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Uterine Phosphatase Concentrations and Their Relationship to Number and Weight of Embryos in the Rat

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ABSTRACT

This investigation is part of an attempt to determine the factors involved in embryonic mortality and the maintenance of viable embryos during gestation in swine. The multiparous rat has been used in this laboratory since 1951 to study certain reproductive processes before trying similar studies on farm animals.

A total of 112 mature female albino rats have been used, of which 100 were pregnant and 12 non-pregnant. The animals were bred and 13 days later they were killed by decapitation. The uterus of each was removed, emptied of its contents and analyzed for alkaline and acid phosphatases by the method of Huggins and Talalay (1945). The weight of the gravid and empty uterus, number of implantation sites, and the number of viable embryos were recorded.

The results show that 28 percent of the corpora lutea were not represented by viable embryos on the thirteenth day of gestation and that 60 percent of this loss occurred prior to implantation. The data also show: (1) The number of implants present on the 13th day had no influence on average embryonic weight. (2) As the number of embryos increased by 1, the uterine weight increased by + .0622 grams. (3) The acid phosphatase concentration exceeded that of the alkaline phosphatase both in pregnant and non-pregnant rats. (4) An increase in uterine weight was paralleled by an increase in acid phosphatase and a decrease in alkaline phosphatase. (5) The acid phosphatase content of the uterus was correlated significantly with uterine weight ($r = +.744$; $P < .01$), the number of implantations, the weight of implantations, and with the number of viable embryos.

The results of this investigation suggest that the uterine contents may influence, either directly or indirectly, their environment (the uterus) but do not rule out an alternative hypothesis that hormones or other factors control the uterine environment which in turn affects the uterine contents.

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INTRODUCTION

As a part of research on litter size in swine, investigators at the Missouri Station have given major consideration during the past few years to embryonic deaths as a contributing factor. Numerous reports in the literature on swine and other litter bearing animals show that less than two-thirds of the ova released during an estrous period result in viable embryos at the end of the first quarter of the gestation period. The results of Squiers *et al.* (1952) suggest that fertilization failures account for but a small fraction of the total loss.

In swine, according to Squiers *et al.* (1952) and Lerner *et al.* (1954), the bulk of the loss occurs prior to the twenty-fifth day of the gestation period and is attributable to a failure of the fertilized ova to implant or to the death of the embryo immediately following implantation. Further, as shown by Squiers *et al.* (1952), among 147 pregnant gilts bred once in early estrus and slaughtered 25 days later, only 65 percent of the ova shed were represented by normal embryos at the time of slaughter. On 65 sows in which the mean number of ova shed was 15.3, a loss of 35 percent of these ova was observed at the twenty-fifth day.

Perhaps, then, a major portion of the loss of fertilized ova at and immediately following implantation may be the result of a natural physiological mechanism in which the number of ova shed and fertilized exceeds the number that the uterine surface available in an animal can accommodate and maintain during gestation. This mechanism would furnish adequate numbers of fertilized ova to assure that the uterus will have available, for implantation and maintenance during a gestation period, its quota of embryos. Similarly, in the male, a great excess of spermatozoa are available at each mating to assure the fertilization of the one or more ova shed by the female.

It would appear, then, that our first endeavor is not necessarily the prevention of early embryonic mortality but, through selection, to provide females with a high ovulation rate and a maximum of uterine surface for the implantation of a greater number of the ova shed.

If the above can be accomplished, a second problem of physiological importance is that of maintenance of the implanted embryos throughout the gestation period. Although many factors, including nutrition and other pro-

duction practices, are essential during gestation, embryonic viability is influenced primarily through the immediate environment of the embryos— the uterus.

Estrogen and progesterone (and possibly other hormones) affect the embryos indirectly through the uterus by influencing enzymes and enzyme systems. Changes in the uterus of a physiological nature would change the environment of the embryos and should be reflected in the enzyme systems. Quantitative measurement of the activity of certain enzymes previously shown to be affected by fluctuations in the concentration of the ovarian hormones should yield data indicative of physiological conditions in the uterus.

Since it is not economically feasible to use farm animals for research of this nature, the multiparous rat was used in investigations that were started at this laboratory in 1951. The first series of investigations was concerned with hormone relationships and their essentiality in maintaining pregnancy in rats ovariectomized a few days after mating. This series was followed by experiments designed to measure the levels of phosphatases in the uterus and to correlate the fluctuations of these enzymes with various levels of administered hormones.

It was concluded, on the basis of these preliminary experiments with ovariectomized female rats, that the levels of these enzymes should be more completely understood in the normal pregnant animal not receiving hormone therapy in order to evaluate correctly the results obtained by such treatments. It appeared highly possible that the number or weight of the embryos might in themselves, directly or indirectly, influence the concentration of phosphatases (or other enzymes) in the uterus.

The investigation reported in this bulletin was an endeavor to determine possible correlations between the number of implantations, viable embryos, or weight of uterine contents and the levels of acid and alkaline phosphatases in the uteri of normal pregnant female rats.

REVIEW OF THE LITERATURE

The phosphatases apparently are present in almost every kind of cell as well as in milk, but despite this rather universal distribution, relatively little is known about them. They may be defined, however, as enzymes which are capable of catalyzing the hydrolysis of various esters of phosphoric acid. Since the phosphatases are quite precise in their affinity for the phosphoric component of the ester, they may be divided into groups, the nomenclature of which is based on the degree of esterification of the phosphoric constituent. There are considered, therefore, to be three general types which are called phosphomonoesterases. phosphodiesterases and pyrophosphatases. In general, phosphomonoesterases are not too specific in regard to the nature of the alcoholic radical and act on many organic phosphates in a similar man-

ner (Baldwin, 1952). The rather general hydrolytic reaction that they catalyze might be considered as follows:

$$R\text{-}OP + H.OH \rightleftharpoons R\text{-}OH + HOP$$

For purposes of classification they may be divided into two groups depending on the pH necessary for optimum activity (Folley and Kay, 1935). Those which have an optimum pH of about 5 are called acid phosphatases, and those with an optimum pH of around 9, alkaline phosphatases. Huggins and Talalay (1945), using human semen, reported the optimum pH for acid phosphatase to be from 5.5 to 6.0 and an extract of cattle intestinal mucosa gave an optimum pH range of from 9.1 to 9.6 for alkaline phosphatase activity.

