HEMATOLOGY OF THE FETAL AND NEWBORN PIG

I. Cellular Populations and Serum Protein Concentrations

Thesis for the Degree of Ph. D.
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Dallas Gene Waddill
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This is to certify that the

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I. Cellular Populations and Serum Protein Concentrations

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HEMATOLOGY OF THE FETAL AND NEWBORN PIG

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by

Dallas Gene Waddill

AN ABSTRACT

Submitted to the School for Advanced Graduate Studies of
Michigan State University of Agriculture and
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ABSTRACT DALLAS GENE WADDILL

One hundred twenty-seven fetuses and 80 newborn pigs from 22 female swine were used in this study. The fetuses were studied at approximately 30, 51, 72 and 93 days post-conception. The newborn pigs were bled immediately after birth. The following measurements were made on the indicated number of pigs:

Measurement	Number of pigs
Red cell counts	196
Hemoglobin	193
Hematocrit	192
Reticulocytes	196
Mean corpuscular volume	189
Mean corpuscular hemoglobin	189
Mean corpuscular hemoglobin concentration	189
Total leucocyte counts	182
White cell differential (Term pigs)	78
Total serum protein	152
Electrophoretic separation of serum proteins	134
Electrophoretic separation of plasma proteins	145

Red cells increased from a mean of 560,000/mm^3 of blood at 32 days of age to a mean of 5.5 million/mm^3 at birth. The slowest increase in number was from 72 to 93 days. The period of greatest increase came between 93 days

Mean corpuscular hemoglobin decreased from 31 micromicrograms at 51 days to 21 micromicrograms at term. All decreases were significant with the exception of 93 days to term. Mean corpuscular hemoglobin concentration varied within a narrow range.

Total white cell numbers increased throughout gestation in the fetal blood. The count increased from $1,433/mm^3$ of blood at 51 days to $6,269/mm^3$ at term. All increases were significant except the increase between 72 and 93 days.

Differential counts showed a relative lymphopenia and neutrophilia in the blood of term pigs. Neutrophils comprised about 60 percent of the total leucocytes and lymphocytes 38 percent. Basophils, monocytes and eosinophils were present in very small numbers.

Total serum protein concentration in the fetal blood decreased significantly from 51 to 72 days ($P < .01$). The concentration increased significantly from 72 to 93 days and from 93 days to term ($P < .01$). The concentration was 2.83 grams percent at 51 days and 2.93 grams percent at birth.

Electrophoretic separation of serum protein components established that at 51 days the protein was largely alpha and beta globulins. The relative percent of alpha and term. The increases at all ages studied were significant ($P<.01$). Sex differences were not significant.

Hemoglobin concentration increased from 6.43 grams percent at 51 days to 8.68 grams percent at 72 days ($P<.01$). The level decreased to 8.65 grams percent at 93 days then increased to 11.69 grams percent at birth. The blood of males was frequently higher in hemoglobin than females but the difference was not significant. Crossbred pigs had higher hemoglobin levels than purebred pigs at birth ($P<.05$).

Hematocrit percentage increased throughout gestation. It increased from 20.4 percent at 30 days to 37.8 percent at term. A significant increase occurred between 51 and 72 days and between 93 days and term ($P<.01$). Sex differences were not significant at any age studied. This was true also of the breed differences.

Reticulocyte percentage was low at all ages studied. The highest mean value was 1.63 percent at 51 days and the lowest .40 percent at 72 days.

Mean corpuscular volume and mean corpuscular hemoglobin decreased throughout gestation in the fetal blood. Mean corpuscular volume decreased from 117 cubic microns at 51 days to 67 cubic microns at term. The decrease between all age increments studied was significant ($P<.01$).

globulin increased to 93 days when it was approximately 50 percent of the total protein. Beta globulin decreased in proportion to this increase. Albumin percentage reached a low of 17 percent at 93 days then increased to 21 percent at birth. Gamma globulin varied between 8 and 11 percent during gestation. Fibrinogen increase was greatest during the last three weeks of pregnancy.

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INTRODUCTION

Hematology is the study of blood in all its relations--anatomy, physiology, pathology and therapeutics. This science has an unusual and interesting history. Even primitive man was quick to realize the life-giving power of blood and noted that death was frequently associated with its loss. Humans were, at one time, sacrificed for their blood which was sprinkled over the ground when vegetables began to wither and die.

Glynn (1948) states that blood may not be the most important tissue of the body but it is certainly the most versatile. Despite homeostasis, this versatile nature reveals many of the body's secrets, providing a closer relationship between the sciences of hematology and nutrition. Many nutritional deficiencies have a pronounced effect on blood and blood-forming organs. Through study of both the formed elements and the plasma, some of these effects may be recognized and used to characterize the deficiencies.

The blood of pigs from birth to maturity has been extensively studied, although the observations at birth are quite conflicting because of differences in time elapsed between birth and sampling of the blood.

Data on normal hematology of the swine fetus are very limited. For this reason and because the intrauterine phase is a particularly critical period of the young pig's life, this study was begun.

REVIEW OF LITERATURE

I. Cellular components

Prenatal hematology

Nicholas and Bosworth (1927) working with rat fetuses and Von Deseö (1929) with beef fetuses apparently were the first to measure mean volume and hemoglobin content of red corpuscles in fetal blood.

Nicholas and Bosworth (1927) found the quantity of hemoglobin present in the fetal blood of the rat to increase gradually during the latter part of the middle third of gestation. The hemoglobin concentration increased rapidly during the early part of the last 1/3 of the gestation period (14 to 17 days after conception). This increase was followed by a slight decrease, then by recovery which resulted in an increased amount of hemoglobin shortly after birth. Wintrobe and Shumacker (1936) found the most rapid hematological change in rat fetuses occurred during the last third of gestation. At birth the number of erythrocytes was roughly 32 percent of the mean adult value, the hemoglobin 61 percent and the hematocrit 63 percent.

Von Deseö (1929) working with 25 beef fetuses ranging in age from two and one-half to nine months of age found an increase in the erythrocyte count from 3.74 to 7.80 million per cubic millimeter of blood and an increase in hemoglobin from 7.5 to 10.83 grams percent as the age of the fetuses advanced. The volume of packed red corpuscles increased from 34.0 to 42.3 percent, whereas, the mean corpuscular volume decreased from 90.9 to 54.2 cubic microns. Mean corpuscular hemoglobin fell from 20.5 to 14.0 micromicrograms and mean corpuscular hemoglobin concentration fluctuated between 20.9 and 25.6 percent.

Zeidberg (1929) measured the erythrocyte count, hemoglobin concentration and percentage of basophilic red cells during the last third of pregnancy in the blood of rabbit fetuses. The hemoglobin concentration was found to increase by 50 percent. The volume of red cells increased roughly 50 percent and the percent of basophilic cells decreased from 28 percent at the 22nd day to only four percent at term. Blood sugar was found to be three times as high at term as at the beginning of the last third of the gestation period.

Wintrobe and Shumacker (1935, 1936) studied many of the blood components in the fetus and newborn of several species of animals. In the pig fetus the most marked changes in the blood took place during the second quarter of gestation (29th to 56th day). The erythrocytes increased from 4.3 percent of the adult value to 40 percent. Mean size of the cells decreased from four times the adult size to only 1.6 times this size. Hemoglobin increased from 14 percent of the adult value to 50 percent and the volume of packed cells from 18 percent of the adult value to 66 percent. Erythroblasts formed 75 percent of cell erythrocytes at the end of the first quarter of the gestation period and dropped to less than three percent at the end of the second quarter. Reticulocytes dropped during this time from 100 percent to 35 percent. Mean corpuscular hemoglobin was higher in the younger fetuses and lower in the older ones. The decrease was rapid at first, but later became more gradual. Mean corpuscular hemoglobin concentrations varied within a very narrow range. This measure was the only character studied which was not related to the length, weight or age of the fetus. The narrow mean corpuscular hemoglobin concentration range suggests that the concentration of hemoglobin within the cell tends to be the same, no matter how large or small the red corpuscles may be. The fetal blood resembled cases of human pernicious anemia in many respects.

Jones <u>et</u> <u>al</u>. (1936) reported a study of fetal blood in the pig. The results of this study, Table 1, were fairly consistent with other workers.

Swenson <u>et</u> <u>al</u>. (1955) showed the sow's ration during pregnancy has a pronounced effect on the hematology of the pig at birth. The erythrocyte counts were significantly higher in pigs from sows fed a balanced ration when

Table 1. Fetal blood picture

Age	42 days	106 days	2-42 days postnatal
Total erythrocyte count, millions/mm^3	0.74	3.0	3.9
Hemoglobin, gms. %	3.6	6.76	9.7
Mean corpuscular volume, μ^3	216.0	128.0	-
Mean corpuscular hemoglobin, gm.x 10^{-12}	56.2	21.0	-
Average cell diameter, μ	8.9	6.01	-
Range of cell diameter, μ	2.0-16.0	4.0-8.5	-
Reticulocytes, percent (1,000 cells counted)	28.8	8.0	-

compared to those from sows fed an inferior ration. The hemoglobin and hematocrit values were not significantly different in the two groups of pigs. Total leucocyte counts were significantly higher in the pigs from sows receiving the poorly balanced ration. Mitchell (1932) found no correlation between the type of gestation diet and the hemoglobin content in blood of newborn rats.

Windle (1941) found changes occurring in the human fetus which were similar to those observed in other animals. The number of red cells and the levels of hemoglobin and hematocrit increased as the fetus grew older. The size of the red cell and the percentage of reticulocytes decreased during this time.

Barcroft and Kennedy (1939) working with sheep found before the 80th day of pregnancy the blood of the fetus contained very little hemoglobin. Between 80 and 100 days the rate of appearance of total hemoglobin in the circulating blood was slow, but from 110 days to term it increased rapidly. No great change in proportion of plasma to cells was found throughout fetal life.

Barcroft and Kennedy (1939) determined the blood volume of the fetus and compared the proportion of the blood present in the fetus to that present in the placenta. During entire fetal life a decreasing proportion of blood volume to fetal weight was found. The ratio of blood in the fetus to that in the placenta increased as time passed, and at 100 days about equal proportions were found in each. Between 110 and 130 days the average distribution was about 67 percent of the blood in the fetus and 33 percent in the placenta, and at 140 days, 80 percent of the blood was in the fetus.

Windle (1941) compared the erythrocyte count and hemoglobin concentration of human infants whose umbilical cords were clamped immediately after birth with those in which the clamping was delayed for a short time. The erythrocyte count and hemoglobin concentration was higher in the infants whose cords were not clamped immediately. This procedure allowed the blood from the placenta to flow back into the infant.

Barcroft and Rothschild (1932) found the amount of blood present in the uterus of rabbits during pregnancy reached a peak near the 27th to the 30th day. Beginning at conception the quantity of blood increased from approximately one to 10 cubic centimeters while the weight of the fetuses was still insignificant. The weight of the placenta seemed to be closely related to the quantity of blood in the uterus. In the latter part of pregnancy the quantity of blood again increased relatively. The maximal quantity of blood, approximately 30 cubic centimeters, seemed to occur near 28 to 29 days and the quantity fell rapidly before parturition.

