OXYGEN UPTAKE IN ATTACHMENT SITE TISSUES OF THE BOVINE PLACENTA

By

John Arthur Holt

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Thesis Approved:

L E McDonald
Thesis Adviser

Larry Ewing

D P Durham
Dean of the Graduate College

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

The placenta performs several functions during pregnancy. Such functions include serving as an area through which substances from the maternal circulation can pass to the fetal circulation and visa versa (Hagerman and Villee, 1960). Another function is to produce and or modify hormones (Diczfalusy, 1964). Little is known about the mechanisms initiating parturition. The placenta is a transient organ that goes through definite biochemical (Villee, 1956) and morphological (Wislocki, 1956) changes during gestation. Suggestions that the placenta, as a consequence of placental senescence with regard to some aspects of carbohydrate metabolism (Villee, 1956), may be the site from which parturition is initiated, are due for re-evaluation in light of more recent studies on placental carbohydrate (Walker et al., 1967) and steroid metabolism (Diczfalusy, 1964). Unique functions of the placenta appear to be the aromatization of estrogens and the production of protein gonadotrophins, which, either directly or indirectly, serve to maintain uterine conditions that are appropriate for gestation and then the expulsion of the fetus (Page, 1967).

Theoretical consideration suggests that the total energy requirements of the placenta would be the sum of energy requirements for all specific functions the placenta contributes in gestation plus energy required for placental maintenance. Changes in placental function could

be expected to result in changes in placental energy requirements. Thus quantitation of oxygen uptake at various stages of gestation might be used as an index of functional changes that occur in the placenta as gestation approaches term.

The studies reported here were designed to determine functional changes that occur in the bovine placenta from the sixth month of gestation to term.

Materials and methods are presented in more than customary detail in order that the thesis might have some value as a reference source for any future manometric studies of attachment site tissues of the bovine placenta.

CHAPTER II

REVIEW OF PERTINENT LITERATURE

Hellman et al. (1950) and Campbell et al. (1966) credit the first few attempts to measure oxygen uptake by the placenta to Rech (1924), Kurstner and Sudentopf (1929), and Budelmann (1929), all of whom used human placenta and a technique whereby the organ was perfused with blood containing a known amount of oxygen. Manometric techniques on the Warburg apparatus were first employed to study placental oxygen uptake and carbohydrate metabolism by Murphy and Hawkins (1925) and Loeser (1932). Since then many other workers have used similar manometric techniques either alone or in combination with techniques measuring other parameters of placental metabolism (Wang and Hellman, 1943; James et al., 1948; Hellman et al., 1950; Villee, 1953, 1956; Kyank, 1954; MacKay, 1958; Friedman and Sachtleben, 1960; Page, 1960; and Baird, 1963). Recently oxygen uptake by individual placental cotyledons in vivo has been measured in the ewe by infusion techniques (Campbell et al., 1966).

Comparisons of absolute values for oxygen uptake from the different studies are difficult to make because of variations in experimental procedures, particularly with regard to species, gas phase, buffer system, age of tissue and manner of tissue preparation. Preferred buffer systems for oxygen uptake by placenta tissue utilize phosphate at pH 7.4 as the primary buffering agent (Hellman et al., 1950; Villee, 1953; Friedman and Sachtleben, 1960). Both human and rat placenta tissue show variations

in glucose uptake, lactic acid production and response to adrenaline depending upon whether a bicarbonate or phosphate buffer is used (Ginsburg and Jeacock, 1967). Stadie-Riggs preparations have been used by at least two groups of investigators (Villee, 1953; Friedman and Sachtleben, 1960), while teased preparations have been used by others (Hellman et al., 1950; MacKay, 1958). Baird (1963) used chopped placental tissue suspensions. Campbell et al. (1966) used isolated intact cotyledons infused in vivo. Representative values for oxygen uptake by term placenta tissue reported in the various studies are 2 µl/mg dry wt/hr (Friedman and Sachtleben, 1960) and 3 µl/mg dry wt/hr (Hellman et al., 1950) for human placental tissue, 0.3 µl/mg tissue dry wt/hr (Baird, 1963) for cow placental tissue, and 0.6 µl/mg tissue dry wt/hr (Campbell et. al., 1966) for sheep placental tissue. In the last case the authors acknowledged that their estimate was probably low.

Use of atmospheric gas instead of gas phases with high oxygen content has been reported to cause an 8% to 16% decrease in oxygen uptake (Friedman and Sachtleben, 1960).

Data obtained by Friedman and Sachtleben (1960) indicate that sample aging may be of significant importance in metabolic studies of the human placenta. Their oxygen uptake rates show a parabolic decrease as time from sample collection increases. The progressive loss of oxygen uptake rose sharply at first (10.4% the first hour) but gradually diminished toward a plateau of 42.5% reduction from the initial metabolic activity. Though refrigeration at 5^{o} C retarded loss of oxygen uptake somewhat (from 16.1% the first hour at 37^{o} C to 12.4%), they considered data from stored tissues to be of questionable value.

CHAPTER III

MATERIALS AND METHODS

Tissue Collection

Fetal (cotyledonary) and maternal (caruncular) tissues were collected from cows at slaughter. The time interval between administration of the stunning blow and placing the tissues on ice ranged from 15 to 40 minutes. On obtaining the uterus, an incision on the outside curve of the pregnant horn was made and the fetus pulled out. The age of the fetus was estimated using crown rump measurement and fetal development criteria by Roberts (1956). The umbilical cord was grasped and the placenta and uterus were turned inside out. Attachment sites which had good vascularization and which were adjacent to the principal arteries and veins were selected for collection. The cotyledon was separated from the caruncle before collection. Following separation, the cotyledon and caruncle tissues were cut free from the remaining tissues and were immediately placed in cold 0.9% saline (150 ml) in 18 ounce polyethylene sample bags (Whirl-Pak)[1] from which excess air was removed. The sample bags were then sealed and buried in ice. Transport of the tissues from the slaughter house to the laboratory required from 75 to 105 minutes.

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[1]W. H. Curtin and Co., Tulsa, Okla.

Tissue Preparation

Preparation of Fetal Tissue

Intact Villi. A 100 or 200 ml round-bottomed flask was covered with a paper towel which was held in place over the round end by elastic bands placed around the flask neck. The separated fetal tissue was recovered from the cold saline and draped, with the villi out, over the round-bottomed flask. The flask was then turned to a horizontal position and cold saline poured over the villi to orient them in a single direction. Using scissors and beginning at the upper edge, villi were cut off at their bases. Cold saline was used to wash the cut villi into a Petri dish resting on ice. Approximately 4 to 6 medium-sized villi were allowed for each reaction vessel.

Chopped Villi Suspension. Preparation of chopped tissue suspension was done with the aid of the McIlwain Tissue Chopper[2] as described by Baird (1963). Intact villi preparations were made as described above and then chopped into 0.156 mm slices by the McIlwain Chopper[3]. The tissues were chopped in two directions, the second direction at 90° from the first. Tissue suspension for the individual reaction vessels was obtained by

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[2]M. Mickle, Gomshaw, Surrey, England.

[3]A doubled-edged stainless steel razor blade (Gillette) mounted in the holding fixture served as the cutting edge. The cutting edge of the razor blade is approximately 3.5 cm long, therefore the tissues to be chopped should lie within a 3 cm^2 area. Satisfactory chopped tissues were obtained by placing the tissues on a 6 x 6 cm x 0.75 mm thick (3 single sheets) pad of saline-soaked paper towels (Garland, Sof-Knit, Single Fold Towels, Fort Howard Paper Co., Green Bay, Wisc.). The paper towels should be firmly pressed together between the palm of the hand and the table top before the tissues are placed on the pad. Failure to obtain a satisfactory cutting pad results in either incompletely cut tissues or extensive amounts of chopped paper toweling in the tissue preparation.

placing approximately 250 mg chopped tissue in 3 ml 0.9% saline at 0° C in 10 x 1 cm culture tubes. The tubes were shaken by hand to obtain an even suspension. A Pasteur Pipette, precalibrated for 1.5 ml with the tip broken off and fire-polished to provide a 3 mm diameter opening, was inserted into the suspension and 1.5 ml suspension withdrawn. The suspension was then emptied in a reaction vessel containing 1.5 ml Baird-(KOH) or Baird-(NaOH) buffer at pH 7.4 (see Appendix A).

Preparation of Maternal Tissue

Caruncular Slices. The caruncle was placed with the medullary portion down on a double layer of paper toweling soaked in cold saline and a portion of the tissue cut out that was approximately 5 cm long, 1.5 cm wide and as deep as the tissue was thick. Excess connective and vascular tissues in the medullary portion were trimmed off. Slices of maternal tissue were cut in the Stadie-Riggs Hand Microtome[4] in such a manner that they were approximately 5 cm long and contained both central and outer cortical tissue. The thickness of the slices was approximately 0.5 mm. Care was taken not to apply excess pressure on the microtome. Slices were removed from the microtome and placed in a Petri dish containing cold 0.9% saline and resting on ice. Large fragments of fetal tissue appearing in the maternal slices were removed as gently as possible. Approximately 2 slices of the prepared tissue were placed in a reaction vessel.

Chopped Caruncle Suspension. Initial steps for preparation of chopped caruncle suspension were identical to those described for the preparation

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[4]Arthur H. Thomas Co., P.O. Box 779, Philadelphia, Pa.

of caruncular slices except that slices 3 to 4 mm thick were cut using the Stadie-Riggs Microtome. These slices were then chopped using the same techniques as with fetal tissue. Tissue suspensions in the reaction vessels were prepared with the same techniques employed with fetal tissue.

Reaction Vessel Preparation

Filter paper (Whatman #1, chromatography grade) wicks 2.5 x 1.3 x 1.3 cm were folded into accordion shape and placed in the center well of all reaction vessels. A thin coating of petroleum jelly was applied to the upper inside surface of the well above the wick. Petroleum jelly-greased stop-cocks were placed in the shut position in the sidearm openings of all reaction vessels and held in place with elastic bands. With minimum delay, freshly prepared buffer (3 ml, room temperature, see Appendix A for buffer preparation) was pipetted into the appropriate reaction vessels; following this 0.1 ml 20% KOH was placed in the center well using a 1 cc tuberculin syringe. If tissues were added they were put into the reaction vessel just before addition of the 20% KOH. Reaction vessels were affixed to the respirometer as soon as possible after addition of the KOH. Variations in reaction vessel preparation were instituted in cases where the experimental design called for it and are noted in Results. Unless otherwise specified the term "control reaction vessel" refers to a reaction vessel that contained all required materials except the tissue. Reaction vessels which received tissue suspensions received only 1.5 ml buffer. The 1.5 ml of tissue suspension added to the 1.5 ml buffer brought the final fluid volume in reaction vessels with suspensions to 3 ml.

Preparation and Incubations on the Gilson Respirometer[5]

Two Model G20 Gilson Medical Electronics differential respirometers[6] were available for the studies on placental respiration. Regardless of the gas phase employed, water bath temperatures were set at 37° C and the bath allowed to equilibrate for a minimum of 1 hour with the stirring motor on. Frequency of the shaking arm was set at 160 cycles per minute with a stroke length of 1 cm. Micrometers were set at an initial reading of 100. The adjustable index lines were placed so the meniscus of the manometer fluid rested on top of the black line. Prior to each use, the manifold side of the manifold-reaction vessel joint was coated with a layer of petroleum jelly.

Time zero of an incubation was marked when vessels were lowered as simultaneously as possible into the 37° C water bath and the shaking motor turned on. A 15 minute equilibration period was allowed with the gassing inlet valve and master valve lever in the up position assuring that the operating valves were open. Equilibration was terminated by closing the gassing manifold valve and simultaneously pushing the master valve lever down thereby closing all the operating valves.

Changes in gas volume were recorded at 15 minute intervals over 1 1/2 hours. Approximately 1 minute prior to reading time the manometer fluid meniscus was adjusted to slightly above the indicating line. Then, at the exact reading time, final adjustments were made. Because of the

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[5]The Model G20 Gilson respirometer has components composed of Tygon tubing. The significance of Tygon tubing with regard to oxygen uptake assays is discussed in detail in Chapter V, "Discussion".

