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The Immunoglobulins of Mice. 4. Serum Immunoglobulin Changes Following Birth. (29914)

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A characteristic pattern of serum changes in 3 immunoglobulins, *i.e.*, the IgG (7S γ_2-globulin, IgA (γ_{1A}-globulin) and IgM (γ_{1M}-globulin), has been described following birth of the human infant(1-3). Quantitative studies of individual immunoglobulin changes after birth, however, have not been described in other species. Four major immunoglobulin classes, the 7S γ_2-globulins, 7S γ_1-globulins, γ_{1A}-globulins and γ_{1M}-globulins were recently identified in mice(4). The present studies were undertaken to determine the serum levels of these immunoglobulin components in the newborn and to characterize the immunoglobulin changes following birth of the normal mouse. Three mouse strains were studied and found to be similar. Both similarities and differences between man and mouse were observed.

Methods. C57BL/6JN, C3H/HeN, and GP (general purpose, white Swiss mice maintained in a closed colony at the NIH) mice were obtained from the Animal Production Section of the NIH at one day of age, one week of age, or at weekly intervals thereafter. Animals were weaned at 3 weeks of age. Ten or more animals were bled at each date to obtain enough serum for the various experiments. All animals were housed and fed in a similar manner throughout the study.

Quantitative serum levels of 7S γ_2-globulin, 7S γ_1-globulin, γ_{1A}-globulin and γ_{1M}-globulin were measured in antibody-agar plates using essentially the same method described elsewhere for measuring the human serum immunoglobulins(5). Briefly, addition of antigen (mouse immunoglobulin) to wells in an antibody-containing agar plate results in antigen-antibody precipitate rings, the diameter of which reflects the concentration of antigen.

Specific antiserums were prepared from antiserums produced in rabbits by immunization with 7S γ_2-myeloma protein (5563), 7S γ_1-myeloma protein (MPC-25), γ_{1A}-myeloma protein (MPC-1) and normal γ_1-macroglobulin(4).

Antibody-agar plates were prepared by pouring a solution containing 8 ml of diluted antiserum and 8 ml of 3% Noble agar onto a 3¼ × 4 inch glass plate. The antiserum dilution for the individual immunoglobulin determinations varied from 1:10 to 1:60 depending on the potency of the antiserum. The highest dilution of antiserum which gave distinct end points (precipitation rings) was used. Thirty-six antigen wells were cut in the antibody-containing agar plates using a plastic template(5). Six of the wells in each plate were used for standard reference samples of known protein concentration. In practice a single serum sample was used as a reference on all antibody-agar plate tests. The level of each immunoglobulin in this serum was determined by comparison with the following purified immunoglobulin preparations: two 7S γ_2-myeloma proteins, two 7S γ_1-myeloma proteins, two $\gamma_{1A}(\beta_{2A})$-myeloma proteins and a preparation of normal mouse γ_{1M}-globulin.

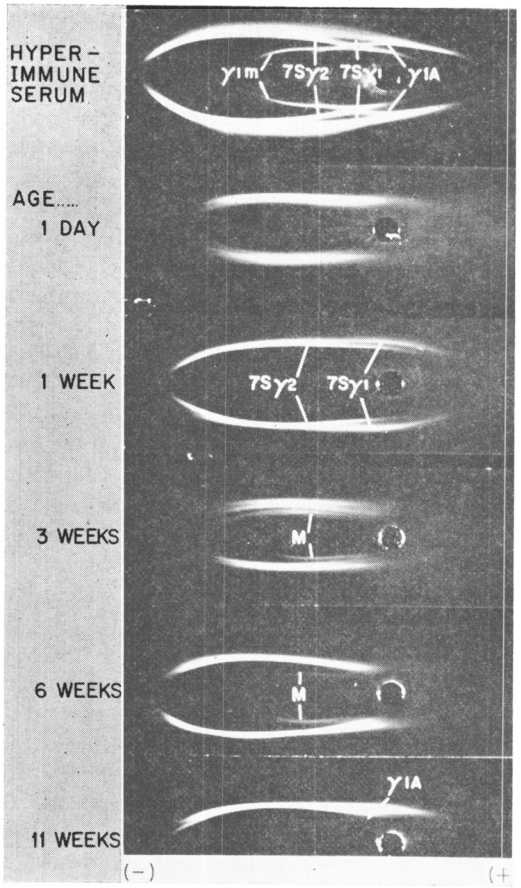

FIG. 1. Immunoelectrophoretic demonstration of immunoglobulin changes in mouse serum at various ages. A polyvalent antiserum reacting with the known classes of mouse immunoglobulin was used in antiserum troughs. Photographs obtained after 48 hr diffusion at 5°C.