Occurrence and Physiological Significance

Pritchard (1947), while investigating the distribution of alkaline phosphatase in the decidua and foetal membranes of the rat throughout pregnancy, discovered that during cellular proliferation and differentiation, the enzyme was predominantly nuclear. Fully differentiated cells engaged in the transfer of materials to and from the fetus showed heavy concentrations of the enzyme in their peripheral cytoplasm. The enzyme was least active in the region of the decidua, trophoblast and metrial gland engaged in glycogen storage. Specifically, the enzyme was found from the first to the fourth days in the uterine epithelium, endothelium and leucocytes. From the fifth to the eleventh days the decidua contained large amounts, and from the eleventh day to term, large quantities were in the placenta and yolk sac. It disappeared from the uterine epithelium on the fifth day and reappeared on the thirteenth.

Wislocki and Wimsatt (1947), claim that the chorionic epithelium absorbs material present in the uterine lumen, including erythrocytes, iron compounds, phosphatase and possibly glycogen. The phosphatase is passed through Reichert's membrane into the yolk sac where it is absorbed by the cells lining the visceral wall. Phosphatase gradually appears and increases steadily in amount as the placental labyrinth ages. The enzyme is localized chiefly in the trophoblastic elements of the endotheliochorial trabeculae.

Acid phosphatase was found to be present in lesser amounts than alkaline phosphatase at all times in the tissues studied by Stafford, McShan, and Meyer (1947). They concluded that phosphatase may play a role in the secretion of steroids by the lutein cell, since phosphatase esterification of several substances is believed to be necessary for the passage of these substances across cell membranes. It seems reasonable that dephosphorylating enzymes such as phosphatases might be of importance in the passage of luteal hormone precursors into the lutein cell and the secretion of the finished product out of the cell.

The kidney tubules' alkaline phosphatase probably functions in dephosphorylating sugar molecules that are removed from the tubular fluid by phosphorylation (Moog, 1946). The enzyme is present in other sites where it might serve in a similar manner.

Phosphatases are commonly found in the cytoplasm of growing, regenerating, and secreting cells in which protein synthesis is in progress. There appears to be a correlation in such cells between content of ribose nucleic acid and phosphatase. In dividing nuclei, phosphatase parallels desoxyribose nucleic acid. Acid phosphatase is found in seminal fluid where it may help to nourish the spermatozoa. In tissues which carry on much traffic in glycogen, acid phosphatase is possibly provided to prevent bone formation. Alkaline phosphatase is indispensible in normal ossification.

By use of various adaptions or ramifications on the basic methods of Kay (1926), Gomori (1940) or Takamatu (1941), it has been shown that both enzymes are in rather uniform distribution in the cell, and never in discrete granules. Specific intracellular localizations may be shown, however.

The occurrence of two common phosphomonoesterases with widely different pH optima may indicate a mechanism for allowing two dephosphorylating steps to go on simultaneously in a single cell.

Chemical Methods of Determining Phosphatase

In 1930, Kay introduced a method of determining quantitatively the alkaline phosphatase activity of the blood, employing sodium beta glycerophosphate as the substrate. Of the many modifications introduced in which this substrate was used, Bodansky's (1933) method has been more generally employed because of its short incubation period and accuracy. The King-Armstrong method also made its appearance (1934) and has been adapted to the acid range by Gutman and Gutman (1940). The King-Armstrong method is based on the use of disodium phenyl phosphate as a substrate, and it measures the phenol that is liberated in the ratio of three phenol to one phosphate.

Shinowara, Jones, and Reinhart (1942) developed a method for the simultaneous determination of serum inorganic phosphate and "acid" and "alkaline" phosphatase activity.

In contrast to the numerous and rather well defined methods of serum phosphatase determinations are the less standardized methods of tissue phosphatase determinations. In 1939, Gomori in the United States and Takamatu in Japan, independently published a histochemical method of determining phosphatases, but most of the present procedures are ramifications of a method developed by Kay (1926). The general procedure in these methods is to mince the tissue and allow the resultant homogenate to autolyze in a solution of water and toluene or chloroform for a specified length of time. After autolysis has occurred, the mixture is subjected to centrifugation and one of

the previously mentioned methods for determining serum phosphatase is used on the clear extract.

One of the more recent methods developed for phosphatase determination is that of Huggins and Talalay (1945). It embodies the use of soluble sodium phenolphthalein phosphate, which is water clear until enzyme decomposition occurs. Phenolphthalein is then liberated and determined colorimetrically in an alkaline solution. The advantages of this method include simplicity, accuracy, a reduced number of technical manipulations, and the elimination of the need for preliminary separation of proteins. It is assumed in this test, as in other quantitative phosphatase tests, that the amount of product formed under standard conditions is proportional to the concentration of the enzyme, as long as an excess of substrate is provided. Among those who have made use of adaptations of this method are Stafford, McShan, and Meyer (1947) who used it while determining acid and alkaline phosphatase in the corpus luteum and corpus luteum free ovarian residue of the rat.

MATERIALS AND METHODS

For the present study, an inbred colony of albino rats was used, the experimental animals all being mature virgin females. The animals to be bred were caged with males and examined every morning for signs of mating. This was done by the vaginal smear technique using a small cotton swab; the presence of sperm cells in the smear constituted a positive test for successful copulation. The morning of the day on which spermatozoa were first observed in the smear was considered the first day of pregnancy. The animals were then removed from the "breeding cages" and isolated until the thirteenth day of pregnancy, at which time they were slaughtered by decapitation.

The thirteenth day of pregnancy was chosen because the enzyme concentration before placentation was to be measured and placental function probably occurs on or after the fourteenth day (Long and Evans, 1922).

After the animals were decapitated, the gravid uterus was quickly removed, placed on moist filter paper, freed of any excess fat or tissue and weighed. A chainomatic type analytic balance was used for all weighings throughout this work. After weighing, each horn of the uterus was slit from the cervix to the distal end and the contents scraped out. The number and location of all embryos were recorded as well as any abnormalities. The empty uterus was then weighed, and the weight of the embryos, their membranes and their fluids found by difference. The uterus was then placed in a tightly stoppered tube and kept at a temperature of -20° C until homogenized. The ovaries were then removed and the corpora lutea of pregnancy counted. The observance of tumors, pathological conditions, or serious abnormalities of any kind resulted in the immediate elimination of the animal.

Prior to about 1945, the methods for determining phosphatase activity in tissues or cells were predominantly histochemical or entailed the use of tissue extract in the hydrolysis of various phosphate esters. Moog (1946), however, pointed out that much of the tissue phosphatase is bound to "heavy particles" in the cell, and that values obtained using tissue extracts would not indicate the true enzyme content of the tissue. It is, therefore, more desirable to use a whole tissue homogenate rather than an extract and thereby achieve a more valid measurement of the phosphatase activity of that tissue.