[6]Gilson Medical Electronics, 3000 West Beltline Highway, Middleton, Wisc.

common reference flask, vessels with considerable changes in gas volume had to be adjusted to their original position, or their change in volume was reflected in the readings of other vessels. Counting equilibration time, reaction vessels were in the water bath for 105 minutes. Vessels with malfunctions occuring during the run were closed off by closing the front lever of the disconnecting valve.

An incubation was terminated by opening the gassing manifold valve and opening the master valve lever. Vessels were lifted from the water bath and examined for evidence of leakage before being dismounted.

Incubation Procedures on the Warburg Apparatus

Calibration of the respirometers at the 150[7] mark was done with mercury as described by Umbreit et al. (1964). Calculations for flask constants were performed as suggested by Umbreit et al. (1964). Brodie's Manometer Fluid (Umbreit et al., 1964) was used in the manometers. Water bath temperature was regulated at 37° C.[8] Reaction vessels were shaken at 132 cycles per minute with a stroke length of 1.4 cm measured at the level of the reaction vessel.

When a gas phase of atmosphere was used respirometers were allowed to equilibrate 15 minutes with the reaction vessels immersed in the 37° C water bath accompanied with shaking and with the manometer stopcocks open. Sidearm stopcocks were in the closed position and remained so. At the end of the equilibration period the manometers were checked to

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[7]Experience showed calibration to the 200 or 250 mark would have been preferable.

[8]Water baths of both the Gilson and Warburg respirometers were within 0.1° C of each other when measured by the same thermometer.

make sure the menisci of both manometer fluid columns were at the 150 mark and the left meniscus was read, then the stopcocks were closed.

In experiments involving reaction vessels that contained gas phases other than atmosphere, the reaction vessels were mounted with sidearm stopcocks open and with the manometer stopcock open vertically.[9] Reaction vessels not to be gassed were mounted with the sidearm stopcocks closed and the manometer stopcocks open to the side outlet. When all respirometers were on the apparatus, a manifold was attached at the top opening of all manometers and the gas turned on sufficiently strong to cause a 100 to 150 mm rise in the Brodie's solution. Individual reaction vessels were checked to see if they were flushing by turning their stopcocks to an off position. If the vessels were flushing, that is if the sidearm stopcocks were open, so as to permit gas to flush through, then the manometer fluid returned to equal heights immediately; if not, that is if the sidearm stopcocks were closed, thereby preventing gas to flush through, the pressure was maintained.

At the end of 10 minutes flushing the gas was turned off at the tank and the gassing hose disconnected at the tank. Sidearm stopcocks were then immediately closed. Five minutes equilibration was then allowed through the gassing manifold and hose. When equilibration time was completed, assay procedures proceeded exactly as described for the Warburg apparatus using atmosphere as the gas phase.

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[9]When mounting these respirometers if the respirometer is viewed from the top the viewer should be able to see down through the stopcock hole and to the Brodie's solution beyond. Respirometers that are not to be gassed are mounted so that as the operator looks in the side opening of the manometer top, the hole of the stopcock should be seen and should open on the other end to the space above the Brodie's solution.

Sample Processing Following Incubation

As the vessels were dismounted, excess petroleum jelly and water were wiped from the reaction vessel neck. Center wells of the discounted flasks were packed with strips of disposable wipers.[10] Intact villi or sliced tissue in the reaction vessels was removed with the aid of small pointed forceps and given two rinses in distilled water contained in Petri dishes. After the rinses in distilled water the tissue was blotted on paper toweling and placed in tared[11] vials (discarded liquid scintillation vials) which were then placed for 18 hours in a drying oven at 110-120° C. At the time the weighted vials were removed from the drying oven they were corked with polyethylene corks[12] and placed immediately into a desiccator for cooling. A minimum of 4 hours was allowed for cooling before dry weights were determined.[13]

Buffer solution remaining in the reaction vessels after removal of tissue was decanted into 1 x 7 cm vials (discarded Sigma DPNH vials) for

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[10]Type 900, Kimberly-Clark Corp., Neenah, Wisc.

[11]All weights in the experiments were determined on a Mettler Balance, Model B6, and could be estimated to the nearest 0.01 mg. Mettler Instrument Corp., Heightstown, N. J.

[12]#1 polyethylene stoppers, cap type, W. H. Curtin and Co., Tulsa, Okla.

[13]An important detail in obtaining repeatable weights consisted of loosening the polyethylene corks to release the vacuum occurring in some of the cooled vials. Failure to release the vacuum resulted in weights 1-5 mg lighter than expected. This phenomenon was first noticed when control vials showed inconsistent weights.

pH determination.[14]

Atmospheric pressure and water bath temperature were noted at the beginning and end of each run.

Procedures for using gas phases of either 95% O_2-5% CO_2 or 95% N_2-5% CO_2 in the Gilson respirometer are given in the footnotes to Tables I and II.

Calculation of Gilson Respirometer Data

The quantity of gas change for an hour in a particular experimental reaction vessel was obtained by subtracting the 30 minute micrometer reading from the 90 minute reading. The average change of control vessels over the same period was then added or subtracted from the experimental vessel to give a corrected hourly gas change. The following formula[15] was used for converting corrected hourly gas change to standard conditions:

$$(\text{corrected reading}) \frac{(273)(P_b - 3 - P_w)}{(t + 273)(760)} = \text{STP } \mu\text{l/hr}$$

where

t = water bath temperature

P_b = barometric pressure (with 3 being subtracted to compensate for the specific gravity of Hg at room temperature)

P_w = pressure of water vapor (at 37° C the value is 47.1).

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[14]Coleman Metrion II pH meter fitted with a Coleman 3-650 electrode, combination tripurpose. Standardization of the meter was performed at 2 pH's, pH 4.0 (Coleman certified buffer tablets Catalog No. 1-020), pH 7 (Buffer Solution, W. H. Curtin and Co., Tulsa, Oklahoma) Coleman Instru., Inc., Maywood, Ill.

[15]Adapted from Gilson Medical Electronics, undated instruction manual.

The STP μl/hr value was then divided by the tissue dry weight to give STP μl/mg tissue dry wt/hr.

Calculation of Warburg Apparatus Data

Data obtained from the Warburg apparatus were converted to STP μl/mg tissue dry wt/hr with the procedures of Umbreit et al. (1964) with the exception that both thermobarometer and experimental reaction vessels were multiplied by their respective flask constants before the experimental reaction vessel was corrected for the thermobarometer reading, thus eliminating the error stemming from differences in flask constants between reaction vessels.

Determination of Per Cent Dry Weight

Fresh tissues, either caruncular slices or intact fetal villi preparations, were blotted on paper toweling and placed in individual preweighed vessels and weighed to the nearest 0.1 mg. Tissues and weighing vessels were then placed in a drying oven at 110-120° C for 18 hours. After 18 hours the tissues and vessels were removed from the oven, loosely capped and placed in a desiccator for cooling, after which weighing was repeated.[16] The dry weight was divided by the wet weight and multiplied by 100 so that the final value represented dry weight as a per cent of the wet weight.

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[16]An important detail in obtaining repeatable weights consisted of loosening the polyethylene corks to release the vacuum occurring in some of the cooled vials. Failure to release the vacuum resulted in weighing 1-5 mg lighter than expected. This phenomenon was first noticed when control vials showed inconsistent weights.

Other Considerations

To obtain data on changes in oxygen uptake by the placenta at different stages of gestation an *in vitro* assay was used. The Gilson differential respirometer was chosen in preference to the Warburg apparatus for assaying oxygen uptake for several reasons. Two Gilson respirometers were available providing a total of 40 reaction vessels as opposed to a single Warburg apparatus having 20 reaction vessels. In studies involving the effects of storage on tissue, or when comparisons between immediately prepartum and postpartum samples are needed, a large assay capacity in a given time interval is mandatory. Mounting and immersion of reaction vessels on the Gilson respirometer are simpler and more rapid than on the Warburg appartus. Closing and opening of valves of all reaction vessels can be performed simultaneously on the Gilson respirometer which facilitates operating two respirometers simultaneously. Operation of the Warburg apparatus requires opening and closing of reaction vessels valves be done to each vessel individually and is a time-consuming operation at a critical time. The reaction vessels on the Gilson respirometer are interchangeable and do not require calibration whereas those of the Warburg apparatus are not and require calibration. On the Gilson respirometer read out is faster than on the Warburg apparatus.

CHAPTER IV

RESULTS

Evaluation of the Oxygen Uptake Assay

Three aspects of the assay system were studied: (1) gas phase in the reaction vessels; (2) buffer used as the suspending medium; and (3) tissue preparation and storage time.

Gas Phase in the Reaction Vessels

Extensive volume changes in the gas phase of control reaction vessels occurred when the Gilson differential respirometer was gassed with 95% O_2-5% CO_2 (Table I) or 95% N_2-5% CO_2 (Table II). Comparatively small gas volume changes occurred in the Gilson respirometer when atmosphere is the gas phase (Tables III and IV).

A comparison of data obtained from the Gilson respirometer with data from the Warburg apparatus when both types of respirometers were operated with gas phases of atmosphere (Table V) indicated no obvious differences of oxygen uptake by bovine placental tissues. Because of Tygon tubing components of the Gilson respirometer[1], experiments to evaluate the effects on oxygen uptake by attachment site tissues under atmosphere as opposed to 95% O_2-5% CO_2 as a gas phase were performed on the Warburg

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[1]Details on the significance of Tygon tubing components of the Gilson respirometer are presented in Chapter V, "Discussion".

TABLE I[1]

GAS VOLUME CHANGES RECORDED FROM CONTROL REACTION VESSELS MOUNTED ON GILSON DIFFERENTIAL RESPIROMETERS AND GASED WITH 95% O_2-5% CO_2[2]

Contents of Control Vessels	Respirometer	n	Cumulative Changes in Gas Volume at 10 Minute Intervals Expressed in µl ± Standard Error[3]					
			10	20	30	40	50	60
empty	E	4	-7.8 ± 1.0	-10.9 ± 1.4	-12.6 ± 1.6	-17.7 ± 1.4	-20.5 ± 1.0	-24.5 ± 0.9
	G	2	-3.0 ± 1.2	-3.5 ± 1.5	-3.0 ± 2.8	-2.0 ± 5.2	-5.8 ± 5.4	-6.8 ± 6.0
0.1 ml 10% KOH in center well	E	5	164.0 ± 9.2	181.6 ± 10.6	182.4 ± 12.0	180.8 ± 13.0	176.8 ± 13.7	173.0 ± 14.4
	G	3	168.6 ± 5.7	185.2 ± 10.0	189.8 ± 13.0	188.6 ± 15.0	184.5 ± 17.1	181.9 ± 17.3
2 ml Krebs-Ringer phosphate Ca^{++} free buffer pH 7.4	E	5	-2.2 ± .7	-6.3 ± 1.6	-10.4 ± 2.1	-17.6 ± 2.0	-24.7 ± 3.1	-31.5 ± 3.4
	G	5	-3.7 ± 1.1	-6.6 ± 2.7	-12.2 ± 3.3	-18.1 ± 3.9	-23.7 ± 4.6	-29.4 ± 4.8
2 ml Krebs-Ringer phosphate Ca^{++} free buffer pH 7.4; 0.1 ml ml 10% KOH in center well	E	3	-3.6 ± 1.8	-10.2 ± 2.2	-16.2 ± 2.8	-23.4 ± 3.7	-31.4 ± 4.4	-38.0 ± 4.8
	G	3	-1.3 ± 2.5	-4.6 ± 4.0	-9.8 ± 5.0	-15.8 ± 6.2	-21.5 ± 6.9	-25.4 ± 8.2

[1]Data of Tables I, II, and III, are presented for comparative purposes in Table IV.

[2]Two Gilson respirometers, E and G, both the same model, were tested separately. Five of the 20 reaction vessels of each respirometer were distributed at random to each of the 4 treatments. Reaction vessels were mounted and immersed with sidearm stopcocks open. Ten minute gassing was done via the gassing valve; the gas was then turned off at the tank and the sidearm stopcocks closed. After 5 minutes equilibration with the gassing valve open, the gassing and operating valves were closed. Thereafter readings were made at 10 minute intervals. Water bath temperature was 37° C.

