METABOLIC ASPECTS OF THE PLACENTA IN THE COW

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TABLE OF CONTENTS

LIST OF TABLES

LIST OF FIGURES

CHAPTER I

INTRODUCTION

This work is the result of a search for information which might serve as a basis for a study of placental retention in the bovine animal. Retention of fetal membranes following term parturition in the cow is of major economic importance. Weight loss, lowered milk production, and impaired fertility may follow placental retention.

A review of the literature reveals that much of the work on placental retention has concerned the clinical management from a practical standpoint, with little attention given to determining the basic cause. Satisfactory management of a pathological process through symptomatic treatment is an achievement worthy of practice, however, the ultimate goal must be an explanation and understanding of the fundamental cause so that more direct management procedures can be instituted. At least one of the reasons for an investigation of a pathological process is to be able to explain in detail the pathogenesis as well as the degree of existing variation from the normal state. Before this is entirely possible it is necessary to establish a physiologically normal pattern for the process involved.

The physiological mechanisms involved when normal detachment of the fetal cotyledon from the maternal caruncle occurs are not well understood. Undoubtedly, however, it is a complex process, possibly

involving a reduction of blood supply, degenerative cellular changes followed by shrinking of maternal and fetal structures, separation, and then strong uterine contractions facilitating expulsion.

The cotyledonary type placentation which is characteristic of the bovine species has certain inherent features on which one may theorize as predisposing to membrane retention. The syndesmo-chorial attachment and the large and deeply penetrating villi of the fetal cotyledon into the convex surface of the maternal caruncle may provide a more stable arrangement of apposition of the fetal and maternal tissues, especially from a physical standpoint. It is tempting, however, to consider these features merely as an arrangement conducive to retention of the fetal membranes in the presence of some underlying physiological or biochemical disorganization.

This study was initiated to gain an insight into one of the normal physiological processes occurring in the placenta during the course of pregnancy. In vitro measurements of oxygen uptake by chopped suspensions of placental tissue should indicate if there are changes in aerobic metabolism as parturition approaches. Furthermore, such a study could be followed by respirometric studies in the immediate post parturient period to determine if retained placentas respire quantitatively differently than placentas which are shed normally.

CHAPTER II

REVIEW OF LITERATURE

Several investigators have found that brucellosis, leptospirosis, vibriosis, and vitamin A deficiency are etiological factors accounting for many cases of placental retention (Palmer 1932, Kennedy 1947, Boyd and Sellers 1948, Ronning et al. 1953). However, it is agreed by these researchers and others that many cases of retained placenta occur without known etiology and in the absence of identifiable infections.

McDonald et al. (1954), in studies of the corpus luteum of pregnancy in the cow, have shown that a relationship exists between corpus luteum ablation and subsequent retention of the placenta following term delivery. It appears that physiological levels of progesterone or a progestin are necessary for normal loosening of the placental attachment.

Venable and McDonald (1958) found that increased frequency and amplitude of uterine contractions followed placental retention in cows with ablated corpora lutea. This indicates that reduced uterine motility is not a contributing factor in experimentally induced retained placenta.

Wang and Hellman (1943) note that the placenta is a rapidly developing organ with apparent full functional activity being reached

in the early months of gestation. Beginning senescence of the human placenta has been described from gross and microscopic studies near the end of gestation. The overall process of aging is non-specifically described as a slowing down of body processes, accompanied by an increase in the rate of degenerative catabolism over that of regenerative anabolism. These changes are descriptive of the organism as a whole, as well as at the organ and cellular levels. The cytology of the human placenta suggests that a progressive decrease in metabolic activity occurs with aging of the placenta.

Two procedures have been used to study the oxygen uptake of the human placenta. Several investigators separately studied oxygen consumption by perfusing the organ with blood containing a known amount of oxygen, and reported variable results (Kustner and Sudentopf 1929, Rech 1924, Budelmann 1929). Loeser (1932) found that the more immature the human placenta was, the greater was the production of lactic acid in an anaerobic vessel. Wang and Hellman (1943) showed that the course of pregnancy was accompanied by a progressive decrease in oxygen uptake by the human placenta with QO_2 values ranging from 5.3 for placentas of two months of age to 1.7 at term. These values are expressed as $mm.^3$ of oxygen consumed per mg. of dried tissue, per hour. It was also shown that oxygen consumption was not influenced by the use of analgesics or anesthetics during labor, and the addition of glucose to the incubation medium did not significantly increase the oxygen consumption. Studies made by Hellman *et al.* (1950) on the oxygen consumption of the placenta using the Warburg apparatus were in agreement with those reported by Wang and Hellman in 1943. Villee (1953) studied the oxygen consumption

of thirty term placentas and thirty-three placentas ranging from six to thirty weeks of age. The QO_2 values at term had decreased to about one-half that of the placentas of six weeks age.

MacKay (1958) studied oxygen consumption of 267 specimens of human placental tissue from normal and abnormal pregnancies. In most instances the experiments were carried out promptly, but in some cases there were unavoidable delays of up to eight hours between collection and oxygen consumption determinations in the Warburg apparatus. Experiments indicated that storage up to eight hours did not influence the rate of oxygen consumption providing the specimens were from clinically normal patients in various periods of gestation, ranging from nine weeks to forty-three weeks. Oxygen consumption was determined in the Warburg apparatus at 37.6°C. in an atmosphere of room air. The reduction in the rate of oxygen consumption of human placental tissue was found to be similar to previous workers' findings, however, the individual QO_2 values were of a lower magnitude. There was a progressive decline in QO_2 values beyond the 18th week of gestation, and the decline was most marked from the 35th to the 43rd week.

