PROGESTERONE SYNTHESIS IN THE ISOLATED PERFUSED PORCINE OVARY OF EARLY PREGNANCY

By

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CHAPTER I

INTRODUCTION

Extensive research effort has been directed to the investigation of the role of the corpus luteum and its major hormonal secretion, progesterone, in the female reproductive system. The cyclic and gestagenic function of progesterone are generally known and comparatively similar in most species. Control of the development, maintenance and function of the corpus luteum is, on the other hand, more variable and less understood.

In vivo attempts to elucidate the physiology of this regulatory system have been hindered because of the difficulty of experimental control and subsequent lack of precision. The *in vitro* techniques offer a useful alternative. In removing tissue from the organism the neural and humoral influence can be eliminated or to some degree manipulated to experimental advantage.

Of the *in vitro* techniques the isolated perfused organ should provide a practical model for investigation of organ function. An organ removed intact from the animal, mounted in an artificial environment and maintained viable, by perfusion of a natural or artificial medium through its vascular system, should function free of interaction with other organs and systems. With proper selection of experimental conditions the physiology of the viable organ should then be estimated more accurately. Although data so obtained would need to be evaluated

in the context of *in vitro* studies this approach would be more physiological than many other *in vitro* methods in that compartmentalization would be maintained and should provide useful information concerning the organ as an intact unit.

With the foregoing in mind, an experiment was designed to determine the ability of the isolated perfused porcine ovary (luteinized and intact) to synthesize progesterone. Citrated whole blood perfusate was used and the perfusate allowed to pass through the ovary only once (monocyclic). The ovaries were taken immediately after slaughter at an abattoir from gilts and sows which were less than three weeks pregnant.

The objective of this study was to determine the rate and quantity of progesterone synthesized by the system compared to other *in vitro* models and *in vivo*, and thereby determine the usefulness of the system as a model to elucidate corpus luteum physiology.

CHAPTER II

LITERATURE REVIEW

In vivo Progesterone Concentrations

Progesterone in the porcine (2) as in many other species (36) is required for normal female reproductive function. Although this steroid hormone is biosynthesized as an intermediate by mammalian steroid producing tissues (36,48), its major synthesis and release, in most species, is a function of the corpus luteum (29,36). In the rabbit, a notable exception, the loci of progestin synthesis also includes the interstitial tissue of the ovary (19). At this time the physiological contribution of these additional progestinogenic (progesterone biosynthesizing) tissues, cannot be completely overlooked.

In addition to progesterone, swine luteal tissue slices are known to produce 20α-hydorxy-Δ^4-pregnen-3-one (20α-OH) in variable but small amounts. This progestin has also been synthesized *in vitro* by human luteal tissue preparations (16,22,36), and by superovulated rat (21) and rabbit ovaries (38), as well as by the mare ovary *in vivo* (37). In the cow, 20α-OH has not been found *in vitro* but its epimer, 20β-hydroxy-Δ^4-pregnen-3-one, has been reported (36). In swine, the minute quantity of 20α-OH that has been found suggests that this progestin may not be as important in this species (6) as for example in the rabbit (20).

Progesterone concentrations in porcine luteal tissue (μg/gm) and

ovarian venous blood (μg/ml) have been determined during the estrous cycle and during pregnancy. Loy et al. (24) assayed progesterone in porcine corpora lutea at day 70 of gestation. They found that total luteal progesterone is a function of the amount of luteal tissue present and the concentration of progesterone in the tissue. There was no correlation between the numbers or weights of corpora lutea and progesterone concentrations in corpora lutea of the same ovary. Likewise, comparison of corpora luteal weights or numbers and luteal progesterone concentrations between right and left ovaries indicated little variation within the luteal tissue of a single animal.

Duncan et al. (8) determined total luteal tissue weight and progesterone concentration in gilts at various reproductive stages. During the estrous cycle, tissue weight increased sharply from days 4-8, gradually peaked at day 12, decreased slightly by day 16 and at day 18 was similar to the weight observed at day 4. Progesterone concentration followed a similar trend though slightly delayed. Concentration increased almost linearly at days 4,8 and 12, then peaked at day 16, but fell to 0 by day 18. During pregnancy the total luteal weight remained slightly higher from days 16-102 than was previously noted for peak day 12 of the cycle. Progesterone concentration was also slightly higher at day 16 of gestation than day 12-16 of the cycle. It increased to peak at day 48 of gestation but subsequently fell to a concentration at day 102 that was similar to that noted for day 4 of the estrous cycle.

Gomes et al. (15) cannulated the utero-ovarian vein of 18 sows during the estrous cycle. Blood was collected daily via a heparin filled exteriorized cannula for 1 to 11 days. Progesterone analysis

of collected fractions and grouping of data in three day means demonstrated an almost symmetrical variation of ovarian venous progesterone concentration during the estrous cycle. Progesterone levels increased from estrus (day 0) to a peak at days 10-12. The concentrations then dropped to near the initial level by days 19-21.

Masuda et al. (25) noted that luteal tissue weight during the porcine estrous cycle increased sharply from day 1 to peak at day 8. The weight was maintained at a slightly lower level through day 16 with a rapid decrease to day 18. If pregnancy occured, the weight was maximal at day 14 but decreased slightly at days 18 and 25. On day 102, weights were increased again and maintained through day 112. Luteal progesterone concentration increased from day 4 to day 8 of the cycle with the maximum at days 8-12. The level dropped slightly to day 14 but was sharply reduced by day 18. During gestation the concentration was maximal at day 18, while at day 25, it was somewhat reduced but still similar to day 14 of pregnancy and days 8-12 of the cycle. By days 102-112 the level was even lower than day 25. Ovarian venous plasma, analyzed for progesterone during the estrous cycle and pregnancy, also exhibited the cyclic changes in progesterone concentration observed in luteal tissue. During the estrous cycle the lowest plasma concentrations occurred at day 1, reached a maximum at days 8-12 and at day 16 fell to the same level observed at day 1. During pregnancy, the level decreased gradually from days 14-25 and was slightly decreased below the day 25 level at days 102-110. These workers concluded that the luteal progesterone concentration measurements provide an indication of the relative progesterone concentrations in ovarian venous blood.

Brinkley and Young (3) also compared the progesterone concentrations of ovarian venous effluent and luteal tissue collected at days 7, 11 and 13 of the estrous cycle in sows. Progesterone secretion rates per ovary were almost identical at days 7 and 13 of the cycle and peaked at day 11. The synthesis rate, adjusted for luteal tissue weight (μg/gm luteal tissue/min), was very similar on days 7 and 11, these being periods representing rapid development and maximum development respectively. The synthesis rate was substantially decreased at day 13 even though luteal tissue weight was maximal. However, this decrease was observed in a single animal and may not be a true representation. Luteal progesterone was maximal at day 13 and minimal at day 7. Progesterone was undetectable in simultaneously acquired arterial blood, collected before it entered the opposite ovary from which the venous blood was collected. This suggests that all progesterone identified was the result of ovarian secretion.

The relative trends in progesterone concentration are quite similar when comparing the results of these experiments. It is evident that the corpus luteum varies in size and progesterone concentration during the estrous cycle. The weight gradually reaches a peak by day 12, is maintained through day 16 and regresses rapidly by day 18. The peak progesterone concentration occurs with peak luteal size but begins to decrease just prior to luteal regression. Similarly, the progesterone concentration of ovarian venous plasma increases with luteal development and progesterone synthesis, peaks with maximum luteal concentration and decreases as the luteal tissue synthesizes less hormone. Though little conclusive evidence is available, the work of Masuda et al. (25) suggests that the same is true during at least the early part

of gestation. It has also been demonstrated in the ovine (10,37) and bovine (14) that a similar relationship exits between the concentration of progesterone in the corpus luteum and in the ovarian venous effluent.

In vitro Techniques

Investigation of organ function and regulation *in vivo* is inherently confounded by experimental error associated with the complexities of inter-organ relationships. The inability to control neural and endogenous humoral factors requires that some studies be conducted with tissues that are removed from the organism (37). In so doing, however, it is in error to infer that because a mechanism is demonstrable *in vitro* that it functions as such *in vivo*. *In vitro* systems are simply tools which provide evidence that may be indicative of *in vivo* activity.

