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Zygote Transfer to Facilitate Altered Expression of Immunoglobulin Light Chain Phenotypes in Homozygous Rabbits* (33643)

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(Introduced by J. E. Kempf)

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Rabbits of genotype b^4b^5, heterozygous for the light polypeptide chains of immunoglobulins, generally have approximately twice as much b4-γG than b5-γG (1, 2).[1] However, a persistent alteration in the quantitive expression of these allelic allotypes occurs in heterozygous rabbits exposed during lethal and neonatal life to antibody specific for the allotype inherited from the gene of the father (3). Thus, when b^4b^5 homozygous mothers are immunized against the paternal b5 allotype the b^4b^5 heterozygous offspring, throughout their lives, have very low serum b5 allotype and relatively high compensatory levels of the b4 allotype; e.g., one rabbit had b4 to b5 ratios of 300:1 at 5 months, 71:1 at 1 year, 63:1 at 2 years and 19:1 at 3 years (4, 5). "Allotype suppression" can be effected either by uterine transfer of antiallotype antibody or by injection of newborn heterozygous rabbits with antiallotype antisera directed against the paternal allotype (3, 5). In heterozygous suppressed animals, the total γG levels appear normal and a compensatory increased production of the allelic allotype accounts for most of the immunoglobulin in the sera (3, 5).

The question arose as to whether all immunoglobulins with light chains controlled at the *b* locus could be suppressed by injection of antiallotype antibody into homozygotes. Would this lead to ahypogammaglobulinemia or would a compensatory increase of light chain synthesis occur at another locus in order to maintain normal levels of immunoglobulins? In fact, 10–30% of the molecules from homozygous or heterozygous rabbits lack allotypic specificities controlled by the *b* locus suggesting that some light chains are controlled by genes other than at the *b* locus (1, 2).

It was not feasible to attack the problem directly since a newborn homozygous rabbit, e.g., b^5b^5, must come from a mother having the b^5 gene and thus at birth will have a relatively large amount of the b5 allotype as a result of uterine transfer. Anti-b5 injected into this newborn would react with the b5 allotype to form antigen–antibody complexes and this may prevent the free antibody from being effective on the cell population synthesizing b5. Dubiski (6) minimized uterine transfer of the b5 allotype by using b5 suppressed b^4b^5 heterozygous mothers. He succeeded thereby in obtaining significant suppression of b5 light chains in the b5 homozygous offspring (6). However, by this precedure, half the offspring are b^5b^5 homozygotes and half are b^4b^5 heterozygotes, making it difficult to distinguish the genotype of the newborn. Furthermore, b5 allotype suppressed rabbits are still producing small

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[1] Allotypic specificities Ab4, Ab5, and Ac7 are abbreviated to b4, b5 and c7; the genes are b^4, b^5, and c^7.

amounts of b5 light chains which might interfere with the full effectiveness of the injected anti-b5 (4, 5).

In this study, homozygous b^5b^5 zygotes were transferred to the uteri of b^4b^4 homozygous mothers thus completely preventing the uterine transfer of b5 immunoglobulin in the developing fetus. At birth, anti-b5 prepared in a b^4b^4 rabbit was injected into such neonatal b^5b^5 progeny from foster uteri to produce suppression of the b5 allotype. These offspring had normal total immunoglobulin levels implying a compensatory increase in synthesis of light polypeptide chains controlled at another locus.

Materials and Methods. The b^5b^5 donor of the zygotes was mated with a b^5b^5 buck. At this time, b^4b^4 recipient was given 2.5 mg of luteinizing hormone (Mann Research Laboratories, New York, N. Y.) intravenously to induce ovulation. After 24–36 hr both does were prepared for surgery (7), anesthetized with Nembutal, and the ovaries were exposed to flank incisions. The oviduct and uterus of the donors were exteriorised and a 30-cm length of polythylene tubing (Intramedic, PE 160) flanged slightly at the end was inserted into the fimbriated end of the oviduct and held in place by a miniature plastic clothespin. A blunted 20-gauge needle was inserted through the wall of the uterus and passed beyond the tubo-uterine junction into the isthmus of the oviduct. With 5 ml of 20% normal rabbit serum, ova were flushed toward the fimbriated end of the oviduct onto a watchglass. The ova were counted and examined for cleavage and abnormalities with a dissecting microscope. The ovaries of the recipient were inspected for ruptured follicles which must be present for a successful transplant. The donor's zygotes were drawn up in a fine glass capillary pipette and deposited 2–3 cm inside the fimbriated end of the oviduct. A small sheet of absorbable gelatin (Gelfoam sponge, Upjohn) was inserted before the incisions were closed.