[3]Calculated using method of Mantel (1951).

TABLE II[1]

GAS VOLUME CHANGES RECORDED FROM CONTROL REACTION VESSELS MOUNTED ON GILSON DIFFERENTIAL RESPIROMETERS GASED WITH 95% N_2-5% CO_2[2]

Contents of Control Vessels	Respirometer	n	Cumulative Changes in Gas Volume at 10 Minute Intervals Expressed in µl ± Standard Error[3]					
			10	20	30	40	50	60
empty	E	5	0.2 ± 0.2	4.3 ± 1.4	7.4 ± 1.5	11.5 ± 1.6	12.6 ± 1.9	17.7 ± 2.1
	G	5	9.4 ± 4.0	18.6 ± 5.7	27.4 ± 7.7	34.8 ± 10.0	40.9 ± 11.4	46.5 ± 12.1
0.1 ml 10% KOH in center well	E	5	Within 40 minutes, all reaction vessels registered more than 200 µl change, the capacity of the system under these circumstances.					
	G	5						
2 ml Krebs-Ringer phosphate Ca^{++} free buffer pH 7.4	E	5	2.3 ± 0.8	5.2 ± 1.5	4.9 ± 1.7	5.4 ± 2.3	4.9 ± 2.3	3.6 ± 2.1
	G	5	7.2 ± 1.3	12.3 ± 1.1	14.7 ± 0.9	15.9 ± 1.0	16.7 ± 1.3	16.5 ± 1.3
2 ml Krebs-Ringer phosphate Ca^{++} free buffer pH 7.4; 0.1 ml ml 10% KOH in center well	E	5	Within 20 minutes all reaction vessels registered more than 200 µl change, the capacity of the system under these circumstances.					
	G	5						

[1]Data of Tables I, II, and III, are presented for comparative purposes in Table IV.

[2]Two Gilson respirometers, E and G, both the same model, were tested separately. Five of the 20 reaction vessels of each respirometer were distributed at random to each of the 4 treatments. Reaction vessels were mounted and immersed with sidearm stopcocks open. Ten minute gassing was done via the gassing valve; the gas was then turned off at the tank and the sidearm stopcocks closed. After 5 minutes equilibration with the gassing valve open, the gassing and operating valves were closed. Thereafter readings were made at 10 minute intervals. Water bath temperature was 37° C.

[3]Calculated using method of Mantel (1951).

TABLE III[1]

GAS VOLUME CHANGES RECORDED FROM CONTROL REACTION VESSELS MOUNTED ON GILSON DIFFERENTIAL RESPIROMETERS WITH A GAS PHASE OF ATMOSPHERE[2]

Contents of Control Vessels	Respirometer	Trial	n	Cumulative Changes in Gas Volume at 15 Minute Intervals Expressed in µl ± Standard Error[3]			
				15	30	45	60
empty	E	1	2	4.1 ± 0.8	4.6 ± 0.2	4.6 ± 0.9	3.7 ± 1.3
		2	1	4.0	4.1	7.5	6.1
	G	1	2	7.7 ± 1.4	8.8 ± 1.6	9.1 ± 1.3	9.1 ± 1.3
		2	2	7.0 ± 1.4	7.8 ± 0.2	8.2 ± 0.0	8.2 ± 0.1
	$\bar{x}$			5.7 ± 0.9	6.3 ± 1.2	7.4 ± 1.1	6.8 ± 1.4
0.1 ml 10% KOH in center well	E	1	2	9.8 ± 1.7	11.2 ± 2.0	10.6 ± 7.1	9.9 ± 8.8
		2	3	12.0 ± 0.9	10.7 ± 0.8	10.3 ± 1.8	7.9 ± 1.3
	G	1	3	23.2 ± 4.2	22.8 ± 5.6	20.6 ± 6.1	19.6 ± 6.9
		2	2	12.4 ± 2.3	12.7 ± 1.7	8.5 ± 2.5	7.2 ± 2.3
	$\bar{x}$			14.4 ± 3.4	14.4 ± 3.0	12.5 ± 2.3	11.2 ± 3.1
3 ml Krebs-Ringer phosphate Ca^{++} free buffer pH 7.4	E	1	2	-1.0 ± 0.9	0.0 ± 0.7	0.7 ± 0.8	1.7 ± 0.2
		2	2	0.4 ± 0.6	1.6 ± 0.6	2.5 ± 1.4	3.5 ± 0.2
	G	1	2	5.8 ± 0.3	7.1 ± 0.2	8.1 ± 0.0	9.0 ± 0.0
		2	2	2.5 ± 0.7	4.3 ± 0.8	4.6 ± 1.7	4.7 ± 2.4
	$\bar{x}$			1.9 ± 1.7	3.2 ± 1.8	4.0 ± 1.8	4.7 ± 1.8
3 ml Krebs-Ringer phosphate Ca^{++} free buffer pH 7.4; 0.1 ml 10% KOH in center well	E	1	3	-3.5 ± 0.7	-5.4 ± 0.7	-6.3 ± 0.6	-6.9 ± 0.9
		2	3	-3.2 ± 0.8	-6.4 ± 1.2	-7.2 ± 1.4	-7.6 ± 1.0
	G	1	3	-0.3 ± 0.1	-3.0 ± 0.2	-3.4 ± 0.2	-4.0 ± 0.5
		2	3	-1.7 ± 0.5	-3.9 ± 0.7	-5.5 ± 0.5	-5.7 ± 0.4
	$\bar{x}$			-2.2 ± 0.8	-4.7 ± 0.8	-5.6 ± 0.7	-6.0 ± 0.9

[1]Data of Tables I, II, and III, are presented for comparative purposes in Table IV.

[2]Two Gilson respirometers, E and G, were tested twice independently. Five of the 20 reaction vessels of each respirometer were distributed at random to each of the 4 treatments. Details of assay procedures are given under Materials and Methods.

[3]Calculated using the method of Mantel (1951).

TABLE IV

SUMMARY OF GAS VOLUME CHANGES IN CONTROL REACTION VESSELS ON THE GILSON DIFFERENTIAL RESPIROMETER FOR THREE TYPES OF GAS PHASES

Contents of Control Vessel	Gas Phase[1]		
	95% O_2-5% CO_2	95% N_2-5% CO_2	Atmosphere
empty	-15.6 ± 8.8[2]	32.1 ± 17.4	6.8 ± 1.4
0.1 ml KOH in center well	177.4 ± 4.4	more than -200.0	11.2 ± 3.1
2-3 ml Krebs-Ringer phosphate Ca^{++} free buffer, pH 7.4	-30.4 ± 1.0	10.0 ± 6.4	4.7 ± 1.8
0.1 ml KOH in center well; 2-3 ml Krebs-Ringer phosphate Ca^{++} free buffer, pH 7.4	-31.7 ± 6.3	more than -200.0	-6.0 ± 0.9

[1]The design of the Gilson differential respirometers is such that only one type of gas phase can be employed at one time, therefore the experiments could not include a design where all three gas phases were tested simultaneously on the same respirometer.

[2]All values are expressed in µl ± standard error and represent total changes for 60 minute incubations. Procedures for the experiments are footnoted to Tables I, II, and III.

TABLE V

OXYGEN UPTAKE[1] BY PLACENTAL ATTACHMENT SITE TISSUES ON TWO TYPES OF RESPIROMETERS UNDER A GAS PHASE OF ATMOSPHERE

Month of Pregnancy			6		7		8		9	
Cow			1	2	3	4	5	6	7	8
Respirometer	Tissue	n								
Warburg	Cotyledon	3	3.0 ± .3	3.9 ± .1	3.2 ± .7	2.9 ± .3	4.4 ± .1	3.7 ± .2	3.4 ± .2	5.2 ± .3
Gilson	Cotyledon	4	3.2 ± .4	4.2 ± .4	3.2 ± .1	2.7 ± .1	4.4 ± .3[2]	3.9 ± .2	3.5 ± .6[2]	4.0 ± .1
Warburg	Caruncle	4	1.2[3]	1.4 ± .3[2]	1.6 ± .1	2.7 ± .2[1]	1.6 ± .3[2]	2.6 ± .6	1.7 ± 1.3	2.4 ± .3
Gilson	Caruncle	4	1.0 ± .1	2.8 ± .2	1.5 ± .1	2.2 ± .2	1.9 ± .2	2.2 ± .1	1.8 ± .1	2.3 ± .1

[1]All values expressed in STP µl/mg dry wt/hr ± standard error (calculated using method of Mantel, 1951). Tissues for any given cow were from the same attachment site. Following tissue preparation, as similar as possible samples were distributed at random between the two respirometers for assay. Incubation was at 37° C using 3 ml Ca^{++} free Krebs-Ringer phosphate buffer pH 7.4 and 0.1 ml 20% KOH in center well with wick. Cotyledon tissue was incubated as intact villi preparation. Caruncle tissue was incubated as slices.

[2]Only 3 samples.

[3]Only 1 sample.

apparatus (Table VI). Fetal tissue assayed under a gas phase of high oxygen concentration (Table VI) exhibited greater oxygen uptake than comparable samples assayed under atmosphere.

Buffer Used as the Suspending Medium

Two aspects, effect on manometer readings, and changes in pH were studied with regard to three buffers in the Gilson differential respirometer: (1) Krebs-Ringer bicarbonate; (2) Krebs-Ringer phosphate; and (3) Krebs-Ringer phosphate without calcium ions (Ca^{++} free phosphate buffer). Two separate trials on each of two Gilson respirometers (total of four trials) were conducted using atmosphere as the gas phase. In each trial the 20 reaction vessels of a Gilson respirometer were distributed at random among the treatment groups as recorded in Table VII. Details of preparation and assay procedures appear under Materials and Methods. A third aspect, that of which buffer provides optimum performance for the tissue under assay conditions is treated in the last part of this section.

Effect of Buffers on Manometer Readings. During the 105 minute period recordings were made, empty vessels averaged 6.5 µl increase in gas volume, approximately 5.0 µl of which occurred in the first 15 minute interval following equilibration (Figure 1). Vessels without buffer solutions, but containing KOH in the center well had a 14.4 µl average increase in volume during the first 15 minute period following equilibration (Figure 1). After the initial increase, reversal of gas volume change occurred with the gas volume gradually decreasing so that at the end of 105 minutes, average net change in gas volume was an increase of 7.7 µl (Figure 1). Vessels containing bicarbonate buffer but with no

TABLE VI

OXYGEN UPTAKE[1] BY PLACENTAL ATTACHMENT SITE TISSUES UNDER GAS PHASES OF EITHER 95% O_2-5% CO_2 OR ATMOSPHERE ON THE WARBURG APPARATUS

Month of Pregnancy			6		7		8		9	
COW			1	2	3	4	5	6	7	8
Tissue	Gas Phase	n								
Cotyledon (intact villi)	Atmos.	3	3.0 ± .3	3.9 ± .1	3.2 ± .7	2.9 ± .3	4.4 ± .1	3.7 ± .2	3.4 ± .2	5.2 ± .3
	95% O_2-5% CO_2	3	4.1 ± .4	4.8 ± .4	3.9 ± .6	5.8 ± .2[3]	6.2 ± .2	4.9 ± .2	3.5 ± .1	5.5 ± .5
Caruncle (slices)	Atmos.	4	1.2[4]	1.4 ± .3	1.6 ± .1	2.7 ± .3[2]	1.6 ± .3[2]	2.6 ± .6	1.7 ± .1	2.4 ± .3
	95% O_2-5% CO_2	4	1.1 ± .5[3]	1.5 ± .3	1.0 ± .3	2.8 ± .1	1.6 ± .5	3.2 ± .2	2.6 ± .4	3.0 ± .3

[1]Values expressed in STP µl/mg dry wt/hr ± standard error (calculated using the method of Mantel, 1951). Tissues from any given cow were from the same attachment site. Twenty manometers were divided at random among the following treatments: Atmosphere controls = 3; 95% O_2-5% CO_2 controls = 3; cotyledon tissue atmosphere = 3; cotyledon tissue 95% O_2-5% CO_2 = 3; caruncle tissue atmosphere = 4; caruncle tissue 95% O_2-5% CO_2 = 4. Incubation at 37° C in 3 ml Ca^{++} free Krebs-Ringer phosphate buffer pH 7.4 and with 0.1 ml 20% KOH in center well with wick. Ten minutes gassing occurred while vessels were submerged in water bath and shaking. Gassing was terminated and the sidearm stopcocks closed allowing 5 min. equilibration through the gassing manifold with the manometer stopcock open following which the manometers were adjusted to initial readings and the manometer stopcocks closed.