In vitro techniques utilizing subcellular fractions, tissue homogenates, tissue slices and minces and intact isolated perfused organs have all been employed to investigate steroidogenesis. Few experiments have been performed with porcine luteal tissue when compared to other species and tissues.

Acetone extracts of the mitochondrial fraction of bovine adrenal cortex were used by Koritz and Hall (23) to study the conversion of 7α-^{3}H-cholesterol to ^{3}H-pregnenolone. Knowing that the pathway for this conversion was intramitochondrial and that further pregnenolone metabolism occurs extramitochondrially, they demonstrated end-product inhibition by pregnenolone at the first step of the reaction sequence in the mitochondrial extract. This evidence readily suggests the possible importance of the intramitochondrial concentration of pregnenolone and the mechanism of pregnenolone transport across the mitochondrial

membrane in steroidogenesis by the adrenal cortex.

Tissue homogenates of bovine corpus luteum, corpus luteum-bearing ovaries and corpus luteum-free ovaries were prepared by Tamaoki and Pincus (41) in an attempt to demonstrate progesterone synthesis. Although they demonstrated side-chain cleavage of cholesterol and 20α-hydroxy-cholesterol in all tissues, only in homogenates containing luteal tissue did the conversion of cholesterol, 20α-hydroxy-cholesterol and pregnenolone into progesterone occur to any extent. Thus, a strong suggestion of the progestinogenic function of luteal tissue is presented. Though both this and the subcellular fraction techniques have their place in studies at the enzymatic level, it can readily be seen that the influence of compartmentalism of the organ, tissue or even the cell itself is totally disregarded. Further consideration of _in vitro_ preparations with more structural integrity is therefore warranted. Tamaoki and Pincus (41) even suggest the possibility that intact tissues might be required to demonstrate gonadotropic responses of the progesterone synthesisizing pathways in this tissue.

Duncan et al. (8) compared the _in vitro_ synthesis of progesterone by porcine luteal tissue slices at various stages of the estrous cycle and pregnancy. Slices from days 4,6,8,12 and 16 of the cycle and days 16,24,48,96 and 102 of gestation all synthesized progesterone during a 2 hour incubation period. However, progesterone was not detected, initially or after incubation, in luteal tissue of cyclic day 18 which represents the period of luteal regression. As discussed previously, the weight and initial progesterone concentration of the luteal tissue varied with the reproductive state of the gilt. Although tissues obtained from cycling animals produced more _de novo_ progesterone than

those tissues obtained from animals during gestation, it must be remembered that the initial progesterone concentration of luteal tissue from early pregnancy was greater than the maximum concentration observed during the estrous cycle. It appears that little difference existed in the total concentration after incubation in either the late cycle or early gestation; thus, those samples of high initial concentration synthesized less progesterone in reaching the final level. These workers concluded that a general parellelism exists between the capacity of luteal tissue to synthesize progesterone and the stage of development of the tissue.

In additional porcine luteal tissue slice incubations, Duncan et al.(9) attempted to investigate the control of progesterone synthesis. The presence of gilt pituitary homogenates, pregnant mare serum (PMS), human chorionic gonadotropin (HCG), relaxin, oxytocic materials or ovine lactogenic hormone had no effect on synthesis in vitro. However, regulator substances were encountered in aqueous filtrates of porcine endometrium. Filtrates from days 12 and 13 of the estrous cycle increased progesterone synthesis while those from days 16 and 18 showed definite inhibitory effects. Although the specificity of these materials has not been determined, the inference of the possible physiological significance cannot be overlooked.

Progesterone synthesis in porcine luteal tissue slices has also been examined recently by Cook et al. (6). This work agrees with previous investigation that progesterone is synthesized _in vitro_ by incubated slices. It was also shown that acetate-1-^{14}C is a precursor to this steroid compound and to very small quantities of 20α-hydroxy-Δ^4-pregnen-3-one. The acetate incorporation is somewhat variable, but with the exception of the first half of the estrous cycle, follows the

trend of progesterone synthesis that occurs during cyclic and gestational luteal maintenance and regression. *In vitro* porcine progesterone synthesis, when compared to ovine and bovine synthesis under similar conditions, is quite variable. This they attributed to the inhomogenious nature of the porcine luteal tissue when compared to other species, and the subsequent lack of sampling uniformity. Conversely to previous reports (9), the porcine luteal tissue was found to respond by increased progesterone synthesis upon the addition of porcine, ovine or bovine lutenizing hormone (LH) to the incubation medium. Porcine follicle stimulating hormone (FSH) and prolactin did not affect synthesis (9). A graded dose response to ovine LH was also demonstrated (porcine and bovine LH were not tested). In general, LH stimulation was represented by 15%, 30% and 40% increases over control synthesis by porcine, ovine and bovine luteal tissue slices respectively. This limitation of porcine tissue was thought, in part, to be the result of the relative low availability of sterol precursors.

The tissue slice technique definitely maintains partial cellular integrity and thus provides an advantage over previously discussed methods. However, the possibility of reaction alteration due to partial tissue damage still exists. Further, and more important, these systems provide no means for constant maintenance of substrate concentration or product removal. The reactions are no doubt affected by substrate availability or product accumulation.

Isolated Organ Perfusion

Isolated organ perfusion provides a practical concept for maintenance of the entire organ *in vitro* without sacrificing histological

and morphological integrity. The substrates of the perfusion medium are supplied to and biosynthetic products are removed from the cell by the natural route of the circulatory system. Hecter (18) has likened the perfused organ to "an organized collection of tissue slices" which is presented substrates at the cellular level via the circulatory system. This technique is clearly a more physiological approach to organ investigation than can otherwise be achieved by previously described in vitro methods.

Though the metabolic or biosynthetic function of perfused organs is independent of the uncontrollable in vivo regulators, it is dependent upon the composition of the perfusion medium (5) and various intrinsic factors such as capillary hydrostatic pressure, maintenance of effective vasculature or, probably most important, cell permeability (18). The assay of perfusate and/or the tissues of organs perfused with medium of known or estimated composition is then a measure of organ function.

The continuously recycling (multicyclic) perfusion system provides information comparable to slice and mince preparations. In this system substrates are constantly removed from the medium while metabolic products are accumulated. The single cycle (monocyclic) perfusion (13, 32), however, provides a different concept. The perfusate does not recirculate through the organ, thus the system is similar to in vivo (18). Substrates are presented at constant concentration and the products are constantly removed. The continuous product removal not only reduces further metabolism of the product by the perfused organ but also permits rapid deactivation of enzyme systems which might promote further metabolism of perfusion products by the perfusate itself (7).

Extensive experimentation by Carrel and Lindberg (5) with a variety of organs and perfusion media determined that most organs could be maintained with insignificant degradation for periods of one to forty days. Using histological examination as criteria, it was evident that the organs remained alive, but the degree of growth or function was dependend upon the substrates of the perfusing media. Carrel and Lindberg (5) and Werthessen (45), after perfusing various tissues, stressed the necessity of asepsis and the detrimental effects of infection in extended term perfusions. Werthessen (45) indicated that in perfusions which last for several days without infection, the tissues are capable of healing cut surfaces or adding new growth. It seems obvious, considering the above, that perfused organs which maintain normal histology and are capable of growth or healing must possess intact enzyme systems which are capable of supporting life.

Accepting the ability of the perfused organ to remain viable, it is logical to assume that the organ's functional capacity might also exist essentially intact. In reference to the steroidogenic endocrine organs, their function is the enzymatic biosynthesis and release of steroid hormones into the blood or perfusate.

A general consideration of steroid synthesizing organs is warranted due to the presence of similar intermediates and similar enzyme systems which are known to exist in their steroidogenic pathways (36,48). Probing adrenal gland function with a multicyclic citrated whole blood perfusion, Hecter (17) demonstrated the ability of bovine and ovine glands to synthesize corticosteroids in response to medium borne ACTH. Further investigations with steroid precursors ultimately revealed active enzyme systems specific for various steps in corticosteroido-

genesis by the perfused adrenal (18). In similar perfusions, the incorporation rate of ^{14}C-labeled acetate, cholesterol and progesterone precursors in the biosynthesis of adrenocorticoids was examined with and without ACTH stimulation. This allowed postulation of the site of ACTH action and the existence of an additional pathway for the synthesis of corticosteroids from acetate (40). It is apparent that in these perfusion studies the enzymatic integrity of the perfused tissue was sufficiently maintained to allow investigation of adrenal corticosteroid biosynthesis.