The method used in these experiments was an adaption of that used by Huggins and Talalay (1945), and quite similar to the one described by Stafford, McShan, and Meyer (1947). The tissue to be analyzed was homogenized in a Potter type homogenizer using a friction drive motor. The homogenizing tube containing the uterus and 5ml. of distilled water was kept in an ice bath throughout homogenization to prevent denaturation of any protein by heat and to prevent any enzyme activity. All tissues were homogenized for approximately 10 minutes, after which they were diluted to a volume of 100 ml. A specific quantity of this homogenate was placed in a test tube containing 2 ml. of the appropriately buffered substrate and diluted to 2.6 ml. The substrate-containing tubes had previously been equilibrated in a water bath at 37° C.

The amount of homogenate finally used was 0.5 ml. for both acid and alkaline determinations. Various quantities were tried in the test, ranging from 0.2 to 0.5 ml., and 0.5 ml. was found to give the most satisfactory results. The amount of substrate used was 2 ml. for both determinations. The acid substrate was buffered at pH 5.4 for the acid phosphatase determination with sodium acetate and acetic acid, while the alkaline substrate was buffered at pH 9.7 for the alkaline phosphatase determination with sodium barbital. All determinations were made in duplicate, since it was found that little was to be gained in the way of accuracy by triplicate determinations.

Tubes containing tissue homogenate and substrate were then incubated in a water bath at 37° C for precisely 1 hour, at the end of which time the reaction was rapidly stopped and the characteristic color developed. This cessation of activity was accomplished by the addition of 2 ml. of glycine buffer containing sodium pyro-phosphate, a phosphatase inhibitor. To further insure rapid retardation of enzyme activity, the tubes were simultaneously plunged into an ice bath. After the addition of glycine buffer (pH 11.2) the pH of the entire mixture was around 11, which is within the range of optimum pH conditions for maximum color development of phenolphthalein. This mixture was then diluted to a final volume of 10 ml. and the tube rotated to insure uniformity in color throughout. The solution was transferred to a clean, dry colorimeter tube and read in a Fisher Electropho-

tometer using a 550 millimicron filter. All readings were taken exactly 2 minutes after the addition of the glycine buffer.

To correct for the slight turbidity of the solutions, all tissue-containing tubes were read against a blank tube containing glycine, homogenate, and water. The relatively small amount of homogenate in the final volume did not warrant centrifugation and practically no color could be recorded on pure substrate blanks due to the presence of free phenolphthalein, so no correction factor was necessary.

A phenolphthalein calibration curve had been previously constructed by plotting known concentrations of phenolphthalein against the colorimeter readings of these concentrations. The straight line which resulted was used subsequently to convert colorimeter readings into units of phosphatase.

A phosphatase unit curve was then determined by the following procedure: Varying amounts of uterine tissue from estrous rats were homogenized and subjected to the preceding test in both acid and alkaline media. Amounts of uterine tissue used ranged from 0.3343 gm. to 2.6745 gm. per 100 ml. The standard phenolphthalein curve was used to convert the electrophotometer readings into amounts of phenolphthalein liberated by these various enzyme concentrations. These values, expressed as mg. phenolphthalein liberated per 100 ml. homogenate, were plotted on graph paper *versus* mg. of tissue per 100 ml. of homogenate. A parabolic curve resulted. The shape of this curve was determined for both acid and alkaline phosphatase. Since it is more convenient to work with a straight line than a parabolic curve, the amounts of phenolphthalein liberated per 100 ml. of homogenate were plotted *versus* the amount of tissue per 100 ml. on log-log paper, and the result was a straight line. Essentially parallel curves were obtained for the acid and alkaline phosphatases which illustrates that the rate of substrate hydrolysis is the same for both enzymes, even though they are present in quantitatively different concentrations. The unitage is defined by Huggins and Talalay (1945) as "10 units of acid or alkaline phosphatase is the amount of enzyme which will liberate the colorimetric equivalent of 1 mg. of phenolphthalein from excess substrate in 1 hour at 37° under optimum conditions of pH." Therefore, a line was drawn parallel to the two lines determined above, but passing through the reference point or point of definition of their units, i. e., 10 units equals 1 mg. of phenolphthalein. This is the unit curve, as shown in Figure 1. The X-axis was then rearranged to represent units of phosphatase instead of mg. of tissue.

When the density of a given sample was obtained on the electrophotometer, it was converted by means of the standard phenolphthalein curve into gamma of phenolphthalein per 10 ml. This value was converted to mg. of phenolphthalein liberated per 100 ml. of homogenate, applied to the unit curve, and the units of phosphatase per 100 ml. of homogenate read directly.