Elliott *et al*. (1934) found that the goat fetus weight increased 19 times between the 10th and 20th week of pregnancy, however, the placental weight increased by slightly less than three times during this period and slowly degenerated, becoming partially atrophic at term. The greatest increase in fetal growth occurred between the 14th and 18th week, when the weight curve was almost vertical. At 14 weeks the fetal weight first exceeded the placental weight. The absolute fetal blood volume increased progressively throughout pregnancy. The percent of blood (expressed as a percentage of fetal weight) circulating in the fetus was very high about the second month of pregnancy and fell to about 14 percent at 14 weeks.

The blood weight maintained a constant level of nine percent of the weight of the fetus plus placenta until shortly before birth. In the last three weeks of pregnancy, the percentage of blood circulating in the fetus and placenta rose. This may have been due to the relative decrease in weight of placenta.

Wyman et al. (1944) reported on the solubility of hemoglobin of calf blood. It was found the hemoglobin from fetal blood was six times more soluble than that of maternal blood. Brinkman et al. (1934) and Brinkman and Jonxis (1935) had reported more than one type of hemoglobin in human blood, one type being more resistant to alkali denaturation.

Postnatal hematology

Data on the newborn animal is widespread and very variable. The great variation seems to be caused by differences in the time elapsed between birth and collection of blood samples.

Fraser (1938) found newborn pig blood to contain 5,800,000 erythrocytes per cubic millimeter of blood. White cells averaged 20,000 per cubic millimeter of blood and reticulocytes three percent. Craft and Moe (1932) found pigs at birth contained 9.69 grams of hemoglobin per 100 ml of blood. This compared favorably with Gardiner et al. (1953) who found 9.42 grams percent of hemoglobin for pigs in one litter and 10.3 grams percent for those in another. This same group also reported the hematocrit to be 34 percent. Total leucocyte counts ranged from 6,700 to 16,000 per cubic millimeter of blood. At birth neutrophils were the most prevalent white blood cell, ranging from 54 to 87 percent. However by one to two weeks of age, the lymphocyte was the most prominant white blood cell. This observation has been made by other workers (Palmer, 1917a; Craft and Moe, 1932; Venn, 1944; Luke, 1953a; and Miller et al., 1960). A variation existed in the percentage of lymphocytes and could have been due in some cases to counting error. Senftleben (1919) found lymphocytes were easily confused with monocytes because of the large size of the latter in pigs. Two types of lymphocytes have been found in the blood of pigs (Venn, 1944), one being much smaller and with differences in the nucleus and staining properties. The similarity between monocytes and lymphocytes in pig blood was also reported by Calhoun and Smith (1958). They felt, however, that the distinction could be made quite accurately.

Effect of stress

Luke (1953b,c) working with swine, Winqvist (1954) and Greatorex (1957a,b) working with cattle have shown that blood changes occur in animals during times of stress, particularly altering the ratio of lymphocytes to neutrophils. During the stress of parturition these changes have been found to occur in the blood of the fetus as well as the maternal blood. This change was explained as being partly due to the stress reaction of the pituitary-adrenal cortical activity of the mother just before parturition. This could influence the fetus through the placental circulation in the case of the calf. Fraser (1938) found the blood of sows contained 45,000 white blood cells per cubic millimeter of blood eight hours after farrowing and neutrophils increased from 40 percent to 93 percent.

Luke (1953c) observed a lymphopenia and neutrophilia with a sharp increase in the total white cell count within two hours following the administration of adrenocorticotrophic hormone and adrenal cortical extract to swine. Palmer (1917b) had shown many years before that this same pattern existed in pigs after exercise and sun exposure.

Variation with sex

Palmer (1917a) found the total white cell count and hemoglobin values were higher in male pigs than in females.

Senftleben (1919) found that male piglets had higher erythrocyte counts than females but that the difference disappeared after weaning. Scarborough (1931) found no sex difference in blood of pigs.

Mitchell (1932) found young female rats had a consistently higher initial hemoglobin and a correspondingly longer period was required to develop anemia. Draper and McElroy (1949) did not find female pigs any more resistant to anemia than males, even though the females had a slightly higher hemoglobin level.

McCay (1931) found male cattle had significantly higher hemoglobin levels than females. Byers _et_ _al_. (1952) and Holman (1955) found no sex difference in cattle. Wintrobe (1956) stated the difference in the human male and female blood is not manifest until puberty and in mammals, in which menstrual loss does not occur, the values are the same for both sexes.

Variation with breeds

Breed differences have been found by some workers. Byers _et_ _al_. (1952) reported a significant difference between blood hemoglobin values of Holstein and Jersey cattle. Greatorex (1957a) found highly significant differences in red and white cell counts, and hematocrit and hemoglobin values between breeds of calves. Swenson _et_ _al_. (1958) reported higher erythrocyte, hematocrit and hemoglobin values in Durocs when compared with Hampshires. The latter differences were observed 36 hours postnatal.

Seasonal variations

McCay (1931) found no seasonal hemoglobin variation in the blood of cattle. Sinclair (1933) reported seasonal variation in pigs. A low point in the blood picture was reached in February with recovery during March and April. Manresa and Orig (1941) reported a double cycle in hemoglobin during each year; the levels rose during the cooler period. Rusoff and Piercy (1946) found no significant seasonal variations occurring in the total white cell count of cattle. Greatorex (1957a) reported no seasonal difference in leucocytes but an increase of erythrocytes, hematocrit and hemoglobin during warm weather (April to September) and a reduction in hemoglobin with cold weather. Greatorex (1957b) reported a wide variation in the total white cell count of calves (4,500-15,000 per cubic millimeter of blood); because of this great fluctuation, seasonal and breed differences were not in evidence.

Variation with environment

The effect of environment on pig blood has been studied by Kernkamp (1932), Venn (1944) and Gardiner *et al.* (1953). Doyle *et al.* (1928) found pigs raised outside showed higher average erythrocytes and hemoglobin than pigs raised inside. Anemia was found to develop much more readily in the pigs raised inside. This observation has been reported in pigs by countless other workers (Craig, 1930; Doyle, 1932; and Gardiner _et al_., 1953). Byers _et al_. (1952) found no difference in hemoglobin level between pasture fed and barn fed cattle.

Table 2 contains a summary of various reports on the blood cellular components of newborn pigs.

II. Serum and plasma proteins

Research in the past 20 years has contributed a large amount of data on the protein composition of serum from many species of animals, including humans. The data from fetuses and newborn pigs are shown in Table 3.

Several of the studies have dealt with different methods of separation (Svensson, 1941; Cooper, 1945; Moore, 1945a; Koenig and Hogness, 1946). Numerous reports have been made on storage effects. Matthews and Buthala (1956), Henry _et al_. (1957) and Miller _et al_. (1960) reported no change in the proteins of serum which was frozen for varying lengths of time. Pensinger _et al_. (1959) reported a change in the serum of young pigs after freezing and thawing.

Many of the variable results from different workers were probably due to differences in the procedures of serum protein separation. The more recent studies have

Table 2. Hemograms reported on newborn swine.

Author	Age	Hemoglobin gm/100 cc	R.B.C. (million/ mm^3)	Hematocrit %	W.B.C. (thousands/ mm^3)	Neutrophils %	Lymphocytes %	Monocytes %	E. %	B. %
Craft & Moe (1932)	Birth	9.69	-	-	-	54.9	44.8	-	0.1	-
Fraser (1938)	Birth	-	5.8	-	20^a	61.0	32.3	3.7	2.9	-
Luke (1953a)	Birth	-	-	-	11,783^b	62.0	38.0	-	-	-
Kohler (1956)	Birth	12.0	5.0	-	6	60^c	30-35	Scanty	-	-
Miller (1960)	Birth	13.3	7.2	42.0	9	70.0	25	3.4	<1	<1

(a) Range 9,000-28,000.

(b) Range 5,850-20,800.

(c) Including eosinophils and basophils.

Table 3. Summary of reported values of swine serum and plasma of fetus and newborn.

Age	Serum or plasma[a]	Total serum or plasma protein (gm/100 ml)[b]	Distribution of serum or plasma protein (%)				Apparatus and buffer[d]	Reference
			γ globulin	β globulin	α globulin[c]	albumin		
<u>Prenatal</u>								
30 mm	P	-	0	25	65	10	T, Ph	Moore (1945b)
130 mm	P	-	0	10	55	35	T, Ph	"
160 mm	P	-	0	5	50	45	T, Ph	"
180 mm	S	-	0	15	40	45	T, Ph	"
250 mm	S	-	0	10	35	55	T, Ph	"
300 mm	P	-	15	5	30	50	T, Ph	"
2½ mo.	S	-	0	34	42	24	P, V	Rutqvist (1958)
3½ mo.	S	-	0	15	68	17	P, V	"
<u>Postnatal</u>								
Birth	P	2.7	4	8	30	50	T, Ph	Foster (1951)
"	S	2.2	0	5	85	10	T, V	Rook (1951)
"	S	2.4	0	19	68	13	P, V	Rutqvist (1958)
"	S	1.8	8[e]	17	64	11	P, V	Nordbring (1957)
"	S	2.2	6.5	15.9	60.8	16.7	P, V	Miller (1960)

(a) S = serum, P = plasma
(b) Values of total protein reported to nearest 0.1 grams percent.
(c) Includes α_2-globulins plus α_1-globulin values reported by several authors and component "x" reported by Rook <u>et al</u>. (1951).
(d) T = Tisellius apparatus, P = paper electrophoresis, Ph = phosphate buffer, V = veronal buffer.
(e) Unable to separate gamma and beta globulins.

utilized methods involving paper electrophoresis. Knill et al. (1958) showed this was a more rapid procedure requiring a smaller amount of serum and giving statistically valid results.

Howe (1921) was one of the early pioneers in the study of animal serum. His study was concerned with the newborn calf and the very rapid change which occurred in serum composition during or shortly after nursing. This change involved an increase in a component which at that time was called euglobulin. Newborn calf blood did not contain any of this component until after nursing and colostrum was ingested. This component is now known as gamma globulin.

Jameson et al. (1942) found serum of newborn calves contained no gamma globulin and only small amounts of beta globulin before the ingestion of colostrum. During the nursing period the composition of calf serum changed rapidly. Both gamma and beta globulins increased in concentration initially and then decreased. The concentration of alpha globulin and albumin decreased initially during the nursing period, followed by an increase of albumin. This work was substantiated again by Hansen and Phillips (1947) when it was reported there was an immediate increase in the blood serum gamma globulins following the ingestion of colostrum during the first 24 hours of life.

Moore et al. (1945b) reported on the blood plasma protein composition of fetal pigs. From four to six components were observed by these workers. Serum albumin has been shown to separate into two fractions (Hewitt, 1936). Barbariak _et_ _al_. (1958) reported alpha globulin also could be separated into two fractions. This may account for the six components found by Moore _et_ _al_. (1945b). Rutqvist (1958) reported only three components in the blood serum of pig fetuses. The components were albumin, alpha globulin and beta globulin. No gamma globulin was found in this study.