The technique for the perfusion of the isolated rabbit testis was developed by VanDemark and Ewing (43) as an alternative to the complexities of physiological interactions _in vivo_. The use of glucose uptake, perfusate flow rate and comparative histology of the control and perfused organs as parameters indicated that defibrinated heterologous rabbit blood would support rabbit testis for 6-10 hrs in a recirculating system (44). Ewing and Eik-Nes (13) subsequently demonstrated that the technique was a feasible _in vitro_ method for study of the biosynthesis and secretion of testosterone by the rabbit testis. Several important concepts which they suggested are: 1) there was no mixing of arterial and venous perfusate in the system, 2) the testosterone in the perfusate was the result of active testicular secretion and not passive leakage, 3) the integrity of the testosterone synthesizing system was maintained for 7 1/2 hrs of perfusion and 4) the secretion of testosterone could be expressed as a rate (testosterone/unit time) which was independent of testis size, perfusate flow rate, or glucose utilization.

Based on gonadotropic response and post perfusion histology,

human testis were also perfused successfully (35). Acetate-1-^{14}C precurson was incorporated into testosterone and Δ^4-androstene-3,17-dione at a basal level which was elevated upon the addition of pituitary gonadotropin. Gonadotropin-primed stallion testis perfused 24 hrs in an aseptic multicyclic system synthesized large stores of estrone and estradiol into which ^{14}C-labeled acetate was incorporated (30). Although the excessive estrogen synthesis reported is contrary to reports in other species, it may only reflect massive steroid production. It is evident that the highly efficient steroidogenic system of the stallion testis, as well as the enzyme systems of both the rabbit and human, were preserved and manifested during isolated organ perfusion.

Ovarian Perfusion

Perfusion of bovine ovaries (26,32,33) has demonstrated that progesterone is synthesized by the perfused corpus luteum of this species. Romanoff and Pincus (33) perfused luteal ovaries multicyclically with citrated whole blood and expressed steroid synthesis as the rate of acetate-1-^{14}C accumulation in the various lipid extracts of fractions of the recirculating perfusate sampled at 15 min intervals. It was noted that in the non-gonadotropin stimulated perfusion, the maximum rate of accumulation in all fractions, including that containing progesterone, occurred within the first 30-60 min of the perfusion but fell for the remaining 5-5 1/2 hrs. It was also observed that the rate of accumulation could be enhanced by equine pituitary gonadotropin.

In subsequent perfusions by Romanoff (32) the monocyclic technique was employed to prevent recirculation of the perfusate through the organ. In these perfusions involving quantitation of progesterone in

serial fractions, the data were expressed as progesterone released per minute and demonstrated that the rate of secretion for each 15 min interval increased for several time periods with an ultimate plateau. Stimulation with FSH (contaminated with LH) resulted in increased height of the plateau. Also noteworthy is the evidence from these perfusions that addition of gonadotropins to the monocyclic perfusion media increased the incorporation of acetate-1-^{14}C into progesterone profoundly and into other progestins variably.

Mills (26) perfused the lutenized bovine ovary in the recirculating apparatus of Morrissette et al. (28). He demonstrated a net progesterone synthesis when either citrated whole blood or a blood substitute containing 10% bovine serum were the perfusing medium. When the blood substitute without the serum was employed, consistent progesterone synthesis was not attained even though the organs appeared viable at the end of the perfusion period. Although enzymatically the organs were intact, a serum factor was evidently required for progesterone synthesis in this perfusion system.

Luteal phase porcine ovaries were perfused multicyclicly by Morrissette et al. (28) with blood substitute containing 40% homologous erythrocytes. When perfused for 1-3 hrs, these organs synthesized progesterone which represented 45% of the total progesterone content of luteal tissue and perfusate at temination (4). Of the de novo progesterone, 88% ± 12% was found in the luteal tissue with the remainder in the perfusate. Acetate-1-^{14}C present in the perfusion medium was incorporated into progesterone in this study at less than 0.5% of the theoretical level. This indicates that plasma acetate is not a major progesterone precursor under the conditions of this system but does not

contradict the possibility that there is more than one acetate precursor pool (12). Exogenous HCG did not affect either progesterone mass synthesis or acetate-1-^{14}C incorporation. Organ morphology and net progesterone synthesis indicated functional integrity. The lack of response to HCG in these perfusions, in light of the gonadotropic response in steroidogenic organs in other species, suggests either that porcine luteal tissue does not respond to HCG or a serum factor is necessary to increase synthesis.

The monocyclic isolated perfusion method could be useful in studying porcine ovarian function. The basic apparatus and techniques for maintaining the viability of this organ in perfusion have already been developed by Morrissette et al. (28). Romanoff (32), similarily, has demonstrated the feasibiltiy of the monocyclic, citrated blood method for the bovine ovary. By using this monocyclic technique with citrated whole blood for perfusing porcine ovaries of early pregnancy, a basic *in vitro* system to demonstrate rate of secretion would be available. This would be useful for future work requiring a knowledge of the rate of secretion as an indication of organ function, of response to luteotropins or lysins, or of luteal metabolism.

CHAPTER III

MATERIALS AND METHODS

Perfusion

Apparatus. The apparatus employed (Figure 1) was basically the design of VanDemark and Ewing (13) as modified by Morrissette et al. (28) for the perfusion of the porcine ovary. Further alterations applicable to this study involved enlargment of the medium reservoir, elimination of the oximeter and addition of an outlet to facilitate continuous collection rather than recirculation of the perfusate. The perfusion medium was continuously oxygenated in the reservoir by bubbling with prehumidified 95% O_2-5% CO_2 gas. The flat bottom of the reservoir allowed continuous agitation with a magnetic stirrer thus maintaining a constant hematocrit with little hemolysis. The medium was withdrawn from the container and pumped through the glass wool filter to the organ by means of a peristaltic perfusion pump[1]. The pump provided arterial pressure which was determined by a mercury manometer, adjusted to 100 mm Hg with the thumbscrew constrictor on the organ by-pass and monitored and recorded on a polygraph[2]. Just prior to the point of organ attachment the system was provided with an infusion valve by which vasodilator was injected when necessary to combat vasoconstriction.

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[1] New Brunswich Scientific Co., Inc., New Brunswich, N.J.

[2] E&M Instrument Co., Houston, Tex.