Friedman and Sachtleben (1960) made a systematic investigation of the effects of various clinical conditions upon placental metabolism. The results suggest that the respirometric observations on placental tissues should be made in the freshest possible state. With a two and one-half hour storage period at 5°C., there appeared to be a reduction of 20% in the oxygen uptake of the placental tissue. These workers also demonstrated by a group of simultaneously run analyses, that a reduction of 12% oxygen uptake occurred when room air was used instead

of 100% oxygen for filling the Warburg vessel. In addition it was suggested that degenerative changes occur rapidly during the manometric procedure precluding the use of consecutive observations in determining the effects of added substrates. It was found that numerous clinical factors such as prolonged duration of ruptured chorio-amniotic membranes, the duration of labor, type of delivery, and certain pathological states affected the QO_2 values. Of significance was the further confirmation of a decline in placental oxygen consumption from early pregnancy to term.

James et al. (1948) studied the effects of certain pharmacologic agents upon the oxygen consumption of fresh human chorionic villi. The placental tissue used was delivered within two weeks of the estimated date of parturition. The QO_2 was determined by conventional manometric methods. Dextrose or pyruvate did not influence QO_2 values, however, succinate and hydroquinone increased the QO_2 in proportion to their concentration. The QO_2 was depressed with in vitro concentrations of merperidine above 2.5×10^{-4}M, Amytal above 2×10^{-4}M, scopolamine above 2.5×10^{-4}M, and diethylstilbestrol at 4 mg. per Warburg vessel. The QO_2 was increased with in vitro concentrations of dinitrophenol above 4×10^{-6}M and Methadon below 1×10^{-3}M. Methadon, however, depressed the QO_2 in concentrations of 2×10^{-3}M and above. In vivo concentrations (after therapeutic dose) of morphine 2×10^{-6}M, merperidine 1.8×10^{-5}M, Methadon 2×10^{-6}M, scopolamine 8×10^{-8}M, and Amytal 5×10^{-5}M caused no change in the QO_2 values.

CHAPTER III

MATERIALS AND METHODS

Eighteen cows were used in this study. Three cows were obtained in each month of gestation from the fourth through the ninth month. Six placentomes were removed from each cow at the time of slaughter (Figures 1 and 2) following antemortem and postmortem inspection to assure healthy cows. The placentomes were placed immediately in plastic bags containing physiological saline, and the bags were stored in crushed ice. After returning to the laboratory the fetal cotyledon was carefully separated from the maternal caruncle. Portions of villi from the cotyledon and a one-eighth inch slice from the caruncle were prepared separately into chopped tissue suspensions. The McIlwain Tissue Chopper was set to chop the tissue into 0.156 mm. slices. The tissue was then turned ninety degrees and cross chopped at the same setting. Approximately one-fourth Gm. of the chopped tissue was suspended in three ml. of normal saline. One and one-half ml. of this preparation was added to a Warburg flask containing one and one-half ml. of phosphate buffer of pH 7.5 (9.923 Gm. KCl, 1.352 Gm. $MgCl_2$, 0.906 Gm. KH_2PO_4 per liter). Two tenths ml. of 30% sodium hydroxide was added to the center well of the Warburg flask which also contained a small strip of filter paper. The Warburg flask was attached to the manometer and flushed with

