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Pre- and Postnatal Normal Mouse Blood Cell Counts* (32926)

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Blood cell counts at any moment may be affected by the rate of production (hemopoiesis), the rate of release, and the length of survival (1). Following the initial formation of blood islands in the 8–9 gestation day mouse embryo (2), there is accelerated formation of the circulatory system and the blood elements in preparation for the adjustments of birth. Nevertheless, the blood of the newborn mouse is quite different from that of the mature mouse of 2 or 3 months of age, with some elements lacking and others being in overabundance, and there are some species variations (3). Even sex is a factor (4). Most pertinent, possibly, is the fact that there are daily diurnal fluctuations in the blood picture so that any continuing study must be made at the same time of day (5–7). This study was designed to determine at weekly intervals the changes in the blood elements of the postnatal mouse, up to 9 weeks at which time a plateau is generally established. Two additional readings were planned, at 12 and at 24 months to see what progressive aging would do to these same blood elements. Studies were always made at the same time of day.

The CF1 strain of laboratory mouse is a readily accessible mammal whose blood maturing changes can be plotted easily. The prenatal mouse is so small, and its blood volume so inadequate for proper study, that samples are difficult to obtain except toward the period of delivery or after day 17. The postnatal data obtained are statistically significant because they were derived from a minimum of 25 mice of each sex, and the same mice were used for the first 3–9 weeks of study. At 12 months one group of 20, and at 24 months another of 13, was examined, all normal controls.

Materials and Method. For complete blood count: (CBC). Blood from mice of 3 or more weeks of age was taken from the tip of the tail. No anesthetic or restraining of the mouse was necessary. Samples for hemoglobin determination were taken first, then samples for white, and red blood cells, and platelets were taken in that order. (Other techniques are available for older mice (8)).

Taking sufficient blood from mice 2 weeks or younger posed some problems. The newborn mouse was held in the left hand, the index finger pressing the head back and the thumb on the lower abdomen. The prominent jugular vein was cut with very fine scissors and three previously prepared pipettes with rubber tubing could be successively controlled by the free hand. Blood is generally plentiful, rich and homogeneous and any mixing with lymph or tissue fluids seemed to be minimal. The total blood removed is about 0.5 ml.

By 2 weeks of age the mice were so strong

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and active that it was necessary to tape them down on their backs, with the head extended to expose the thoracic region. The skin was removed to further expose the jugular vein, and up to 1.4 ml of blood could be obtained. Since clotting was rapid it was usually necessary to make successive cuts to obtain enough blood, after which the mice were sacrificed.

When it was necessary to obtain larger amounts of blood from older mice it was found best to take it from the orbital sinus. A micro blood collecting tube of 3 mm diameter, heparinized, was inserted through the soft tissue just behind the eye. Mouse blood tends to coagulate rapidly. Altogether 3–5 capillary tubes of blood may be taken from the two eyes of a single mouse, totalling 1.0 ml. Cardiac puncture or femoral vein blood were not found to be satisfactory.

Hemoglobin. A Leitz photometer was used for all of the mice to obtain the hemoglobin values. The machine was checked against a Hematrol standard at various times, and the values were recorded in gm/ml from 0.02 ml of blood taken with a hemoglobin pipette. With the younger mice micropipettes holding 0.01 ml calibrated in 5 equal portions were used. In any case the sample was mixed with 5 ml of 0.04% ammonium hydroxide, introduced into the machine and read. With less than 0.02 ml of blood the results were multiplied properly to obtain the correct value.

White and red cell and platelet counts were all made in the usual hematological manner.

Reticulocyte count. Fresh blood was drawn up into the WBC diluting pipette and diluted with fresh 0.5% methylene blue with potassium oxalate to the 1.0 mark, giving equal volumes of blood and dye. Both were then drawn into the bulb, mixed by rotation, and allowed to stand for about 15 min. One drop was expelled on a slide and two smears were made in the usual way. At least 1000 RBC were examined in the two smears, and the number of reticulocytes then counted.

Differential counts. One or two drops of freshly drawn blood was sufficient to make several good smears, stained with Wright's stain and dried for 4–5 min, buffered for 6–8 min, and washed with tap water. A minimum of 100 white cells were counted from top to bottom of the slide to avoid duplication.