Pedersen (1944) isolated a new globulin from the blood of calves under two weeks of age. It was reported that serum from fetal calves and sheep contain a large amount of fetuin which makes up the largest part of the total globulin. Rabbit and human fetal blood serum contain very little fetuin.

Barboriak _et_ _al_. (1958) reported on the electrophoretic separation of plasma proteins in fetal sheep and goats. They revealed that the fetal plasma lacks gamma globulin and exhibited an additional peak in the area of alpha$_1$ globulin. This peak probably corresponded to fetuin. The quantitative concentration of fetal blood plasma proteins seemed to depend largely on the age of the fetus. In the goat fetus, the percent of total proteins and of plasma albumin increased and fetuin decreased with progressing age. The remaining plasma proteins did not seem to be affected. In fetal sheep the percentages of total plasma proteins, plasma albumin and beta globulins increase; levels of fetuin and alpha$_1$ globulin decreased with age. The percentage of alpha$_2$ globulins remained unaffected.

Meschia (1955) compared the colloidal osmotic pressure of fetal blood in goats and sheep with that of the maternal blood. The colloidal osmotic pressure in the fetal blood plasma at 64 days of age was ca. 100 mm H_2O; as age advanced the pressure gradually increased to the value of ca. 250 mm H_2O near term.

Koenig and Hogness (1946) reported fibrinogen was contaminated with various globulin components and a small amount of albumin in adult swine plasma. They compared different buffers and found that the resolution of the beta globulin, fibrinogen and gamma globulin in phosphate buffer was superior to veronal buffer; however, the resolution of the alpha$_1$ globulin and albumin was superior in veronal buffer. It was also noticed that in veronal buffers two alpha globulins and two beta globulins were resolved; however, the second beta globulin migrated with the fibrinogen. In phosphate buffer only one alpha globulin and one beta globulin were resolved with a possibility that alpha$_1$ globulin migrated with the albumin. Deutsch and Goodloe (1945) reported fibrinogen comprised 13.9 percent of the total plasma protein of pigs. They found the characteristic tall peak of fibrinogen lay in close proximity to that of beta globulin. In some of the plasma samples studied it was impossible to ascertain the amount of particular globulins present, because they were not sufficiently separated. Differences in their mobility in an electric field were too small for good resolution. This was often true in the case of fibrinogen, in which evidences of this protein's presence were sometimes seen in the form of a sharp peak superimposed on a mass of other protein. Stenhagen (1938) reported fibrinogen in human blood plasma was an electrophoretically well-defined protein migrating more slowly than beta globulin but faster than gamma globulin.

Appreciable and characteristic specific differences in electrophoretic patterns have been reported for blood plasma from several species under comparable conditions (Moore, 1945a). The pattern obtained depended considerably on the buffer used and it was established that patterns should not be compared unless the conditions of the experiments were similar. Perk and Lobl (1959) found a difference in the serum protein between Holstein-Friesian cattle and native Damascene cattle in Israel. The high milk producing Holstein-Friesian cattle showed a higher total protein and gamma globulin, but a lower albumin/globulin ratio than the hot and dry climate resistant Damascene cattle. In both breeds, alpha and beta globulin patterns were similar.

The albumin fraction of the plasma, being responsible for most of the effective osmotic pressure (West and Todd, 1957), constitutes the factor governing the water-retaining capacity of blood plasma. This led Perk and Lobl (1959) to the conclusion, that the prolonged thirst endurance in the Damascene cow may be partly due to higher blood albumin levels as compared with the Holstein. Liquid taken up by drinking and pasture feeding was probably retained in the blood of the Damascene over a longer period.

Cartwright et al. (1948) showed a prolonged dietary restriction of protein results in a marked diminution in both the relative and absolute amount of albumin, while a relative increase occurs in globulin, especially alpha globulin.

Many investigators have published studies on protein composition of the serum from human infants during fetal life and following birth. Rappoport et al. (1943) found the blood fibrinogen levels to be constant at all ages, and equal to adult values. Total serum protein values increased with increasing maturity. Both the albumin and globulin fractions were involved in the increase, but there was a proportionately greater increase in the globulin fraction. Throughout all of infancy there was a reduction in certain of the globulin fractions, probably gamma globulin.

Longsworth et al. (1945) found both the absolute and the relative concentrations of fetal gamma globulin were higher than the maternal values. Orlandini _et al_. (1952) found the mean gamma globulin level in newborn infants slightly higher than in the mothers but the difference was not significant. Orlandini _et al_. (1955) reported mean cord blood levels of gamma globulin were significantly higher than the level in the mother's blood. The relative gamma globulin concentration after birth showed a definite decrease in the first month of life. Moore _et al_. (1949) reported serum albumin and gamma globulin increased rapidly during the development of the human fetus, whereas the alpha and beta globulins remained at a low level. The high gamma globulin level in newborn infants decreased markedly during the first month or two of life, whereas the other serum components increased.

The relatively high level of fetal gamma globulin in humans was due to the type of placenta. Osborn _et al_. (1952) reported the human placenta near term was permeable to many antibodies. Pedersen (1944) reported placental transfer of gamma globulin in rodents and man in which the maternal blood was separated from the fetal blood by only one to three layers of cells. In ruminants and swine there are five and six layers of cells respectively separating the maternal and fetal blood (Arey, 1954).

Dancis _et al_. (1955) reported evidence for the production of gamma globulin but not of albumin by the human placenta. Dancis et al. (1957) reported the liver from human fetuses of 3-4 months of age was already capable of synthesizing plasma proteins, exclusive of gamma globulin. They also concluded that at no stage of pregnancy does the placenta synthesize proteins that are electrophoretically identifiable as albumin or gamma globulin. It was concluded that under normal circumstances the placenta does not contribute significantly to the plasma proteins of the fetus.

MATERIALS AND METHODS

One hundred twenty-seven fetuses and 80 newborn pigs from 22 female swine were included in this study. Eighteen, nulliparous, Yorkshire gilts, all of approximately the same age and weight were selected from the college station herd. The other four females were Duroc sows which were being used in a Vitamin A study and which had previously given birth to one litter. These sows were being fed an adequate control ration, and were therefore considered normal. The Yorkshire gilts were all self-fed, in confinement (on concrete), ration 1 shown in Table 4a. The Duroc sows were also fed in confinement but were hand-fed ration 2, Table 4b.

The Yorkshire gilts were all bred twice during a single estrus to boars of their own breed. Three of the Duroc sows were bred to Yorkshire boars and the other to a Hampshire boar. After conception had been assured by the absence of further estrus, the pregnant individuals were selected at specific periods during gestation, and brought to the Michigan State Veterinary Hospital where caesarean sections were performed to remove the fetuses. The operations were performed at intervals of approximately 30, 51, 72 and 93 days after breeding. The pigs to be studied at term were farrowed naturally and blood samples taken

Table 4a. Ration 1 - self-fed to Yorkshire gilts.

Ingredient	Percent
Corn	67.8
Oats	10.0
Alfalfa meal	10.0
Soybean meal (44%)	11.5
Meat and bone scrap	3.5
Limestone	0.4
Super trace mineral salt	0.5
Dicalcium phosphate + zinc	0.2
Vitamin B-supplement[a]	1.0 lb /ton
Vitamin A and D mix[b]	0.5 lb /ton
B_{12} supplement[c]	1.0 lb /ton

(a) 2 gm riboflavin, 4 gm pantothenic acid, 9 gm niacin and 10 gm choline per pound of supplement.
(b) 4,450,980 I.U. Vitamin A and 1,264,074 I.U. Vitamin D per pound.
(c) 9 mg per pound of supplement.

Table 4b. Ration 2 - hand-fed to Duroc sows.

Ingredient	Percent
Oats	34.4
Wheat	50.0
Soybean meal (44%)	8.0
Meat and bone scrap	4.0
Dried corn distillers solubles	2.6
Trace mineral salt	0.5
Limestone	0.5
Vitamin B supplement[a]	2.0 lb /ton
B12 supplement[b]	1.0 lb /ton
Vitamin D supplement[c]	4.5 gm /ton
Vitamin A supplement[d]	

(a) 2 gm riboflavin, 4 gm pantothenic acid, 9 gm niacin and 10 gm choline per pound of supplement.
(b) 9 mg per pound of supplement.
(c) 142,000 I.U. per gm.
(d) 16 micrograms per kilogram bodyweight daily.

immediately after birth and before nursing. The number of litters taken at each fetal age varied slightly (30 days - 3 litters; 51 days - 4 litters; 72 days - 4 litters; 93 days - 7 litters). The term litters included three Yorkshire litters and the four crossbred litters from the Duroc sows.

The gilts were anesthetized with an intravenous injection of Surital sodium (thiamylal sodium)* into the marginal auricular vein. Anesthesia was maintained throughout the operation with ether or with slow intravenous injections of Surital.

The fetuses were removed from the uterus, their position in the horn recorded and a hemostat placed on the umbilical cord to prevent loss of blood. The pig was weighed and a blood sample taken as quickly as possible.

The method of sampling the blood from the fetuses varied with their age, because it was very difficult to obtain blood from the very young fetuses. The data from the 30-day-old fetuses were limited because of the extremely small amount of blood present, therefore, it was necessary to obtain blood from these pigs before separating them from the maternal circulation, by incising the umbilical vessels and removing a sample of blood with a heparinized capillary tube.

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*Parke Davis and Co. - Detroit, Mich.

Blood was much easier to obtain from 52-day-old fetuses. After weighing, the thoracic cavity was opened, exposing the heart, and blood samples were taken directly with pipettes and capillary tubes.

The 72 and 93-day-old fetuses, as well as the term pigs were bled from the anterior vena cava according to the method described by Carle and Dewhirst (1942). The pigs were restrained in a dorsal, recumbent position and the blood taken with a 10 cubic centimeter syringe and a 20 or 22 gauge, one inch needle.

Each sample was divided into two aliquots; one portion was heparinized and the other was allowed to clot. The unheparinized blood was rimmed to hasten clotting and placed in a warm room at 40° C. for a short time to allow complete clot retraction. The samples were placed in an International centrifuge, Model V, size 2, for 30 minutes at 3,000 rpm and the serum removed. If the serum was to be used within one week, it was stored in a walk-in cooler at 4° C. If the length of storage was to be longer than one week, the samples were stored at minus 20° C.

The heparinized sample was used for complete blood counts, hemoglobin and hemocrit determinations, while the serum was used for electrophoretic and total serum protein studies.

Red cell counts

For red cell counts the heparinized blood was drawn to the 0.5 mark of a "Zero error Hellige tru count" pipette and diluted to the 101 level with .85% saline. The cells were distributed uniformly through the diluted blood by at least a 10-minute rotation on a Bryan-Gary pipette rotor. The counts were made on a Neubauer chamber according to the method of Ham (1956). Duplicate counts were made and repeated if they varied more than seven percent of the lower count.