The offspring were divided into 3 groups: four animals were each given a total of 5 ml of antiserum (4.5 mg of anti-b5 antibody) distributed among 4 injections at 1, 3, 5, and 7 days of age; two other animals were each injected according to the same schedule with 5 ml of normal b4 serum; two animals were left untreated. Blood samples were obtained at 2 weeks of age and at regular intervals thereafter for analysis.

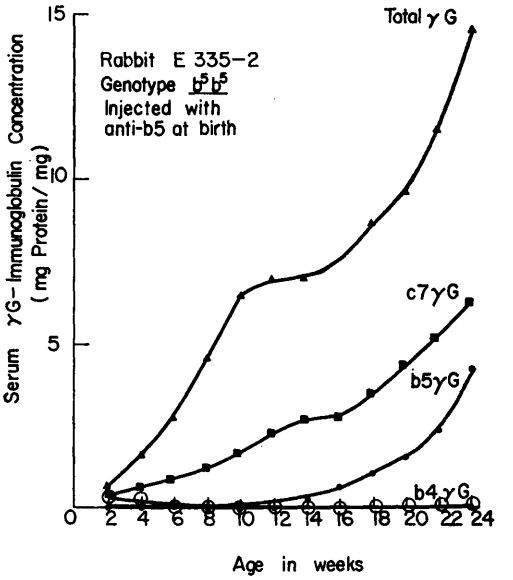

FIG. 1. The b4, b5, c7, and total γG-immunoglobulin concentrations (mg of protein/ml) in the sera of a b^5b^5 homozygous offspring fostered in uteri of a b^4b^4 homozygous mother. The synthesis of b5 was suppressed in the newborn rabbit by the neonatal injection of anti-b5 serum made in a b^4b^4 homozygous rabbit.

The total γG, b5γG, and b4γG concentrations in the sera of the progeny at different ages was determined by radial diffusion analysis (8) using goat antirabbit Fc fragment, rabbit anti-b5 and rabbit anti-b4. Highly purified γG-immunoglobulins (4) were used as standards.

Results. In the b^5b^5 homozygous rabbits injected with anti-b5 (Fig. 1), b5γG synthesized by the neonatal rabbit, was first detectable at 12 weeks (1% of total γG) and then increased in concentration at about the same rate as the total γG. At 24 weeks, the b5γG was 27% of the total γG. In marked contrast, in the control animals (Figs. 2, 3) b5 was present at 2 weeks and then rose rapidly in concentration, and approximately 90% of the γG had b5 specificity at all dates tested.

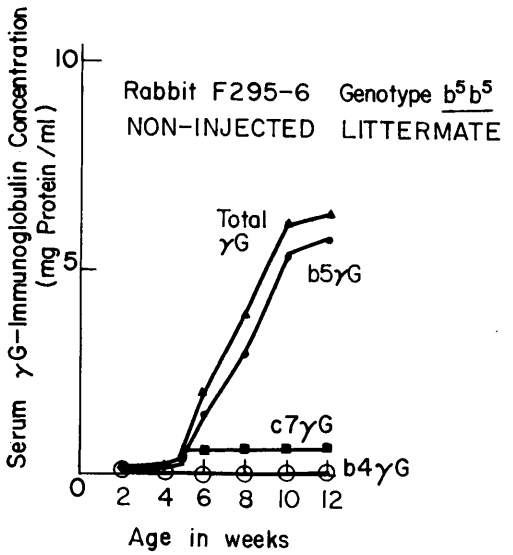

FIG. 2. The b4, b5, c7, and total γG-immunoglobulin concentrations (mg of protein/ml) in the sera of an uninjected b^5b^5 homozygous offspring fostered in uteri of a b^1b^1 homozygous mother.

The b4γG present initially as a result of maternal transfer and the injection of normal b^4b^4 serum could be detected somewhat longer, 4 weeks, in the injected animals (Figs. 1, 3), compared to 2 weeks in the uninjected animals (Fig. 2). The total γG concentrations in the sera of the injected animals (Figs. 1, 3) was initially higher than the uninjected animals (Fig. 2) due to the large amount of b4 which was introduced with the injected serum, but after 4 weeks no marked difference was observed.

The sera were subsequently tested for the presence of c7γG (9),[2] an immunoglobulin which constitutes about 4% of the total γG concentration in serum of c^7c^7 homozygotes (Figs. 2, 3). In the suppressed animals, the c7γG levels (Fig. 1) increased at the same rate as the total γG levels until at 24 weeks, the c7γG accounted for 44% of the total γG. Thus approximately 29% of the molecules (100-27-44) lack the b5 or c7 allotypic specificities. The compensatory increase of c7γG suggests that the c7 allotypic specificity is on the light chain in agreement with recently obtained direct evidence (10).