Preparation of smears from other tissues for photomicrocrographic identification. Bone marrow. This was obtained largely from the femurs. With the newborn and very young mice, one end of the femur was cut off and the very fluid marrow was gently squeezed out with forceps. In larger mice or where the marrow was less fluid, both ends of the femur were cut, a syringe applied to one cut end and the marrow blown out into either serum or 0.5–0.8% sodium citrate.

Spleen, liver, and lymph. These organs were excised, cut transversely, and gently squeezed with forceps and touched lightly to a clean slide, thus causing some cells to become adherent to the slide. It is also possible to suspend these cells in sodium citrate or serum.

These smears were made to confirm the CBC and neutrophil counts.

Observations. The data can be presented most concisely in the form of graphs, each point representing an average of about 25 readings.

The white blood cells of the newborn mouse are at an average of between 3000 and 4000 but during their first several weeks of postnatal life they drop further to an extremely low level at 2 weeks to 1500. Following this there is a steady climb with an unexplained brief drop at 6 weeks prior to establishing a plateau at 9 weeks. This level is also seen at 12 months, and only the females showed a drop of significance by 24 months. Leukopoiesis seems to get underway at about 3 weeks of age after an extremely low level at 2 weeks.

The red blood cells, on the other hand, start at a low level and gradually climb until at 9 weeks the normal value is reached. By 12 months of age there is a slight drop to the 8–9 million range, and by 24 months to 7–8 million cells. The hemoglobin values do not follow the red cell curve. They start between 10 and 11 gm, drop during the first 2 weeks to about 7 gm, and then there is a gradual but consistent rise to a plateau at 7–9 weeks of age. From the third and the fourth week on, female mice consistently had higher red cell and hemoglobin counts than did males.

The platelet counts start at birth at about 600,000 and rise rather consistently to a peak at about 5 weeks, then gradually decline but at 12 and 24 months there is no further decline.

Due largely to changes in clotting time much more blood can be taken from the newborn than from the tail of the 2-week-old mouse.

The lymphocytes, in contrast with the total white blood cell counts, start low (at about 10% of the total white count) and climb rather rapidly, so that by 6 weeks of age they have reached their average normal plateau in the neighborhood of 60%. At ages 12 and 24 months this level has been depressed to about 40%.

Finally, the neutrophil counts are given as percentages of the total white cells. They begin at a high level of more than 70% and drop steadily until at 2 weeks of age they are at about the 30% level which they maintain through the first few months. At 12 months there is a rise, and at 24 months a slight further rise in the neutrophils. It must be pointed out, however, that the absolute count of the neutrophils (actual no./mm^3) is approximately the same for the newborn as for the adult mice, with a decline at first and second weeks of postnatal life. The adult value of neutrophils, achieved by about 1 month of age, ranges from 2000 to 3000 cells/mm^3 and thus the curve for white blood cells is followed until 1 month by these cells. When the lymphocytes are evaluated on an absolute basis, there is a gradual and continuous increase in the blood until the adult maximum is reached at approximately 2 months of age. This is between 5000 and 7000 cells/mm^3, as opposed to 300–400 for the new born. With age there is a definite drop in lymphocytes to approximately 2500–4000/mm^3 while there is the increase in neutrophils to 3500–5500/mm^3. In general, adult female mice have a higher hemoglobin and red cell count than do the adult males, a situation which seems to be opposite to that of the human.

For comparison a group of 17 gestation day mouse fetuses was studied for complete blood count and this is given in tabular form (Table I). It will be noted that many of the cell types found in the fetus are absent from normal adult blood, and may be considered as peculiar to the fetus.

TABLE I.