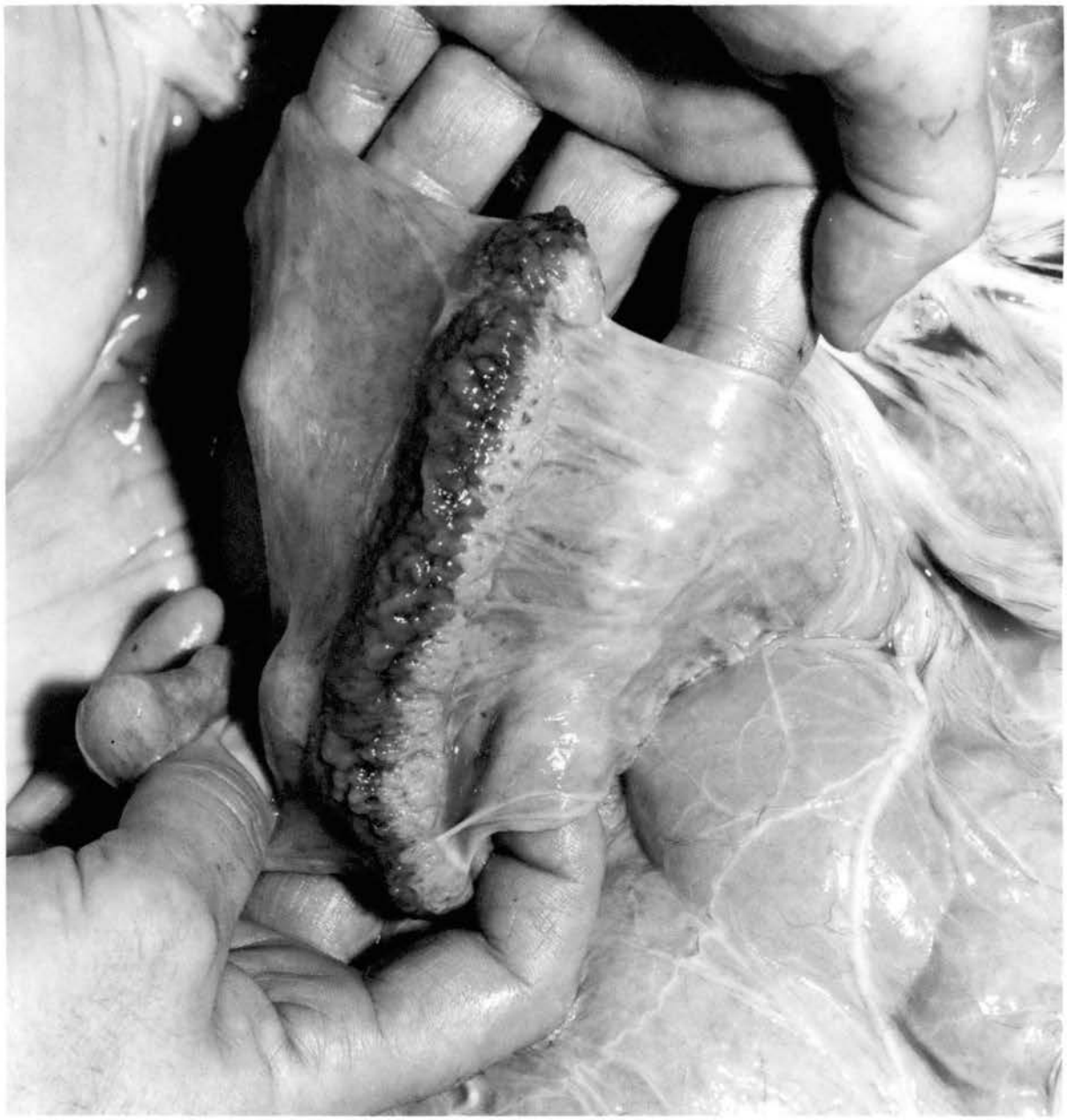

Figure 1. Placentome Still Attached to Uterus and Placenta.