White cell counts

For white cell counts the heparinized blood was drawn to the 0.5 mark of a "Zero error Hellige tru count" pipette and diluted to the 11 mark with three percent acetic acid, producing hemolysis of all the nonnucleated erythrocytes. The cells were distributed through the diluted blood in the same method as the red cells. Two sides of the Neubauer chamber were counted and differences were not acceptable if they exceeded 10 percent of the lower count. Nucleated red cells could not be distinguished from leucocytes in the counting chamber and were counted as white cells in the total white cell count. Swenson *et al.* (1958) experienced the same difficulty. To correct the white cell counts for the presence of nucleated red cells, 200 cells in each smear were identified as either leucocytes or nucleated erythrocytes. The number of nucleated red cells per 200 cells counted gave a correction factor.

White cell differential

Differential counts were performed on each of the term pigs. The smears were made as quickly as possible after obtaining the blood from the pig and were stained with Wright's stain according to the procedure of Wintrobe (1956). Phosphate buffer (pH 6.8) containing equal volumes of M/15 Na_2HPO_4 and M/15 KH_2PO_4 was used. The leucocytes were differentiated into lymphocytes, monocytes, eosinophils, basophils, non-segmented neutrophils and polymorphonuclear neutrophils. Two counts of one hundred cells each were made on blood smears from each pig. The method of counting was that described by Ham (1956). The number of normoblasts observed while counting 100 cells was enumerated and the correction for total white cell counts was made by use of the following formula:

$$\text{Corrected white cell count} = \text{Total white cell count} \times \frac{100}{100 + \text{number normoblasts}}$$

Hematocrit determinations

Capillary tubes 75 mm long and 1.2-1.4 mm in diameter were used in hematocrit determinations. Because of the small amount of blood available in the 52-day-old fetuses, one heparinized tube and one plain capillary tube was filled. The samples were spun in an International "hemacrit" centrifuge for five minutes. The hematocrit was determined with an International micro-capillary reader. The serum and plasma from the capillary tubes were used for total serum protein and serum protein electrophoresis.

Hemoglobin determination

Hemoglobin was determined by the cyanmethemoglobin method of Crosby *et al.* (1954). The blood was taken with a Sahli pipette (.02 ml) and transferred into five milliliters of the diluent solution. The diluent used was Drabkin's (1949) solution which contained the following:

$NaHCO_3$	1.0 gm
KCN	50 mg
$K_3Fe(CN)_6$	200 mg

Diluted to 1 liter with double distilled water.

The readings were made immediately on a Bausch and Lomb Spectronic 20.

Reticulocyte counts

The procedure described by Ham (1956) was used for the reticulocyte counts. A drop of brilliant cresyl blue solution was spread smoothly on one end of a slide and allowed to dry. The thin film was polished, face downward on smooth paper. A drop of blood was placed over the brilliant cresyl blue, mixed with the edge of a slide, and a smear made. The smear was counterstained with Wright's stain.

The counts were made under an oil immersion lens (100X). A cross line reticule was used which divided the field into four quarters. One thousand cells were counted on each slide again using the method of Ham (1956).

MCV, MCH, MCHC[a]

(a) The mean corpuscular volume was calculated by the following formula:

$$\text{MCV} = \frac{\text{Hematocrit (percent) x 10}}{\text{Red cell count } (10^6/\text{mm}^3)}$$

which expressed the average volume of the individual red cells in cubic microns.

(b) Mean corpuscular hemoglobin was calculated by the following formula:

$$\text{MCH} = \frac{\text{Hemoglobin } [(\text{gm}/100 \text{ ml}) \text{ x } 10]}{\text{Red cell count } (10^6/\text{mm}^3)}$$

which expressed the average content of hemoglobin of the individual red cell in micromicrograms.

(c) Mean corpuscular hemoglobin concentration was calculated by the following formula:

$$\text{MCHC} = \frac{\text{Hemoglobin } [(\text{gm}/100 \text{ ml}) \text{ x } 10]}{\text{Hematocrit (percent)}}$$

which expressed the average hemoglobin concentration per 100 milliliters of packed red cells in grams.

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[a] Formulas from Ham (1956).

Serum and plasma proteins

The protein fractions were separated on a Spinco, Model R, paper electrophoresis system (Spinco Technical Bulletin 6027A) at room temperature. A constant current of three milliamperes per cell was maintained for 16 hours on Spinco number 300-846 paper strips using veronal buffer of pH 8.6 and an ionic strength of 0.075. This buffer was made up of 2.26 grams of di-ethyl barbituric acid and 15.4 grams of sodium diethyl barbiturate in one liter of distilled water. When the buffer was used more than one time the current was reversed within the two cells.

After 16 hours, the strips were dried for 30 minutes in a forced draft oven at 110° C. The temperature and time were kept constant because these factors were found to be very critical by Henry _et_ _al_. (1957). These workers reported that for every degree change in temperature between 100-120° there was a one percent increase in the albumin/globulin ratio.

Spinco number 300 pipettes were used for filling the applicator with serum for application to the strips. Approximately .006 milliliter of serum was applied to each strip.

One serum sample and one plasma sample were run from each pig. The plasma sample was used to determine the amount of fibrinogen present and served as a check on separation of the other protein fractions.

The serum present in the capillary tube from the 52-day-old fetuses was used for both electrophoresis and total serum protein. In order to get the serum into the pipettes, the capillary tube was broken just above the level of packed cells after making a mark at this point with a small file. The amount of serum necessary for electrophoresis was removed and the tube resealed with a flame.

The staining method used was described in the Spinco Technical Bulletin 6027A. The dye used was brom phenol blue (one gram brom phenol blue in one liter of methanol). The rinse consisted of five percent acetic acid. The strips were then blotted and placed in the oven at 110° C. for 15 minutes. After development of basic color, by use of NH_4OH, relative intensities of the separated proteins were determined by scanning the stained strip with the Spinco Model RB Analytrol with number five cam.

Total serum proteins

Because of the small amount of serum available, the biuret determination of serum protein was unsatisfactory. Measurement of light absorption at 280 mμ, the approximate position of an absorption maximum for proteins has been used in the quantitative estimation of protein concentrations. The method used in this study was a simple ultraviolet spectrophotometric method, first described by Waddell (1956), which permitted attainment of greater sensitivity, accuracy, and specificity because of the use of shorter wave lengths. This method required only small amounts of serum and has been shown to agree closely with results of other methods.

The serum was withdrawn from the capillary tubes with a five lambda pipette and diluted to five milliliters (1:1,000) with 0.9 percent NaCl. The readings were made on a Beckman Model DU ultraviolet Spectrophotometer. Readings were made at wavelengths of 215 mμ and at 225 mμ. The absorbance at 225 mμ was subtracted from that at 215 mμ. This difference multiplied by 144 gave the protein concentration in the solution expressed in micrograms per milliliter. This value was then converted to grams percent.

RESULTS AND DISCUSSION

I. Cellular components

Results of analysis of variance are summarized in Table 5. Analyses were run on the effect of age and sex on blood composition. The crossbred litters were not included in the graphs but are included separately in the tables. Table 6 is a summary of the cellular studies, showing the number of observations, averages of all values for the various ages and ranges of these values.

Red cell counts

The red cell counts increased steadily from a mean of 560,000/mm^3 of blood at 30 days of age to a mean of approximately 5.5 million/mm^3 at birth. In Table 6 the normal values for the red corpuscles at various ages are presented. The increase in red cell count can be more easily perceived from Figure 1, which shows the increase to be rapid but steady to 72 days followed by a slower rate of increase to 93 days and then a rapid rise from 93 days to birth.

The difference in red cell counts between males and females was not significant although there was a tendency for the males to exhibit slightly higher concentrations at the respective age increments with the exceptions of 72 days where they were equal and 30 days where sex was not determined.

Table 5. Summary of variance analysis of cellular component differences[a]

Comparison	R.B.C.	Hemo-globin	Hemato-crit	Mean corpuscular values M.C.V.	Mean corpuscular values M.C.H.	Mean corpuscular values M.C.H.C.	W.B.C.
F vs. M	N.S.D.	N.S.D.	N.S.D.	N.S.D.	N.S.D.	N.S.D.	$+^{*b}$
<u>Age (days)</u>							
30 vs. 51	$+^{**}$	...	N.S.D.	...	...	...	...
51 vs. 72	$+^{**}$	$+^{**}$	$+^{**}$	$-^{**}$	$-^{**}$	$+^{**}$	$+^{*}$
72 vs. 93	$+^{**}$	N.S.D.	N.S.D.	$-^{**}$	$-^{**}$	N.S.D.	N.S.D.
93 vs. Term	$+^{**}$	$+^{**}$	$+^{**}$	$-^{**}$	N.S.D.	$+^{**}$	$+^{**}$

(a) "+" indicates second value greater than first.
"-" indicates first value greater than second.
** Significantly different ($P < .01$).
* Significantly different ($P < .05$).
N.S.D. - nonsignificant difference.
(b) Significantly different only at 93 days and term.

Table 6. Normal values for red corpuscles at various ages

Age (days)	Sex	No. of animals	Red cell count (millions/ mm^3)	Range	No. of animals	Hemoglobin (gm per 100 ml)	Range	No. of animals	Hematocrit (%)	Range
Prenatal										
30	-	3	.56(.22)[a]	.45- .74	-	-	-	2	20.4(.50)	19.9-20.9
51	F	14	1.88(.18)	1.11-3.30	13	6.01(.51)	3.80- 9.32	13	22.3(1.8)	12.2-33.2
	M	13	2.25(.16)	1.26-2.92	13	6.86(.49)	3.80- 8.83	12	26.3(1.7)	15.7-33.9
72	F	18	3.43(.13)	2.17-4.26	19	8.66(.33)	6.01-11.26	18	30.5(.90)	23.9-39.0
	M	24	3.43(.70)	2.04-4.92	24	8.69(.30)	5.12-10.87	23	30.3(1.8)	19.1-39.0
93	F	29	3.96(.11)	2.98-5.08	29	8.60(.54)	5.71-11.99	29	30.9(.80)	21.5-40.3
	M	17	4.06(.22)	1.84-5.62	17	8.74(.41)	4.70-11.38	17	32.5(1.5)	17.9-40.8
Newborn[b]										
Y	F	15	5.63(.25)	3.00-6.87	15	11.50(.51)	6.27-15.65	15	36.9(1.6)	18.8-48.4
	M	19	5.64(.15)	4.48-6.75	19	11.84(.23)	9.88-13.57	19	38.4(.70)	34.2-44.9
X	F	24	6.06(.18)	4.63-7.54	24	12.36(.31)	8.44-15.17	24	39.6(.90)	29.9-46.7
	M	20	6.08(.21)	4.78-8.42	20	12.54(.26)	10.30-14.55	20	39.7(.70)	33.7-44.9

(a) Numbers in parentheses are standard errors of the mean.
(b) Y = Yorkshire; X = Crossbred.

Table 6. continued. Normal values for red corpuscles at various ages.