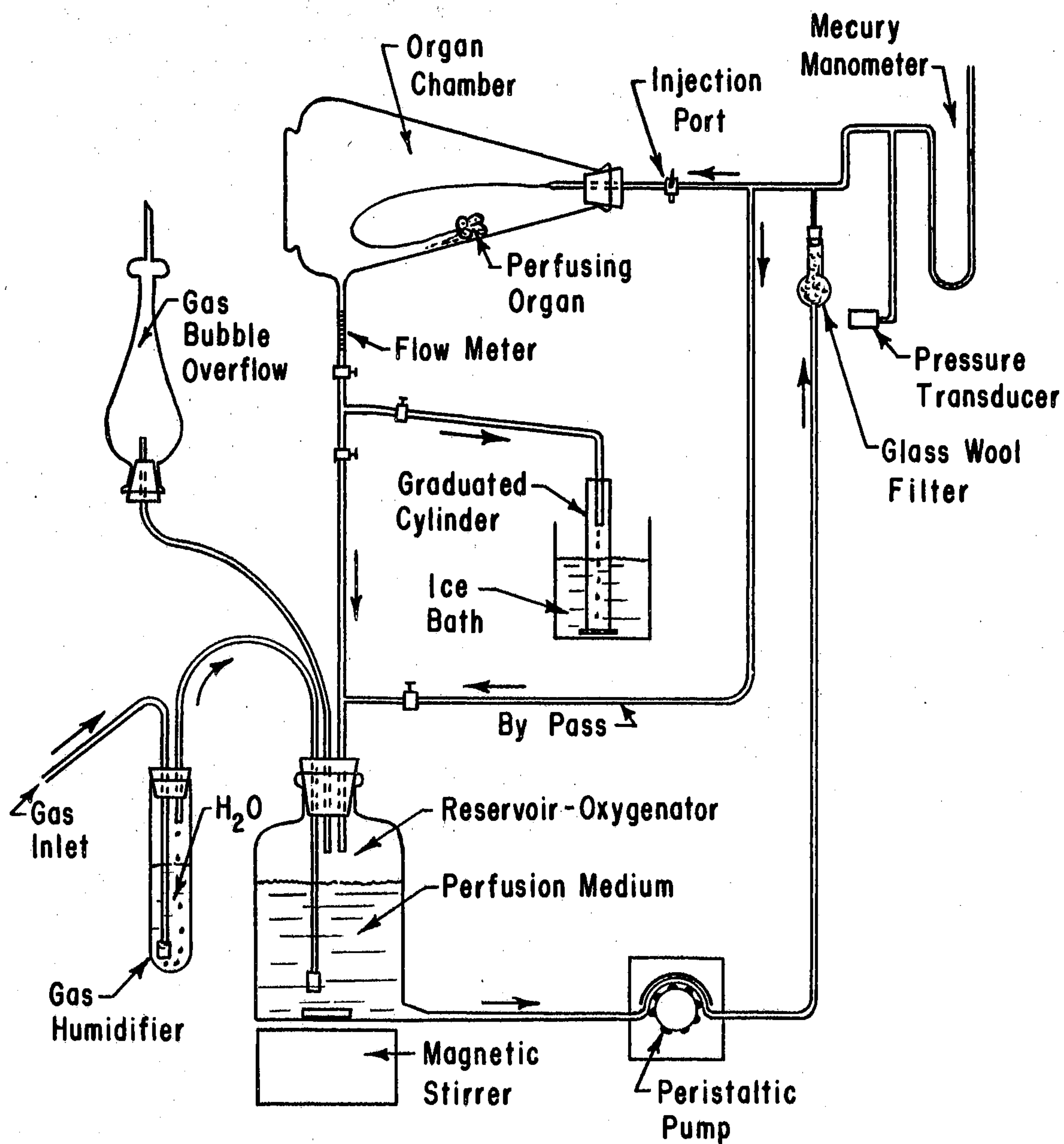

Figure 1. Monocyclic Perfusion Apparatus

As the venous effluent drained from the organ chamber, a 1 ml flowmeter allowed the periodic determination of perfusate flow rate. The outlet below the flowmeter allowed recirculation of the medium when filling the perfusion tubing prior to organ attachment or total collection of the effluent as the organ was perfused; thus the monocyclic system. The entire apparatus was housed in a small constant temperature cabinet maintained at 38°C. Glass doors to the cabinet provided continuous observation and easy access for necessary manipulations.

Blood Collection and Perfusion Medium Preparation. A homologous blood sample was obtained from a barrow or an apparently non-pregnant gilt at an abattoir[3]. Approximately 1300-1400 mls of blood were citrated to prevent clotting by gently mixing with 240 mls of concentrated ACD solution (see Appendix). The citrated blood containing 200,000 IU procaine penicillin and 250 mg equivalent of dihydrostreptomycin base[4] to avert infection was stored on ice until used 3-4 hrs later.

At the time of blood collection the donor gilts were earmarked for identification at evisceration. Subsequent examination of the reproductive tract allowed estimation of stage of the estrous cycle and approximation of plasma gonadotropin concentrations based on previous work conducted in the same laboratories (1).

In the laboratory the citrated blood was filtered through glass wool to remove debris and 500 ml aliquots were obtained. To each aliquot 1.43 gm dextran[5] (m.w. 75,000), 430 mg pilocarpine hydro-

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[3] Wilson and Co., Oklahoma City, Okla.

[4] Jensen-Salsbery Laboratories, Kansas City, Mo.

[5] Sigma Chemical Co., St. Louis, Mo.

chloride[6] and 15 mg sodium acetate[7] were added. The dextran aided in maintaining osmotic pressure, the pilocorpine reduced vasoconstriction and the sodium acetate served as a general steroid precursor. Each aliquot of this final perfusion medium was warmed to 35°C and placed in a prewarmed apparatus. Oxygenation, agitation and recirculation of the perfusate were begun immediately to assure final conditioning and warming of the system in preparation for the organ.

Organ Collection and Preparation. The general procedure for organ collection was that of Morrissette et al. (28). Intact ovaries and uteri of pregnant sows and gilts of unknown reproductive history were obtained from the abattoir processing line 25-30 min post-slaughter. Upon examination, tracts were chosen from apparently healthy sows and gilts less than 40 days pregnant, based on embyro length (42), and with five or more prominent corpora lutea per overy. Immediately after selection the ovarian artery of the selected ovary was located and partially denuded of connective tissue. The artery was cannulated approximately 1 cm distal to the point of ramification with a 12" x .034" ID x .050" OD polyethylene tubing[8] attached to a 20 ga hypodermic needle. Excess broad ligament was ligated and excised without interfering with venous return. The entire ovarian vascular system was then flushed via the cannula with 20-30 mls of chilled dilute ACD solution (see Appendix) to remove blood and chill the tissue. Thoroughness of the flush was judged by the paleness and chill of the corpora lutea. Flushing was

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[6] Sigma Chemical Co., St. Louis, Mo.

[7] J.T. Baker Chemical Co., Phillipsburg, N.J.

[8] Clay-Adams, Inc., New York, N.Y.

occasionally facilitated by very gentle massaging of the organ. The infundibulum and oviduct were then ligated and excised. The flushed organ with cannula and minimum of broad ligament attached and the contralateral ovary, excised through the mesovarium, were sealed in a plastic bag containing chilled dilute ACD and transported to the laboratory in an ice chest.

While the medium was recirculating and warming in the apparatus the ovary was infused with room temperature perfusion medium to insure an adequate flow and facilitate the location of leaks which were ligated as necessary. An organ satisfactorily prepared was mounted in the perfusing chamber, attached to the medium outlet and the perfusion was started. Two organs were perfused simultaneously in independent apparatus but with medium from a single donor.

The Monocyclic Perfusion System. During the initial 15 min warmup period venous effluent collection was begun and perfusion pressure adjusted to 100 mm Hg. As the organ began to function the characteristic bright red color of the oxygenated arterial medium was obviously absent in the venous perfusate. A definite arterial-venous color differential became more apparent as metabolism increased. After the first 15 min most organs were functioning adequately as judged by the arterial-venous color difference, the maintenance of constant arterial pressure and the establishment of venous flow. Those organs not functioning properly within 15 min were replaced. Throughout each perfusion arterial pressure was observed and flow rates were periodically checked. Evidence of vasoconstriction, as determined by either or both the above criteria, warranted the administration of 1/2-2 mls of 3% pilocarpine hydrochloride solution. By occasional injection of this

vasodilator, satisfactory flow rates were consistently maintained.

Following the warmup period, perfusate fractions were collected for two 1 hr intervals or in eight 20 min serial fractions. The perfusate samples were accumulated in graduated cylinders packed in ice. The chilled sample volumes were determined immediately following collection and the sample was stored at 4°C until all samples of the perfusion were collected.

Perfusions treated with standard gonadotropins[9] received ovine NIH-LH-S11 and/or NIH-FSH-S1 at the end of the first one hour collection. Sodium acetate-1-^{14}C [10] was added to the medium of six serially sampled perfusions just prior to organ attachment.

Shortly after termination of the perfusion the hematocrit and pH were recorded for each collected sample. The samples were centrifuged[11] for 20 min at 7500 x g at 5°C, the cellular component discarded, and the clear plasma supernatant stored at -20°C until extracted. The intact perfused and the corresponding contralateral non-perfused ovaries were also stored at -20°C until extracted.

Progesterone Analysis

Plasma and luteal tissue samples were extracted for progesterone with selected solvents, were purified by solvent partition and thin layer chromatography and quantitated by gas liquid chromatography. All

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[9] Endocrinology Study Section, National Institutes of Health, Bethesda, Md.