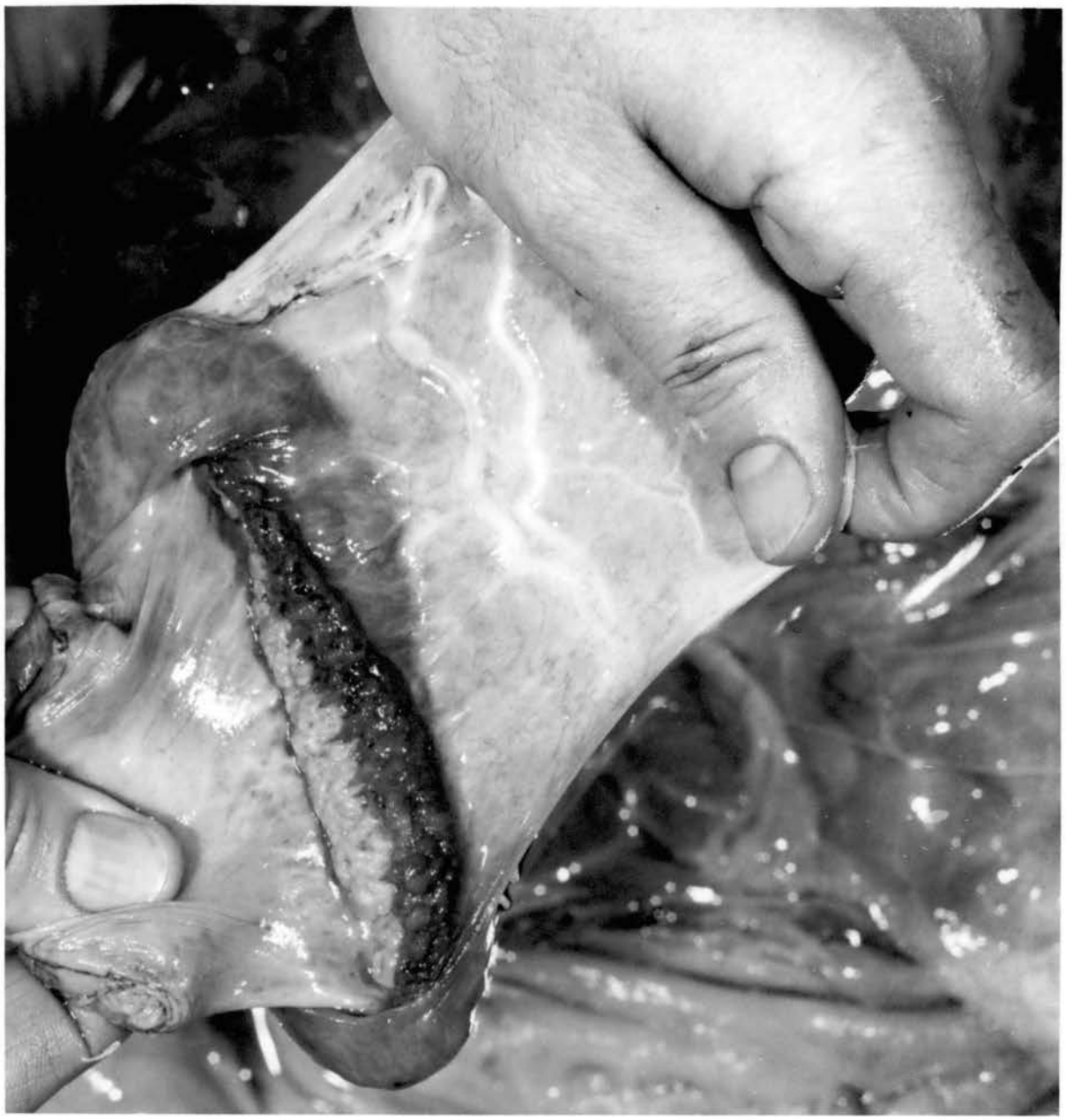

Figure 2. Placentome Detached from Uterus and Placenta and Ready for Refrigerated Storage.

medicinal oxygen for five minutes. Following oxygenation the manometers were attached to the Warburg apparatus and allowed to equilibrate along with the thermobar at 37°C. for seven minutes. At this time all stopcocks on the manometer and flask were closed and readings were taken every ten minutes for a thirty minute period. After completing the three readings, the tissue contents of the Warburg flasks were emptied into shell vials and dried in an oven at a constant 150°C. for twenty-four hours. The dry tissue weight in the vial was determined and the QO_2 was computed on the basis of dry weight, and expressed as $mm.^3$ of oxygen uptake per mg. of dry tissue per hour.

The lapse of time between collection of the placentomes and the beginning of timed Warburg recordings was approximately two and one-half hours, $\pm$ fifteen minutes. The tissue preparations were kept just above freezing until oxygenation was begun. There was no problem in preparing pure fetal membrane preparations, however, considerable precaution was exercised to remove macroscopic fragments of the fetal membrane villi from the maternal caruncular slices prior to preparing the chopped suspension. The length of the gestation period for each cow was determined from size and age characteristics of the fetus (Roberts 1956).

Figure 3. Separating the Cotyledon from the Caruncle.

CHAPTER IV

RESULTS AND DISCUSSION

The mean QO_2 of the maternal placental tissue declined rather steadily from the fourth through the ninth months of pregnancy (Table I and Figure 4). The mean QO_2 of the fetal placental tissue declined during the experimental period except during the seventh month of pregnancy (Table II and Figure 5). The number of samples (18) at each month should minimize the effects of individual variation, but the large standard deviation at each monthly period complicated analysis of results. Perhaps one should look at the general decline in O_2 uptake with less emphasis on individual sample or month of gestation, in which case the decline in O_2 uptake is apparent. A regression analysis of maternal QO_2 values, on the basis of an average of six placentomes for each of three cows at monthly intervals, shows the decreasing QO_2 values are linear and the decline is significant at the 1% level (Figure 4). Furthermore, the data can be fitted to a parabolic curve and this quadratic effect is significant at the five per cent level. This same analysis of the fetal QO_2 values shows the linear effect to be significant at the five per cent level (Figure 5), but the quadratic effect is not significant at this level. Most of the extreme variation in fetal placentome QO_2 values was confined to a few animals.

TABLE I

MEAN OXYGEN UPTAKE VALUES DURING VARIOUS STAGES OF PREGNANCY -- MATERNAL TISSUE

Pregnancy Duration	4 mo.	5 mo.	6 mo.	7 mo.	8 mo.	9 mo.
Mean QO_2 Values	.48	.52	.50	.39	.25	.18
Standard Deviations	.12	.16	.11	.15	.09	.15

TABLE II

MEAN OXYGEN UPTAKE VALUES DURING VARIOUS STAGES OF PREGNANCY -- FETAL TISSUE

Pregnancy Duration	4 mo.	5 mo.	6 mo.	7 mo.	8 mo.	9 mo.
Mean QO_2 Values	.49	.34	.35	.44	.26	.23
Standard Deviations	.24	.19	.13	.18	.14	.15

