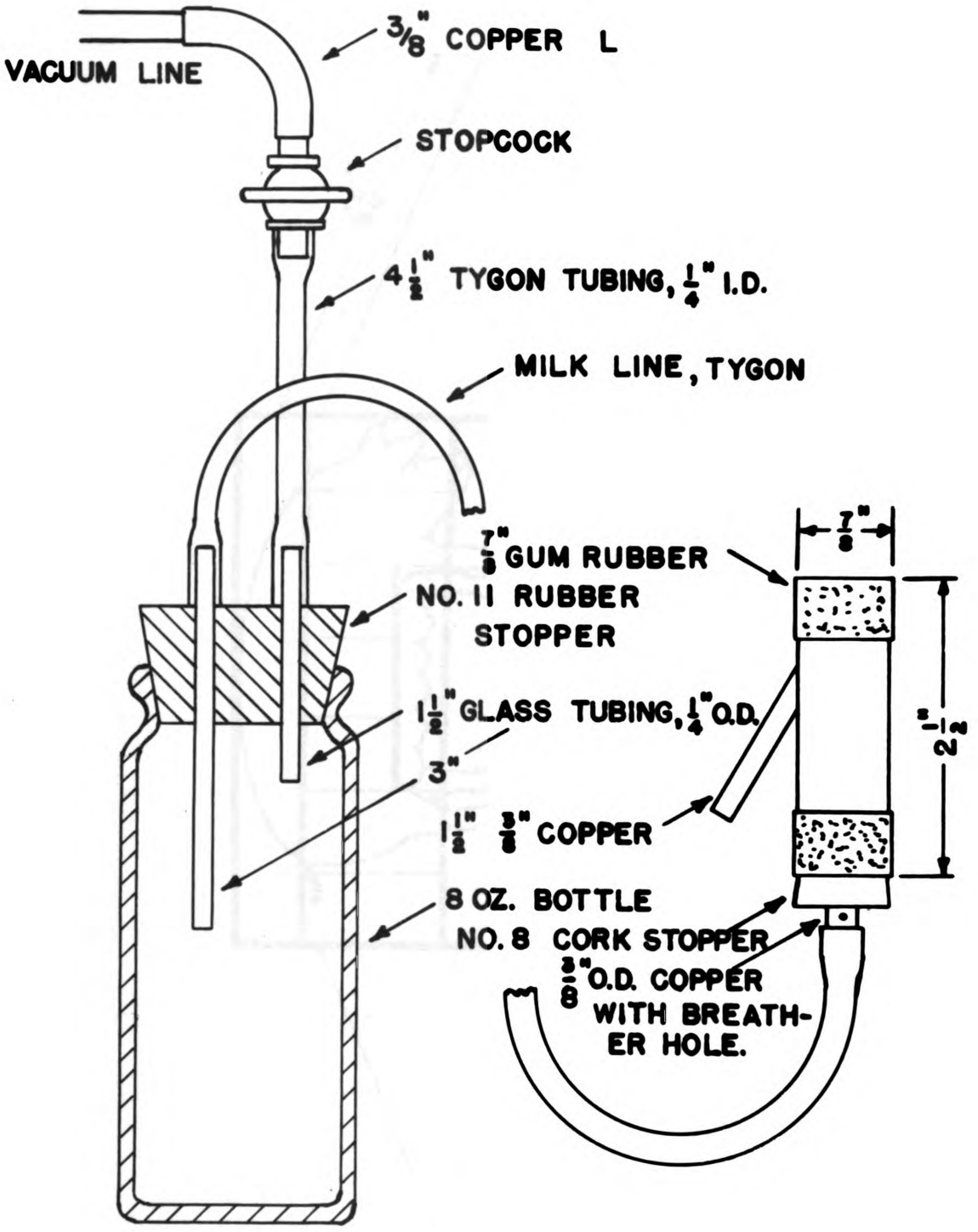

FIG. 2. DETAILED DIAGRAM OF COLLECTION BOTTLE & TEAT CUP ASSEMBLIES.