Age (days)	No. of animals	Reticulocytes (% of R.B.C.)	Range	Mean corpuscular values				
				Sex	No. of animals	M.C.V. (μ^3)	M.C.H. ($10^{-6}\gamma$)	M.C.H.C. (%)
Prenatal								
30	8	1.10	0.1-2.18	-	-	-	-	-
51	27	1.63	0-18.72	F	12	117(4.37)[a]	31(1.22)	27(.84)
				M	12	117(4.98)	31(1.06)	27(.54)
72	42	.40	0-3.86	F	18	90(2.86)	26(.68)	29(.69)
				M	23	91(3.39)	26(1.18)	29(.67)
93	44	.94	0-7.10	F	29	79(1.58)	22(.53)	28(.54)
				M	17	81(2.57)	22(.71)	27(.43)
Newborn[b]								
Y	34	.87	0-2.20	F	15	65(1.60)	21(.59)	32(.93)
				M	19	69(1.45)	21(.14)	31(.14)
X	41	.87	0-4.40	F	24	66(1.96)	21(.66)	31(.39)
				M	20	66(2.06)	21(.63)	32(.32)

(a) Numbers in parentheses are standard errors of the mean.
(b) Y = Yorkshire; X = Crossbred.

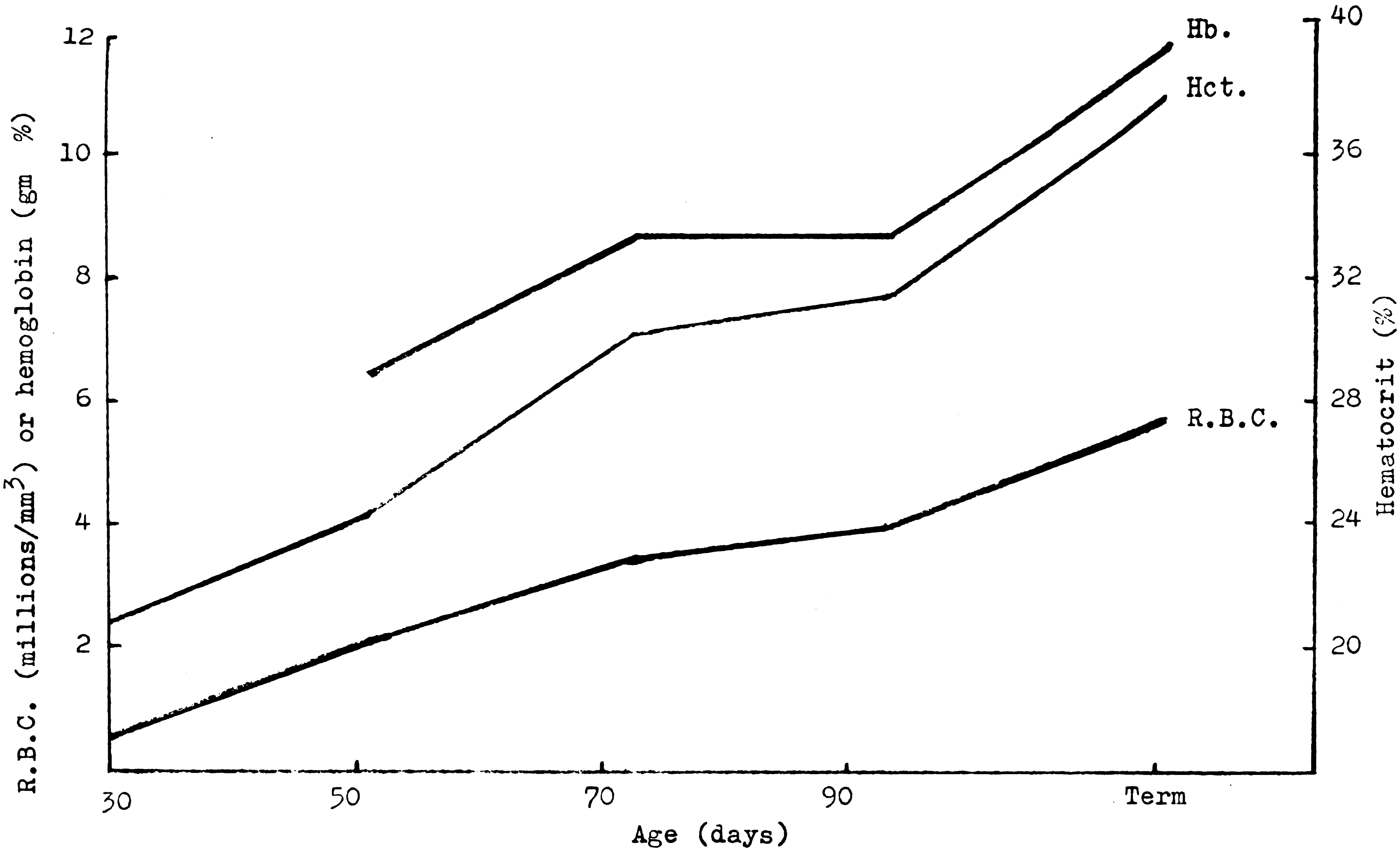


Figure 1. Concentrations of hemoglobin, hematocrit and erythrocytes at different ages.

The blood of crossbred pigs at birth was found to contain higher red cell concentrations than that of purebred pigs. This difference was significant at the five percent level.

Hemoglobin determinations

The concentration of hemoglobin exhibited a rapid, highly significant ($P < .01$) rise from the 51st day of gestation to the 72nd day (Figure 1). This rise was followed by a slight decrease to the 93rd day and then by a very rapid rate of increase to birth. These values are all shown in Table 6.

No significant differences in hemoglobin concentration occurred between males and females of any age. The blood of males was frequently higher in hemoglobin but the differences did not reach significant levels. The crossbred pigs did, however, have a significantly ($P < .05$) higher level of hemoglobin than the purebred pigs at birth.

Hematocrit determinations

The volume of packed red blood cells increased during all stages of gestation (Figure 1). The volume increased steadily from 30 days to 51 days followed by a more rapid rise to 72 days. The rate of increase was much slower between 72 days and 93 days followed by a very rapid increase at term (Table 6).

Statistical analysis revealed the volume of packed red cells was not significantly different in the 51-day-old fetuses when compared with the 30-day-old fetuses. The increase of the packed cell volume between 51 and 72 days and between 93 days and at term were significant at the one percent level. No significant difference was found between males and females of any age or between crossbred and purebred pigs.

Reticulocyte counts

Reticulocyte percentage was low at all ages studied (Table 6). The highest mean value was 1.63 percent at 51 days and the lowest was .40 percent at 72 days.

Because of the highly variable values with considerable overlapping between age groups, statistically significant differences were not evident.

Mean corpuscular volume

Mean corpuscular volume decreased rapidly at first followed by a steady decline (Figure 2). The greatest decrease was from 51 to 72 days, dropping from 117 cubic microns to 90, and finally to a low of 67 cubic microns at term (Table 6). The decrease between each age interval was highly significant ($P < .01$). No significant sex or breed differences were found.

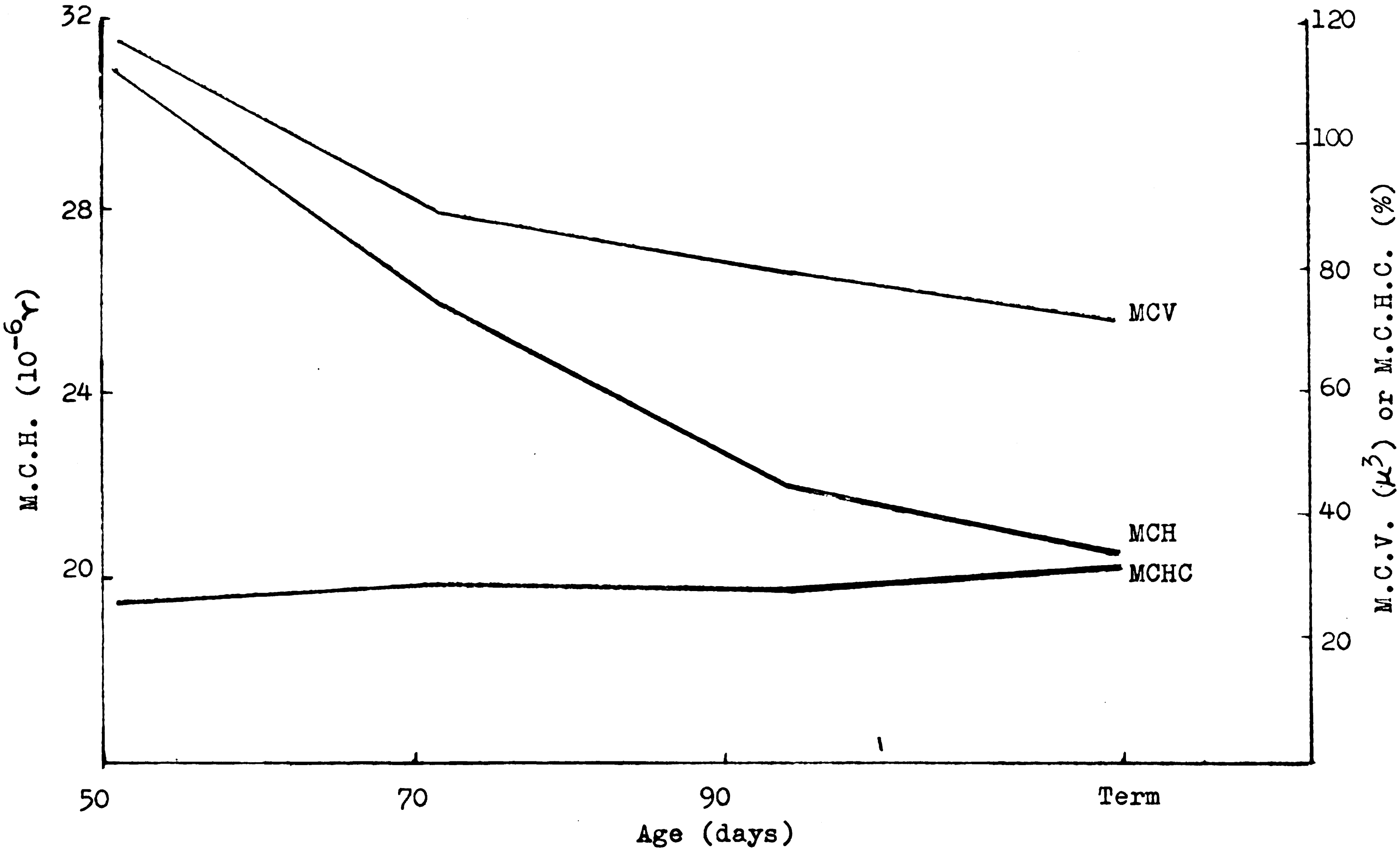


Figure 2. Changes in mean corpuscular volume, mean corpuscular hemoglobin and mean corpuscular hemoglobin concentration with age.

Mean corpuscular hemoglobin

Mean corpuscular hemoglobin decreased rapidly from 51 to 93 days followed by a more steady decline until birth (Figure 2). The decreases between 51 and 72 days and between 72 and 93 days were highly significant ($P < .01$). The decrease from 93 days to birth was not significant. No significant differences were found between males and females or between breeds.

Mean corpuscular hemoglobin concentration

The mean corpuscular hemoglobin concentration varied within a narrow range (Figure 2), from a low of 27 percent at 51 days to a high of 32 percent at birth (Table 6).

The differences between 51 and 72 days and between 93 days and term were found to be significant. The changes can be more easily perceived from Figure 2. No significant differences were found between sexes or breeds.