[10] New England Nuclear Corp., Boston, Mass.

[11] International High-Speed Refrigerated Centrifuge Model HR-1, International Equipment Co., Needham Heights, Mass.

analyses were corrected for efficiency of recovery by adding labeled progesterone to the samples prior to extraction and then measuring the percent of labeled steroid recovered by liquid scintillation counting technique. All solvents employed were of analytical reagent grade unless otherwise specified. To estimate sample recover, 3×10^{-3} μc 4-^{14}C-progesterone [12], used as received, was added to all samples not containing acetate-1-^{14}C; otherwise, 2.0×10^{-2} 7α-^{3}H-progesterone [13], repurified by thin layer chromatography, was employed.

Extraction Method I. The plasma samples from Experiment I were extracted using a modification of the hot acetone method described by Cardeilhac et al. (4). Each known plasma volume of 100 mls or less was extracted with 15 volumes of 50-55°C acetone. The extract was then flash evaporated[14] under reduced pressure and the residue saponified with 10% NaOH and extracted with diethyl ether. After discarding the aqueous layer the ether phase was washed with water several times and dried under N_2. This residue was dissolved in 90% methanol and partitioned against equal volumes of ligroin in a six tube countercurrent system. The combined methanol fractions were dried under N_2 and stored at -20°C until thin layered in a chloroform-methanol (99:1) solvent system.

Extraction Method II. The extraction method used by Stabenfeldt (39) was modified and employed to extract the luteal tissue and serial

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[12] New England Nuclear Corp., Boston, Mass.

[13] Nuclear-Chicago, DesPlaines, Ill.

[14] Rotovapor Model VE-50, Rinco Instrument Co., Inc., Greenville, Ill.

plasma samples of Experiment II. Corpora lutea of perfused and non-perfused ovaries were extirpated and weighted. The tissue was homogenized with a Ten Broeck type hand homogenizer and the volume adjusted to 20 mls with 0.9% saline.

The isotopically labeled progesterone was added to a known volume, 20 mls or less, of plasma or homogenate. Each sample was extracted three times with 2 1/2, 2 1/2 and 2 volumes of dichloromethane. The solvent from the combined extracts was removed by flash evaporation under reduced pressure. After saponification of the residue with 1N NaOH and extraction with ether, the aqueous phase was discarded. The remaining ether phase was washed twice with water and dried under N_2. This dried residue was then stored at -20°C until purified by thin layer chromatography in a benzene-ethyl acetate (4:1) solvent system.

Thin Layer Chromatography. All samples were purified by thin layer chromatography using Silica Gel G-HR/UV[15] and solvents systems of chloroborm-methanol (99:1) or benzene-ethyl acetate (4:1). Authentic progesterone[16] was chromatographed in an isolated lane on each plate with one to four samples. Fluorescent bands or spots which, when observed under ultraviolet light, corresponded to the fluorescent progesterone standard were eluted with diethyl ether. The samples were dried under N_2 and stored at -20°C until quantitated by gas chromatography.

The 7α-^{3}H-progesterone used as tracer to estimate recovery was subjected to thin layer chromatography in a benzene-ethyl acetate (4:1) solvent system. Subsequent counting of the chromatograms on a Packard

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[15]Machery, Nagel and Co., Brinkman Instrument Inc., Westbury, N.Y.

[16]Sigma Chemical Co., St. Louis, Mo.

Model 7201 Radiochromatogram Scanner[17] revealed a single peak corresponding to authentic progesterone; thus, the compound was considered pure.

Gas Liquid Chromatography. Two to 20% of each purified sample was chromatographed on one of several 3 ft glass columns packed with 1%QF-1 liquid phase on 100/200 mesh Gas Chrom Q[18] solid support. Dual Model 5121 hydrogen flame ionization detectors on a Series 5000 Barber-Coleman Gas Chromatograph[19] were employed. Injector and column bath temperature settings of 250°C and 220°C respectively and a N_2 carrier gas flow rate of 48 ml/min allowed a progesterone standard retention time of 11 minutes. With air and hydrogen adjusted to optimum pressures, a detector temperature of 230°C, and an attenuation setting of 100, a standard curve linearity range of .2-2.5 μg was attainable. Peak areas were determined by planometry.

Sample peaks corresponding to authentic progesterone were collected from several perfusion fractions. A detector bath splitter diverting approximately 10% to the detector and 90% to the collection device was utilized. The column effluent was trapped at room temperature in a 8 mm OD x 300 mm glass wool packed glass tube. The collected peak was eluted into a scintillation vial with absolute diethyl ether which was subsequently evaporated under N_2.

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[17] Packard Instrument Co., La Grange, Ill.

[18] Applied Science Laboratories, College Station, Penn.

[19] Barber-Colman Co., Industrial Instruments Division, Rockford, Ill.

<u>Liquid Scintillation Counting</u>. Five to 20% of each sample was counted for estimation of the recovery rate just prior to, or in conjuction with, gas chromatography. The Packard Tricarb Model 314-E liquid scintillation counter[20] was set to count ^{14}C at 79.6% efficiency alone. For mixed isotope counting the counter was set to count ^{14}C at 14.5% on channel red and 30.6% on channel green and ^{3}H at 15.4% on channel red and .006% on channel green. Counted simultaneously all counts on the green channel were assumed to be ^{14}C. the fluor in which the samples were counted was 4.0 gm PPO [21] and 0.3 gm POPOP[22]/L of toluene.

Experimental Design

The study was composed of two parts within which twenty-six monocyclic, luteal phase, swine ovarian perfusions were performed. Perfussions 1 to 14 (Experiment I) were investigations to determine if a variation in the average rate of progesterone secretion could be detected between an initial 1 hr collection period and a second 1 hr collection period. At the beginning of the second 1 hr period LH and FSH, LH alone or no gonadotropin was added as treatment.

Perfusions 15 to 26 were non-treated perfusions in which the effluent was collected serially for 20 min intervals. The objective of these perfusions was to determine the dynamic variation in the rate of progesterone secretion that occurs during the course of a 2 1/2 hr perfusion. Total synthesis was also determined to compare the biosyn-

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[20]Packard Instrument Co., Inc., La Grange, Ill.

[21]Packard Instrument Co., Inc., Downers Grove, Ill.

[22]New England Nuclear Corp., Boston, Mass.

thetic efficiency of intact perfused ovaries to other *in vitro* systems and *in vivo*. Acetate-1-^{14}C was added to the perfusion medium of perfusions 21-26 to determine if that compound was an important precursor in the system and would be incorporated into synthesized progesterone.

CHAPTER IV

RESULTS AND DISCUSSION

Authentication of Progesterone

The evaluation of organ function by chemical determination requires that the identity of the isolated compound be reasonably confirmed. Perfusion samples similar to those of this study were extracted for progesterone by Cardeilhac et al. (4) with Method I. Purification of the compound by thin layer chromatography, quantitation by gas liquid chromatography, preparation of the 2,4-dinitrophenylhydrozone derivative and examination of the ultraviolet absorption spectrum at 240mμ revealed that the isolated compound performed exactly as did authentic progesterone prepared similarily. A coworker (27) recently utilized Method II to extract plasma from bovine ovarian perfusions. After purification by thin layer chromatography and quantitation by gas-liquid chromatography, mass spectrometric analysis verified that the isolated compound was indeed progesterone.

In the course of this study all samples were purified and compared to authentic progesterone by thin layer chromatography. Quantitation of all samples by gas liquid chromatography on 1% QF-1 liquid phase produced chromatograms with only one major peak and the retention time of the peak corresponded exactly to that of standard progesterone. Several samples were developed on additional thin layer systems as

described previously or injected into 1% SE-30 columns on the gas chromatograph. In each case the retention time of the isolated compound was exactly the same as the progesterone standard. In light of the foregoing, it was concluded that the isolated compound being measured was progesterone.