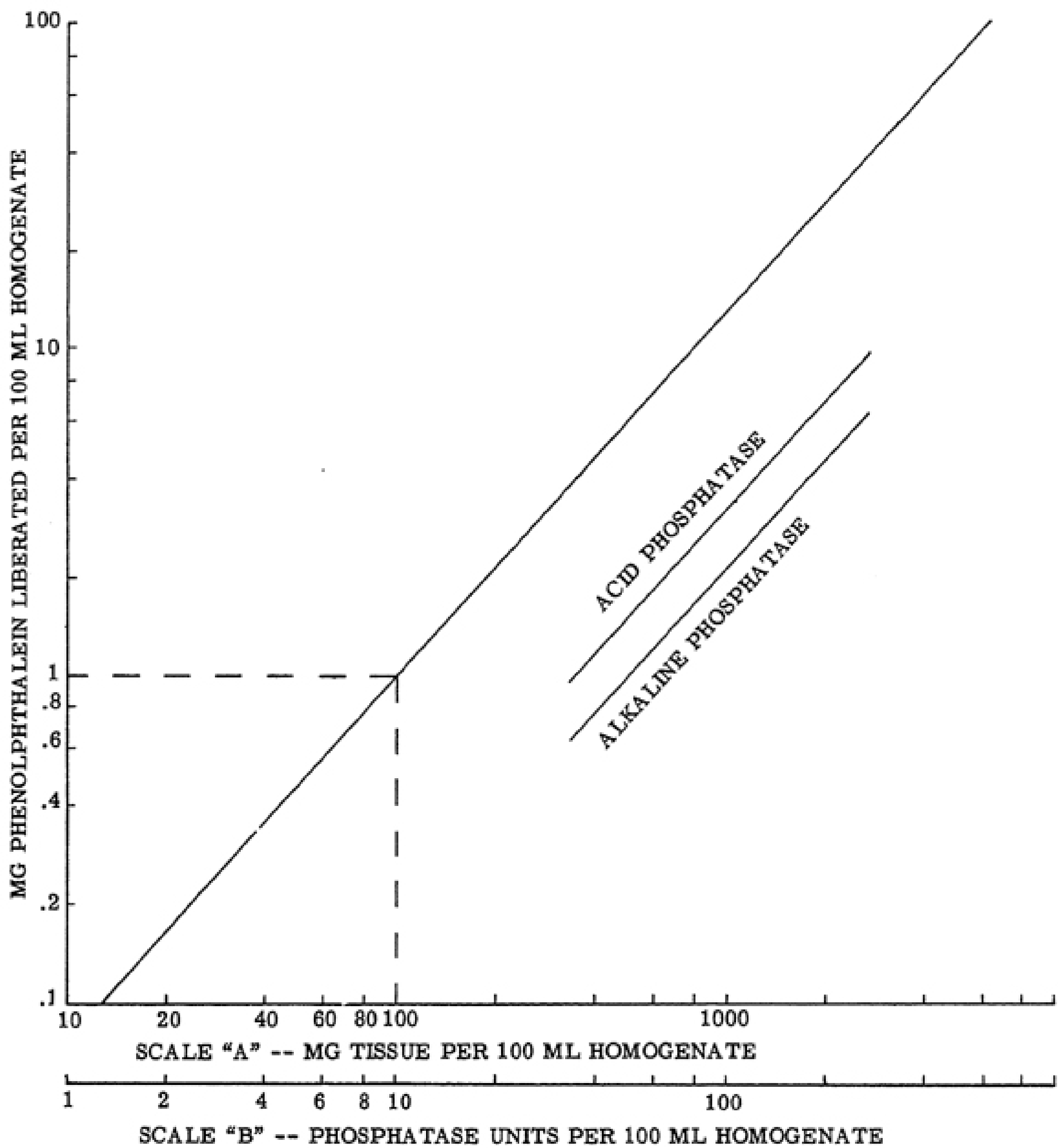

Figure 1—Log-log plot of phenolphthalein liberated in acid and alkaline media by increasing concentrations of uterine tissue homogenate (Scale "A"), and phosphatase unit curve (Scale "B").

All enzyme concentrations in this paper are expressed as units of phosphatase per gram of fresh tissue. These values were calculated from units of phosphatase per 100 ml. of homogenate, since the amount of tissue per 100 ml. of homogenate was known.

RESULTS AND DISCUSSION

A total of 112 rats were used in this investigation. Of this number, 100 were pregnant and 12 were used as normal non-pregnant controls.

The weight of the gravid uterus and of the empty uterus, the number of implantation sites, and the number of viable embryos were recorded for all of the animals but only those concerning rats which were subjected to the enzyme analyses are reported. In addition to these data, the concentration of acid and alkaline phosphatase was determined in 57 of the experimental animals and the number of corpora lutea present was obtained on 61 of the animals. These data are presented in Tables 1, 2, and 3 in Appendix.

One of the first facts observed was the increase in uterine weight with increasing numbers of implantation sites or viable embryos. Statistical analysis revealed a highly significant positive coefficient of correlation of 0.845 between the uterine weight and the number of viable embryos. Figure 2 represents this analysis based on 57 animals; the points plotted are averages.

An unexpected result was that no significant correlation existed between the number of implantations and the average weight of the implantation. This average weight was determined by dividing the weight of the uterine contents by the number of implantation sites. Since the number of implantations and the number of viable embryos did not differ in a substantial number of instances out of the 57 animals analyzed, no analysis was made between the number of viable embryos and their average weight. It should be remembered that these observations were made on the thirteenth day of pregnancy, and though no relationship could be established at this stage in the gestation period, there is some evidence indicating that the number of viable young delivered at parturition does affect their birth weights substantially. Apparently, at least in the rat, this inhibition of growth or reduction in average birth weight by an increased number of embryos does not occur until the second half of pregnancy. This may be partially explained by the fact that up to the thirteenth day there is no obvious crowding effect caused by a large number of viable embryos, hence this inhibiting factor is not present. After the middle of pregnancy, however, it is a force that probably should receive consideration.

Data on 100 animals showed an average of slightly over 9 implantations and an 8 plus average for number of viable embryos.

After the amounts of acid and alkaline phosphatase had been determined, and expressed as units per gram of fresh tissue, a number of interesting relationships were revealed. In all 57 determinations, the acid phosphatase was higher than the alkaline, with an average of 40.59 units and 13.14 units per gram of fresh tissue, respectively.

In comparison to these enzyme levels, the 12 non-pregnant control animals averaged 26 units per gram of fresh tissue for the acid, and 20 units