Total white cells

The number of white cells present increased uniformly from 51 to 72 days followed by a slower rate of increase to 93 days and a very rapid increase from 93 days to term (Figure 3). The number rose from a low of 1,433/mm^3 of blood to a high of 6,269/mm^3 (Table 7).

The increase from 51 to 72 days was significant at the five percent level. The change from 72 to 93 days was

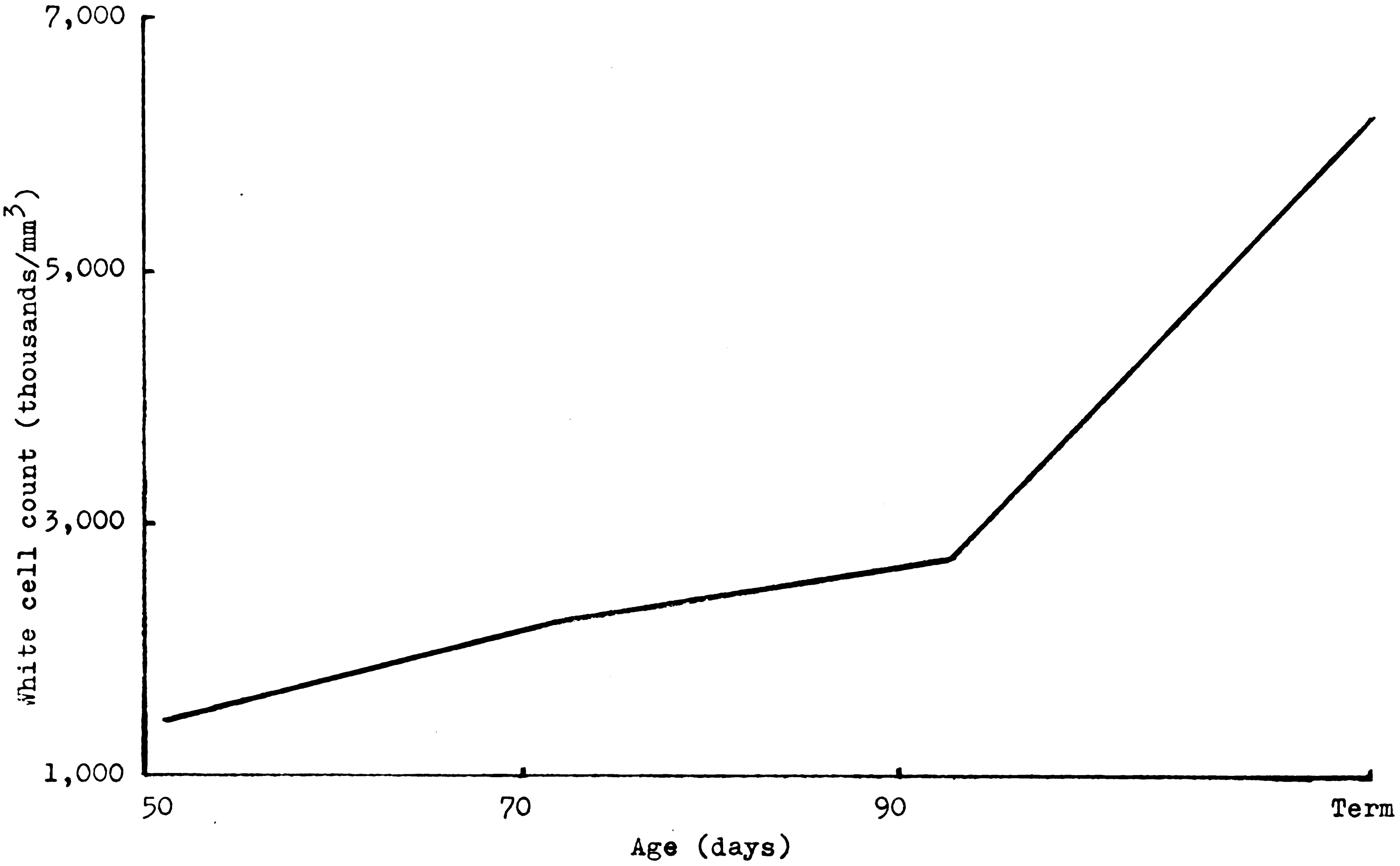


Figure 3. Changes in leucocyte population during fetal life.

Table 7. Total leucocyte counts at different ages.

Age (days)	No. of animals	Sex	W.B.C./mm^3
51	11	F	1,112(251)[a]
	12	M	1,726(324)
72	18	F	2,204(419)
	23	M	2,256(295)
93	26	F	2,785(300)
	15	M	2,739(159)
Term[b]	15	F	5,173(520)
	18	M	7,182(699)
Term[c]	24	F	3,924(277)
	20	M	4,525(314)

(a) Standard error of the mean.
(b) Purebred Yorkshires.
(c) Crossbred pigs.

not significant but the increase from 93 days to term was highly significant (Table 5).

Significant sex differences were found only in the term purebred litters where the white cell concentration of males was significantly higher ($P < .01$) than that of females.

The purebred pigs had significantly higher total white cell concentration than the crossbred pigs. This difference was significant at the one percent level.

Differential count

The blood of pigs at birth was found to exhibit a distinct lymphopenia and neutrophilia. Neutrophils were by far the most numerous cell seen in the counts, comprising about 60 percent of the total leucocytes (Table 8). Lymphocytes represented approximately 38 percent and the basophils, monocytes and eosinophils combined represented only two percent.

No statistical differences were found between sex or breed in percentages of lymphocytes and neutrophils. The other white cells were present in such highly variable concentrations the differences were judged nonsignificant.

Red blood cell concentration, hemoglobin and the volume of packed red blood cells followed the same pattern of development during fetal growth of the pig. Increase of these blood components was slow during the first third of gestation followed by a more rapid rate of increase during the middle third (38 to 76 days). The most rapid increase occurred during the last third of gestation or more specifically the last three weeks (93 days to term) during which time the number of red blood cells increased

Table 8. White cell hemogram of newborn pigs.

Age	No. of animals	Sex	White cell differential (%)					
			Lymphocytes	Total Neutrophils	Non-segmented Neutrophils	Monocytes	Basophils	Eosinophils
Birth								
Yorkshire								
	24	F	39(2.60)[a]	59.75(2.45)	.67	.75	.29	.17
	20	M	37(3.55)	61.65(3.52)	1.15	1.10	.05	.25
Crossbred								
	15	F	38.3(3.15)	60.67(3.12)	1.07	.80	.07	.13
	19	M	38(2.64)	61.32(2.62)	.89	.63	.05	.00

(a) Standard error of the mean.

from approximately 4,000,000/mm^3 of blood to approximately 5,600,000/mm^3, an increase of 40 percent. Hemoglobin during this same period increased from 8.65 grams percent at 93 days to 11.69 grams percent at birth, an increase of 35 percent. The volume of packed red cells increased 20 percent from 31.5 percent at 93 days to 37.8 percent at birth.

Mean corpuscular volume which is a measure of the size of red blood cells decreased rapidly from 51 to 72 days followed by a more steady decline until birth. Mean corpuscular hemoglobin decreased rapidly from 51 to 93 days followed by a more steady decline until birth. Mean corpuscular hemoglobin concentration, which is a measure of the percent of each red cell occupied by hemoglobin, varied within a rather narrow range, being slightly higher at birth than at 51 days.

Even though the size of red blood cells was decreasing rapidly, this change did not keep pace with the increasing number of red cells. The volume of packed red cells, representing the total mass of red corpuscles (controlled by number as well as size), increased quite rapidly.

During the first one-half of gestation the placenta is growing faster than the fetus as shown by Warwick (1928) in pigs, Elliott _et al_. (1934) in goats and by

Barcroft et al. (1939) in sheep. The growth of the pig fetus (expressed in grams body weight) was slow between 30 and 51 days (Figure 4), a little faster between 51 and 72 days followed by a rapid increase in body weight from 72 days to term. Thus it seems that for more than half the gestation period the fetus is gravimetrically the less important intra-uterine structure. Warwick (1928) found the greatest weight increase in pig fetuses occurred in the last 20 days of gestation.

Erythrocyte numbers, hematocrit values and hemoglobin concentrations keep pace with the fast developing placenta and slow growing fetus up to 72 days when placental development is near completion. At this time, fetal growth becomes rapid and the blood components do not keep pace, suggesting the growth of fetal body structures becomes the predominant intra-uterine phenomenon. The proportion of blood present in the fetus in comparison to that in the placenta has been found to increase as the fetus grows older (Barcroft and Kennedy, 1939). Blood can be driven through the placenta at a faster rate as pregnancy proceeds because of higher fetal blood pressure. Subjective examinations suggest that more blood in proportion to weight was present in the pig fetuses at 72 days than at the 51 day period. The blood is flowing more rapidly through the placenta, therefore, the 72-day-old fetus does not require

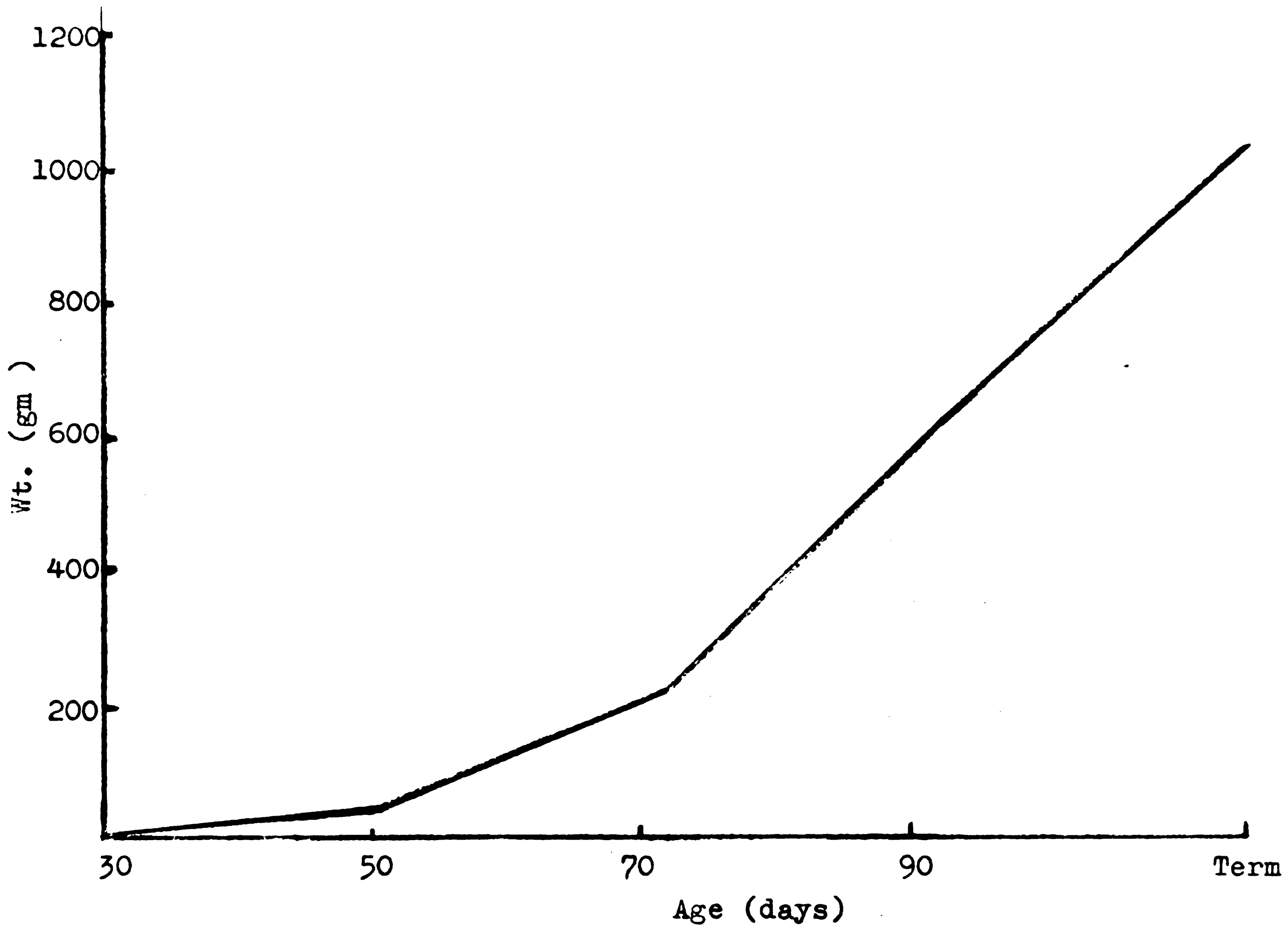


Figure 4. Relationship between fetal weight and age.