A semilogarithmic plot of the protein concentration *vs* the diameter of the ring precipitates provided a reference curve(5). Concentrations of the immunoglobulin in the test serums were determined from this curve using the diameter of the precipitates formed by the reference and test samples 24 to 48 hours after antigen addition to the well.

Many sera were also tested for total 7S γ-globulin (7S γ_2 and 7S γ_1) and for γ_{1A}-globulin levels by an isotopic immune inhibition test similar to that employed for human immunoglobulins(6). Comparable values were obtained by this and the antibody-agar plate technic, and only values from the latter measurements are recorded.

Results. Four immunoglobulins (7S γ_2-, 7S γ_1-, γ_{1A}- and γ_{1M}-globulin) were studied by immunoelectrophoresis (Fig. 1) and quantitative immunochemical methods (Fig. 2). At one day of age all immunoglobulin levels were low. The subsequent changes in the 3 strains studied (C3H/HeN, C57BL/6JN and GP) were similar and are considered together.

γ_{1M}-macroglobulins were not found at birth but became detectable at one week of age in one strain and by 2 and 3 weeks in the other 2 strains. Adult levels of γ_{1M}-globulins were present at 8 weeks of age in both C3H/HeN and C57BL/6JN strains.

$\gamma_{1A}(\beta_{2A})$-globulins were first detectable between 4 and 6 weeks and levels rose gradually over the next 4 weeks. Adult levels were reached between 3 and 4 months of age.

Only 7S γ_2- and 7S γ_1-globulins were detectable in newborn mouse serum. The levels of these globulins were low. By one week, however, serum levels had risen sharply, almost to adult levels (Fig. 2). These levels remained elevated for 1 to 2 weeks, then rapidly declined (Fig. 2). The 7S γ_2- and 7S γ_1-globulins remained at low levels after

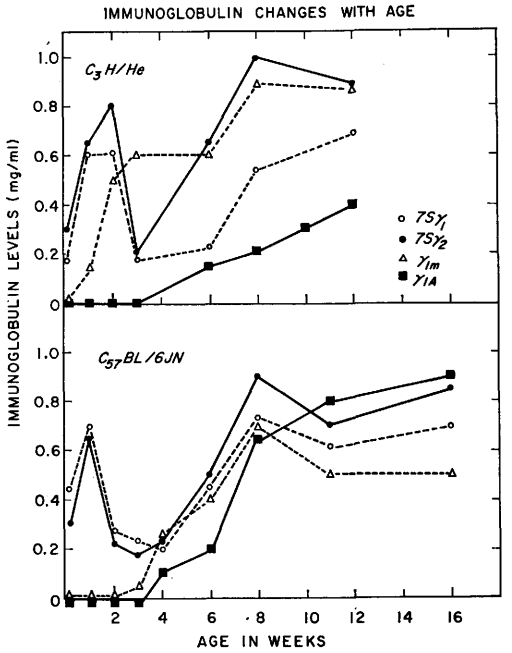

FIG. 2. Quantitative immunoglobulin changes after birth. Quantitative determinations were performed by the antibody in agar plate technic.

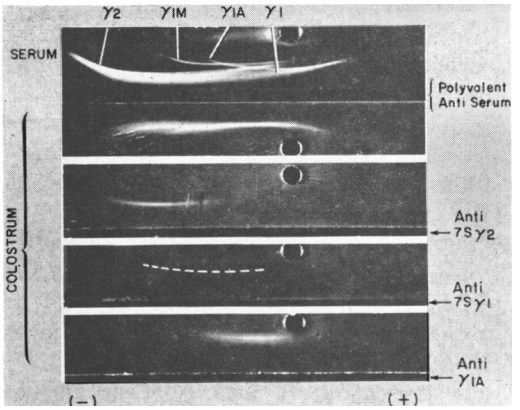

FIG. 3. Immunoelectrophoretic demonstration of immunoglobulins in mouse colostrum. Polyvalent antiserum reacting with all known categories of mouse immunoglobulins was used in the top test, and antiserums specific for immunoglobulin components were used below. Reinforcement with a dashed white line was used where the precipitin lines were too weak to reproduce clearly. No γ_{1M}-globulin was detected, so illustration of the test using specific antiserum for mouse γ_{1M}-globulin is not included.

weaning was completed. At about 6 weeks of age the levels began to rise and achieved adult values between 2 and 3 months of age.