	Mouse fetus at 17 days gestation	
	Male	Female
Hemoglobin	8.9	8.3
White blood cells	1720	1533
Red blood cells	3,438,666	3,244,000
Platelets	453,333	454,000
Total neutrophils	33.8	33.7
Myelocytes	0.1	0.1
Juveniles	1.5	0.8
Stabs	10.3	7.1
Segmentids	21.9	25.1
Young eosinophils	.5	0.0
Mature eosinophils	2.5	1.0
Young monocytes	0.9	1.1
Mature monocytes	18.7	19.7
Young lymphocytes	2.1	2.4
Mature lymphocytes	31.8	30.4
Blasts	0.5	0.8
Histiocyte or mesenchyme cell	0.3	0.1
Triple nucleus	0.0	0.0
Unidentifiable	2.0	0.4
Atypical	0.0	0.0
Nuclear fragmentation	0.0	0.0
Nucleated erythrocytes	48.6%	53.7%

Summary and Conclusions. A study of 17 day fetal mouse blood revealed that almost all cell types were much lower than even in the newborn (2–3 days later) with the exception of the nucleated erythrocytes which constituted about 50% in both the males and females. The graphs of blood counts represent the averages of 25 mice for each age up to 9 weeks, and at 12 months there were no less than 20 mice, and at 24 months no less than 13 mice examined. Females tended to show a higher red cell and hemoglobin count than did males of the same age, and the males after 2 weeks had the higher leukocytes counts and after 5 weeks showed higher platelet counts. With respect to the neutrophils and lymphocytes there was little difference between the sexes. Red cell production is progressively increased in male and female mice from birth until a plateau is reached at 8–9 weeks of age, while the hemoglobin shows a drop from birth through 3 weeks. Thereafter the hemoglobin values parallel those of the red cells possibly because of the decrease of red cell size and of polychromatophilia. Peak production in hemoglobin and red cell production was reached at 8 weeks of age, and this was generally a bit higher than the plateau established for the

fully matured mouse at 12 months of age or the early senile mouse at 24 months. The average counts from week to week were rather steadily altered except for the leukocytes which curiously showed a decline at 6 weeks for both sexes. The decrease of leukocytes in both sexes from birth to the lowest point at 2 weeks of age, and the slight dip at 6 weeks, is caused by the steep drop in the neutrophils while the lymphocytes showed a steady increment from birth to a plateau at 8–9 weeks of age. Both the 12 and 24 months old mice showed a considerable decline in lymphocytes and an increase in neutrophils in their general circulation.

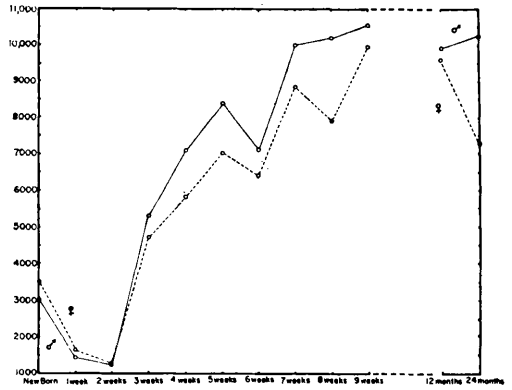

FIG. 1. Normal postnatal blood counts (mouse) white blood cells.

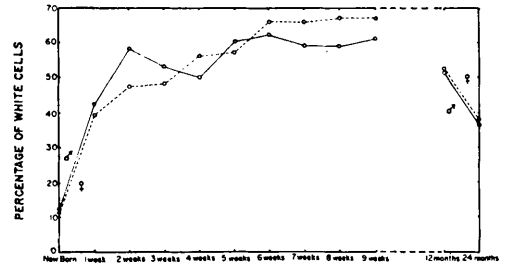

FIG. 2. Normal postnatal blood counts (mouse) lymphocytes.

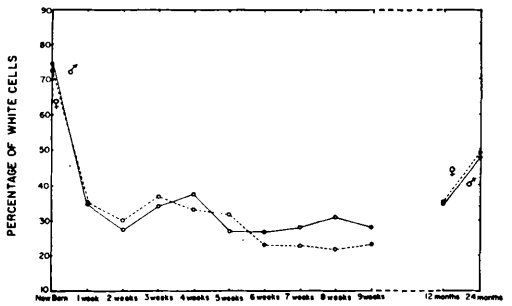

FIG. 3. Normal postnatal blood counts (mouse) total neutrophils.