Perfusion

Experiment I. In this preliminary study fourteen porcine ovaries (perfusions 1-14) were utilized to investigate the rate of progesterone secretion by perfused luteal tissue during a two hour period. The organs were perfused monocyclically, and the perfusates were collected in two 1 hr fractions. The first six perfusions were performed to establish techniques and protocol for use in the remaining perfusions. Citrated barrow blood was used as the perfusion medium in the six preliminaries. After collecting the initial one hour fraction ovine NIH-LH-S11 and ovine NIH-FSH-S1 were added to the medium to attain a concentration of 5 μg/ml of medium. Several perfusions of this series were lost due to technical problems; thus an accurate comparison of progesterone secretion during control and treatment periods was not possible.

Prior to the next series of perfusions (7-14) it was decided that gilt blood would be a more suitable medium source since the plasma LH level could be estimated (1) and the total gonadotropin concentration would probably be lower than in barrow blood. On the eight perfusions four were treated with 1 μg NIH-LH-S11/ml perfusion medium after the first one hour fraction was collected. The remaining four were not treated. This should have provided a comparison of progesterone

secretion with and without gonadotropin stimulation. It was observed (Table I) that not only did the rate of secretion (μg progesterone/gm luteal tissue/hr) vary from one perfusion to another but also from one hour to the next within the perfusion. Mean progesterone secretion during the second hour was decreased 34.5% and 27.4% respectively in the treated and non-treated groups. The addition of LH did not produce a significant increase in progesterone secretion. Similar observations were made by Cardeilhac et al. (4) using HCG to stimulate progesterone secretion in multicyclic porcine ovarian perfusions and Duncan et al. (9) using various gonadotropins with porcine luteal tissue slices. Romanoff (32), however, did stimulate progesterone secretion with gonadotropins in the bovine ovary perfused monocyclically. Cook et al. (6), in contrast to Duncan et al. (9), recently demonstrated that porcine luteal tissue slices would respond to LH of porcine, bovine or ovine origin by increased progesterone synthesis but would not respond to FSH or prolactin. They reported that the response was only slight, approximately 15%, and was so variable that large sample numbers were necessary to produce a statistically significant increase. Further, they concluded that the tissues needed to be collected and incubated within minutes after slaughter.

Current perfusions did not necessarily prove progesterone systhesis but provided evidence of progesterone secretion. Nor did they explain the dynamic changes in secretion which evidently must occur. Further perfusions were required to produce a more complete concept of the activity of the organ in this system.

Experiment II. In light of the previous results this study was designed to provide data to estimate net progesterone synthesis and

TABLE I

PROGESTERONE SECRETION IN EXPERIMENT I

Perfusion No.	Blood Donor	Estimated Endogeneous LH* (ng/ml plasma)	Added LH (μg/ml plasma)		Progesterone in Perfusate (μg/gm luteal tissue/hr)		
					Control Period	Treatment Period	Difference**
7	Gilt-2	45	1		19.7	10.3	- 9.4
8	Gilt-2	45	1		37.5	46.0	+ 8.5
9	Gilt-3	41	1		49.2	23.4	-25.8
10	Gilt-3	41	1		37.4	14.5	-22.9
				Mean	35.9	23.6	-12.4
				±S.E.	7.0	9.2	8.1
11	Gilt-5	67	none		16.5	8.6	- 7.9
12	Gilt-5	67	none		32.5	12.1	-20.9
13	Gilt-7	67	none		20.0	19.7	- 0.3
14	Gilt-7	67	none		29.6	30.5	+ 0.9
				Mean	24.4	17.7	- 6.7
				±S.E.	4.4	5.6	5.8

* LH level approximated for each donor gilt based on the estimated day of the estrous cycle at slaughter (1).

** (+) = increase; (-) = decrease.

demonstrate the dynamic changes in the progesterone secretion rate during the monocyclic porcine ovarian perfusions. The venous effluent from twelve ovaries of perfusions 15-26 were collected in eight 20 min serial fractions following an initial 15 min collection. All perfusate samples and the corresponding corpora lutea of the perfused and non-perfused ovaries of each perfusion were assayed for progesterone. The pre-perfusion progesterone concentration of the perfused luteal tissue was assumed to be approximately the same as the progesterone concentration of the non-perfused tissue (24). The total of progesterone in the perfused luteal tissue and the perfusate samples which exceeded the approximate initial luteal tissue level represented the estimated net progesterone synthesis (μg/gm luteal tissue).

Eleven organs survived the perfusion period and demonstrated a net synthesis of progesterone (Table II) even after 3-4 hrs at 12°C without circulation. The mean de novo progesterone of the eleven perfusions (120.3 μg/gm luteal tissue) represented a 104% increase over the mean initial progesterone level (115.9 μg/gm luteal tissue). Of the estimated net progesterone 35% (40.1 μg/gm luteal tissue) was found in the luteal tissue. In the multicyclic perfusions of Cardeilhac et al. (4) the mean estimated net progesterone synthesized during the 3 hr perfusions was 82% of the mean estimated initial luteal level. Eighty-eight percent of the estimated net synthesis was luteal progesterone. Comparison of these monocyclic (Experiment II) and multicyclic (4) perfusion values indicates that a greater increase of progesterone occurred in the monocyclic perfusions but a smaller percentage of the increase remained in the luteal tissue. Apparently in the monocyclic perfusions more progesterone was synthesized and secreted by the luteal tissue

TABLE II

ESTIMATED NET PROGESTERONE SYNTHESIS IN EXPERIMENT II

Perfusion No.	Blood Donor	Estimated* Endogenous LH (ng/ml plasma)		Luteal Progesterone: Contralateral non-perfused (μg/gm luteal tissue)	Luteal Progesterone: Perfused (μg/gm luteal tissue)	Luteal Progesterone: Estimated Increase (μg/gm luteal tissue)	Progesterone in Perfusate (μg/gm luteal tissue)	Estimated Net Progesterone (μg/gm luteal tissue)
15	Gilt- 9	48		137.1	167.5	30.1	62.5	92.6
16	Gilt- 9	48		77.5	111.1	33.6	49.6	83.2
17	Gilt-10	50		61.5	73.9	12.4	68.8	81.2
18	Gilt-10	50		76.9	116.7	39.8	113.0	152.8
19	Gilt-13	45		72.7	56.4	-15.9	60.7	44.8
20	Gilt-13	45		35.9	154.8	118.9	135.6	254.5
21	Gilt-14	67		154.7	221.1	66.4	82.8	149.2
22	Gilt-14	67		144.3	181.5	37.2	53.0	90.2
23	Gilt-16	67		130.2	166.3	36.1	37.5	73.6
24	Gilt-16	67		147.7	207.9	60.2	68.1	128.3
26	Gilt-17	23		236.4	258.4	22.0	150.5	172.5
			Mean	115.9	156.0	40.1	80.2	120.3
			±S.E.	17.2	18.1	10.1	11.1	17.9

* LH level approximated for each donor gilt based on the estimated day of the estrous cycle at slaughter (1).

and/or less was metabolized by the perfusate.

A comparison of progesterone synthesis during the perfusions reported herein and the porcine luteal tissue slice incubations of Duncan et al. (8) and Cook et al. (6) is presented in Table III. The estimated net progesterone in each experiment is adjusted to an average rate (μg/gm luteal tissue/hr) of progesterone synthesis for comparison. The perfused organs synthesized more progesterone than did the slices and, based on the rate of synthesis, the slice preparations were only 73.5% and 63.6% as efficient as the perfusions. However, if the initial luteal progesterone concentration of each tissue is considered, the increase of progesterone (%/hr) is really not very different among the three works. Values of 35.8%, 36.8% and 35.2%/ hr were determined for the perfusions of Experiment II and the slice studies of Duncan et al. (8) and Cook et al. (6) respectively. Apparently the rate of *in vitro* synthesis in the perfusions and the slice incubations (6,8) was dependent on the intrinsic progestinogenic capacity of the tissues involved rather than the *in vitro* system employed.