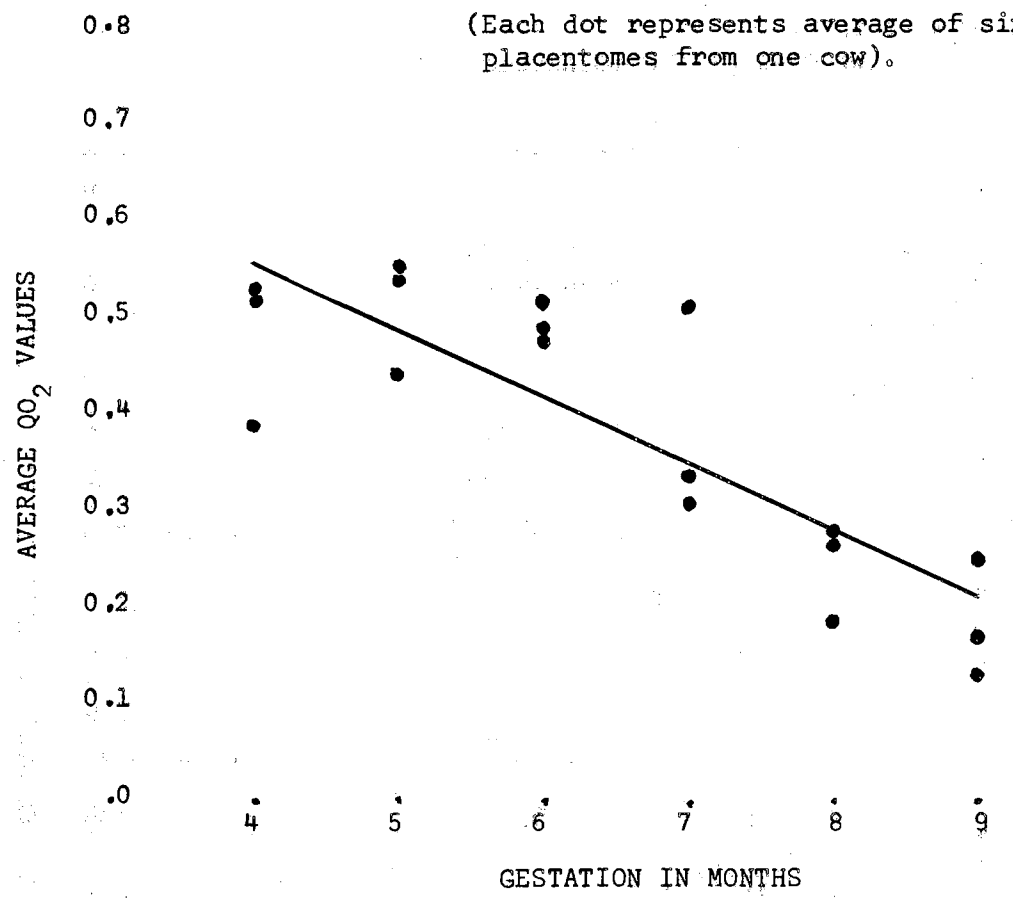

Figure 4. QO_2 Values for Maternal Placental Tissue During Various Stages of Pregnancy.

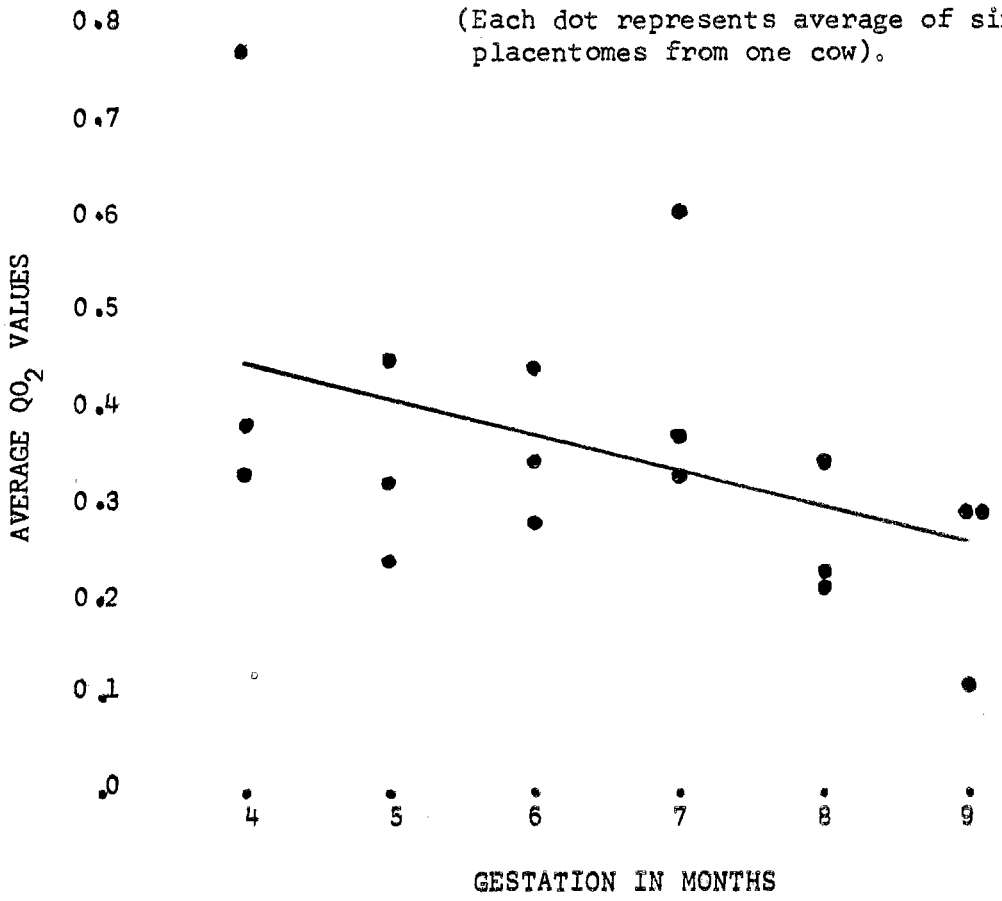

Figure 5. QO_2 Values in Fetal Placental Tissue During Various Stages of Pregnancy.