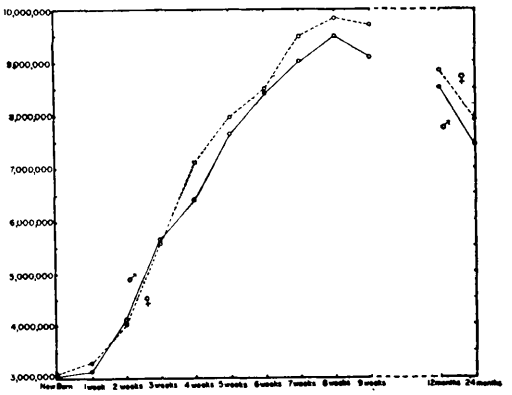

FIG. 4. Normal postnatal blood counts (mouse) red blood cells.

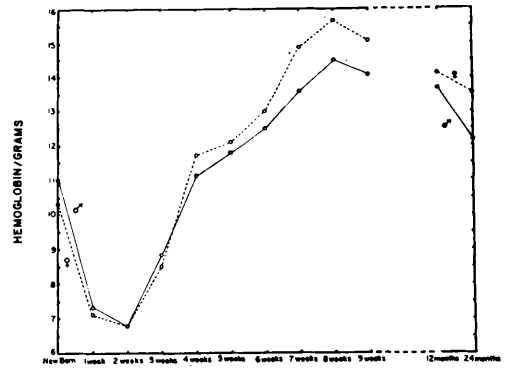

FIG. 5. Normal postnatal blood counts (mouse) hemoglobin.

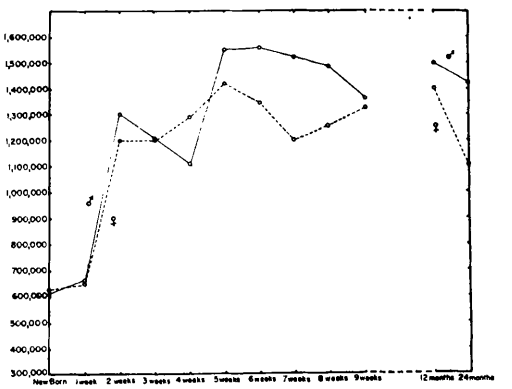

FIG. 6. Normal postnatal blood counts (mouse) platelets.

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Fertilization, Embryo and Fetal Survival Rates in Rabbits Isoimmunized with Semen, Testis, and Conceptus* (32927)

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Evidence in the mouse (1,2), guinea pig (3,4), rabbit (5), and cow (6) has indicated that immunization of females with homologous semen, spermatozoa, or testis will cause infertility. The infertility in mice was reported due to inhibition of fertilization (2). The exact nature of the infertility in other species, however, has not been clearly demonstrated to be fertilization failure, embryo mortality, fetal mortality, or a combination of these factors. The objective of the present study was to determine the effects of isoimmunization with semen, testis, 9-day-old conceptus, and seminal plasma on fertilization rate, embryo survival, and fetal survival in rabbits.

Materials and Methods. The rabbits used in the trials were sexually-mature estrous females of mixed breeding. The antigenic materials consisted of: semen from the pooled ejaculates of 2–4 male rabbits, seminal plasma from 2 vasectomized rabbits, testis material from mature rabbits that was homogenized with an equal volume of physiological saline, and homogenized conceptus material from rabbits 9 days pregnant. The antigenic materials were mixed with an equal volume of Freund's complete adjuvant and 0.5–0.75 ml of this mixture was given per injection. Rabbits injected with adjuvant and physiological saline served as controls.

In Trial 1, 25 rabbits were given 3 weekly intramuscular and subcutaneous injections of the antigen–adjuvant mixtures. One week after the last injection the females were artificially inseminated with 0.5 ml of diluted semen containing 15 to 25 million live sperm. Ovulation was induced by intravenous injection of 100 IU human chorionic gonadotropin (HCG) at the time of insemination. Embryo survival was determined 9 days after breeding by laparotomizing the rabbits and noting the number of embryos and corpora lutea present. Fetal survival was based on the number of viable fetuses at 28 days after breeding or the number of young born compared with the number of normal embryos at 9 days. Rabbits bred a second time were artificially inseminated from 4 to 6 weeks after an infertile breeding or from 3 to 4 weeks postpartum after removal of the litter.

In Trial 2, 30 rabbits were given 3 weekly injections of the antigen–adjuvant mixtures followed in 3 weeks by a fourth injection. One week after the last injection the rabbits were artificially inseminated and injected with

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