The serial fractions of the venous perfusate collected at 20 min intervals throughout the perfusion, and analyzed for progesterone, provided a means of evaluating the dynamic changes in the rate of ovarian progesterone secretion. The mean rate of secretion of ten organs (excluding number 19, Table II) during each collection period is presented in Figure 2. The rate of secretion was highest (579.8 ng/gm luteal tissue/min) during the first 20 min period. The rate declined rapidly for the following 40 min, increased during the next 20 min, then for the remaining 80 min, gradually but steadily declined to the minimum rate of 342.8 ng/gm luteal tissue/min. The large standard error

TABLE III

IN VITRO PORCINE LUTEAL PROGESTERONE SYNTHESIS BY ISOLATED PERFUSED OVARIES AND INCUBATED LUTEAL TISSUE SLICES

Source	Progesterone Levels (μg/gm luteal tissue)			Average Synthesis Rate (μg/gm luteal tissue/hr)	Efficiency Relative to Perfusion (%)	Progesterone Increase/hr (%)
	Initial Luteal	Final*	Estimated Increase			
Experiment II**	115.9	236.2	120.3	41.5	--	35.8
Duncan et al. (8)†	84.0	145.0	61.0	30.5	73.5	36.8
Cook et al. (6)‡	75.0	154.0	79.0	26.4	63.6	35.2

* Luteal progesterone for slice studies; luteal and perfusate progesterone for perfusions.
** Mean values, eleven 175 min monocyclic perfusions; organs day 25-40 of gestation.
† Tissue day 24 of gestation; 2 hr slice incubation.
‡ Average of the mean values of five 3 hr luteal tissue slice incubations from four animals at day 35 of gestation.

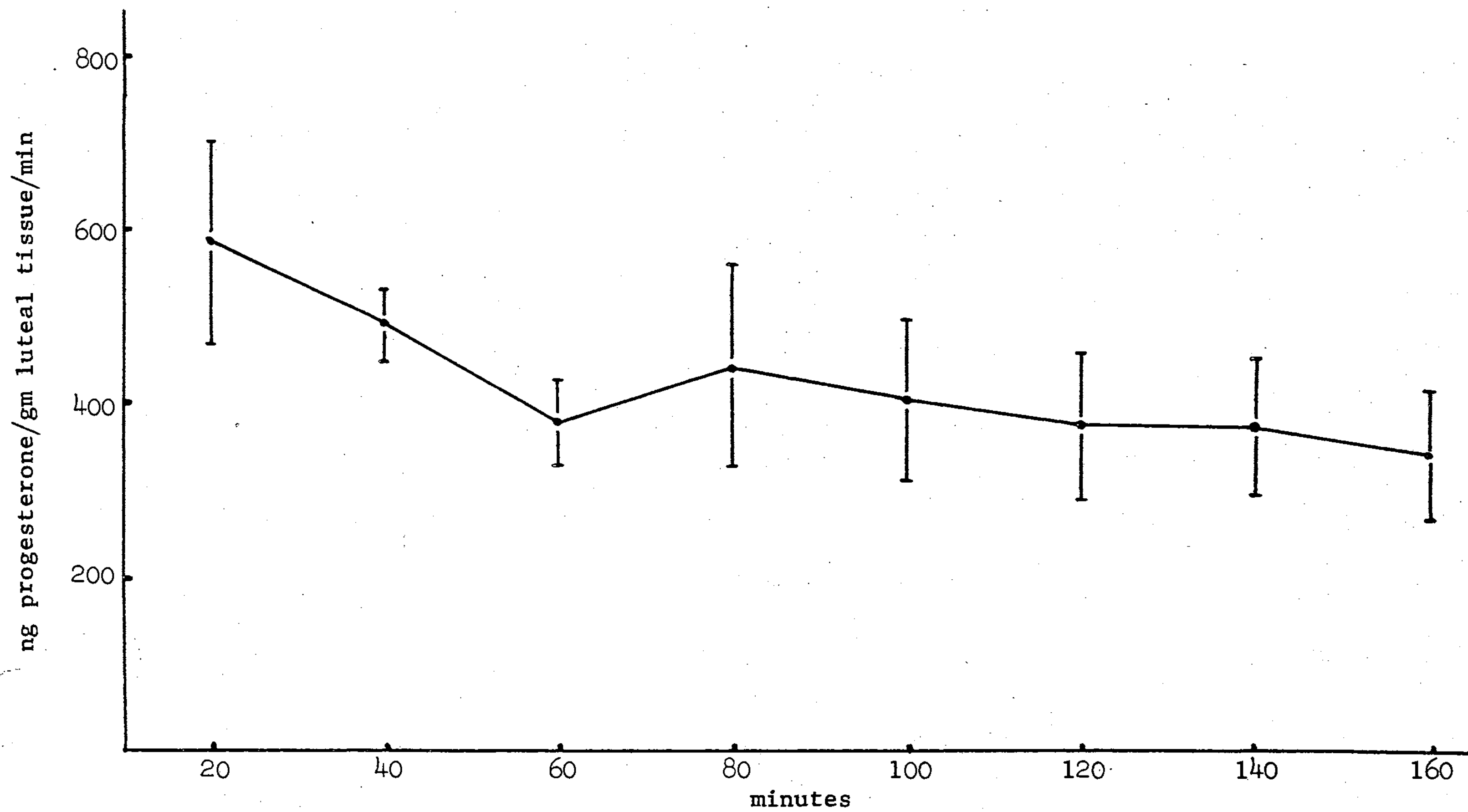

Figure 2. Mean Progesterone Secretion Rate (±S.E.) in Each Perfusate Fraction

of the means is due in part to the variability of the biosynthetic capacity of the individual organs. By expressing the progesterone secretion for each serial fraction on the basis of percent of the estimated net progesterone synthesized, a very similar mean trend of secretion was demonstrated and the S.E. of the means was much smaller (Figure 3). This method of presentation also accentuates the inference that secretion gradually decreased throughout the perfusion even though an unexplained low value was noted for the third 20 min serial fraction. Eik-Nes (11) observed a similar decrease in venous testosterone in the infused dog testis during a 2 hr collection period and Ewing and Eik-Nes (13) noted a gradual decrease of testosterone secretion during the 7 1/2 hr perfusion in the perfused rabbit testis. In light of the fact that the rabbit's testicular capacity for testosterone synthesis was still intact after 7 1/2 hr and that gonadotropins added to the perfusate increased synthesis, they postulated that the decline might have been due to an inadequate gonadotropin level in the perfusate. In this ovarian perfusion study, however, there was no evidence that the endogenous LH level had any effect on net synthesis (Table II) or secretion rate.

Brinkley et al. (3) investigated the _in vivo_ rate of progesterone secretion (μg/gm luteal tissue/min) into the ovarian venous effluent of sows during the estrous cycle. At peak luteal phase they reported 3.96 μg/gm luteal tissue/min. In Experiment II, however, the average secretion rate for a 175 min perfusion period was .458 μg/gm luteal tissue/min. This indicated that the perfused organs were 8.6 times less active than comparable _in vivo_ organs.

Flow rates of the perfused organs averaged 2.1 ml/min throughout the perfusion and fluctuated very little during collection of the eight