The reasons why the QO_2 values in this study are lower than those previously obtained from human placentas are open to speculation. Smaller QO_2 values for the cow's placenta may be due in part to the species weight differential. This would be expected according to Brody (1945) whose basal metabolism studies revealed that basal heat production, per unit body weight, in homeotherms decreases rapidly with increasing weight. As part of the "surface law" it is said that oxygen consumption, heat production, and heat loss are proportional to the square of the corresponding dimensions of the animal under comparison. Brody reports that a 500 Kg. Hereford cow has a basal metabolic rate of 6600 Cal/day, whereas a 60 Kg. human female has a basal metabolic rate of 1370 Cal/day. In other words the cow is metabolizing at the rate of 12.3 Cal/Kg. per day, whereas the human is metabolizing at the rate of 22.8 Cal/Kg. per day, an eighty-five per cent increase.

There are approximately seventy-five to one hundred and twenty placentomes in the uterus of the pregnant cow. These studies indicate an individual variation in O_2 uptake of different placentomes within the same uterus. If, at a specific time, there are variations in activity from one placentome to another in the cotyledonary type placenta, then a source of variation becomes apparent. The time lapse from tissue collection to Warburg studies should be considered as a factor possibly contributing to lower QO_2 values. The two and one-half hour interval in this study is longer than the time lapse reported by Wang and Hellman (1950) or Villee (1953), which amounted to approximately one-half hour. Because of conflicting data on the effect of storage upon the oxygen uptake of placental tissue it seems appropriate to await further study of this issue.

The breeding dates of the experimental cows were unknown, thus the duration of gestation had to be estimated. Even with considerable experience in estimating the age of the fetus, an error of two weeks is certainly possible. This means that the results for an individual sample could or should have been recorded in the preceding or the following month. Such errors contribute to a large standard deviation.

CHAPTER V

SUMMARY AND CONCLUSIONS

Six placentomes from each of three cows in the fourth, fifth, sixth, seventh, eighth, and ninth months of pregnancy were removed at slaughter. Warburg studies of oxygen uptake were determined approximately two and one-half hours post removal. Results indicate a variable decline in oxygen uptake by both caruncular and cotyledonary tissues. This decline in oxygen uptake toward the end of pregnancy supports the view that the placenta is maturing or aging in late pregnancy. If this reduction in O_2 uptake is a characteristic feature of aging in the placenta, then further work in the study of the mechanisms involved is indicated. Also further studies should be made to determine the oxygen uptake of placental tissue after delivery, including retained placentas of spontaneous or experimental occurrence.

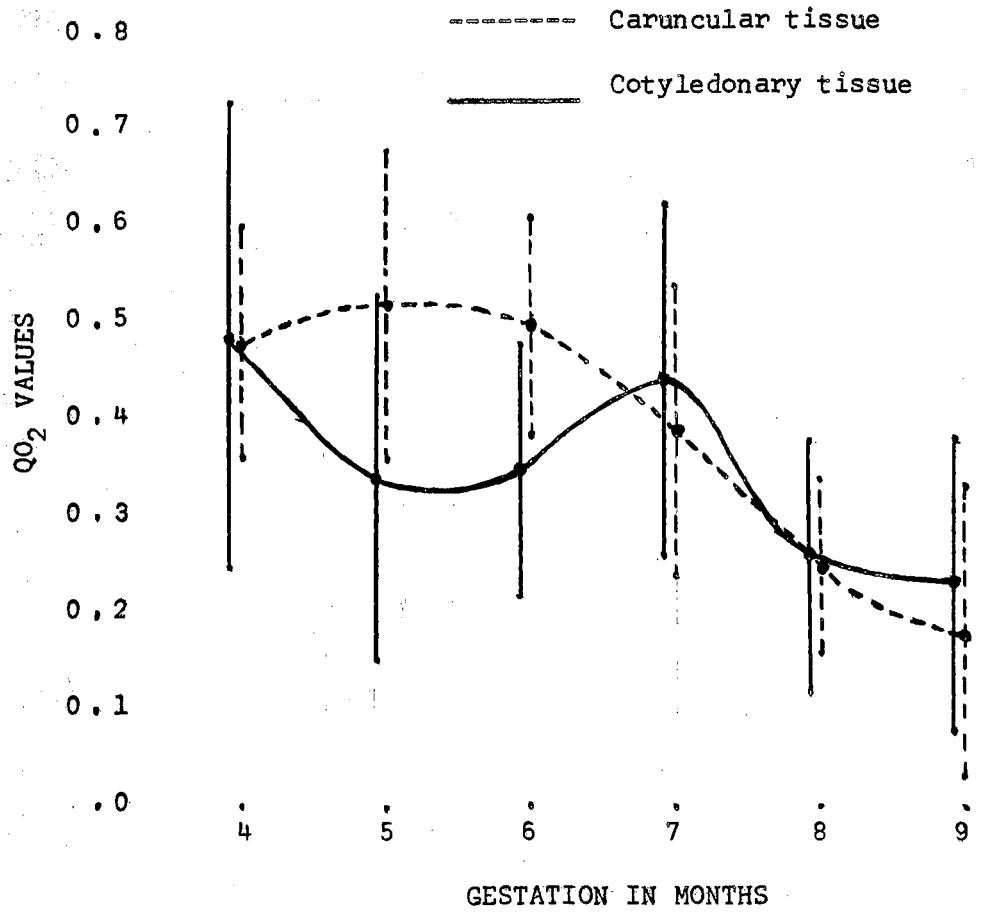

Figure 6. Mean Maternal and Fetal QO_2 Values and Standard Deviation During Various Stages of Pregnancy.