[2]3 samples.

[3]2 samples.

[4]1 sample.

TABLE VII

TREATMENT GROUPS IN STUDIES TO EVALUATE pH STABILITY AND INFLUENCE ON MANOMETER READINGS BY THREE TYPES OF BUFFERS

Number of Vessels[1]	Contents of Vessel
2	empty
3	0.1 ml 20% KOH in center well
2	3 ml Krebs-Ringer bicarbonate, pH 7.6
2	3 ml Krebs-Ringer phosphate, pH 7.4
2	3 ml Krebs-Ringer Ca^{++} free phosphate, pH 7.4
3	0.1 ml 20% KOH in center well 3 ml Krebs-Ringer bicarbonate, pH 7.6
3	0.1 ml 20% KOH in center well 3 ml Krebs-Ringer phosphate, pH 7.4
3	0.1 ml 20% KOH in center well 3 ml Krebs-Ringer Ca^{++} free phosphate, pH 7.4

[1]Reaction vessels and mounting positions on the respirometer were assigned at random. Four replicates of the experiment were performed, the results of which are given in Figures 1, 2, and 3, and Table VIII. Preparations and assay procedures are given under Materials and Methods.

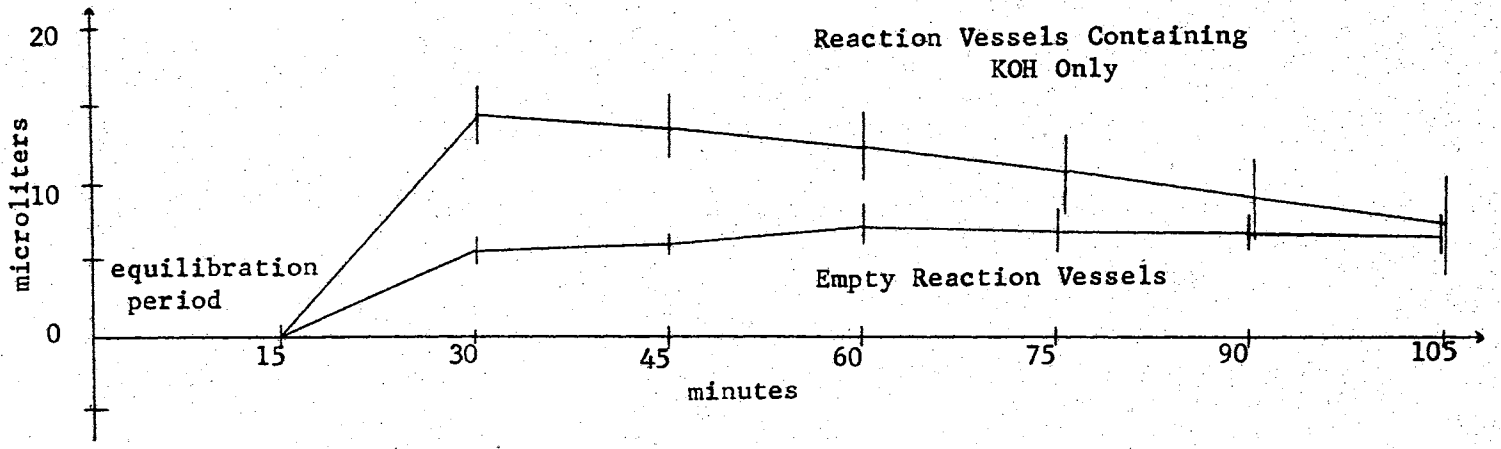

Figure 1. Manometer Readings ± Standard Error in Empty Reaction Vessels and Reaction Vessels Containing KOH Only Incubated on the Gilson Respirometer at 37° C With a Gas Phase of Atmosphere

KOH in the center well averaged 15.6 µl increase of gas volume over the 105 minute period; approximately 11.1 µl of this increase occurred in the first 15 minute interval following equilibration (Figure 2). For the same 105 minute period, vessels with phosphate and Ca^{++} free phosphate buffers but having no KOH in the center well, average 3.9 and 5.6 µl increases respectively (Figure 2). In the 105 minute period recordings were made, vessels with bicarbonate buffer and having no KOH in the center well averaged a 93.4 µl decrease in gas volume, approximately 47.6 µl of which occurred in the first 15 minute time interval following equilibration (Figure 3). Over the same 105 minute period vessels with phosphate and Ca^{++} free phosphate buffers and having KOH in the center well averaged 7.3 and 7.5 µl decreases respectively (Figure 3).

Phosphate buffers either with or without KOH in the center well appear to stabilize gas volume changes in vessels when compared to gas volume changes in vessels containing KOH only, empty vessels, or vessels containing bicarbonate buffer with or without KOH in the center well (Figures 1, 2, and 3).

Changes of pH. Bicarbonate buffer became noticeably alkaline (pH 9.17 and 8.53) during incubation in the reaction vessels (Table VIII). Vessels with KOH in the center well had increased alkalinity (pH 9.17) compared to vessels without KOH in the center well (pH 8.53). Bicarbonate buffer stored on the bench at room temperature in an aluminum foil-covered 50 ml Erlenmeyer flask for 3 1/2 hours became alkaline (ph 8.35) as did bicarbonate buffer stored in a similar manner at 5^{o} C, though to a lesser extent (pH 8.20). The pH of bicarbonate buffer left on the bench top for 10 hours at room temperature in an unstoppered container rose to pH 8.80.

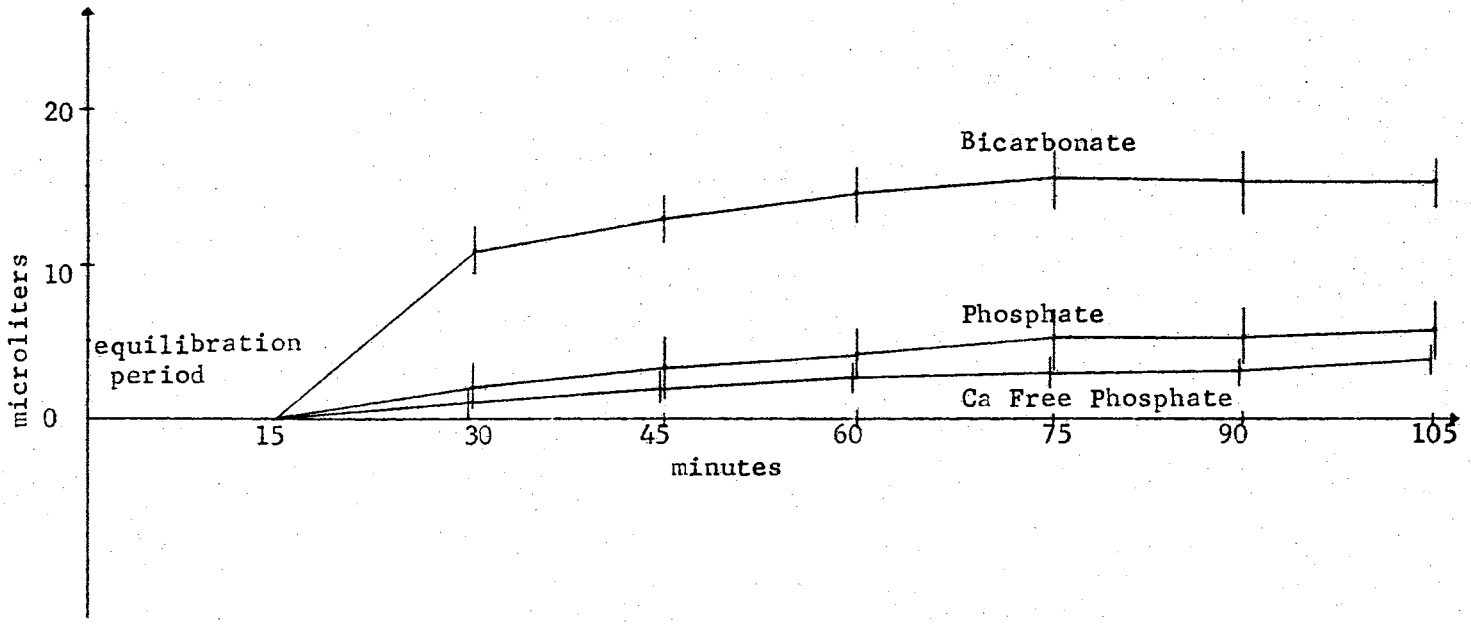

Figure 2. Manometer Readings ± Standard Error in Reaction Vessels Containing Buffers Only Incubated on the Gilson Respirometer at 37° C With a Gas Phase of Atmosphere

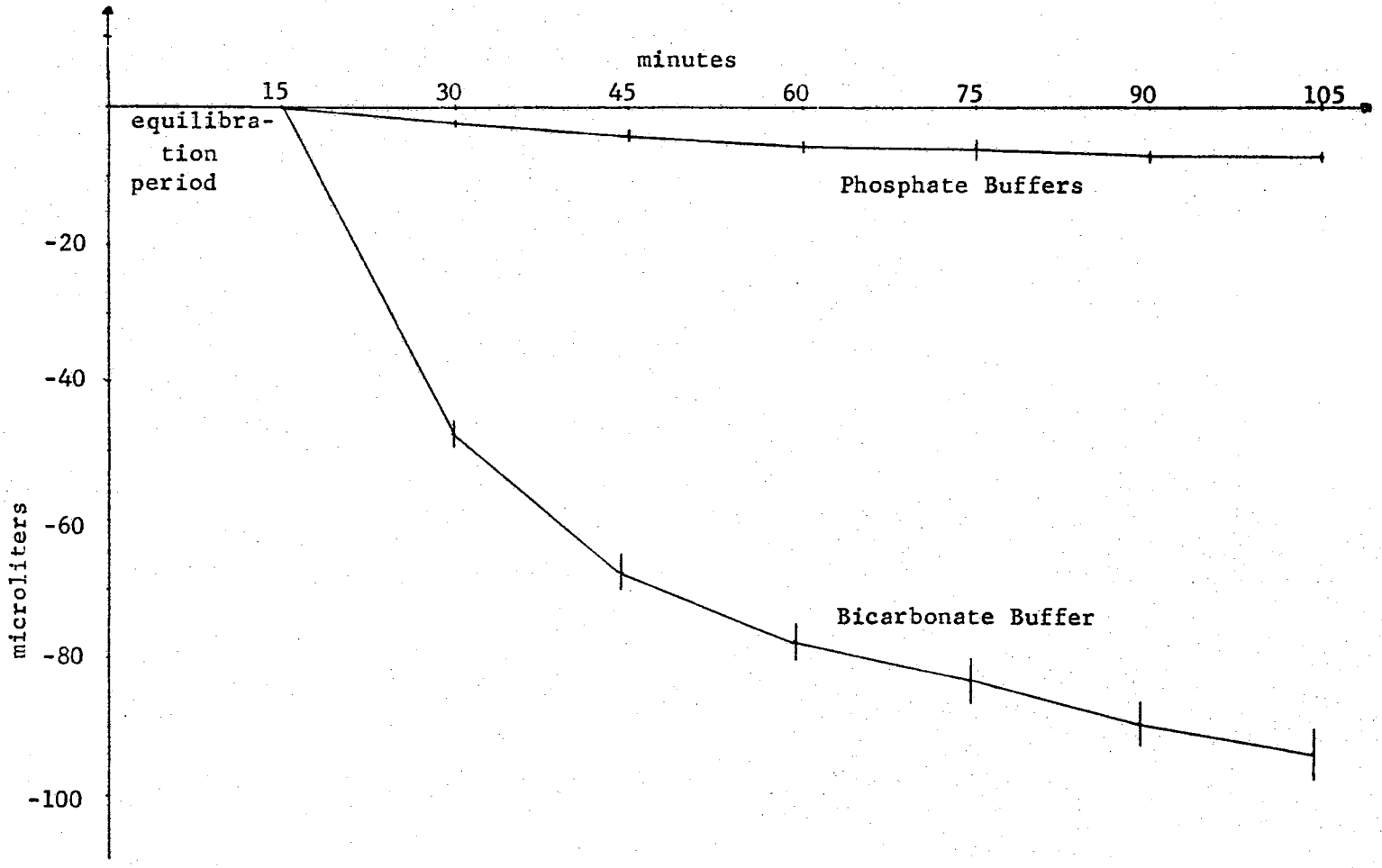

Figure 3. Manometer Readings ± Standard Error in Reaction Vessels Containing Buffers Plus KOH in the Center Well Incubated on the Gilson Respirometer at 37° C With a Gas Phase of Atmosphere