[2] The c7 was called p when originally identified in Ref. (9).

Discussion. A zygote transfer technique is described which permits the study of the effects of "allotype suppression" in homozygous rabbits, free from the problem of uterine transfer of maternal allotypic immunoglobulin of the same phenotype as synthesized by the offspring. Administration of anti-b5 serum to neonatal homozygous b^5b^5 rabbits, fostered in the uteri of b^4b^4 homozygous mothers, resulted in a marked inhibition of the synthesis of b5γG. The total γG levels were normal and an increased synthesis of light chains controlled at another locus accounted for most of the immunoglobulins in the sera. These results are similar to those observed previously by Dubiski (6) in homozygous rabbits which were the offspring of b5 suppressed b^4b^5 heterozygous mothers. Normally, in b^5b^5 homozygotes, molecules lacking the allotypic specificities controlled at the *b* locus constitute only a small percentage of the total γG-immunoglobulins. Since the levels of *b*-negative immunoglobulins are markedly increased in the b^5b^5 homozygous suppressed animals, this technique will be valuable in the investigation of their structure and function (11).

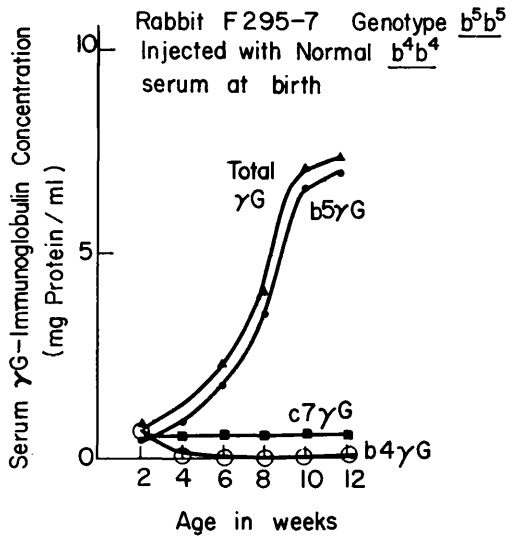

FIG. 3. The b4, b5, c7, and total γG-immunoglobulin concentration (mg of protein/ml) in the sera of a b^5b^5 homozygous offspring fostered in uteri of a b^4b^4 homozygous mother. The newborn rabbit was injected with normal b^4b^4 serum.

Summary. By the techniques of zygote transfer and allotype suppression, b^5b^5 homozygous rabbits were produced with an altered expression of immunoglobulin light chain allotypes; i.e., at 24 weeks of age, only 27% of the total γG was b5γG compared to 90% in nonsuppressed homozygotes. Of the 73% b-negative molecules, 44% were c7γG and 29% were both b- and c-negative. These results suggest that there may be at least three "loci" which control the synthesis of light chains.

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Hepatic Lipidosis Associated with L-Asparaginase Treatment* (33644)

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Although fatty livers have long been recognized, it is only comparatively recently that the factors related to the deposition of fat in this organ have been understood. This condition has been referred to as a fatty degeneration, a fatty metamorphosis, and as a fatty infiltration. According to most viewpoints, fatty infiltration results from an abnormal accumulation in this organ of lipids, predominantly triglycerides, which have been transported there from other tissues.

In late March of 1967 a biopsy specimen was obtained from patient F. H., a terminal acute lymphatic leukemia patient who had received some 200,000 units of *E. coli* L-asparaginase over an interval of 32 days, during which time he had shown a dramatic response and excellent remission (1). Light microscopy of the specimen revealed what appeared to be fatty infiltration of the liver. These results of fatty metamorphosis of the liver associated with L-asparaginase therapy were reported to the leukemia chemotherapeutic task force by Dr. J. M. Hill of this institute in May of 1967. These findings served as the starting point of this investigation.

The production of fatty liver by deficiency (2) or imbalance (3) of amino acids has long been recognized. Many other agents, ethionine (4), orotic acid (5), tetracycline (6), aureomycine (7), ethanol (8), carbon tetrachloride (9), and bacterial endotoxins (10), can also produce fatty liver. However, the production of fatty liver by L-asparaginase, an enzyme, has only recently been suggested. The present studies show that low specific activity L-asparaginase induces a fatty liver in normal male C3H mice and that the accumulated lipids are essentially all triglycerides. On the other hand, enzyme of rather high specific activity does not show the same effects.

Materials and Methods. Liver tissues obtained at autopsy of several L-asparaginase-treated patients were suspended in physiolog-

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