that the red cells and hemoglobin maintain the same concentration in the blood relative to body weight as do 51-day-old fetuses which have a much smaller proportion of blood that is moving more slowly.

The rapid increase in red cells, hemoglobin and hematocrit during the period of 93 days to term is necessary because during this period the placenta is beginning to degenerate (Warwick, 1928 and Elliott _et_ _al_., 1934) and the fetal size is increasing rapidly (Figure 4). As the fetus exceeds more and more the weight of the placenta, the amount of oxygen and other nutrients necessary for life of the fetus and the need for excretion of larger amounts of waste becomes more of a problem. Therefore, during the last stage of gestation (93 days to term) the erythrocyte number and hemoglobin level must increase rapidly in order to counteract the relatively anoxemic conditions of late fetal life.

The number of erythroblasts was very high at 30 days, making up the majority of red blood cells present at this age. The number decreased rapidly as the fetus developed. Reticulocytes, on the other hand, were present in small percentages at all ages studied. Some of the earlier workers (Wintrobe and Shumacker, 1936 and Jones _et_ _al_., 1936) reported very high percentages of reticulocytes at 32 days of age. The ages of these fetuses were estimated from crown-rump lengths, therefore, the estimated age might vary from the real age by a few days. Also in these studies the percentage of both erythroblasts and reticulocytes were reported. The total of these two exceeded 100 percent in some cases. It is apparent the reticulocyte values reported were expressed as a percentage of the non-nucleated erythrocytes. In the present study the percentages of reticulocytes were determined by counting 1,000 red cells including all stages. Thus the large discrepancy between the data presented here and earlier studies was probably due to error in estimating age or differences in the method of expressing the count. In the present study, only those cells in which the basophilic material in the cell was precipitated, appearing as a blue network or reticulum, were counted as reticulocytes.

The total white cell count followed the same pattern as the red cells and hemoglobin, increasing gradually to 93 days followed by a very rapid increase from 93 days to term. The 93 day count of 2,768 mm^3 increased to 6,269 at term, an increase of 126 percent. This large increase was probably due largely to stress. The fetus had become quite large by this time and the placental membranes had become smaller in comparison, which undoubtedly resulted in a stress upon the fetus and its hematopoietic organs, which were quite well developed by this age. The increase in total white cells appears due largely to an increase in neutrophils which have been shown to increase during stress (Palmer, 1917b; Luke, 1953b; Gardiner *et al.*, 1953 and Winqvist, 1954). Kindred and Corey (1930) reported the same increase in white cells of rats during the last few days of fetal life. The term purebred pigs were farrowed during May, June and July and had significantly higher leucocyte concentration than the term crossbred pigs which were farrowed in October and November.

The differential counts on the newborn pigs showed a definite lymphopenia and neutrophilia. Luke (1953c) reported the same pattern after injection of adrenocorticotrophic hormone and adrenal cortical extract in pigs. The high neutrophil count at birth was perhaps due to stress reacting through the adrenal gland.

II. Serum and plasma proteins

The values together with standard errors of the means for total serum proteins are presented in Table 9. In Table 10 the relative percentages of the electrophoretically separated components of serum and plasma are presented. Total serum protein change with age as well as the change in the separated components can be more easily perceived from Figure 5.

Total serum proteins at 51 days were present at the rate of 2.83 gm per 100 ml of serum, composed mainly of

Table 9. Total serum protein

Age	No. of animals	Sex	Total serum protein gm/100 ml
51 days	8	F	2.86(.14)[a]
	9	M	2.80(.09)
72 days	19	F	2.19(.06)
	21	M	2.30(.06)
93 days	11	F	2.48(.06)
	9	M	2.57(.05)
Term[b]	14	F	2.93(.10)
	19	M	2.92(.05)
Term[c]	23	F	2.99(.06)
	19	M	2.93(.05)

(a) Standard error of mean.
(b) Purebred Yorkshires.
(c) Crossbred pigs.

alpha and beta globulins with somewhat smaller amounts of the other components. Levels of serum gamma globulin varying between 8 and 11 percent were found in the blood of fetal pigs at all ages studied, as well as in the pigs at birth. No gamma globulin was found in the blood of fetal pigs by Rutqvist (1958) or by Moore *et al*. (1945b).

Table 10a. Serum protein distribution

Age (days)	No. of animals	Sex	Distribution of serum proteins (%)			
			γ-globulin	B-globulin	α-globulin	albumin
51	11	F	9.0(1.1)[a]	36.7(1.9)	35.1(2.6)	19.2(2.0)
	8	M	8.8(1.0)	31.7(1.0)	31.2(1.7)	28.3(0.7)
72	19	F	9.7(1.0)	27.7(1.3)	41.3(1.4)	21.4(0.8)
	20	M	11.4(1.0)	26.5(1.4)	39.6(1.3)	22.5(0.8)
93	27	F	8.6(0.8)	24.0(1.3)	49.4(1.4)	18.0(1.0)
	17	M	10.7(0.6)	21.1(1.1)	52.4(1.8)	15.8(1.6)
Term	13	F	11.3(0.9)	20.4(1.1)	48.9(2.1)	18.2(1.8)
	19	M	10.0(0.4)	19.3(0.8)	47.7(1.2)	23.0(0.8)

(a) Standard error of mean.

Table 10b. Plasma protein distribution

Age (days)	No. of animals	Sex	Distribution of plasma proteins (%)			
			γ-globulin and fibri-ogen	B-globulin	α-globulin	albumin
51	6	F	8.4(1.0)[a]	33.5(1.9)	35.8(2.7)	22.4(1.6)
	5	M	7.6(2.1)	39.0(3.3)	32.0(4.3)	21.5(3.4)
72	12	F	12.4(1.1)	36.8(5.1)	33.6(3.2)	17.2(2.0)
	12	M	10.0(0.8)	35.6(4.1)	34.4(2.8)	19.8(1.5)
93	21	F	9.9(0.9)	21.6(1.6)	53.4(1.4)	15.1(1.0)
	12	M	9.9(1.1)	20.7(1.0)	55.2(1.0)	14.2(0.9)
Term[b]	15	F	18.6(2.0)	19.8(1.2)	43.7(2.2)	17.9(1.5)
	19	M	18.4(0.9)	20.8(1.1)	40.2(1.1)	20.6(0.9)
Term[c]	23	F	13.6(1.2)	19.4(1.2)	47.5(1.3)	20.4(1.0)
	20	M	13.4(0.8)	19.5(1.2)	45.9(1.8)	21.1(1.1)

(a) Standard error of mean.
(b) Purebred Yorkshire pigs
(c) Crossbred pigs.

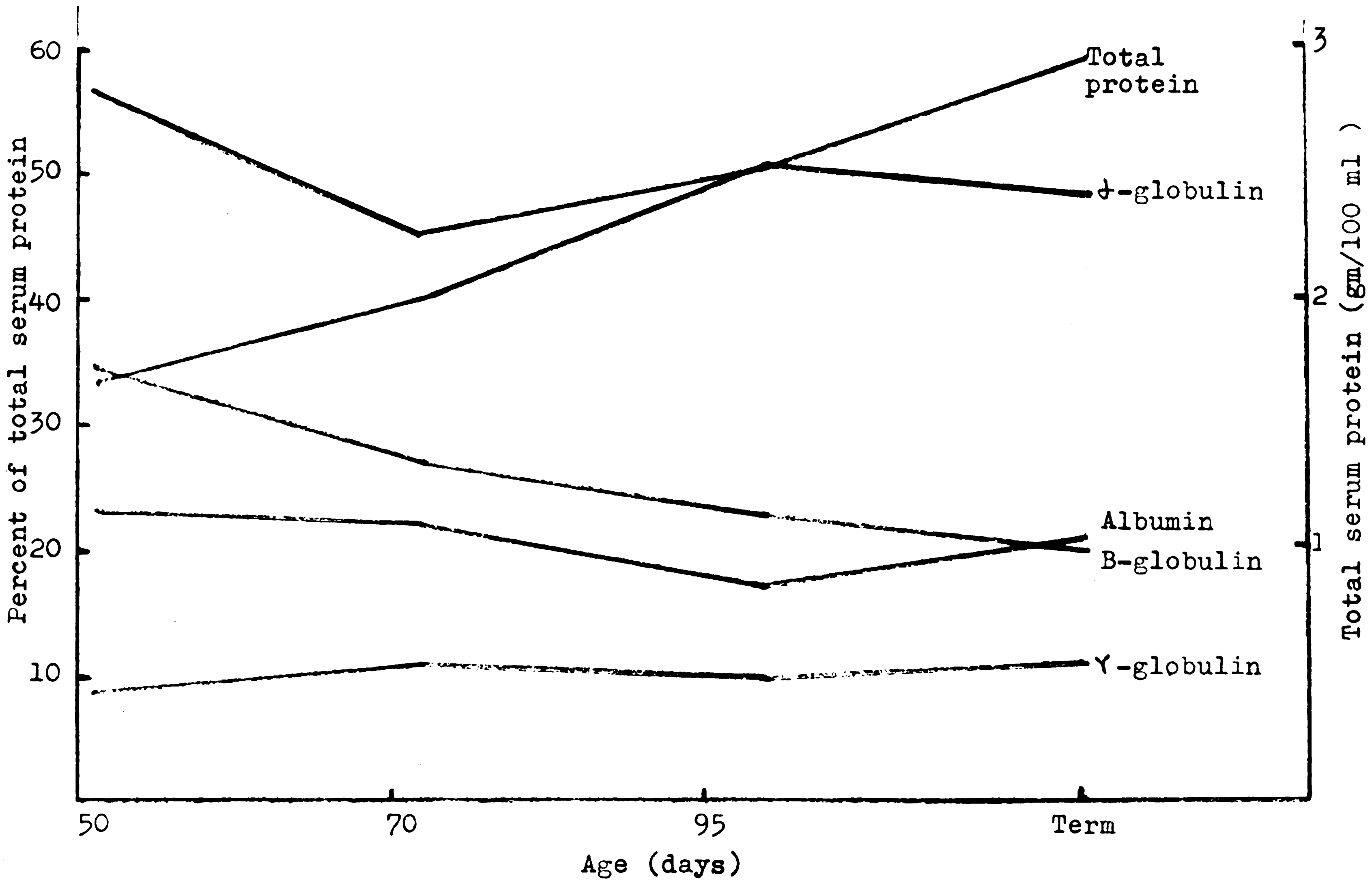


Figure 5. Relationship between fetal age and serum proteins.