TABLE VIII

CHANGES IN pH OCCURRING IN KREBS-RINGER BICARBONATE (KRB), KREBS-RINGER PHOSPHATE (KRP), AND Ca^{++} FREE KREBS-RINGER PHOSPHATE (Ca^{++} FREE KRP)

			Treatment[3]			
			1. incubated		2. stored	3. stored
Buffer	Trial	Initial pH	0.1 ml 20% KOH in well[1]	no KOH in well[2]	stored 3½ hr. at room temperature[2]	stored 3½ hr. at 5° C[2]
KRB	I	7.60	9.18	8.60	8.30	8.10
	II	7.55	9.18	8.48	8.40	8.20
	III	7.60	9.15	8.55	8.30	8.20
	IV	7.65	9.18	8.50	8.40	8.30
	$\bar{x}$	7.60	9.17	8.53	8.35	8.20
KRP	I	7.40	7.20	7.20	7.30	7.30
	II	7.40	7.17	7.15	7.30	7.30
	III	7.40	7.15	7.15	7.25	7.30
	IV	7.40	7.20	7.20	7.30	7.20
	$\bar{x}$	7.40	7.18	7.18	7.29	7.28
Ca^{++} free KRP	I	7.40	7.48	7.37	7.40	7.30
	II	7.40	7.40	7.40	7.40	7.30
	III	7.40	7.40	7.38	7.40	7.35
	IV	7.40	7.40	7.40	7.40	7.40
	$\bar{x}$	7.40	7.42	7.39	7.40	7.34

[1]The value for each run is an average of 3 reaction vessels.

[2]The value for each run is an average of 2 reaction vessels.

[3]Treatments: 1) incubation at 37° C for 105 minutes in reaction vessels, 2) and 3) storage in capped 50 ml Erlenmeyer Flasks.

Phosphate buffer pH decreased from pH 7.4 (Table VIII) to pH 7.18 following incubation and pH 7.29 following storage. Neither the presence of KOH nor cooling appeared to have great influence on phosphate buffer pH changes. The pH of phosphate buffer stored at room temperature for 10 hours in an unstoppered container fell from pH 7.40 to pH 7.15.

Ca^{++} free phosphate buffer remained near the initial pH 7.40 in all treatments except storage at 5^{o} C for 3 1/2 hours which was accompanied by a small decrease in pH. Thus both phosphate buffer and Ca^{++} free phosphate buffer maintained pH closer to the initial pH 7.40 than did bicarbonate buffer; however, the drop to pH 7.18 of incubated phosphate buffer was noteworthy.

All buffer solutions remained clear except phosphate buffer in which a fine white precipitate began to develop approximately 15 minutes following buffer preparation. 0.1 N HCl readily dissolved the precipitate. The precipitate was insoluble in 0.1 N NaOH.

Effects of Buffers on Tissue Oxygen Uptake. Experiments were run to obtain data on oxygen uptake by slices of caruncular tissue and intact fetal villi preparations suspended in Krebs-Ringer bicarbonate and Krebs-Ringer phosphate (Table IX) prepared as described in Umbreit et al. (1964).

Other possible suspending media besides Krebs-Ringer bicarbonate and phosphate as described in Umbreit et al. (1964) include Ca^{++} free Krebs-Ringer phosphate and Baird's (1963) buffer formula. If Baird's formula for making buffer is followed, a pH of approximately 4.5 results and considerable OH^{-} must be added to bring the pH to 7.4 (see Appendix A). Unfortunately Baird (1963) did not indicate whether NaOH, KOH, or what other source of OH^{-} should be used to adjust the pH. Baird's

TABLE IX

OXYGEN UPTAKE AND TERMINAL pH IN REACTION VESSELS WITH EITHER FETAL OR MATERNAL TISSUE INCUBATED ON THE GILSON RESPIROMETER UNDER ATMOSPHERE IN EITHER KREBS-RINGER BICARBONATE OR KREBS-RINGER PHOSPHATE

Vessel Contents	Fetal (intact villi)						Maternal (Stadie-Riggs slices)						Control Vessels					
Buffer	Krebs-Ringer Phosphate			Krebs-Ringer Bicarbonate			Krebs-Ringer Phosphate			Krebs-Ringer Bicarbonate			Krebs-Ringer Phosphate			Krebs-Ringer Bicarbonate		
Trial[1]	n	gas uptake[2]	pH	n	gas uptake[2]	pH	n	gas uptake[2]	pH	n	gas uptake[2]	pH	n	gas uptake[3]	pH	n	gas uptake[3]	pH
1	4	3.5	7.5	4	1.4	9.0	6	1.9	7.1	5	0.9	9.1	8	4.1	7.4	8	27.6	9.0
2	6	4.5	6.8	5	1.3	9.0	5	2.5	6.8	6	0.8	9.0	8	2.0	6.8	8	27.0	9.0
3	5	4.7	7.2	5	1.5	9.0	4	1.6	7.0	5	0.7	9.0	6	0.0	7.3	5	19.1	9.0
4	5	3.5	6.8	5	1.4	8.7	5	1.6	6.9	5	1.0	8.7	4	2.2	-	4	23.2	8.3
$\bar{X}$	4	4.0	7.1	4	1.4	8.9	4	1.9	7.0	4	0.8	9.0	4	2.1	7.2	4	24.2	8.8
std. error[4]		±.3	±.2		±.0	±.1		±.1	±.1		±.1	±.1		±1.0	±.2		±2.1	±.2

[1]Tissues from a given trial were all from the same cow and attachment site.

[2]Gas uptake expressed as µl/mg dry wt/hr and represents the average uptake over the hour beginning 15 minutes after equilibration and is corrected for control vessel gas volume changes over the same time period.

[3]Values expressed as µl/hr gas volume change.

[4]Calculated using procedure of Mantel (1951).

formula is noticeably lacking in Na^{+} and has an excess of K^{+} (see Appendix B).

To obtain information as to a buffer that would provide optimum tissue response, trials on intact fetal villi preparations were performed using the 3 buffers, Ca^{++} free Krebs-Ringer phosphate pH 7.4, Baird's formula adjusted to pH 7.4 with 1 N KOH, and Baird's formula adjusted to pH 7.4 with 1 N NaOH (Table X).

Of the 5 buffers, the data in Tables IX and X indicate Ca^{++} free Krebs-Ringer phosphate probably allows for best response of intact fetal villi preparations with respect to oxygen uptake.

Tissue Preparation and Storage

Trials were conducted on both fetal and maternal tissues using two types of preparation to determine the effect on oxygen uptake (Table XI). Fetal tissue prepared as intact villi and maternal tissue prepared as slices had greater oxygen uptake values than did similar tissues prepared as chopped suspensions.

The effects of storage on ice was studied as a possible source contributing to variations in oxygen uptake by attachment site tissues (Talbe XII). Storage on ice had no detrimetal effects on either fetal or maternal tissues with regard to oxygen uptake.

Oxygen Uptake During the Last Four Months of Gestation

Fetal villi showed highly significant changes in oxygen uptake over the last four months of gestation (Table XIII and Appendix C). The fetal villi exhibited a highly significant decline in oxygen uptake from the sixth to seventh months followed by an insignificant rise in oxygen

TABLE X

OXYGEN UPTAKE AND TERMINAL pH IN REACTION VESSELS WITH FETAL TISSUE INCUBATED ON THE GILSON DIFFERENTIAL RESPIROMETER UNDER ATMOSPHERE IN EITHER Ca^{++} FREE KREBS-RINGER PHOSPHATE, BAIRD-(NaOH) OR BAIRD-(KOH)

Vessel Contents	Fetal (intact villi)						Control Vessels					
Buffer	Ca^{++} free Krebs-Ringer Phosphate			Baird-(NaOH)			Ca^{++} free Krebs-Ringer Phosphate			Baird-(NaOH)		
Trial[1]	n	gas uptake[2]	pH	n	gas uptake[2]	pH	n	gas uptake[3]	pH	n	gas uptake[3]	pH
1	8	4.6	7.3	8	3.7	7.2	2	-0.1	7.4	2	+1.0	7.5
2	8	4.0	7.2	8	4.0	7.3	2	+1.5	7.4	2	+0.8	7.5
					Baird-(KOH)						Baird-(KOH)	
3	8	4.3	7.3	8	3.6	7.3	2	+0.2	7.5	2	+1.1	7.4
4	7	4.5	7.3	7	4.0	7.3	2	+0.4	7.5	2	+6.0	7.5
5	7	3.0	7.3	8	2.8	7.2	2	+6.4	7.4	2	+4.2	7.4
$\bar{X}$	5	4.1	7.3	5	3.6	7.3	5	1.5	7.4	5	2.6	7.5
std. error[4]		±.3			±.2			±1.3			±1.0	

[1]Assays were performed on Gilson differential respirometers with 3 ml of the buffers indicated and a gas phase of atmosphere as described under Materials and Methods. Each trial represents tissue from a single attachment site similar samples of which were placed in reaction vessels containing 1 of 2 buffer types. Four control vessels, 2 for each type of buffer were used in each trial. Each trial was run on a single respirometer with the reaction vessels and treatments assigned at random to mounting positions.

[2]Values expressed in STP µl/mg dry wt/hr ± standard error.

[3]Values expressed as µl/hr gas volume change.

[4]Calculated using procedure of Mantel (1951).

TABLE XI

OXYGEN UPTAKE[1] BY DIFFERENT PREPARATIONS OF PLACENTAL ATTACHMENT SITE TISSUES

Tissue	Fetal		Maternal	
Trial[2]	1	2	1	2
Tissue Preparation n	8	7	8	8
Chopped Suspension[3] (McIlwain Chopper)	1.2 ± .23	1.4 ± .08	0.6 ± .02[5]	0.5 ± .04
Slices (Stadie-Riggs[4] Hand Microtome)			2.1 ± .08	1.6 ± .13
Intact Villi[4]	3.8 + .07	3.8 + .09		

[1]Values expressed in STP µl/mg dry wt/hr ± standard error (calculated using the method of Mantel, 1951). Each respirometer had either cotyledon or caruncle tissue. The different preparations were distributed at random among the reaction vessels of each respirometer. Four control vessels, 2 for each type of buffer, were assigned at random on each respirometer. Details of tissue preparation and assay procedures are given under Materials and Methods.

[2]Trial 1 tissues from a single attachment site obtained from a placenta at 7 months gestation. Trial 2 tissues from a single attachment site obtained from a placenta at 9 months gestation.

[3]Suspended in 1.5 ml Baird-KOH buffer pH 7.5 + 1.5 ml 0.9 % saline.

[4]Suspended in 3 ml Ca^{++} free Krebs-Ringer phosphate buffer pH 7.4.

[5]7 samples.