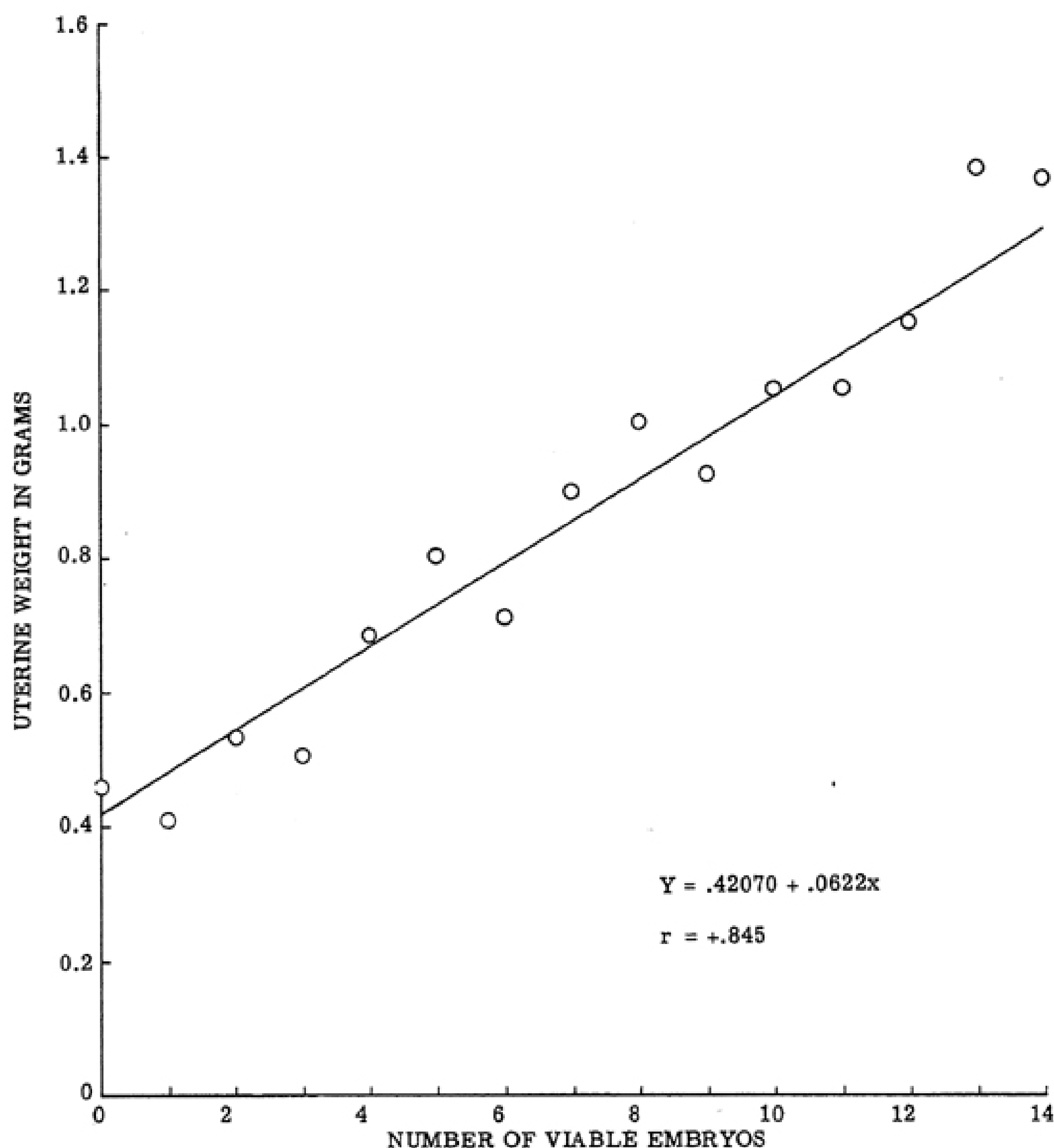

Figure 2—Regression line of number of viable embryos on uterine weight.

per gram of fresh tissue for the alkaline phosphatase. In only one instance did the alkaline phosphatase concentration exceed that of the acid phosphatase. The reason for this one exception is not known.

Analysis of the acid and alkaline phosphatase values showed a negative coefficient of correlation between the two of -.105, which is not statistically significant. While this correlation is not large, it may indicate a trend; mainly that when acid phosphatase is high, the aklaline phosphatase may have a tendency to be low. Of course this statement cannot be positively stated on the basis of these data alone.

The amount of acid phosphatase was compared with the uterine weight, total weight of the uterine contents, average implantation weight, number of implantations, and number of viable embryos. This last relationship is represented graphically in Figure 3. In each instance, a positive, statistically significant coefficient of correlation was established, with the highest one being between acid phosphatase and uterine weight ($r = +.744$) and the lowest between the enzyme concentration and average weight of implantation ($r = +.334$).

It had been shown earlier that there was a close relationship between the number of viable embryos or implantation sites and total uterine weight, as illustrated by the regression line in Figure 2. If, then, a positive, significant correlation is obtained between the amounts of acid phosphatase and number of viable embryos, one might suspect a similar relationship between the enzyme concentration and uterine weight because the uterine weight seems to depend to a large extent on the number of embryos it contains. This anticipated correlation was found.

Since the average weight of the embryo, determined in the manner described previously, was not correlated with the number of implantations, one could say that the weights of the individuals were independent of the number contained in the uterus. An analysis therefore was made to determine whether there was any relationship between the average embryonic weight and the level of acid phosphatase. A statistically significant r value was obtained, +.334, but this number was not as high as the one which resulted when the total weight of the uterine contents was compared to the acid phosphatase concentration. From the latter analysis a coefficient of correlation of +.727 resulted.

By comparing the acid phosphatase concentration to the number of implantations and the number of viable embryos, positive coefficients of correlation of 0.616 and 0.635, respectively, were obtained.

Thus, we see that in all instances, with an increase in uterine weight, weight of uterine contents, average embryonic or implantation weight, number of implants, or number of viable embryos, an increase in acid phosphatase also occurs.

Following this, the alkaline phosphatase values were subjected to the same analyses as the acid phosphatase (correlated with uterine weight, weight of the uterine contents, average weight of the embryos or implantations, number of implantation sites and number of viable embryos). The striking fact noted here, though not statistically significant in every instance, was that a negative coefficient of correlation was obtained in every analysis.

A low coefficient of correlation was found between the enzyme concentration and average weight of implantation, the r value being -.135. Values approaching significance resulted from comparing the alkaline phos-