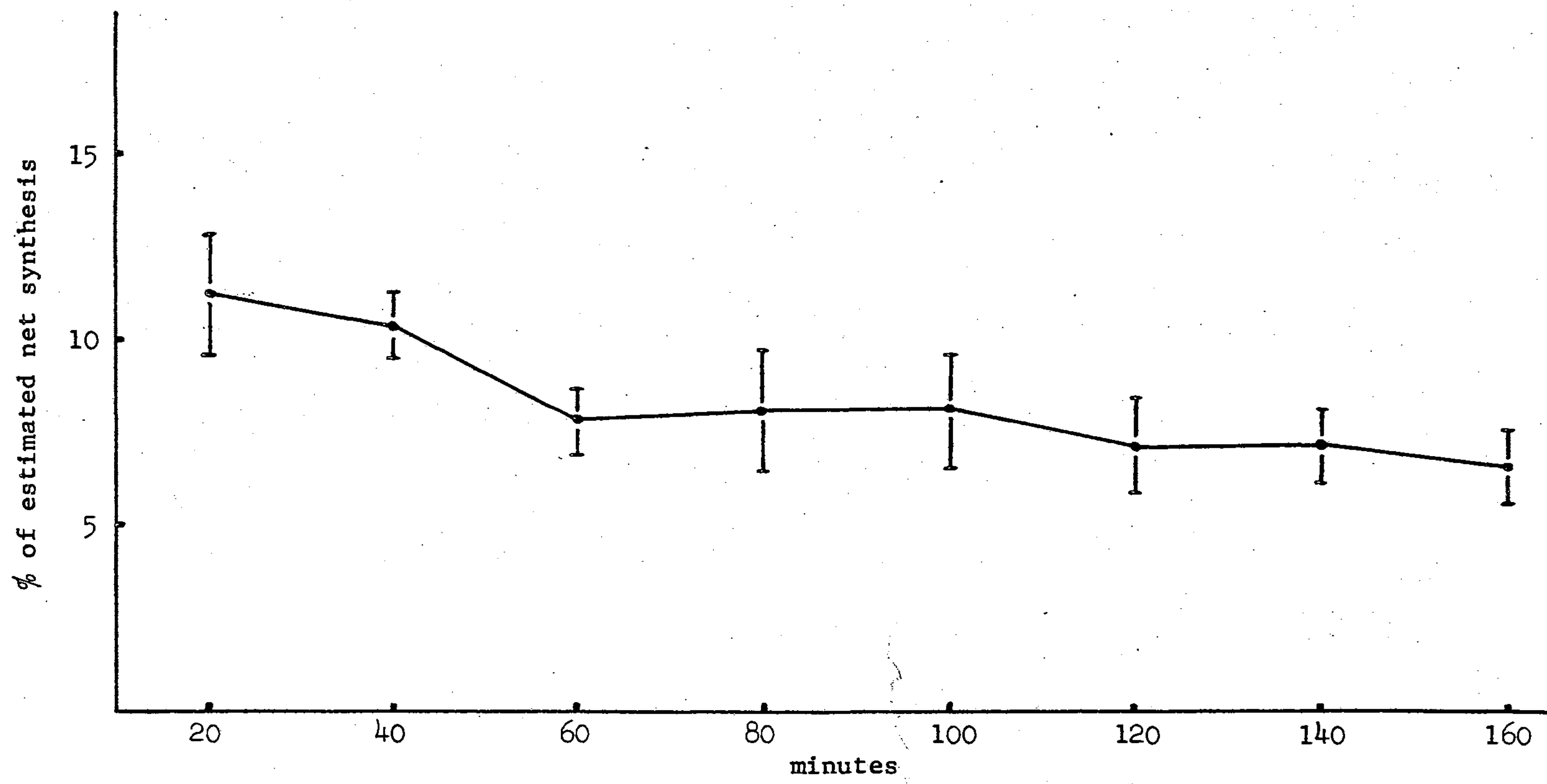

Figure 3. Mean Percent (±S.E.) of Net Progesterone Secreted in Each Perfusate Fraction

serial samples. By contrast, the in vivo flow rates reported by Brinkley et al. (3) ranged from 4.5 to 16.9 ml/min. If the general reduction of flow rate is considered for the perfused organs, the secretion rate of progesterone can be based on the volume of perfusate or venous plasma collected rather than period of the collection. Values of progesterone secretion of .59 and .20 μg/gm luteal tissue/ml perfusate were reported by Brinkley et al. (3) and in Experiment II respectively. On this basis the activity of the perfused ovary is only 2.9 times less than the activity of organs in vivo. Similarily, .86 and .30 μg progesterone/gm luteal tissue/ml venous plasma were observed by Masuda et al. (25) (gilts 25 days pregnant) and in this experiment respectively. The activity relationship indicates that the in vivo organ (25) secretes progesterone 2.8 times more actively than does the perfused organ. The similarity of the activity relationships based on volume of perfusate or venous plasma suggests that progestinogenesis in the isolated perfused organs may have been inhibited by reduced flow rates.

An attempt to study the incorporation of acetate-1-^{14}C into progesterone by the addition of .2 μc/ml of the salt to the perfusion media of perfusions 21-26 was inconclusive. Gas chromatographic progesterone peaks of several perfusate serial samples were captured as described previously. Subsequent differential isotope counting by liquid scintillation revealed only occasional counts of ^{14}C. The insufficient number of counts of ^{14}C in conjuction with the excessively large $^{3}H/^{14}C$ ratio (31) required in this case to estimate sample recovery reduced the precision of the study drastically. The only observation that can be made is that sodium acetate-1-^{14}C at the perfusate level was not extensively incorporated into progesterone in this system. It is possible, however,

that the labeled acetate did not enter the proper precursor pool (12).

CHAPTER V

SUMMARY

Corpus luteum function has been examined *in vivo* and by various *in vitro* techniques. Though these methods have supplied useful information, a study of these results suggests that maintenance of the intact organ *in vitro* by perfusion might combine some of the advantages of both. It was the objective of this investigation to examine the applicability of the monocyclic perfusion technique to the study of progesterone synthesis and secretion in the isolated porcine ovary of early pregnancy.

Twenty-six perfusions with citrated gilt or barrow blood were performed in two experiments. During the first fourteen perfusions, the perfusates were collected in two 1 hr fractions. Analysis of these fractions indicated less progesterone was secreted during the second hour of perfusion than during the first. Gonadotropins added to the medium of some perfusions during the second hour had no effect on progesterone secretion.

Eleven of twelve additional non-gonadotropin treated perfusions were completed successfully. The perfusates from these perfusions were collected at 20 min intervals and these serial fractions and the perfused and contralateral non-perfused corpora lutea were assayed for progesterone. Mean synthesis of 104% of the estimated initial endogenous level was observed for the eleven perfusions. Thirty-five

percent of this estimated net synthesis was accumulated in the luteal tissue at termination of the perfusions. The increase in luteal progesterone levels over the initial estimated luteal tissue level was 35.8%/hr in these perfusions and did not differ on this basis from reported values for porcine luteal tissue slice incubations by other workers (6,8). On the basis of average rate of synthesis (μg/gm luteal tissue/hr) the slice preparations were only 73.5% (8) and 63.6% (6) as efficient as the perfused tissue preparations.

The rate of progesterone secretion decreased throughout the perfusions but this decline could not be explained by the estimated endogenous gonadotropin level of the perfusion medium. A decrease in the perfusate flow rate was not the cause of decreased progesterone secretion, for flow rates varied only slightly throughout the experiment.

The isolated perfused porcine ovaries of early pregnancy were 8.6 times less active than comparable organs _in vivo_ (3) when the rate of activity was based on the average secretion of progesterone/gm luteal tissue/min. When based on the secretion of progesterone/gm luteal tissue/ml of perfusate or plasma the perfused organs were only 2.8-2.9 times less active than similar organs _in vivo_ (3,25). Although _in vitro_ systems are not usually as efficient as _in vivo_ systems the low flow rate of the perfused ovary when compared to reported _in vivo_ flow rates (3) may offer an explanation for the considerably reduced biosynthetic capacity of the perfused organs.

The perfused organs did, however, synthesize progesterone even after being held for prolonged periods at reduced temperatures and nutrient support, and this synthesis appeared to be as efficient as in other _in vitro_ systems. It is probably that with further experimenta-

tion the efficiency of the system could be greatly improved. This basic information concerning the activity of the isolated perfused porcine ovary of early pregnancy indicates that this technique could be used to study the regulation and biosynthetic activity of endocrine organs.

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APPENDIX

CONCENTRATED ACD SOLUTION

Sodium Citrate	77.0 gm
Citric Acid	28.0 gm
Dextrose	85.7 gm

Dilute to 3,500 ml with distilled water

pH adjusted to 7.4

DILUTE ACD SOLUTION

Dilute 480 ml of concentrated ACD solution with 2,520 ml of 0.85% NaCl. Buffer with 60 ml of phosphate buffer. pH adjusted to 7.4

PHOSPHATE BUFFER

Solution #1. KH_2PO_4 (m.w. 136). Dissolve 9.08 gm in 1 L of distilled water.

Solution #2. Na_2HPO_4 (m.w. 141). Dissolve 9.47 gm in 1 L of distilled water.

For 60 ml of phosphate buffer, mix 11.5 ml of Soln. #1 with 48.5 ml of Soln. #2.

VITA

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