TABLE XII

OXYGEN UPTAKE BY PLACENTAL ATTACHMENT SITE TISSUES AFTER STORAGE IN ICED SALINE

Tissue		Fetal (intact villi)				Maternal (Stadie-Riggs slices)			
cow	attachment site	n	not stored[2]	n	stored[3]	n	not stored[2]	n	stored[3]
1	a	2	2.2 ± .2	2	3.1 ± .3	4	1.7 ± .2	3	2.3 ± .1
	b	3	2.9 ± .2	3	3.4 ± .1	3	2.3 ± .2	4	2.1 ± .2
2	a	3	2.9 ± .4	3	3.2 ± .2	4	2.1 ± .1	4	2.0 ± .1
	b	4	3.3 ± .4	4	3.3 ± .1	4	2.3 ± .1	4	2.2 ± .0
3	a	4	3.0 ± .2	3	3.1 ± .2	3	1.8 ± .1	4	1.8 ± .0
	b	4	3.1 ± .1	3	3.6 ± .2	4	2.0 ± .0	4	2.2 ± .0
4[4]	a	4	3.4 ± .1	3	2.9 ± .3	4	2.1 ± .2	3	1.3 ± .1
	b	3	3.6 ± .2	3	2.1 ± .2	4	2.1 ± .1	2	1.8 ± .2
$\bar{X}$		8	3.0 ± .2	8	3.1 ± .2	8	2.0 ± .1	8	2.0 ± .1

[1]Values expressed in STP µl/mg dry wt/hr ± standard error (calculated using method of Mantel, 1951). Assays were performed on the Gilson respirometer with 3 ml Ca^{++} free Krebs-Ringer phosphate buffer pH 7.4 and a gas phase of atmosphere as described under Materials and Methods.

[2]Tissues were kept in iced-saline 2 1/2 - 2 3/4 hr before being mounted on the respirometer.

[3]Tissues were kept in iced-saline 4 1/2 - 5 hr before being mounted on the respirometer.

[4]Tissues obtained at cesarean section; "not stored" tissue on ice 50 minutes; "stored" tissue on ice 4 hr. Initial buffer pH 7.4, terminal pH 6.6; performed in Krebs-Ringer phosphate.

TABLE XIII

EXPERIMENTAL DESIGN AND RESULTS FOR OXYGEN UPTAKE[1] BY PLACENTAL ATTACHMENT SITE TISSUES DURING THE LAST FOUR MONTHS OF GESTATION

Month of Gestation		6	7	8	9
Number of Cows		6	6	6	6
Number of Attachment Sites per Cow		2	2	2	2
Number of samples of each tissue per site		4	4	4	4
Number of samples contributing to the mean	Fetal	48	48	48	48
	Maternal	48	48	48	48
Tissue					
Fetal		4.5 ± .4	3.3 ± .3	3.7 ± .3	3.2 ± .3
Maternal		2.0 ± .2	1.9 ± .1	2.2 ± .1	1.8 ± .2

[1]All values are expressed in STP μl/mg dry wt/hr ± standard error. Assays performed on Gilson differential respirometer, with 3 ml of Ca^{++} free Krebs-Ringer phosphate buffer pH 7.4 and a gas phase of atmosphere as described under Materials and Methods. Four samples of each tissue type from each attachment site from each cow were assayed to give a mean O_2 uptake for either tissue type of a particular attachment site. All tissues from any given cow were assayed simultaneously on a single respirometer. Four control reaction vessels containing everything but tissue were run with each assay. Reaction vessels and treatments were assigned at random to mounting positions on the respirometer.

uptake from the seventh to the eighth months; this was followed by a significant decline to the ninth month (Table XIII and Appendix C). Oxygen uptake by fetal villi in the ninth month was significantly lower than that of the sixth month at the 1% level of probability. A statistical analysis revealed that no significant changes of oxygen uptake by slices of maternal tissue occurred during the last four months of gestation (Appendix C).

Examination of the data showed oxygen uptake by fetal villi was significantly higher than maternal tissue during all of the last four months of gestation (Appendix C). Furthermore the changes in fetal villi oxygen uptake appeared to be independent of that by the maternal tissue. The difference in pattern was examined by expressing oxygen uptake by slices of maternal tissue as a per cent of fetal villi oxygen uptake, at each of the last four months and then performing an analysis of variance to detect changes in this per cent between the last four months (Table XIV, Appendix C). Expressed on a dry weight basis, oxygen uptake by slices of maternal tissue ranged from 44% to 62% that of fetal villi (Table XIV).

Five assays for oxygen uptake by attachment site tissues were performed on samples obtained at or following calf delivery (Table XV). The data show oxygen uptake rates comparable to that observed in the four months preceeding delivery.

Dry Weight as Per Cent Wet Weight During the Last Four Months of Gestation

The dry weight of fetal tissue expressed as per cent wet weight was significantly smaller than that of maternal tissue during the last four

TABLE XIV

OXYGEN UPTAKE[1] DURING THE LAST FOUR MONTHS OF GESTATION BY SLICES OF MATERNAL TISSUE EXPRESSED AS PER CENT[2] OF OXYGEN UPTAKE[1] BY FETAL VILLI

Month of Gestation	6	7	8	9
Number of Cows	6	6	6	6
Average Oxygen Uptake by Maternal Slices as a Per Cent of Fetal Villi Oxygen Uptake ± Standard Error	44 ± 2	62 ± 5	59 ± 5	56 ± 3

[1]Oxygen uptake calculated as STP μl/mg dry wt/hr.

[2](STP μl/mg dry wt/hr maternal slices)/(STP μl/mg dry wt/hr fetal villi) x 100.

TABLE XV

OXYGEN UPTAKE[1] BY PLACENTAL ATTACHMENT SITE TISSUES COLLECTED[2] AT OR FOLLOWING PARTURITION

Source	Cow	Attachment Site	n	Fetal Oxygen Uptake	pH	n	Maternal Oxygen Uptake	pH
Cesarean Section	1[3]	1	4	3.4	6.8	8	2.1	6.8
		2	3	3.6	6.8	8	2.4	6.8
	2[3]	1	4	4.5	6.8	4	2.5	6.8
		2	4	4.1	6.8	4	2.3	6.8
		x	4	3.9			2.3	
At Normal Parturition	3[4]	1	2	4.6	7.4	2	2.3	7.4
		2	2	4.7	7.4	2	2.2	7.4
		x	2	4.6			2.3	
30 min. Post Partum	3[4]	1	2	6.2	7.4	2	2.2	7.4
		2	2	4.6	7.4	2	2.4	7.4
		x	2	5.4		2	2.3	
6 - 7 hr. Post Partum	4[3]	1			7.1	7	1.8	7.1
		2			7.1	7	1.6	7.1
		x				2	1.7	

[1]Incubations performed on Gilson respirometers under a gas phase of atmosphere at 37° C. Oxygen uptake values expressed in STP µl/mg dry wt/hr.

[2]Tissues were mounted on the respirometers in less than 45 minutes after the tissue had been removed from the cow.

[3]Krebs-Ringer phosphate used as the suspending medium. Initial pH 7.4. pH value presented represents pH in reaction vessels at end of incubation.

[4]Ca^{++} free Krebs-Ringer phosphate used as the suspending medium. Initial pH 7.4. pH value presented represents pH in reaction vessels at end of incubation.

months of gestation (Table XVI and Appendix C). Both tissues exhibited significant increases in dry matter over the last four months of gestation. Analysis of regression coefficients showed no significant differences between the slope of rise for the two tissues.

TABLE XVI

DRY WEIGHT AS PER CENT WET WEIGHT ± STANDARD ERROR OF BOVINE ATTACHMENT SITE TISSUES DURING THE LAST FOUR MONTHS OF GESTATION

Month of Gestation	6	7	8	9
Number of Cows	6	6	6	6
Number of Attachment Sites per Cow	1	1	1	1
Number of Samples of Each Tissue per Site	2	2	2	2
Number of Samples Contributing to the Mean				
Fetal	12	12	12	12
Maternal	12	12	12	12
Tissue				
Fetal	13.3 ± .3	14.5 ± .7	15.3 ± .4	15.5 ± .3
Maternal	17.5 ± .1	18.2 ± .4	18.2 ± .1	18.6 ± .5

CHAPTER V

DISCUSSION

The Assay System

While the Warburg apparatus has been used extensively in metabolic studies of tissues with a variety of assay procedures (Umbreit et al., 1964) the Gilson differential respirometer is a relatively new instrument. Unsatisfactory results of preliminary experiments to characterize bovine placental oxygen uptake in vitro on the Gilson differential respirometer employing methods adapted from Umbreit et al. (1964) and Baird (1963) indicated a closer study of the assay system and procedures was required before satisfactory data pertaining to placental physiology could be obtained. The principal cause of the unsatisfactory data was the permeability of Tygon tubing components of the Gilson differential respirometer to oxygen, carbon dioxide and nitrogen.

The permeability of Tygon to gases was not known to the investigator when efforts were begun to develop a technique for assaying oxygen uptake by bovine placenta attachment site tissues. Information[1] available in the Department of Physiology and Pharmacology and Oklahoma State University Library made no mention that the Tygon tubing used in the Gilson

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[1]Directions for Operation of the Gilson Respirometer, undated manual provided by Gilson Medical Electronics, Middleton, Wisc.; Umbreit, W. W., R. H. Burris and J. F. Stauffer. Manometric Techniques, 4th ed., Minneapolis, Minn,: Burgess Publishing Co., 1964, p. 104.

respirometers was permeable to gases; there were no indications of difficulties that could occur if incubations on the Gilson respirometers were carried out using gas phases other than atmosphere. The discussions of operating procedures for the Gilson respirometers indicated that gassing procedures on the Gilson respirometer were simple, straight-foreward operations, and required no particular adjustments in equipment or calculations. From the early experiments in Dr. McDonald's laboratory (Tables I and II), it soon became evident that considerable gas volume changes occurred in empty reaction vessels that had been gassed with either 95% O_2-5% CO_2 or 95% N_2-5% CO_2. Meticulous examination revealed no mechanical aspect of the Gilson respirometer that could account for the undesireable gas volume changes.

In the first part of August, 1966, a member of the faculty, Dr. G. H. Stabenfeldt, was requested to telephone Gilson Medical Electronics and inquire if the manufacturer could provide information that might be helpful in determining the cause of the gas volume changes in question. Gilson Medical Electronics informed Dr. Stabenfeldt that after their respirometer had been placed on the market, it had become evident that the Tygon tubing which connected the manometers with the flasks was permeable to hydrogen, oxygen, nitrogen and carbon dioxide. After Gilson Medical Electronics recognized that the Tygon tubing components of their respirometer rendered their respirometer not applicable for use with gases which pass through Tygon, an all glass volumometer was designed to overcome the problem. The Gilson Medical Electronics catalogue that was distributed in December, 1966, was their first catalogue that offered the all glass volumometer and described why such an all glass system was required. A copy of this catalogue was received by the Department of

Physiology and Pharmacology, O.S.U., in February, 1967. A separate description of the all glass volumometer offered in the catalogue and that described why an all glass system was required, was received by Dr. Stabenfeldt after his telephone conversation with Gilson Medical Electronics.

The data (Tables I and II) and information from Gilson Medical Electronics indicate that the Gilson respirometer model available in the Department is not suitable for manometric studies of bovine placental tissues as described herein involving gas phases of either 95% O_2-5% CO_2 or 95% N_2-5% CO_2.

Incubations performed under 95% O_2-5% CO_2 on the Warburg apparatus (Table VI) apparently had higher oxygen uptake than those performed under atmosphere, particularly by fetal tissue. These results are similar to those observed by Friedman and Sachtleben (1960) with human placental tissue. Though speculative, it is suggested that results of oxygen uptake during the last four months of gestation (Table XIII) would probably have exhibited the same trends had assays been performed under 95% O_2-5% CO_2 instead of atmosphere.

The primary purpose of a suspending medium is to protect the integrity of the cells. Of the various suspending media available, investigators frequently choose a medium that gives optimum tissue response even though ionic composition of the suspending medium may be considerably different from that of interstitial fluid (Umbreit et al., 1964). Different tissues including placentae show variations in response to different suspending media (Umbreit et al., 1964; Feinstein and Stare, 1940; Ginsburg and Jeacock, 1964, 1967). Human placenta incubated in phosphate buffer with 11.1×13^{-3} M glucose had a 50% higher lactic acid production

than when incubated in bicarbonate; however, lactic acid production increases similarly in both suspending media upon addition of adrenaline (Ginsburg and Jeacock, 1964, 1967). In the rat placenta there is little difference in glucose uptake or lactic acid production between incubations done in phosphate and bicarbonate buffers. The data for oxygen uptake by the cow placenta (Tables IX and X) suggest optimum oxygen uptake occurred in Ca^{++} free phosphate buffer.