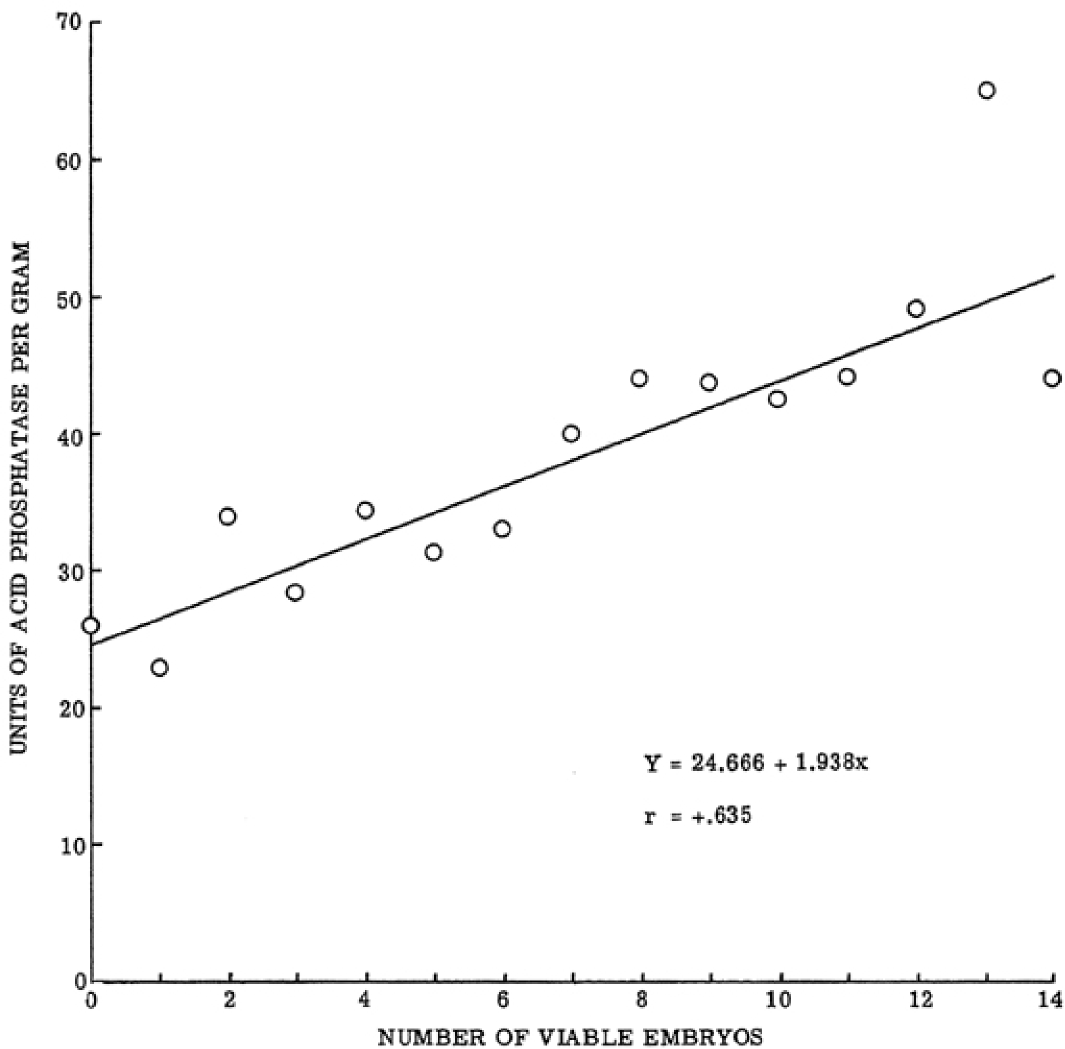

Figure 3—Regression line of number of viable embryos on units of acid phosphatase.

phatase level to weight of uterine contents and number of viable embryos, but more data are necessary before any positive statement can be made concerning these relationships. Figure 4 represents this last correlation.

The two statistically significant coefficients of correlation were found between the enzyme concentration and uterine weight ($r = -.324$) and the alkaline phosphatase level and the number of implantations ($r = -.354$). Thus, it is seen that as the number of implantations increased, and also the uterine weight, the amount of alkaline phosphatase in the uterus decreased.

The dependence of the uterine mass on the number of implantation sites, as previously discussed, is again illustrated by the fact that the only significant correlations were between the enzyme concentration and the

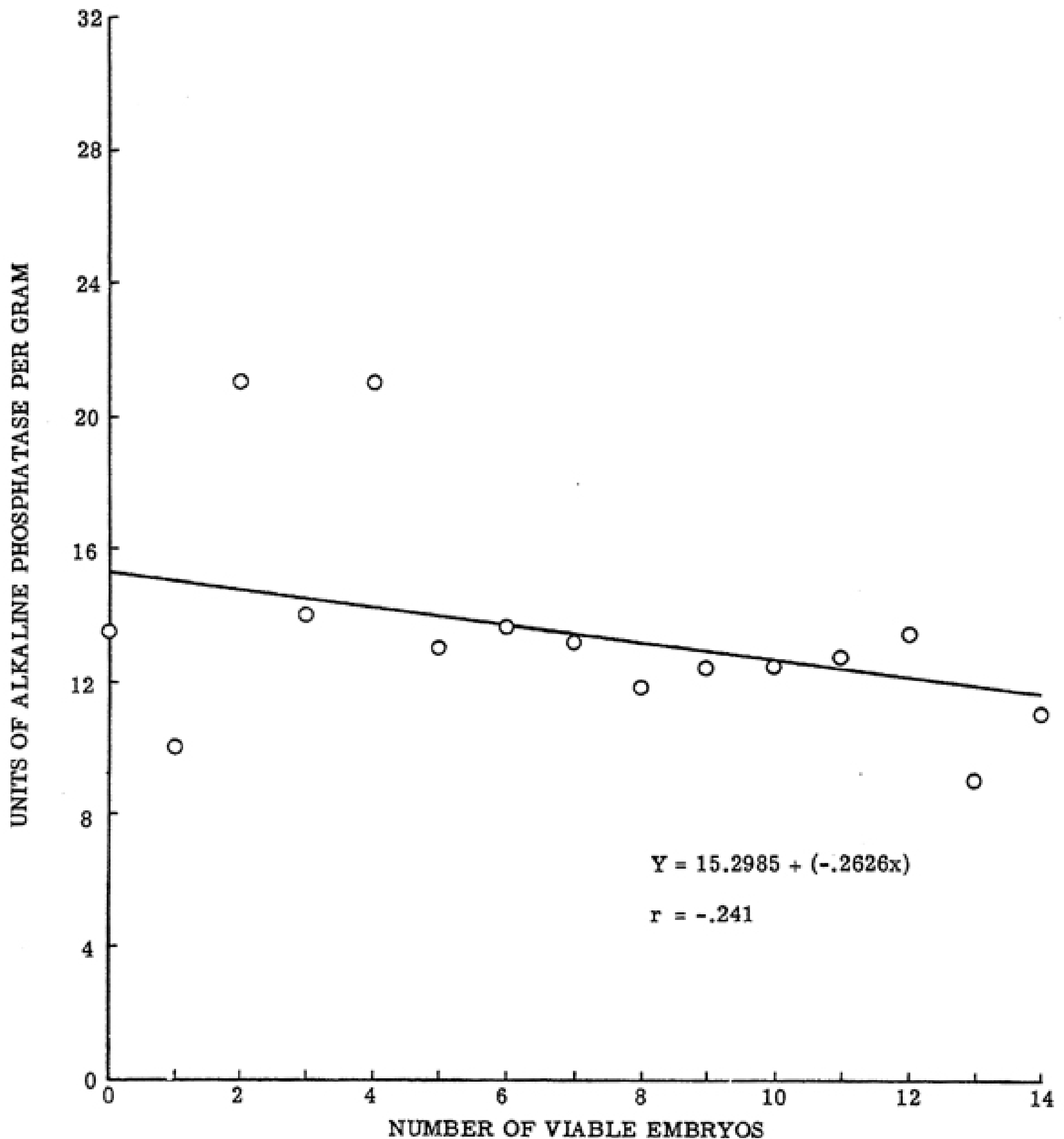

Figure 4—Regression line of number of viable embryos on units of alkaline phosphatase.

number of implantations and in turn with the uterine weight, which is materially affected by the quantity of future individuals found in the uterus.

Though no significance can be placed on the mathematical expression of the relationship of alkaline phosphatase to the number of viable embryos, it seems reasonable to assume that more data would confirm the existence of such an interrelationship, insofar as one does exist between this phosphatase and the total number of embryos. At this time, however, the data do not support concrete assertions.