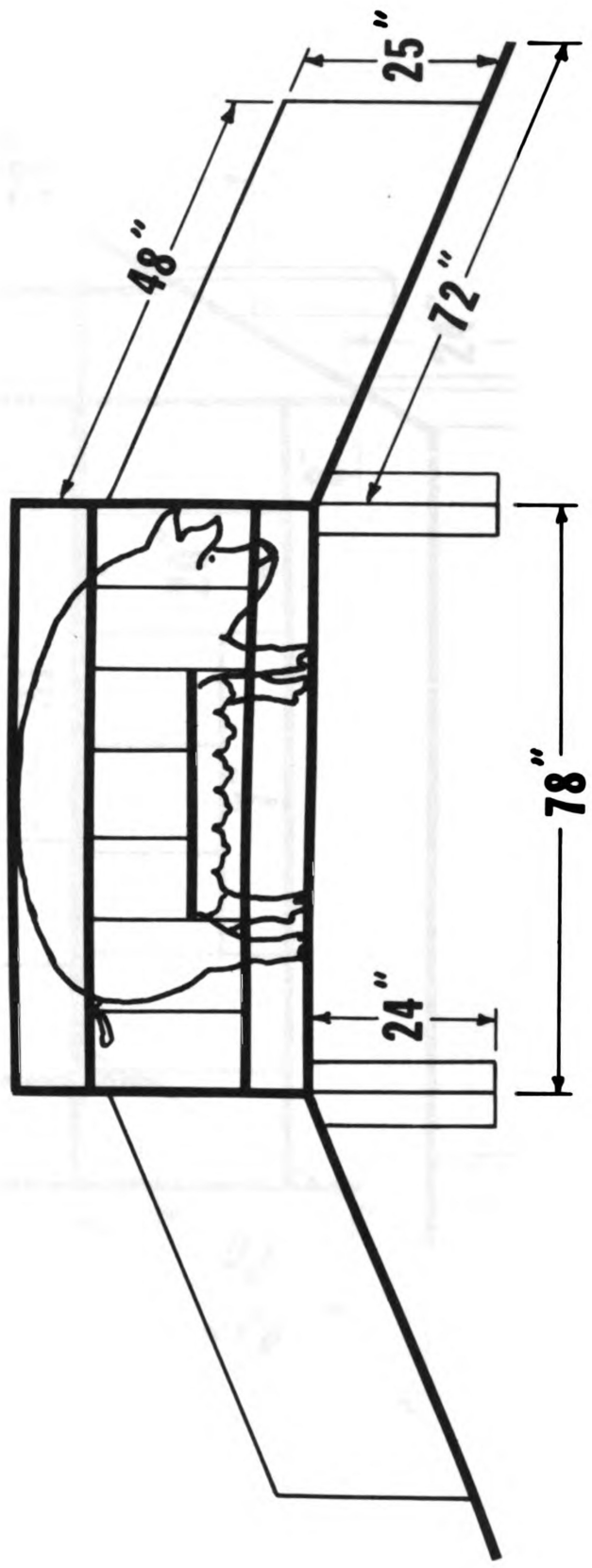

FIG. 3. DIAGRAM OF MILKING PARLOR WITH ENTRY & EXIT RAMPS

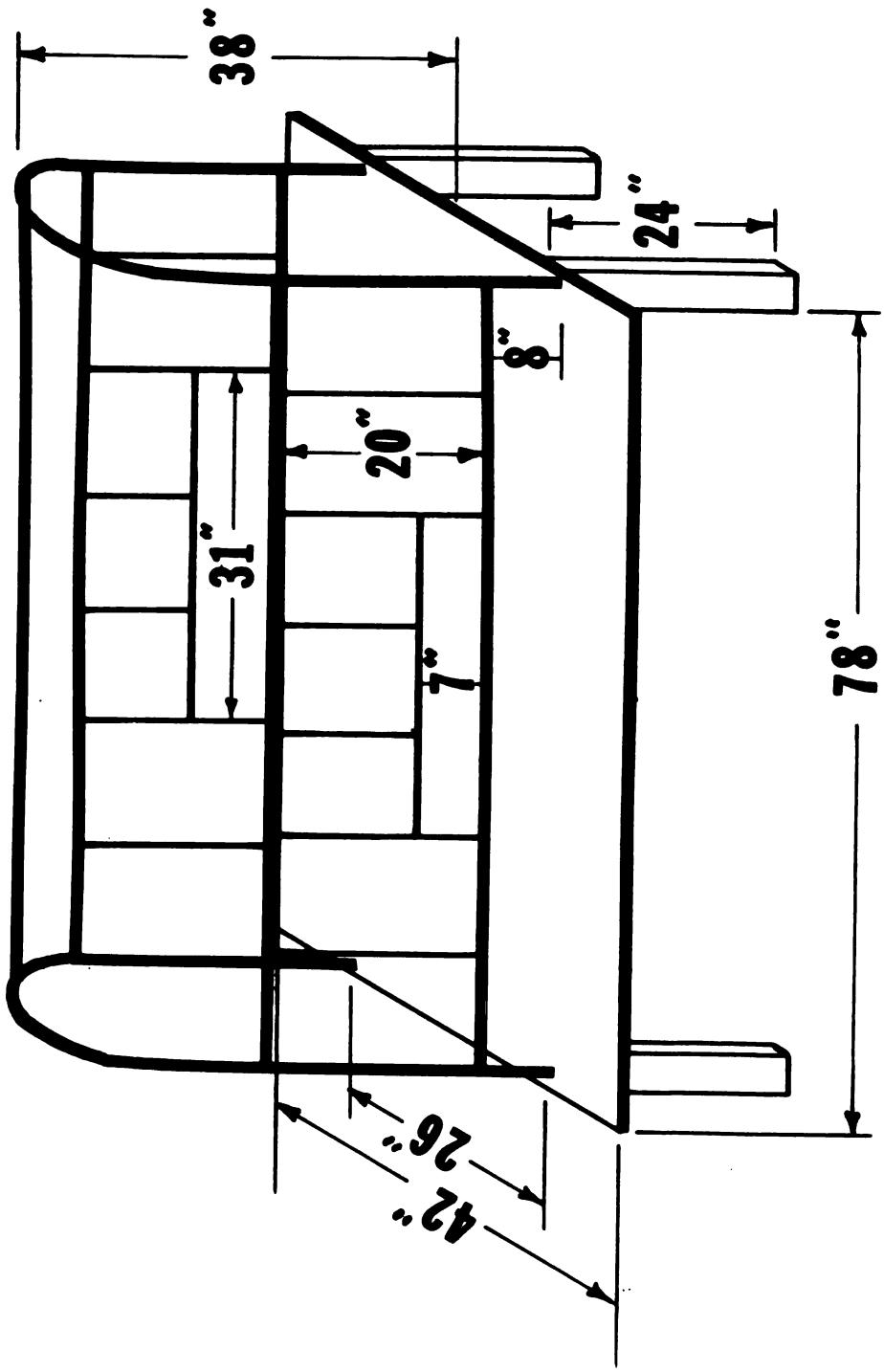

FIG. 4. DETAILED DIAGRAM OF MILKING PARLOR

RESULTS

In the present study delayed parturition was in most cases accompanied by death of fetuses. Examination of fetuses by Caesarean operation during delayed parturition or subsequent to their expulsion following the end of treatment, demonstrated that the death of the young occurred at intervals during delayed parturition, or during the labor process. For these reasons a simple description of fetuses as being alive or dead is not meaningful. A scheme was devised to aid in the differentiation of fetuses as to their time of death and condition (Table 2).

Table 2. CLASSIFICATION OF FETAL CONDITION

Group	Classification	Description
A	Live at birth	
B	Death during labor	1. skin color normal 2. fresh blood in umbilicus 3. hair tight
C	Death prior to onset of labor	1. skin color grey 2. necrotic 3. no blood in umbilicus 4. hair loose In extreme cases 1. putrefaction 2. partial reabsorption (usually of facial region)

The first experiment was designed to determine whether the milking stimulus and partial mammary evacuation would result in an increase in the levels of MAP required to block parturition, Nellor (1963a) reported that levels of 0.6 to 0.7 mg. MAP/lb. B.W. daily successfully delayed parturition in gilts. The initial dosage level in the present study was 0.7 mg. MAP/lb B.W. daily. The results of this experiment are presented in Table 3.

Gilt 68-1 was administered 0.7 mg. MAP/lb. B.W. daily and mammary stimulation and partial glandular evacuation were performed. This dosage level proved inadequate for blocking parturition. The gilt delivered normally while on MAP treatment. Dysphagia did not occur prior to labor and therefore complete ingestion of the MAP had occurred. Gilts 63-7 and 66-1 received a daily dose of 0.8 and 0.9 mg. MAP/lb. B.W., respectively. The expulsion of the fetuses did not occur at the physiological due date although signs of uterine contraction were noticed while the treatment was continued. Following cessation of treatment parturition was accomplished. The fetuses had died prior to the onset of labor. In addition the fetuses were delivered in conjunction with their placentae.