A possible explanation for gas volume changes in vessels on the Gilson respirometer containing bicarbonate buffer, either with or without KOH in the center well, and the pH changes in these vessels, may be the escape of the carbon dioxide from solution. During the equilibration period when the vessels are warmed to 37° C, CO_2 comes out of solution and displaces atmosphere in the vessel. The displaced atmosphere can escape from the open gassing valve. When the equilibration period is terminated by closing the system, vessels containing bicarbonate buffer with KOH in the center well have a significant decrease in volume because the CO_2 is then absorbed by the KOH-soaked wick.

If CO_2 leaves the bicarbonate buffer, then an increase in pH of the buffer would be expected. An increase in pH did occur and was greater (pH 9.17) in vessels containing KOH in the center well than in those without KOH in the center well (pH 8.53, Table VIII). Evidently the KOH in the center well traps gaseous CO_2 that might otherwise act to keep CO_2 in solution. Bicarbonate buffer left for 10 hours in an unstoppered container at room temperature goes from an initial pH of 7.5 - 7.6 to pH 8.8 which also suggests the escape of CO_2 from the buffer is an aspect of the bicarbonate buffer instability.

With regard to changes in pH of Krebs-Ringer phosphate it should be

noted that the buffering action of phosphate solutions is dependent upon the equilibrium of 3 species of orthophosphoric acid, the completely deprotonated of which will combine with calcium to give a white precipitate in alkaline solution as illustrated by the equilibrium formula[2] below.

$$(H_3\ PO_4)_2 \rightleftharpoons (H_2\ PO_4^-)_2 \rightleftharpoons (HPO_4^{=})_2 \rightleftharpoons (PO_4^{\equiv})_2 + 3Ca^{++} \xrightarrow{\text{ppt}} Ca_3(PO_4)_2 \quad .$$

Krebs-Ringer phosphate is 0.0012 M in $CaCl_2$ and 0.0031 M in Na_2HPO_4 (see Appendix A). Exact predictions of pH changes resulting from calcium trapping the completely deprotonated phosphate species are difficult because: (1) activity coefficient complexity stemming from other ions in solution; (2) the fact that phosphoric acid is triprotic; and (3) the temperature changes occurring when buffer solutions are raised from room temperature to 37° C (King, 1959). However, the net effect of removing $PO_4^{\equiv}$ from solution would be to increase the hydrogen ion concentration.

Studies with Krebs-Ringer bicarbonate (Table IX) are plagued with the possibility of interactions between the incubated tissue and the buffer which influence manometer readings in a manner that cannot be corrected for with controls. Lactic acid production by the tissues would drive more CO_2 out of solution. Comparisons of oxygen uptake by either maternal or fetal tissues in Krebs-Ringer phosphate to that in Krebs-Ringer bicarbonate when performed on the Gilson respirometer must be made with caution. Attempts to interpret differences as a function of

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[2]Adapted from King (1959).

tissue performance should be tentative. The high terminal pH in reaction vessels containing bicarbonate (Table IX) might be expected to reduce cellular uptake of oxygen by acting as a hostile milieu. The somewhat lower than physiologic terminal pH observed for most trials in reaction vessels containing Krebs-Ringer phosphate (Table IX), agrees with other evidence (Table VIII), that indicates Krebs-Ringer phosphate has limited ability to buffer changes in pH which could reduce oxygen uptake by the tissue preparations (Table IX).

Variation between cows combined with an insufficient number of replicates reduces the possibility of reaching definitive conclusions from comparing oxygen uptake by fetal tissue in Ca^{++} free Krebs-Ringer phosphate with that occurring in Baird-(KOH) or Baird-(NaOH) (Table X). Incubations in both Baird-(KOH) and Baird-(NaOH) tend to exhibit lower oxygen uptake than those performed with Ca^{++} free Krebs-Ringer phosphate. The effects of the high concentrations of potassium in the Baird suspending media (Appendix B) might have on cellular uptake of oxygen are difficult to evaluate.

In chopped tissues and minces the degree of cellular destruction could be expected to be greater than in intact fetal villi preparations and slices of maternal tissue. This maybe an explanation for the low oxygen uptake values (Table XI) observed when tissues were prepared by Baird's (1963) procedures. That the difference between fetal and maternal placenta oxygen uptake is real and not merely the result of preparation is suggested by the fact there is a difference between fetal and maternal oxygen uptake in those assays where the two tissues were incubated as similar preparations (chopped tissues). Values for maternal tissue in Table XI are approximately those found by Baird (1963).

Because Baird used 95% O_2-5% CO_2 as a gas phase the oxygen uptake by attachment site tissue in Baird's (1963) assays might be expected to have been slightly greater, particularly for the fetal suspension. Table XI values for intact fetal villi preparations are three to four times those observed by Baird.

Lengthened periods of storage on ice did not appear to be detrimental to the oxygen uptake by the tissues. Tissues obtained at cesarian section and at normal parturition (Table XV), when the tissues were put immediately on ice had unexpectedly high oxygen uptake values compared to tissues collected at the slaughter house where they remained at body temperature for 40 minutes after slaughter. This suggests that holding tissue at normal body temperature results in decreased oxygen uptake values.

While the assay system developed in this study has recognizable limits (gas phases other than atmosphere can not be used) results for oxygen uptake measured by this system fall within the range of values found or reviewed by other investigators (Campbell et al., 1966). The method appears sensitive enough, provided enough samples are processed, to measure differences in oxygen uptake similar to those reported in the literature (Wang and Hellman, 1943; Villee, 1953, 1956; MacKay, 1958; Friedman and Sachtleben, 1960).

Oxygen Uptake During the Last Four Months of Gestation

Data of Table XIII show a significant decrease in oxygen uptake by tissue during the last four months of gestation. The general pattern is the same as that found by Baird (1963) except that in the present studies fetal and maternal tissues were shown to have independent changes with

regard to oxygen uptake. Other workers who have observed decreases in oxygen uptake as gestation approaches term include Wang and Hellman (1943), Villee (1956), MacKay (1958), and Friedman and Sachtleben (1960). In all of these studies oxygen uptake was expressed in terms of tissue dry weight.

Interpretation with regard to physiologic significance of decreases in oxygen uptake as gestation approaches term expressed in terms of tissue dry weight should be done with care. Examination of dry weight and wet weight ratios (Table XVI) indicate that dry matter content goes up as gestation approaches term. Similar increases in dry matter as gestation approaches term were found by Villee (1956) in human placentas and by Behrman et al. (1964) in the rhesus monkey placenta. Behrman et al. (1964) noted that increases in dry matter content of the rhesus monkey placenta are less toward the end of gestation than the increase in dry matter content of the fetus. If the data of Table XIII (oxygen uptake expressed on a dry weight basis), are converted to a wet weight basis by multiplying them by the per cent dry weight data (Table XVI), the fetal tissues exhibit a 17% reduction in oxygen uptake from the sixth to ninth months in contrast to the 30% reduction obtained on a dry weight basis. Conversion of maternal tissue oxygen uptake from a dry weight basis to a wet weight basis reduces the amount of variation from 1.8 - 2.2 μl/mg dry wt/hr to 0.3 - 0.4 μl/mg wet wt/hr. Conversion calculations also have the effect of reducing the per cent difference in oxygen uptake between fetal and maternal tissues by an average of 14.6% over the last four months of gestation. No data are available for expressing oxygen uptake in terms of DNA and RNA content of placental tissues, which would provide the most valid information on changes in cellular function of

attachment site tissue with respect to oxygen uptake.

Present studies showed that fetal tissues have almost twice the oxygen uptake that maternal tissues have. This is to be expected since electron microscope studies (Wynn and Davies, 1965; Burgos and Rodriquez, 1966) and immunofluorescent studies (Sciarra et al., 1963; Pierce and Midgley, 1963) suggest strongly that the trophoblast is the major site of active transport and protein and steroid hormone synthesis in the placenta. There is considerable evidence in the literature to suggest that cellular function of attachment site tissues should not be expected to decrease as gestation approaches term. In the sheep, oxygen consumption by the placental membranes and cotyledons has been proposed to act in regulating oxygen tension in fetal blood (Campbell et al., 1966). Walker et al. (1967) studied the several enzymes in the glycolytic pathway in the placentas of rats, guinea pigs, rabbits, monkeys and humans and found little change in activities as gestation approached term, regardless of whether activities were calculated on a dry or wet weight basis.

The growth of mammalian fetuses, after an initial lag phase, conforms to a cubic law of growth, differences in the size of offspring being determined by the rate of transport of nutrients to the fetus across the fetal vascular endothelium (payne and Wheeler, 1967). The same cubic law describes the growth of tumours (Payne and Wheeler, 1967). As the placenta is the site through which most nutrients are thought to be transported from the maternal circulation to the fetus (Hagerman and Villee, 1960; Dancis, 1960) it could be expected that the transport function of the placenta would not decrease as gestation approaches term, particularly since in most species of mammals the ratio of placental mass

to fetal mass does not decline toward term (Behrman et al., 1964). In some breeds of sheep after the 120th day the total weight of attachment site tissues falls from about 600 g/fetus to 200-300 g/fetus at term (Dawes, 1956). How nutrients cross the placenta is poorly understood (Hagerman and Villee, 1960; Dancis, 1960; Howard and Krantz, 1967). It is possible that with increasing age the vascular surface area of placental tissues increases, thereby increasing efficiency (Harrison, 1956).

Additional evidence that placental function does not decrease with age is the suggestion in current literature that the fetus and placenta form a unit with regard to endocrine function (at least in the human and sheep), particularly with regard to steroid hormones (Diczfalusy, 1964; Klevit, 1966; Ryan, 1966; Sitteri and MacDonald, 1966; Liggens et al., 1967). An extensive review of estrogen excretion in the cow by Mellin and Erb (1965) compiled evidence that the urinary excretion of estrogens increases dramatically during the terminal stages of pregnancy, reaching a peak immediately before parturition, followed by a steady decline postpartum. Concentrations of estrogens increased in the bovine fetal attachment site tissues during pregnancy (Mellin and Erb, 1965). Production of estrogens during human pregnancy (Smith et al., 1966) follows a pattern similar to that described by Mellin and Erb (1965) for the cow and appear to be aromatized by the placenta from precursors produced by the fetus and mother (Klevit, 1966; Sitteri and MacDonald, 1966). The source of progesterone during the final months of gestation is not established. Ryan et al. (1966), Lurie et al. (1966), and Schreiner and Villee (1965) have shown that the human placenta can synthesize progesterone from precursors obtained from either fetal or maternal sources. The placenta of the cow does not appear to provide sufficient progesterone

to maintain pregnancy if the ovaries are removed before the 200th day of gestation (McDonald et al., 1953); after the 200th day the placenta does produce sufficient progesterone to maintain pregnancy.

The preceeding paragraphs present supplementary evidence in support of the suggestions by Hugget (1956) that little functional senescence should be expected in the placenta as gestation approaches term, even though there is evidence of anatomic aging (Wislocki, 1956).

Data from the oxygen uptake assay in the present studies contains elements of both functional changes and anatomic changes. The functional changes are associated primarily with oxygen uptake, the anatomical changes are associated primarily with dry weight. The data presented for the oxygen uptake during the last four months of gestation (Table XIII) are therefore a function of three variables: age, cellular function, and anatomic changes. The variable of age was defined by using Roberts' (1956) parameters for estimating age (fetal crown rump measurement and body development criteria) of the fetus. In practice, application of Roberts' parameters doubtlessly introduced some error. An attempt to define the limits for the variable of changes in dry matter content was made by collecting the per cent dry weight data (Table XVI) though admittedly these data were subject to the error in estimating age of the fetus.

Taking into consideration the variables of age and dry matter content the data indicate that there exists a decline of approximately 17% in oxygen uptake by fetal placental tissues from the sixth through ninth months of gestation. Variables considered, caruncle tissue does not have a decrease in oxygen uptake as gestation approaches term.

CHAPTER VI

SUMMARY AND CONCLUSIONS

An oxygen uptake assay system was developed to measure oxygen uptake in both maternal and fetal aspects of the bovine placenta attachment site tissues. Oxygen uptake by fetal villi, calculated on a basis of dry weight or wet weight declined during the last four months. Maternal tissue showed no comparable change in oxygen uptake. Maternal tissue oxygen uptake in terms of dry weight ranged from 44% - 62% that of fetal tissue. Patterns of oxygen uptake by the two types of tissue were not similiar to each other. The decline in oxygen uptake by fetal tissues was discussed with respect to findings by others.