When the acid and alkaline phosphatase content of the uterus was considered as a unit, expressed as total phosphatase content, and compared to

the various weights (uterine, uterine contents, and average implantation) and numbers (implantations and viable embryos), positive correlations were found in every instance. Thus, we see that the high positive correlations found between the acid enzyme and these various factors is not to be denied, since the relationships between total enzyme concentrations more closely approximate those found for the acid phosphatase than the negative ones obtained for the alkaline enzyme.

By comparing the number of corpora lutea on both ovaries and the number of implantations, it was found that an average of 17 percent of the eggs shed were not implanted. It may be noted in a few instances that the number of implantation sites exceeded the number of corpora observed. This may be due to a failure to count all of the corpora, two ova shed by one corpus luteum, or to twinning. From the data in Table 3, it can be seen that the average number of corpora lutea for 61 animals was 11.67.

From the data presented in Table 3, it was calculated that 83 percent of all the ova shed were fertilized and underwent nidation, which indicated a 17 percent loss between ovulation and implantation. All of the eggs which were fertilized originally, however, were not viable after 13 days, and analysis of these data revealed that only 72 percent of the ova released at ovulation were viable embryos at this stage in gestation. This is a further loss of 11 percent between implantation and placentation, and results in a total death loss of 28 percent. This calculation, of course, is based on the supposition that all of the normal appearing embryos at this stage would continue to thrive and produce living young. It seems feasible that a further, though slight, death loss may occur between placentation and parturition as has been reported in swine by Squires, *et al.* (1952), which would increase this value. An over-all death loss of 28 percent occurred between ovulation and placentation in the 61 animals under observation; 17 percent occurred between ovulation and implantation, and 11 percent between implantation and placental functioning. Thus, it appears that the more important reduction in number of living young occurred between ovulation and implantation, as 60 percent of the total death loss occurred during this time.

In swine, although the embryonic death loss of 35 percent does not differ markedly from the 28 percent observed in rats, the major portion of the early embryonic mortality occurred between fertilization and the twenty-fifth day of gestation with fertilization failures accounting for but a small fraction of the total loss. In the rats under investigation, 60 percent of the total loss could be attributed to fertilization failures and/or factors operating prior to implantation.

From the foregoing it can be seen that in rats the levels of acid and alkaline phosphatase change considerably with the advent of pregnancy. It might also prove interesting to follow the fluctuations in these two enzymes throughout gestation in order to gain a more complete concept as to their

exact function in the cell. As yet, the exact cause-effect relationship is not clearly understood. Whether the increased enzyme content enables a greater number of future offspring to survive in the uterine environment, or whether the number of embryos regulates the phosphatase level appears to be unknown. Perhaps future investigations may contribute additional knowledge concerning the important relationships between embryonic viability, uterine environment as reflected in enzyme systems, and factors influencing the uterine environment, especially the hormones of pregnancy.

SUMMARY

1. The results of this investigation on 112 female rats showed that as the number of embryos increased by one, the uterine weight was increased by 0.0622 grams.

2. The number of implantations present in the uterus of the rat on the thirteenth day of pregnancy had not as yet materially affected the average weight of the future offspring. If a large number of young in the uterus retards their average birth weights, this inhibition evidently occurs during the latter part of gestation.

3. In 11 out of 12 determinations using normal, non-pregnant rats, the acid phosphatase exceeded the alkaline phosphatase level. The acid phosphatase ranged from 20 to 32 units per gram of fresh tissue with an average of 26 units, while the alkaline phosphatase averaged 20 units per gram of fresh tissue, with a range of from 15 to 30 units per unit weight.

4. By the thirteenth day of pregnancy the enzyme concentration was increased to an average of 40 units for the acid with but 13 units for the alkaline phosphatase per gram of tissue. In 57 determinations the acid phosphatase was higher than the alkaline phosphatase.

5. An increase in uterine weight was paralleled by an increase in acid phosphatase concentration and by a decrease in the alkaline phosphatase concentration. The value for the former coefficient of correlation was +.744 and for the latter -.324. The total phosphatase content followed the pattern taken by the acid phosphatase, having an r value of +.612 for its relation to uterine weight. All of these correlations were highly significant at the 1 percent level.

6. There was a highly significant positive correlation between the weight of the uterine contents and the acid phosphatase and total phosphatase content of the uterus. The alkaline phosphatase, however, was found to be correlated in a negative manner with the weight of the uterine contents, but the degree of this relationship was not significant.

7. The acid phosphatase content of the uterus was found to be significantly correlated with the average implantation weight, number of implantations, and number of viable embryos. All of these statistical analyses resulted in r values that are classified as highly significant at the 1 percent level.

8. A statistically sound negative correlation was found between the aklaline phosphatase content of the uterus and the number of implantations, but no significant relationship could be established between the concentration of this enzyme and the average implantation weight or number of viable embryos.

9. The total phosphatase content of the uterus again followed the trend taken by the acid enzyme, being positively related to the average weight of implantation, number of implantations, and number of viable embryos in a positive statistically significant manner.

10. In the rat there was an embryonic death loss of 28 percent between ovulation and the establishment of a functional placenta. Sixty percent of this mortality loss occurred between ova release and nidation and 40 percent of the total loss during the interval between implantation and the thirteenth day of pregnancy.