Rook et al. (1951) and Rutqvist (1958) found no gamma globulin present in the serum of pigs at birth, however, Foster et al. (1951) reported a small percentage and Nordbring and Olsson (1957) obtained an average of 7.9 percent. Miller et al. (1960) found a similar percentage in newborn pigs. The difference between the present study and other work reported may have been due to differences in methods of separation, protein denaturation at point of application, or to differences in distinguishing between the gamma globulin and slow moving beta globulin. The point of separation was quite arbitrary and required considerable personal judgment.

As shown in Figure 5, the amount of total serum protein decreased rapidly from 51 to 72 days. This decrease was highly significant ($P<.01$) and was followed by a highly significant increase to 93 days. The increase from 93 days to term was also highly significant. The significant drop in serum protein from 51 to 72 days was likely due to the change in the amount of blood in the fetal circulation. At 51 days the fetus contained very little blood when compared to the amount present at 72 days of age, therefore, even though the total amount of protein present in the serum may have increased the concentration per 100 ml of blood decreased because of dilution with greater amounts of blood.

As shown in Figure 5, during the period from 51 days to 72 days, alpha globulin increased whereas, beta globulin decreased quite rapidly. Pierce (1955) reported that fetuin possibly has a substituting function for albumin in newborn animals. If fetuin corresponds to the α_2-globulin as suggested by Nordbring and Olsson (1957) and Rook *et al.* (1951), then the alpha globulin during fetal life may replace albumin as the primary factor maintaining osmotic pressure of the blood. During this period when alpha globulin increased rapidly the blood volume increased rapidly also. Meschia (1955) found that colloidal osmotic pressure increased greatly during the middle of the second third of the gestation period. Thus it is reasonable that there is a direct relationship between the increase in blood volume of the swine fetus during the second third of gestation and the colloidal osmotic pressure, resulting from the increasing level of alpha globulin present in the blood during this same period. Albumin and gamma globulin remained quite constant during this period.

From 72 to 93 days the fetus made rapid growth (Figure 4), exceeding the weight of the placenta (Warwick, 1928 and Elliott *et al.*, 1934). Alpha globulin production was rapid during this period in the fetal pig becoming the dominant serum protein component at 93 days as shown in Table 10. Total serum protein increased during this period due entirely to the increase in alpha globulin. Albumin, which is produced by the fetal liver, decreased during this period.

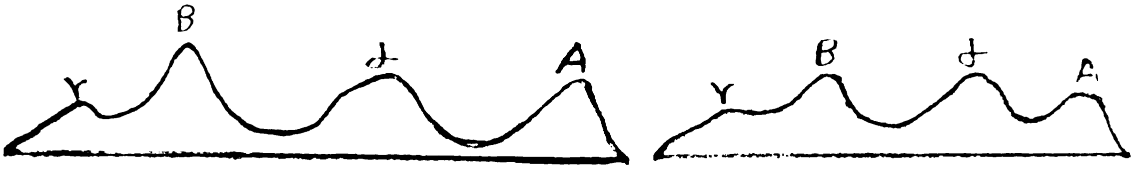
Total serum protein increased significantly from 93 days to term reaching its highest level of 2.95 grams per 100 ml at birth. Relative levels of alpha and beta globulins decreased during this time whereas the relative amount of albumin increased. This increase in albumin accounts for the slight decrease in alpha globulin percentage. Alpha globulin production did not change much during this time although the relative serum concentration did. Beta globulin production did, however, seem to decrease slightly.

The relative amount of gamma globulin present throughout fetal life is either produced by the fetus itself, the fetal placenta, or passes from the maternal circulation into the fetus. The anatomical arrangement of the placental membranes of the pig prevents the transfer of large amounts of gamma globulin. The pig has the epitheliochorial type placenta in which there are several barriers to the transfer of substances across the placental membranes. The human fetus has been found to contain a higher percentage of gamma globulin than the maternal blood (Longsworth *et al.*, 1945 and Orlandini *et al.*, 1952) and the placenta has been found to be highly permeable to maternal antibody (Osborn *et al.*, 1952) especially in the latter part of pregnancy. Humans have the hemo-endothelial placenta (Arey, 1954) in which there is little barrier between maternal and fetal bloods. This accounts for the relatively high level of circulating gamma globulin and albumin. Gamma globulin and albumin have been found to be the fastest increasing protein components in the blood of the developing human fetus (Moore _et_ _al_., 1949).

Paper electrophoresis of plasma was found to be completely unsatisfactory as a measure of the percentage of fibrinogen or of the separated protein components present. The differences obtained between serum and plasma can be readily seen by comparing the values in Table 10. The results varied quite widely between serum and plasma and the variation was due largely to the fibrinogen present and perhaps in part to the fact that the small amount of plasma present in the capillary tubes could not be mixed before applying to the strips. The same general pattern can be seen in comparing the plasma and serum patterns. Fibrinogen seemed to increase greatly during the last three weeks of gestation as shown in Table 10. Fibrinogen migrated very slowly and was found in close proximity to the gamma globulin. It was impossible to distinguish between these two protein components.

Alpha globulin was not separated into two fractions in this study. Miller _et_ _al_. (1960) separated the alpha fraction from pig blood into two distinct fractions. This phenomenon had been observed by Nordbring and Olsson (1957). Rook *et al*. (1951) referred to the $alpha_1$ fraction as component "X". Pedersen (1944) found a high relative concentration of a component in the blood of young calves which was called fetuin. This component has been found in large percentages in the fetal blood of goats and sheep (Barboriak *et al*., 1958) with the same electrophoretic mobility as the $alpha_1$ globulins. Nordbring and Olsson (1957) and Rook *et al*. (1951) suggested that the $alpha_1$ fraction may correspond to the fetuin component of Pedersen (1944) and Barboriak *et al*. (1958). Any of this compound evident in the present study was included in the alpha globulin fraction. Figure 6 shows the electrophoretically separated pattern at the various ages studied.

No significant differences were found in total serum protein between males and females. The albumin was significantly higher at 51 days and at term in the male pigs than in the female pigs. No significant differences were found between purebred and crossbred pigs in either total serum protein or the separated components.



51-day fetus 72-day fetus

93-day fetus Newborn pig

Figure 6. Electrophoretic patterns of serum proteins from different age fetuses.

Total serum proteins at 51 days constituted a concentration of 2.83 grams per 100 ml of serum composed mainly of alpha and beta globulins with somewhat smaller amounts of the other components, as shown above the relative amount of gamma globulin changed very little. The most pronounced change was the α-globulin which increased from 33.5 percent at 51 days to 48.3 percent in the newborn pigs. The percent of B-globulin decreased throughout fetal life. The relative amount of albumin decreased slightly during the gestation period.

The total serum protein decreased from 51 to 72 days then increased to term at which time the level was 2.95 grams per 100 ml.

SUMMARY

One hundred twenty-seven fetuses and 80 newborn pigs from 22 female swine were used in this study. The fetuses were studied at approximately 30, 51, 72 and 93 days post-conception. The newborn pigs were bled immediately after birth. The following measurements were made on the indicated number of pigs:

Measurement	Number of pigs
Red cell counts	196
Hemoglobin	193
Hematocrit	192
Reticulocytes	196
Mean corpuscular volume	189
Mean corpuscular hemoglobin	189
Mean corpuscular hemoglobin concentration	189
Total leucocyte counts	182
White cell differential (Term pigs)	78
Total serum protein	152
Electrophoretic separation of serum proteins	134
Electrophoretic separation of plasma proteins	145

Red cells increased from a mean of $560{,}000/mm^3$ of blood at 32 days of age to a mean of 5.5 $million/mm^3$ at birth. The slowest increase in number was from 72 to 93 days. The period of greatest increase came between 93 days and term. The increases at all ages studied were significant ($P < .01$). Sex differences were not significant.

Hemoglobin concentration increased from 6.43 grams percent at 51 days to 8.68 grams percent at 72 days ($P < .01$). The level decreased to 8.65 grams percent at 93 days then increased to 11.69 grams percent at birth. The blood of males was frequently higher in hemoglobin than females but the difference was not significant. Crossbred pigs had higher hemoglobin levels than purebred pigs at birth ($P < .05$).

Hematocrit percentage increased throughout gestation. It increased from 20.4 percent at 30 days to 37.8 percent at term. A significant increase occurred between 51 and 72 days and between 93 days and term ($P < .01$). Sex differences were not significant at any age studied. This was true also of the breed differences.

Reticulocyte percentage was low at all ages studied. The highest mean value was 1.63 percent at 51 days and the lowest .40 percent at 72 days.

Mean corpuscular volume and mean corpuscular hemoglobin decreased throughout gestation in the fetal blood. Mean corpuscular volume decreased from 117 cubic microns at 51 days to 67 cubic microns at term. The decrease between all age increments studied was significant ($P < .01$).

Mean corpuscular hemoglobin decreased from 31 micromicrograms at 51 days to 21 micromicrograms at term. All decreases were significant with the exception of 93 days to term. Mean corpuscular hemoglobin concentration varied within a narrow range.

Total white cell numbers increased throughout gestation in the fetal blood. The count increased from 1,433/mm^3 of blood at 51 days to 6,269/mm^3 at term. All increases were significant except the increase between 72 and 93 days.

Differential counts showed a relative lymphopenia and neutrophilia in the blood of term pigs. Neutrophils comprised about 60 percent of the total leucocytes and lymphocytes 38 percent. Basophils, monocytes and eosinophils were present in very small numbers.

Total serum protein concentration in the fetal blood decreased significantly from 51 to 72 days ($P < .01$). The concentration increased significantly from 72 to 93 days and from 93 days to term ($P < .01$). The concentration was 2.83 grams percent at 51 days and 2.93 grams percent at birth.

Electrophoretic separation of serum protein components established that at 51 days the protein was largely alpha and beta globulins. The relative percent of alpha globulin increased to 93 days when it was approximately

50 percent of the total protein. Beta globulin decreased in proportion to this increase. Albumin percentage reached a low of 17 percent at 93 days then increased to 21 percent at birth. Gamma globulin varied between 8 and 11 percent during gestation. Fibrinogen increase was greatest during the last three weeks of pregnancy.

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