Dry matter content of fetal villi is less than that of maternal tissue. For both tissues, the dry matter content increases toward term.

Consideration of placenta function with regard to transport and protein and steroid hormone production as gestation approaches term suggests caution must be used in relating *in vitro* oxygen uptake to *in vivo* placental physiology.

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APPENDIX A

TABLE XVII

BUFFER COMPOSITIONS

Krebs-Ringer Phosphate pH 7.4 (Umbreit et al., 1964):

			parts	dilution	final molarity
0.90%	NaCl	(0.154 M)	100	100/128	0.1203
1.15%	KCl	(0.154 M)	4	4/128	0.0048
1.22%	$CaCl_2$	(0.110 M)	3	3/128	0.0012
3.82%	$MgSO_4 \cdot 7H_2O$	(0.154 M)	1	1/128	0.0156
17.79%	$Na_2HPO_4 \cdot 2H_2O$	(0.100 M)			
0.07%	HCL	(0.020 M)	20	20/128	0.0031
		total parts	128		0.144

Ca^{++} Free Krebs-Ringer Phosphate pH 7.4 (Umbreit et al., 1964):

Same as Krebs-Ringer Phosphate except substitute distilled H_2O for the $CaCl_2$.

Krebs-Ringer Bicarbonate pH 7.4 (Umbreit et al., 1964):

			parts	dilution	final molarity
0.90%	NaCl	(0.154 M)	100	100/130	0.1185
1.15%	KCl	(0.154 M)	4	4/130	0.0047
1.22%	$CaCl_2$	(0.110 M)	3	3/130	0.0025
2.11%	KH_2PO_4	(0.154 M)	1	1/130	0.0012
3.82%	$MgSO_4 \cdot 7H_2O$	(0.154 M)	1	1/130	0.0012
1.30%	$NaHCO_3$	(0.154 M)	21	21/130	0.0249
		total parts	130		0.154

Baird-(NaOH) Incubation Solution pH 7.5 (Baird, 1963)[1]:

		parts	dilution	final molarity
stock solution Baird-(NaOH)		1	½	
0.9923% KCl	(0.1333 M)			0.0667
0.1352% MgCl	(0.0141 M)			0.0070
0.0906% KH PO	(0.0067 M)			0.0033
7 ml 1 N NaOH = 0.0280% NaOH	(0.0070 M)			0.0035
0.90% NaCl	(0.154 M)	1	½	0.0770
	total parts	2		0.157

Baird-(KOH) Incubation Solution pH 7.5 (Baird, 1963)[1]:

Same as for Baird-(NaOH) Incubation Solution except KOH is substituted for NaOH.

7 ml 1 N KOH per liter buffer = 0.03935 KOH	(0.0070 M)	0.0035

[1]If Baird's buffer was prepared as described by Baird (1963), the resulting solution required 7 ml of 1 N KOH to obtain a pH of 7.5 which is approximately equivalent to the presence of 0.392 g KOH/per liter of buffer. Baird (1963) makes no mention of the fairly substantial amount of base required to bring the buffer to pH 7.5 nor of the type of base (NaOH or KOH) that should be employed to obtain a 7.5 pH. Therefore buffers of two types (Baird-KOH and Baird-NaOH) were prepared. It should be noted that the final suspension of tissues was in a solution made up 50% of either Baird-(KOH) or Baird-(NaOH) and 50% 0.9% NaCl. A table comparing the final molarities of ions in the various buffers is provided (Appendix B).

APPENDIX B

TABLE XVIII

COMPARISON OF FIVE BUFFER SOLUTIONS AND INTERSTITIAL FLUID WITH REGARD TO MILLIEQUIVALENTS IONS PER LITER

Ion	Interstitial Fluid[1]	Baird(NaOH) Incubation Sol.	Baird(KOH) Incubation Sol.	Krebs-Ringer Bicarbonate	Krebs-Ringer Phosphate	Ca^{++} Krebs-Ringer Phosphate
Na^{+}	145.1	80.5	77.0	143.4	135.9	135.9
K^{+}	4.1	70.0	03.5	5.9	4.8	4.8
Mg^{++}	1.3	14.1	14.1	2.4	2.4	2.4
Ca^{++}	3.5	--	--	2.6	2.6	--
Cl^{-}	115.7	157.8	157.8	128.2	133.4	127.2
HCO_3^{-}	29.3	--	--	24.9	--	--
$PO_4^{\equiv}$	2.3	9.9	9.9	3.6	46.8	46.8
$SO_4^{=}$	--	--	--	2.4	2.4	2.4
Other	6.7	--	--	--	--	--

[1](Ruch and Patton, 1965).

APPENDIX C

TABLE XIX

ANALYSIS OF VARIANCE OF OXYGEN UPTAKE[1] BY FETAL TISSUE FOR THE LAST FOUR MONTHS OF GESTATION

Source	d.f.	Sum of Squares	Mean Square	F value
Cows	23	30.95		
Months	3	12.77	4.27	
Cows X Months	20	18.18	.91	4.96**
Sites X Cows	24	3.96	.16	
Total	47	34.91		

[1] µl STP O_2/mg dry wt/hr.

TABLE XX

DUNCAN'S NEW MULTIPLE-RANGE TEST[1] APPLIED TO MEAN FETAL OXYGEN UPTAKE[2] FOR EACH OF THE LAST FOUR MONTHS OF GESTATION

Month	9th	7th	8th	6th
Mean	3.16	3.29	3.72	4.48

Value of p (d.f. = 20)	5% 2	5% 3	5% 4	1% 2	1% 3	1% 4
SSR	2.95	3.10	3.18	4.02	4.22	4.33
LSR ($s_{\bar{x}}$ = .123)	.36	.38	.39	.50	.52	.53

[1] Steel and Torrie (1960).

[2] µl STP O_2/mg dry wt/hr.

TABLE XXI

ANALYSIS OF VARIANCE OF OXYGEN UPTAKE[1] BY MATERNAL TISSUE FOR THE LAST FOUR MONTHS OF GESTATION

Source	d.f.	Sum of Squares	Mean Square	F value
Cows	23	6.42	0.28	
Months	3	1.19	0.40	
Cows X Months	20	5.23	0.26	1.54
Sites X Cows	24	1.28	0.05	
Total	47	7.70		

[1] µl STP O_2/mg dry wt/hr.

TABLE XXII

ANALYSIS OF VARIANCE OF OXYGEN UPTAKE[1] COMPARING FETAL WITH MATERNAL TISSUE FOR THE LAST FOUR MONTHS OF GESTATION

Source	d.f.	Sum of Squares	Mean Square	F value
Month	3	0.83	0.28	
Tissue	1	5.87	5.97	49.75**
Error	3	0.35	0.12	
Total	7	7.15		

[1] µl STP O_2/mg dry wt/hr.

TABLE XXIII

ANALYSIS OF VARIANCE OF PER CENT DRY WEIGHT COMPARING FETAL WITH MATERNAL TISSUE FOR THE LAST FOUR MONTHS OF GESTATION

Source	d.f.	Sum of Squares	Mean Square	F value
Month	3	3.25	1.08	
Tissue	1	24.36	24.36	135.33**
Error	3	0.53	0.18	
Total	7	28.18		

TABLE XXIV

ANALYSIS OF VARIANCE OF MATERNAL OXYGEN UPTAKE[1] AS A PER CENT OF FETAL OXYGEN UPTAKE FOR THE LAST FOUR MONTHS OF GESTATION

Source	d.f.	Sum of Squares	Mean Square	F value
Month	3	1,058	353	9.05**
Cow	5	528	105	1.18
Error	15	1,341	89	
Total	23	2,927		

[1] μl STP O_2/mg dry wt/hr.

TABLE XXV

ANALYSIS OF VARIANCE OF PER CENT DRY WEIGHT OF FETAL TISSUE FOR THE LAST FOUR MONTHS OF GESTATION

Source	d.f.	Sum of Squares	Mean Square	F value
Cows	23	62.71	2.73	
Months	3	40.06	13.35	11.22**
Cows X Months	20	22.65	1.19	
Sample X Cows	24	68.60	2.86	
Total	47	131.31		

TABLE XXVI

DUNCAN'S NEW MULTIPLE-RANGE TEST[1] APPLIED TO MEAN FETAL PER CENT DRY WEIGHT FOR EACH OF THE LAST FOUR MONTHS OF GESTATION

Month	6th		7th	8th		9th
Mean	13.26		14.46	15.27		15.53
Value of p (d.f. = 20)	2	5% 3	4	2	1% 3	4
SSR	2.95	3.10	3.18	4.02	4.22	4.33
LSR ($s_{\bar{x}}$ = .44)	1.30	1.36	1.40	1.77	1.86	1.90

[1]Steel and Torrie (1960).

TABLE XXVII

ANALYSIS OF VARIANCE OF PER CENT DRY WEIGHT OF MATERNAL TISSUE FOR THE LAST FOUR MONTHS OF GESTATION

Source	d.f.	Sum of Squares	Mean Square	F value
Cows	23	15.45	0.67	6.20**
Months	3	7.44	2.48	
Cows X Months	20	8.01	0.40	
Samples X Cows	24	1.54	0.06	
Total	47	16.99		

TABLE XXVIII

DUNCAN'S NEW MULTIPLE-RANGE TEST[1] APPLIED TO MEAN MATERNAL PER CENT DRY WEIGHT FOR EACH OF THE LAST FOUR MONTHS OF GESTATION

Month	6th		7th	8th		9th
Mean	17.50		18.15	18.20		18.63
Value of p (d.f. = 20)	5%			1%		
	2	3	4	2	3	4
SSR	2.95	3.10	3.18	4.02	4.22	4.33
LSR	0.78	0.81	0.83	1.04	1.10	1.12
($s_{\bar{x}}$ = .26)						

[1]Steel and Torrie (1960).

TABLE XXIX

ANALYSIS OF VARIANCE TESTING FOR DIFFERENCE BETWEEN REGRESSION COEFFICIENTS[1] OF PER CENT DRY WEIGHT FOR FETAL AND MATERNAL TISSUE

Source	$\frac{(\Sigma xy)^2}{\Sigma x^2}$	d.f.	Residual Sum of Squares	d.f.
Fetal	2.90	1	0.22	2
Maternal	0.59	1	0.07	2
Two Regressions	3.49	2	0.29	4
Fetal + Maternal (one regression)	3.06	1	25.08	5
Regression Coefficients (two regressions vs one)	0.43	1	$F = \frac{0.43}{0.29/4} = 5.93$ d.f. 1,4	

[1]Steel and Torrie (1960).

VITA

John Arthur Holt

Candidate for the Degree of

Master of Science

Thesis: OXYGEN UPTAKE IN ATTACHMENT SITE TISSUES OF THE BOVINE PLACENTA

Major Field: Physiology

Biographical:

Personal Data: Born in Washington, D.C., May 29, 1941, the son of John Bradshaw and Elizabeth Gilmore Holt.

Education: Attended U. S. Army grade school in Berlin, Germany, and Anglo-American Community Grade School, Salonika, Greece; attended eighth through tenth grades in public schools of the District of Columbia; completed the last two years of high school at Le College Cevenol, Haute Loire, France; received the Bachelor of Arts with a major in Biology from The College of Wooster, 1964; completed the requirements for the Master of Science degree in Physiology from Oklahoma State University, July, 1968.

Professional Experience: Employed as an Interpreter with Programs Evaluation Office of U. S. Operations Mission, Vientaine, Laos, September, 1959, to January, 1960; employed by Medico, Muoung Sing, Laos, as Interpreter and Liason Officer, January to May, 1960; worked in the Pathology Laboratory, Veterinary Science, Ohio Agricultural Experiment Station, June to September, 1964; attended the University of Vermont as a NASA Predoctoral Trainee in the Zoology Department, September, 1964, to February, 1966; served as Graduate Teaching Assistant, Department of Physiology and Pharmacology, Oklahoma State University from September, 1966, to June, 1967; served as a Graduate Research Assistant, Department of Physiology and Pharmacology, Oklahoma State University, June, 1967, to July, 1968.