Gilts 80-2 and 9-5 received 1.0 mg. MAP/lb. B.W. daily. Gilt 80-2 produced seven live fetuses and two which died during labor following the cessation of treatment. Placentae and fetus were expelled as one unit. The labor of gilt 9-5 was prolonged and all fetuses died during labor. In all cases, the presentation of a placental segment preceded preceded its corresponding fetus. The first fetus was presented abnormally and with considerable difficulty, as noted by intense and frequent contractions prior to presentation through the vagina.

Levels of 1.3 and 1.5 mg. MAP/lb. B.W. daily were administered to gilts 60-3 and 66-4 respectively. Uterine contractions were noted during treatment. All fetuses had died prior to labor and showed a graded death reaching the extreme condition of putrefaction and partial reabsorption. Fetuses and placentae of gilt 60-3 were extruded as single units. Caesarean section performed on gilt 66-4 revealed a pale, flaccid uterus and ovaries containing non-functional corpora lutea 0.3 to 0.6 cm in diameter. There were no follicles in the ovaries larger than 0.5 cm in diameter.

Table III. EFFECT OF PROGESTIN LEVELS UPON PARTURITION IN PROLONGED GESTATION

Gilt No.	Dosage[1] (MA)	Treatment[2] Pre Let-Down	Treatment[3] Post Let-Down	Let Down[4]	Birth	Live	Dead
68-1	0.7	7	0	4-27	4-27	5	0
63-7	0.8	65	4	6-19	6-24	0	3
66-1	0.9	15	7	6-30	7-9	0	5
80-2	1.0	74	4	6-16	6-21	7	2
9-5	1.0	13	4	7-8	7-14	0	10
60-3	1.3	10	5	6-24	7-1	0	5
66-4	1.5	13	4	6-3	7-3	0	10

[1]Amount of 6-methyl-17-acetroxyprogesterone (MAP)/ lb. B.W. Daily.

[2]The number of days prior to milk let-down (approximated from the calculated due date).

[3]The number of days following milk let-down.

[4]The day of the spontaneous copious let-down of milk in mammary gland ducts and cisterns.

Table IV. EFFECT OF PROGESTIN AND DIETHYLSTILBESTROL UPON PARTURITION IN PROLONGED GESTATION.

Gilt No.	Dosage[1]		Treatment[2] Pre Let-down		Treatment[3] Post Let-down		Let-down[4]	Birth	Live	Dead
	MAP	DES	MAP	DES	MAP	DES				
63-4	1.0	0.0266	16	7	4	4	8-2	8-7	0	11
10-1	1.0	0.0186	8	6	4	4	7-25	7-30	0	9
64-3	1.0	0.031	10	2	11	11	7-27	8-9 (Caesarean)	0	12
13-3	1.0	0.028	8	0	4	4	7-25	7-29 (Caesarean)	3	0
27-1	1.0	0.028	6	0	5	5	11-1	11-6 (Caesarean)	9	2
27-2	1.0	0.028	8	0	4	5	11-4	11-9	0	11
60-3	1.0	0.028	37	0	12	13	11-2	11-15	0	7
6-10	1.0	0.028 0.056	8	0	4	4 +3	4-3	4-11	1	7
6-11	1.0	0.028 0.056	8	0	15	3 +12	4-4	4-18 (Caesarean)	7	7
24-1	---	0.028	---	1	---	0	11-7	11-7	15	0

[1]Amount of 6-methyl-17-acetoxyprogesterone (MAP) and diethylstilbestrol (DES)/lb. B.W. Daily.

[2]The number of days during which treatment has been administered prior to milk let-down and approximated from the expected due date.

[3]The number of days following milk let-down during which treatment was continued.

[4]The day of the spontaneous copious let-down of milk in mammary gland ducts and cisterns.

In an attempt to modify the responses encountered in group 1, a second experiment was devised in which 1.0 mg. of MAP was administered simultaneously with varying quantities of diethylstilbestrol (DES) (Table 4).

Gilt 10-1 received 1.0 mg. MAP and 0.0186 mg. DES/lb. B.W. daily beginning six days prior to the calculated due date. As reported for group 1, the placental attachment continued to remain weak, as noted by fetal and placental delivery as a single unit during parturition following the cessation of treatment. Fetuses died during labor. The duration of labor was 44 hours.