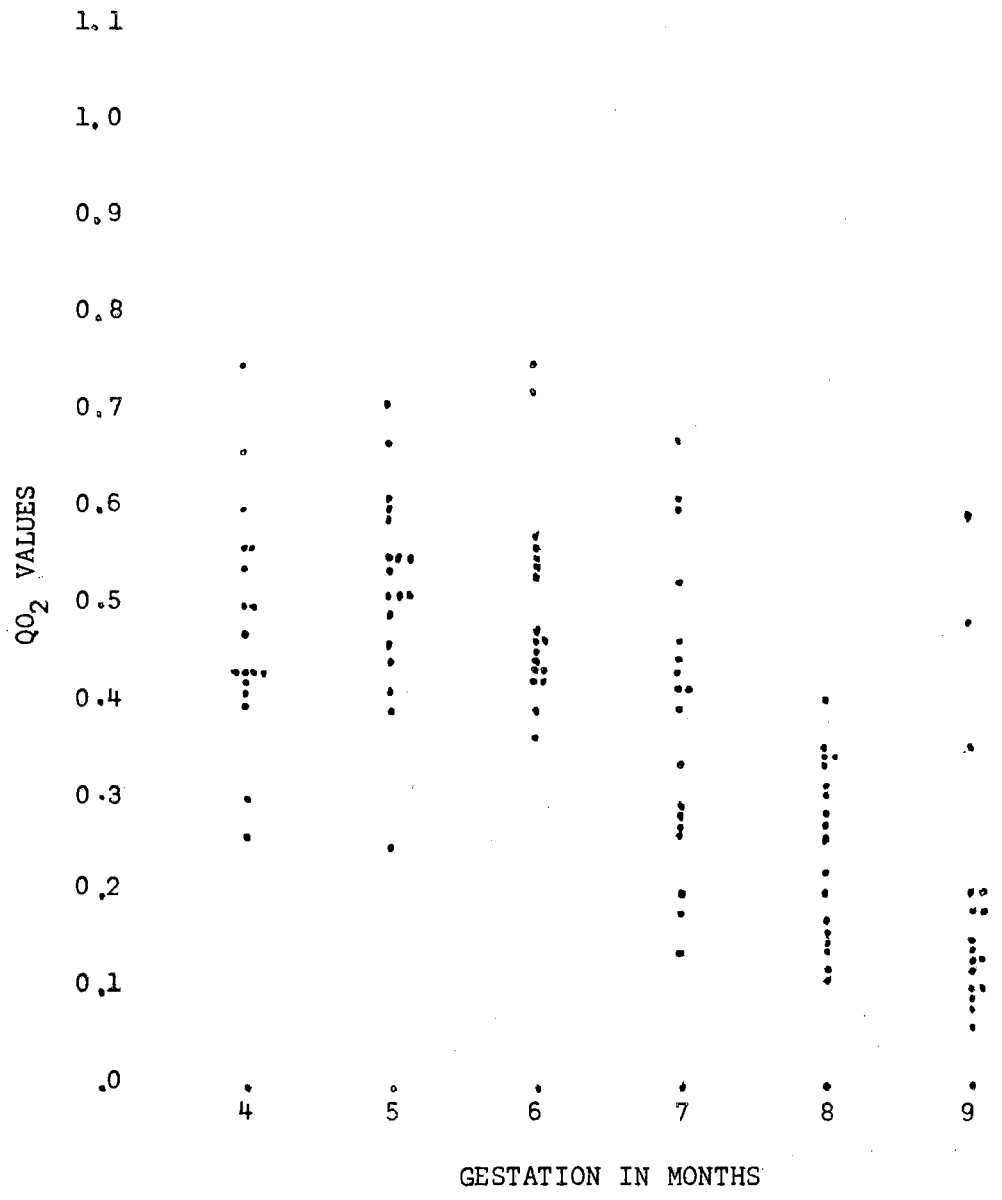

Figure 7. The Scatter of the QO_2 Determinations for Individual Maternal Tissues During Various Stages of Pregnancy.