The marked rise in 7S γ_2- and 7S γ_1-globulins between 1 and 7 days of age indicated that these 2 immunoglobulins might be of maternal origin and pass into the fetus *via* the maternal colostrum and the fetal gastrointestinal tract. Colostrum was obtained from C3H/HeN mice* and examined by immunoelectrophoresis (Fig. 3). Specific antisera showed the presence of both 7S γ_1- and 7S γ_2-globulins. High levels of γ_{1A}-globulin were also present in colostrum but γ_{1M}-macroglobulins were not detectable by immunoelectrophoresis.

Discussion. The 7S γ_2- and 7S γ_1-globulins are present in low but detectable amounts in newborn mice. These proteins are presumed to have been transmitted across the placenta or yolk sac. The analogous proteins (7S γ_2- and 7S γ_1-globulins) in the guinea pig(7) and the 7S γ-globulins of rabbit(8) and man(9) also are transmitted from the maternal to fetal circulation.

The absence of detectable quantities of $\gamma_{1A}(\beta_{2A})$ and $\gamma_{1M}(\beta_{2M})$-globulins in newborn mice would indicate that these proteins are not transmitted across the placenta in the mouse. Similar observations have been made in man(1-3).

Transfer of immunoglobulins *via* the colostrum and newborn gastrointestinal tract also appears to be a selective process. The rapid rise in serum levels of 7S γ_2- and 7S γ_1-globulins following onset of nursing and the identification of these proteins in colostrum indicates that these proteins were transferred from mother to newborn *via* the colostrum and gastrointestinal tract. Plentiful amounts of γ_{1A}-globulin were also identified in the colostrum but there was no rise in the neonatal serum level of this protein, indicating that γ_{1A}-globulins were not transferred or were digested or were very rapidly catabolized after transfer.

Selectivity in placental transfer and in gastrointestinal transfer of the 7S γ_2- and 7S γ_1-globulins and the absence of γ_{1A}- and γ_{1M}-globulin transfer reflects structural differences in these proteins. In studies in the rabbit Brambell and associates(10) showed that piece III (Fc fragment) of 7S γ-globulin is needed for selective gastrointestinal transfer of this protein. Recent studies(11) have show that the difference between mouse 7S γ-globulin and γ_{1A}-globulin is based on properties of the Fc fragment (*i.e.*, the F fragment obtained by papain digestion which is analogous to piece III of rabbit γ-globulin). The present experiments are consistent with the hypothesis that the Fc fragment of mouse, as well as of rabbit, γ-globulin is responsible for the active placental transfer of these molecules. Direct experiments to test this hypothesis are indicated. It also will be of interest to determine whether 7S γ_2- and 7S γ_1-globulins share the same chemical configuration and are transferred by the same mechanism or whether they are transferred by different mechanisms based on different molecular specificities.

The rapid fall in serum 7S γ_2- and 7S γ_1-globulin levels after 2 weeks of age raises the possibility that these proteins were no longer present in the mother's milk or that they were no longer able to pass from the gastrointestinal tract into the nursing mouse. The mouse gut has been shown to decrease

* Colostrum obtained from Dr. William Feller, Nat. Cancer Inst.

markedly its permeability to mouse antibody between 14 and 17 days of age(12,13). The low serum 7S immunoglobulin levels at age 3 and 4 weeks are probably due to an absence of maternal supply *via* the colostrum and gut plus a poorly developed capacity for endogenous 7S γ-globulin synthesis.

The possibility that markedly increased rates of catabolism of 7S γ_2- and 7S γ_1-globulins at age 3 and 4 weeks caused the low levels must be considered. Other studies with labeled 7S γ-globulin, however, have showed that catabolism is decreased (not increased) in mice with low 7S γ-globulin levels(14). These studies included 4-5-week-old mice with low 7S γ-globulin levels and rule out the possibility that increased catabolism is responsible for the hypogammaglobulinemia at this age.

The serum immunoglobulin levels in the newborn mouse differ from the human newborn in several respects. The human fetus has received abundant quantities of 7S γ-globulin *via* the placenta and the serum 7S γ-globulin (IgG) levels at birth are near the normal adult levels(1-3). In contrast, mouse 7S γ-globulins (7S γ_2- and 7S γ_1-) are very low at birth. Transfer of 7S γ-globulin from the mother to the newborn *via* the colostrum and gastrointestinal tract soon bring the mouse abreast of his human counterpart. After 2 weeks of age similar changes occur to the immunoglobulins of mice and man. The 7S γ-globulin levels fall in both species (placental and colostrum sources no longer being available) until increasing endogenous synthesis of 7S γ-globulins gradually raises serum levels toward normal adult levels. Meanwhile, in both species, increased serum levels of γ_{1M}-globulins (IgM) and then γ_{1A}-globulins (IgA) become evident.