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APPENDIX

TABLE 1 -- THE WEIGHT OF THE GRAVID UTERUS, EMPTY UTERUS, AND UTERINE CONTENTS

Animal number	Wt. (gm.) of gravid uterus	Wt. (gm.) of empty uterus	Wt. (gm.) of uterine contents
1.0	2.0231	0.7818	1.2413
1.2	2.3684	0.8117	1.5567
3.2	1.7524	0.6658	1.0866
3.3	3.3276	0.9660	2.3616
4.0	1.2589	0.6748	0.5841
4.1	2.9494	0.8846	2.0648
5.0	1.1694	0.5478	0.6216
5.1	2.5498	1.1210	1.4288
6.1	3.0145	0.8447	2.1698
7.0	0.9483	0.4712	0.4771
7.1	3.8023	0.8655	2.9368
7.2	2.2835	0.7973	1.4862
8.0	1.5551	0.6196	0.9355
8.1	3.4616	1.1013	2.3603
8.2	2.1566	0.7536	1.4030
9.2	2.3273	0.9001	1.4272
9.3	0.6583	0.4975	0.1608
9.4	2.9174	0.9195	1.9979
9.5	2.6798	0.9007	1.7791
10.0	1.3657	0.4691	0.8966
10.1	3.5766	1.1999	2.3767
12.0	3.8069	1.2730	2.5339
12.1	0.9379	0.5335	0.4044
12.2	2.9875	1.0642	1.9233
13.1	1.6854	0.6539	1.0315
14.1	3.0218	1.0135	2.0083
14.2	3.1711	0.9527	2.2184
14.3	2.7353	0.9507	1.7846
15.0	3.6920	1.1011	2.5909
16.1	2.6195	0.9810	1.6358
16.2	0.6628	0.4103	0.2525
16.3	2.0107	0.8892	1.1215
18.0	3.7343	1.2867	2.4476
19.0	3.7734	1.0571	2.7163
19.1	2.9536	0.9224	2.0312
20.0	4.2324	1.3602	2.8722
22.0	3.6111	1.2711	2.3400
23.0	1.3937	0.7138	0.6799
24.0	3.3583	1.1059	2.2524
21.1	2.5052	0.8829	1.6223
25.0	3.4287	1.1318	2.2969
23.1	2.8603	1.1084	1.7519
25.1	3.5290	1.1467	2.3823
19.2	2.4570	0.9585	1.4985
22.2	3.5332	1.0060	2.5272
15.1	0.4838	0.4326	0.0512
24.1	3.4297	1.1559	2.2738
21.2	2.9477	1.0391	1.9086
28.0	3.0721	0.9984	2.0737
26.0	2.5926	0.8677	1.7249
20.1	4.2520	1.3878	2.8642

TABLE 1 -- CONTINUED

Animal number	Wt. (gm.) of gravid uterus	Wt. (gm.) of empty uterus	Wt. (gm.) of uterine contents
18.2	3.1780	1.0451	2.1329
22.1	4.8057	1.2508	3.5549
32.0	3.3811	1.1408	2.2403
29.2	3.0589	1.0913	1.9676
62.0	3.3214	1.0909	2.2305
33.0	3.0149	1.0549	1.9600

TABLE 2 -- NUMBER OF IMPLANTATION SITES AND VIABLE EMBRYOS, AVERAGE IMPLANTATION WEIGHTS AND UNITS OF UTERINE PHOSPHATASE

Animal number	Number of implantations	Number of viable embryos	Avg. wt. of implantation (gm.)	Phosphatase units per gm. of fresh tissue	
				acid	alkaline
1.0	11	11	.1128	40	13
1.2	8	8	.1946	26	12
3.2	6	6	.1811	38	12
3.3	10	7	.2362	38	10
4.0	6	6	.0974	21	10
4.1	10	10	.2065	38	13
5.0	3	3	.2072	26	12
5.1	12	12	.1191	41	17
6.1	9	9	.2411	38	14
7.0	3	3	.1590	31	16
7.1	7	7	.4195	49	16
7.2	11	9	.1351	34	10
8.0	6	6	.1559	37	23
8.1	11	11	.2146	44	15
8.2	8	6	.1754	39	10
9.2	7	7	.2039	51	15
9.3	3	0	.0536	24	17
9.4	11	4	.1816	35	11
9.5	9	7	.1977	34	11
10.0	5	4	.1793	34	31
10.1	10	10	.2377	53	15
12.0	10	10	.2534	42	13
12.1	2	2	.2022	34	21
12.2	10	8	.1923	39	11
13.1	5	5	.2063	26	8
14.1	10	10	.2008	34	13
14.2	11	11	.2017	41	14
14.3	11	11	.1622	25	12
15.0	10	10	.2591	39	13
16.1	10	10	.1636	38	12
16.2	1	1	.2525	23	10
16.3	9	6	.1246	30	13
18.0	11	11	.2225	44	11
19.0	12	12	.2264	41	15
19.1	11	11	.1847	46	11
20.0	14	14	.2052	44	11
22.0	12	12	.1950	55	13

TABLE 2 -- CONTINUED

Animal number	Number of implantations	Number of viable embryos	Avg. wt. of implantation (gm.)	Phosphatase units per gm. of fresh tissue	
				acid	alkaline
23.0	5	5	.1360	27	17
24.0	11	11	.2048	45	14
21.1	8	7	.2028	31	14
25.0	12	9	.1914	49	12
23.1	12	12	.1460	45	11
25.1	11	11	.2166	51	13
19.2	10	10	.1499	44	10
22.2	11	9	.2297	52	13
15.1	8	0	.0064	28	10
24.1	11	11	.2067	55	13
21.2	10	8	.1909	52	11
28.0	10	10	.2074	44	12
26.0	10	9	.1725	46	13
20.1	13	13	.2203	65	9
18.2	8	5	.2666	41	14
22.1	12	12	.2129	64	11
32.0	11	11	.2037	50	11
29.2	8	8	.2460	54	12
62.0	13	10	.1716	50	12
33.0	9	8	.2178	49	13

TABLE 3 -- NUMBER OF IMPLANTATION SITES, VIABLE EMBRYOS AND CORPORA LUTEA OF PREGNANCY

Animal number	Number of implantations	Number of viable embryos	Number of corpora lutea
29.3	14	12	13
30.0	1	1	7
29.4	6	6	11
34.0	13	11	14
33.1	12	11	11
33.2	10	9	10
34.1	14	13	15
32.1	10	10	17
33.3	13	10	14
34.2	13	11	13
32.2	12	8	16
29.5	4	3	12
27.2	8	8	11
37.1	7	6	12
35.0	9	6	10
22.3	2	1	7
30.1	12	12	12
34.3	11	8	12
36.2	10	10	12
36.3	12	10	13
39.0	8	7	12
36.5	13	11	13
31.1	9	9	15
35.1	13	7	14
38.1	11	9	14

TABLE 3 -- CONTINUED

Animal number	Number of implantations	Number of viable embryos	Number of corpora lutea
31.2	9	9	10
40.1	9	9	9
37.3	10	9	11
39.2	9	9	9
35.2	13	11	13
39.3	6	2	12
40.2	9	9	9
41.0	10	8	11
41.1	10	8	13
41.2	8	7	9
42.0	3	3	10
39.4	11	9	10
45.0	7	7	7
44.0	8	8	11
40.4	10	8	10
41.3	10	10	10
43.2	8	8	8
31.3	7	6	7
21.1	8	7	11
25.0	12	9	13
23.1	12	12	19
25.1	11	11	13
19.2	10	10	10
22.2	11	9	12
15.1	8	0	10
24.1	11	11	11
21.2	10	8	12
28.0	10	10	11
26.0	10	9	11
20.1	13	13	14
18.2	8	5	15
22.1	12	12	11
32.0	11	11	12
29.2	8	8	12
62.0	13	10	15
33.0	9	8	11