The level of 1.0 mg. MAP was continued and diethylstilbestrol was increased to 0.0266 mg./lb. B.W. daily in gilt 13-4. Administration of DES was initiated eight days prior to the calculated due date. No overt differences from the response of gilt 10-1 were observed.

Gilt 13-3 was treated with 1.0 mg. MAP/lb. B.W. daily 8 days prior to the calculated due date and 0.028 mg. DES/lb. B.W. daily was administered with MAP from the day of milk let-down to discontinuation of treatment four days later. Sixteen hours later a portion of placenta was passed and remained partially hanging from the vagina. At this stage signs of discomfort or labor were not evident. Caesarean section revealed three living fetuses. The tone of the uterine musculature and the degree of placental attachment were assessed as excellent. Each ovary contained functional corpora lutea. In order to determine the effects of further increase in DES, 0.31 mg. DES and 10. mg. MAP/lb. B.W. daily were administered to gilt 64-3 beginning two days prior to let-down. Following a 13 day prolongment of gestation, and while on treatment a caesarean section was performed. Fetuses were all dead and one half of these were putrefied and partially reabsorbed. Although the uterus appeared to have satisfactory tone and color, the ovaries contained corpora albicans and follicles less than 0.5 cm in diameter.

A treatment consisting of 1.0 mg. MAP and 0.028 mg. DES/lb. B.W. daily beginning of the day of milk let-down was further studied with gilts 27-1, 27-2 60-3, 6-10 and 6-11. Gilt 27-1 was not milked and regression of the mammary gland occurred. Five days following the physiological due date caesarean section was performed. Nine fetuses were alive and two had died either during surgery or just prior to this time. As noted in gilt 13-3 uterine tone and blood supply were excellent; placental attachment was apparently normal; and both ovaries contained healthy, functional corpora lutea. The same treatment was initiated with gilts 6-10 and 6-11. The dose of DES was increased to 0.056 mg/lb. B.W. four and three days after milk let-down. Parturition occurred 24 hours later. The first pig was presented alive but with considerable difficulty resulting in severe damage to the piglet. The following seven pigs died during labor. A caesarean section was performed upon gilt 6-11 fourteen days after let-down. Excellent uterine tone and blood supply were observed. Of the 14 fetuses removed during the operation seven were alive and the remaining seven appeared to have died at different intervals extending from the time of surgery to more than 48 hours prior to this time.

Mammary evacuation was performed on gilt 27-2 during the delay period. Four days following let-down, MAP administration was removed and DES treatment was administered. The following labor process required a total of 21 hours. Improved placental integrity was apparent in that fetuses were expelled while the placenta was retained in all but the first three fetuses delivered. Although a graded death was prevalent when comparing the first three fetuses to the remaining eight, all fetuses appeared to have died less than 48 hours prior to delivery.

Gestation of gilt 60-3 was prolonged 14 days and mammary evacuation was

not accomplished. The treatment of 1.0 mg MAP and 0.028 mg DES per lb. B.W. daily was discontinued 13 days after milk let-down. Thirty hours prior to labor 30 mg DES dissolved in Sesame oil was injected intramuscularly into the ham. This procedure was repeated 14 hours prior to parturition. A regime of 80 I.U. of oxytocin intramuscularly repeated every 45 minutes was initiated 12 hours after the final DES injection. Immediately following the third injection of oxytocin fetuses one and two were presented. This procedure was continued for four hours following the first fetal presentation at which time the uterus had been thoroughly evacuated. Seven fetuses were delivered over a 3.5 hour period. Fetal condition revealed that a graded death had occurred in utero. The five fetuses presented along with their respective placentae died more than 48 hours prior to parturition, the remaining two apparently died during labor.

Gilt 24-1 was administered 0.028 mg DES/lb. B.W. daily. No MAP was administered. A normal labor with 15 live fetuses occurred.