Brambell(15) has recently pointed out that a similar mechanism may be involved in skin sensitization for passive cutaneous anaphylaxis and in transmission of 7S γ-globulin across the gut wall. The present studies and investigations of passive cutaneous anaphylaxis with mouse immunoglobulins(16-18) are relevant to this hypothesis. (It will be assumed that serum levels of several immunoglobulins at 2 weeks of age reflect gut transfer, although additional study of this matter is needed.) The colostrum γ_{1A}-globulins do not pass through the gut wall and serum γ_{1A}-globulins do not sensitize homologous (*i.e.*, mouse) skin(16-18), in accord with Brambell's hypothesis. The 7S γ_1-globulins do sensitize homologous skin and do pass through the gut wall, also in accord with Brambell. A possible difficulty may exist, however, in the correlations with mouse 7S γ_2-globulin. These proteins have little or no capacity to sensitize homologous skin(16-18) but do appear to be transferred across the gut. Additional data on rate of gut transfer of 7S γ_2-globulin, especially data relative to 7S γ_1-globulin, would be helpful in this comparison. More specific studies, also, will be needed to establish whether the same part of the molecule is responsible for passive anaphylactic sensitization and for transmission of immunoglobulin from mother to young.

Summary. The changes in 7S γ_2-, 7S γ_1-, γ_{1A}- and γ_{1M}-immunoglobulin components following birth were studied in 3 mouse strains. Evidence for transfer of immunoglobulins *via* the placenta or yolk sac and *via* the colostrum and neonatal gastrointestinal tract were evaluated. Low levels of 7S γ_2- and 7S γ_1-globulins were found in the newborn indicating transplacental or yolk sac transfer of these proteins. Marked increase (to adult levels) of these 2 immunoglobulins occurred at 1 and 2 weeks of age. Both 7S γ_2- and 7S γ_1-globulins were present in the colostrum and appeared to be supplied *via* the gastrointestinal tract until 2 weeks of age. Subsequently, serum 7S γ_2- and 7S γ_1-globulin levels fell until age 4 weeks, when neonatal synthesis began to produce an increased serum level of these proteins. The γ_{1M} (IgM)-globulins were detected in the serum at 1-3 weeks of age. The γ_{1A} (IgA, β_{2A})-globulins were not detected in the serum until 4-6 weeks of age. Although γ_{1A}-globulins are present in the colostrum, little or no γ_{1A}- (or γ_{1M}-) globulin appeared to be supplied *via* the colostrum and neonatal gastrointestinal tract. Adult levels of the 4 immunoglobulins are reached at 2-3 months of age.

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Blood Volume in Relation to Body Weight of the Male Rat Using Radio-Iodinated Serum Albumin.* (29915)

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An accurate means of estimating total blood volume in the rat is useful in laboratory investigation. This report is the result of blood volume determinations in 122 normal male albino rats of varying body size. The method described is simple and the results provide a constant means of estimating total blood volume in relation to body weight.

Method. One hundred twenty-two white male rats ranging in weight from 140 to 294 g were used. The animals were anesthetized with 2% sodium pentobarbital given intraperitoneally in dosage of 0.2 cc per 100 g body weight and the femoral artery was then cannulated with a no. 50 polyethylene catheter. Radio-iodinated serum albumin (RISA) was used to determine blood volume. Approximately 1.0 microcurie of RISA in a volume of 0.25 to 0.5 cc was injected into the femoral artery, then the cannula was flushed with 0.4 to 0.5 cc of heparinized saline to insure complete injection of RISA. Initially a few animals received the RISA intravenously, but it was soon found that intraarterial injection gave equal values and satisfactory curves. Arterial injections eliminated the additional step of cannulating a vein. Samples of blood of 0.2 cc each were obtained from the femoral artery at 5, 10 and 15 minute intervals following injection. Before each sample was collected the arterial cannula was drained of its heparinized saline and after each collection the tube was flushed with 0.2 cc of saline. The blood samples were pipetted into test tubes containing 1 cc of saline and counted in a well-type scintillation counter. Standards for each determination were prepared by injecting from the same syringe used in the rat, an equal amount of RISA in a dilution of 100 ml into a volumetric flask. After thorough mixing of this standard, 2 cc was pipetted into a test tube and this was counted in the well counter. The counts per minute of the 3 blood samples were plotted on linear graph paper. The curve was extrapolated to zero time and this zero

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