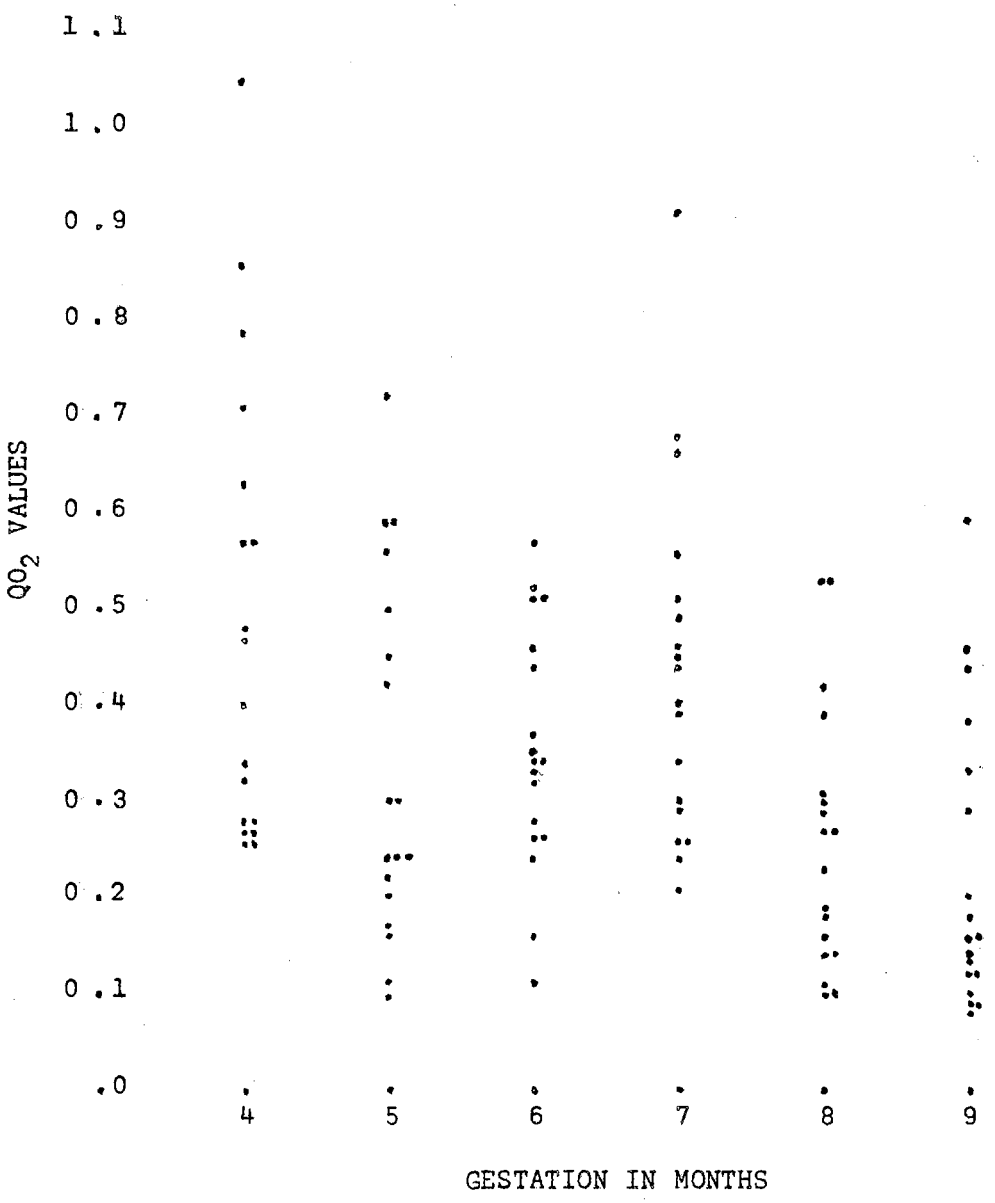

Figure 8. The Scatter of the QO_2 Determinations for Individual Fetal Tissues During Various Stages of Pregnancy.

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APPENDIX

INDIVIDUAL QO_2 VALUES FOR EACH PLACENTOME - 6 PLACENTOMES FROM EACH OF 3 COWS IN VARIOUS STAGES OF PREGNANCY

M = Maternal Tissue F = Fetal Tissue

4th Month		5th Month		6th Month	
M	F	M	F	M	F
.41	.57	.44	.24	.42	.57
.54	.63	.71	.56	.45	.26
.56	1.05	.55	.17	.57	.52
.43	.71	.55	.59	.46	.44
.60	.86	.54	.30	.54	.34
.66	.79	.46	.10	.42	.51
.42	.57	.59	.59	.46	.37
.43	.26	.51	.50	.55	.24
.47	.47	.67	.24	.43	.32
.75	.28	.41	.20	.39	.33
.56	.27	.61	.45	.56	.16
.50	.40	.49	.72	.75	.28
.26	.27	.51	.11	.36	.11
.43	.32	.25	.24	.43	.34
.43	.28	.51	.16	.47	.35
.50	.48	.60	.42	.44	.46
.40	.34	.55	.22	.72	.26
.30	.26	.39	.30	.53	.51

INDIVIDUAL QO_2 VALUES FOR EACH PLACENTOME -
6 PLACENTOMES FROM EACH OF 3 COWS IN
VARIOUS STAGES OF PREGNANCY

M = Maternal Tissue F = Fetal Tissue

7th Month		8th Month		9th Month	
M	F	M	F	M	F
.46	.40	.33	.18	.13	.59
.67	.66	.31	.31	.20	.13
.52	.56	.14	.14	.13	.18
.41	.44	.30	.16	.35	.14
.60	.91	.35	.30	.48	.29
.44	.68	.20	.27	.20	.38
.33	.45	.34	.23	.10	.08
.14	.26	.22	.39	.14	.10
.39	.26	.40	.19	.06	.09
.61	.21	.27	.42	.12	.12
.18	.30	.11	.27	.18	.16
.39	.51	.34	.53	.18	.09
.20	.39	.12	.10	.00	.46
.28	.29	.28	.11	.09	.12
.41	.46	.15	.14	.59	.20
.27	.34	.26	.53	.15	.16
.43	.24	.17	.29	.08	.33
.26	.49	.16	.10	.10	.44

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