DISCUSSION

Induced delayed parturition in swine following the administration of 6-methyl-17-acetoxyprogesterone was reported by Nellor (1963a). A graded fetal death was noted to occur beginning 12 days after physiological term. Fetal death at this time was assumed due to placental insufficiency. It was apparent from these studies that the normal birth mechanism was upset subsequent to delayed parturition. When parturition was delayed longer than 48 hours and treatment was discontinued, normal birth did not result. Uterine contractions started 24 hours following cessation of treatment at which time the fetal death had occurred. Dilation of the cervix did not occur at this time. Fetuses were not expelled until 12 hours to 5 days later. Concommitant with this critical 48 hour period is the time required for the mammary gland to become insensetive to manual stimulation and incapable of milk ejection. Since oxytocin is required for both milk ejection and a normal birth sequence in multiple bearing animals, the possibility presented itself that the simultaneous failure of both mechanisms might be related. The present study was concerned with the effect of mammary evacuation on the birth mechanism of swine in prolonged gestation.

The first experiment was designed to determine whether partial mammary evacuation would increase the quantity of orally administered MAP required to delay parturition. An initial dosage of 0.7 mg. MAP/ lb. B.W. daily was found to be insufficient and normal parturition resulted during treatment. An increase of MAP dosage to 0.8 and 0.9 mg./lb. B.W. was capable of delaying parturition. It is significant, however, that uterine contractions did occur, as observed by abdominal palpation while on treatment. The fetal death observed following cessation of treatment and parturition, obviously occurred prior to labor, it became apparent that the contractions

which had occurred while on treatment may have resulted in fetal mortality. Sub-threshhold or assymetric contraction (Reynolds, 1935,; Csapo, 1956b; Goto and Csapo, 1959; Csapo and Lloyd-Jacob, 1961) which were capable of causing fetal death but not expulsion of the fetuses appeared to have given these results. Therefore, a further increase of MAP was indicated. Gilts 80-2 and 9-5 were administered 1.0 mg. MAP/lb. B.W. daily. At this level uterine contractions did not occur prior to removal of the progesterone block. The fact that 80-2 delivered seven living fetuses and two dead while 9-5 had 100 percent fetal mortality during labor will be discussed below.

Levels of 1.3 and 1.5 mg. MAP were apparently harmful to fetal viability. This assumption was made since marked uterine contractions did not occur in either gilt 60-3 or 66-4, and yet in both cases fetal death occurred while upon treatment. Verification of this possibility deserves attention.

When considering this experimental group as a single unit, certain abnormalities of the labor mechanism were consistent. In general, the duration of labor was increased significantly from the normal 3 hours in untreated animals to 6 hours in the treated animals. The most outstanding anomaly was the loss of placental integrity, prior to birth, as noted in the presentation of the fetus with the umbilicus and placenta as a single unit. The fetuses of 80-2 were delivered at intervals of less than five minutes. If the rapid delivery had not occurred it would appear logical that the fetal death encountered might be due to suffocation, since apparently, placental separation occurred prior to fetal expulsion. This assumption, if reliable, would account for fetal death in gilt 9-5. In this case the improper presentation of the first fetus caused intense labor contractions and could have been the cause of fetal fatality. The caesarean

section performed on gilt 66-4 revealed the presence of a flacid, pale uterus which apparently lacked muscular tone.

It appears reasonable that the placental ability to produce estrogen might decrease following normal term and that during delayed parturition a condition resulted where the uterus would be under the complete control of progesterone, that is, it would lack the tone necessary to affect parturition. It has been reported that estrogen and progesterone act synergistically to maintain a healthy uterine epithelium and secretory glands (Everett, 1962). The maintenance of placental integrity might also be due to a similar steroid balance. Estrogen is reported to increase myometrial actomyosin and contractile strength (Csapo, 1948; Csapo, 1950; Csapo and Corner, 1953; Marshall, 1959; and Goto and Csapo, 1959). It was for these reasons that DES was administered in conjunction with 1.0 mg. MAP in an attempt to correct the abnormalities which occurred in group 1 discussed above.

Levels of 0.0186 and 0.0266 mg DES/lb. B.W. daily and 1.0 mg MAP resulted in no significant variation from the results obtained with 1.0 mg. MAP alone. Gilt 13-3 was administered 1 mg. MAP to block parturition and 0.028 mg DES/lb. B.W. daily, the latter treatment being initiated on the day of milk let-down. Manual mammary evacuation was accomplished and was continued for five days following milk let-down at which time a caesarean section was accomplished. The abnormalities in uterine tone, blood supply, and placenta integrity were all apparently corrected by the administration of this level of DES. It was also of interest that both ovaries contained active corpora lutea. This treatment regime was further investigated. Gilt 27-1 was administered the same treatment as 13-3. The only variation from the scheme with gilt 13-3 was that the milking procedure

was not utilized and that gilt 27-1 was allowed to dry up. Caesarean section performed five days post let-down revealed identical conditions as in gilt 13-3. Although milking maintained oxytocin release there was no apparent change of the ovaries and uterus.

Due to the apparently normal ovarian and genital tract conditions revealed by surgery on gilt 27-1, gilt 27-2 received 1.0 mg MAP to block parturition and 0.028 mg. DES daily beginning at physiological term. The secretory activity of the mammary glands was maintained by the milking procedure. Four days after initial milk let-down, MAP treatment was discontinued while DES treatment was continued. The labor which followed was accompanied by 100% fetal mortality. In all but the first two fetuses presented the placenta was retained until all fetuses had been expelled. Labor lasted 21 hours. The fact that 20 hours elapsed from the onset of dysphagia to presentation of the first fetus is quite critical. It is assumed that the estrogenic effects of diethylstilbestrol were not of a prolonged nature and had disappeared prior to the actual labor sequence. Oral administration of this compound was unsatisfactory in inducing increased uterine tone.

The same procedure utilized with gilt 27-2 was again repeated with gilt 60-3. In addition the intramuscular injection of 30 mg. DES in sesame oil followed in 12 hours by I.M. injections of oxytocin at 45 minute intervals was accomplished in an attempt to shorten the duration of labor to a length of time normal to the untreated, undelayed gilt. This procedure shortened labor to 3.5 hours, suggesting that a lack of sufficient oxytocin and estrogen during delayed parturition had been responsible for the lack of both cervical dilation and properly coordinated expulsive uterine contractions.

A further increase in DES to 0.031 mg/lb. B.W. resulted in death of all fetuses in utero during treatment. Milking was not performed and death may be presumed associated with the administration of high levels of estrogen.

Gilt 24-1 was utilized as a control animal receiving DES alone beginning two days prior to the calculated due date. Since birth did occur on the day of milk let-down, it seems likely that 0.028 mg. DES/lb. B.W. daily is not capable of delaying parturition.

The second experiment tends to clarify the ineffectiveness of DES as a source of estrogen in a study of this nature. Diethylstilbestrol did, apparently, correct the loss of placental integrity which occurs when MAP is administered alone although at the same time it failed to shorten the abnormally prolonged periods of labor. It is possible, however, that the ineffectiveness in shortening labor is due to the short duration of action of DES as suggested above.

The physiological role of the estrogenic metabolites estriol, estradiol-17-beta in parturition require further study. It is possible that the site and mode of action of these metabolites do vary as to whether affecting the myometrium or placental integrity. Since it has not been resolved as to which metabolite DES mimics, it is not possible to speculate upon the role of DES in parturition. DES might be questionable as a source of estrogenic activity in an experiment of this type; more desirable results might occur if natural estrogen metabolites were administered.

It has been proposed that oxytocin has the action of a gonadotrophin releasing factor (Shibusawa et al., 1955; Armstrong and Hansel, 1958; Armstrong and Hansel, 1959; Martini et al., 1959; Hansel and Wagner, 1960; and Staples et al., 1961). Strong circumstantial evidence from this study

supports the role of oxytocin as a releasing factor.

The studies of Nellor (1963a) have revealed that parturition may be successfully delayed in the unmilked gilt by levels of 0.6-0.65 mg. MAP/lb. B.W. daily, by the oral route. The level of 1.0 mg. MAP/lb. B.W. daily was found in these experiments to be the lowest level of MAP capable of maintaining uterine quiesence during prolonged gestation in gilts in which mammary stimulation and partial evacuation was accomplished. Treatment with 0.7 mg. MAP/lb. B.W. daily showed overt signs of uterine contractions when milking was initiated at the physiological due date. Parturition was blocked but the contractions were sufficient to cause fetal death. Since the progesterone dominated uterus will not contract in response to either electrical stimulation or due to the administration of oxytocin it appears that uteri of gilts treated with 0.7 to 0.9 mg. MAP/lb. B.W. daily overcame the progesterone block and became estrogen dominated. Gilts blocked with 6.5 mg. MAP/lb. B.W. daily were injected with 80 I.U. of oxytocin. This is four times the amount advised by veterinarians to affect complete uterine evacuation. This dosage, however, when injected into a pregnant gilt with a progesterone blocked uterus did not evoke noticeable uterine contractions. The physiological actio resulting from a dosage of this order is further pointed out by the observation that 40 I.U. of oxytocin induced sows to show seven to eight waves of active milk ejection while the normal nursing procedure only results in one milk ejection wave. In view of the increased uterine tone and contractility evoked in progestational blocked gilts by the milking stimulus, the thesis suggested is that oxytocin release and/or activity results in an endogenous production and/or release of estrogenic compounds.

It is known that the suckling stimulus causes the reflex release of oxytocin and of lactogenic hormone. Furthermore, the release of oxytocin is

of a more continuous nature than the massive injections described above. It is unlikely that oxytocin acts directly on tissues to increase estrogen release. It is more likely that the estrogen response is secondarily due to an increase of endogenous gonadotrophin.

The sources of endogenous gonadotrophin and estrogen which are in question are unknown. Pituitary, adrenal, ovarian, placental and fetal sources are all possible. It should be noted that caesarean sections in the progestational blocked mammary stimulated gilts revealed ovaries containing small definitive follicles, follicles presumably unable to supply sufficient estrogen to dominate the uterus. The circumstantial evidence observed indicates that further studies are merited in order to determine if oxytocin or the nursing stimulus in some yet undisclosed means can act to cause the release of gonadotrophin.

Prior studies have demonstrated that estrogen is capable of maintaining the corpus luteum. Heckel and Allen (1939) reported that estrogen administration on the day prior to parturition causes maintenance of corpora lutea in the rabbit. This report was supported by the caesarean sections on gilts administered DES from the day of physiological term where corpora lutea were maintained. Gilt 64-3 wad administered DES two days prior to let-down and caesarean section did not reveal the presence of maintained luteal tissue. The reason for these differences is not obvious.

The copious let-down of milk which has been utilized as a characteristic of physiological term is at present not understood. It is apparent that an intrinsic factor creates a build up and release of massive amounts of oxytocin on the physiological due date. Manual milking of gilts on the first day of let-down requires a period of time exceeding ten minutes at each milking before

active ejection ceases. This period of milk ejection gradually shortens until active ejection is of 30 seconds to 1 minute duration by approximately three days following the first day of let-down. This time sequence is characteristic of both delayed and non-delayed gilts. Although parturition wad delayed the procedures utilized in this study abolished this intrinsic factor is of great significance. It is quite possible that future studies disclosing the nature of this oxytocin build up and release mechanism will shed important and still unknown knowledge on the physiological factors controlling parturition.

CONCLUSIONS

1. A dose of 1.0 mg. MAP/lb. B. W. daily is required to create a state of uterine quiesence during delayed parturition in gilts subjected to daily mammary stimulation and evacuation on the physiological due date.
2. Levels of 1.3 and 1.5 mg., MAP/lb. B. W. daily result in fetal mortality in utero during delayed parturition.
3. When parturition is delayed with MAP for a period greater than 48 hours following milk let-down a loss of placental integrity occurs.
4. Prolonged gestation by the administration of MAP results in a prolonged parturitional sequence following the removal of the progestin block.
5. A combination of 0.028 mg. DES and 1.0 mg MAP/lb. B.W. daily maintains placental integrity during delayed parturition but fails to shorten the prolonged labor following the discontinuation of treatment.
6. Diethylstilbestrol administration on the day of physiological term in progestin blocked gilts resulted in the maintenance of corpora lutea of pregnancy.
7. Circumstantial evidence suggests that oxytocin may indeed act as a gonadotrophin releasing factor.
8. The milking stimulus decreases the length of fetal survival in gilts, in utero during progestin prolonged gestation, when compared to the gilt not subjected to the milking stimulus during progestin prolonged gestation.
9. Cervical relaxation does occur subsequent to cessation of treatment in gilts milked during induced prolonged gestation.
10. It seems fitting in concluding a discussion on the mechanisms of parturition to utilize the always humbling words of W. F. Barlow, 1847:

"At present, we must confess how much is to be learned respecting parturition ere we can explain many of its phenomena. As it is, to what a variety of queries must the candid physiologist at once reply 'I do not know,' words which should never be difficult of utterance to any one who feels that a confession of ignorance is a step to its removal, and who is a sincere inquirer after truth."

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