in the activation of the substrate. Sphingomyelin and phosphatidylethanolamine were not studied. None of the other serum nonlipoprotein components (albumin, α_1-glycoprotein, γ-globulin) or whole serum from which lipoproteins were removed by ultracentrifugal flotation at density greater than 1.21 (Spinco Model L, 114,000*g*, 24 hours, 16°C) were active either alone or after mixing with sols of HDL phospholipids. Modification of the substrate by ionic surfactants (sodium octyl to hexadecyl sulphate and benzyldimethyloctyl to nonadecyl ammonium chloride) in the presence or absence of apoprotein, was also ineffective in eliciting enzyme activity.

All of the enzyme preparations used produced comparable and reproducible results. As observed by Korn (*6*) they were inactivated by NaCl and showed a decline in activity upon prolonged storage at 4°C.

The results indicate that partial structural restoration of HDL apoprotein by addition of phospholipids is accompanied by restitution of function. This observation may prove useful in studies of the structure of HDL and in the analysis of its mode of formation. The mechanism of substrate activation by HDL must await isolation of lipoprotein lipase in pure form.

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Immunoglobulin M Allotypes of the Rabbit: Identification of a Second Specificity

Abstract. *Rabbit antiserums, originally prepared to react specifically with rabbit immunoglobulin allotypes Ab5 and Ab6, also react with some normal rabbit serums that clearly do not contain Ab5 or Ab6 allotypes. This reaction is due to antigenic specificity present on rabbit immunoglobulin M (IgM) and not on rabbit immunoglobulin G. Serums that contain this IgM allotype do not react with an antiserum that reacts with the known rabbit IgM allotype, Ms1. This specificity may therefore be identified as a second rabbit IgM allotype, Ms2.*

Rabbit immunoglobulin allotypes are genetically controlled antigenic specificities which may differ from one individual to another. Immunization of a rabbit not having a given allotypic specificity with the serum (or immunoglobulin) of another rabbit bearing the given allotypic specificity results in the formation of antibodies in the serum of the immunized rabbit which react with the given allotypic specificity (*1*). To date, six different allotypic antigenic specificities have been well defined. Allotypes Aa1, Aa2, and Aa3 are controlled by one chromosomal locus "a," and are located on the H-chain of rabbit immunoglobulin G (IgG); allotypes Ab4, Ab5, and Ab6 are controlled separately by a second chromosomal locus "b" and are generally thought to be located on the L-chain of rabbit IgG (*2*). The determinants controlled by both loci are present on serum IgG and immunglobulin M (IgM) and on immunoglobulin A (IgA) and IgG in colostrum (*3*).

An allotypic determinant present on rabbit IgM and not on rabbit IgG has been identified and has been named Ms1 (*4*). The hypothetical allele or alleles for the IgM allotype Ms1 controlled by the gene locus Ms, or any other allotypic determinant of IgM, has not previously been reported. I now report preliminary identification of a second allotypic antigenic determinant on rabbit IgM.

The allotypic specificities of the immunoglobulins of a given rabbit are determined in this laboratory by "double-diffusion-in-agar" precipitin reactions between the appropriate specific antiserums to an allotype and the serum obtained from the rabbit being tested. During the course of allotyping the serums of rabbits with antiserums to the six well-characterized immunoglobulin allotypes Aa1, Aa2, Aa3, Ab4, Ab5, and Ab6 (*5*), faint precipitin bands with antiserums directed against Ab5 and Ab6 appeared 2 to 3 days after the major precipitin bands (Fig. 1) appeared.

With one Ab4,5 serum two lines were observed with the antiserum to Ab5. One was the result of a reaction with the Ab5 antigenic determinant, whereas the second faint line was probably the result of a different group of molecules as the line passed through the precipitin line formed by the reaction of antiserum to Ab5 with Ab5 (Fig. 1). Thus an intraspecies antigenic difference present on a serum protein other than IgG was tentatively identified. According to current terminology this antigenic determinant could be called an allotype.

The next step was to ascertain on which serum protein this allotypic determinant was located. The late appearance of the precipitin band, the location close to the antigen well, and the tendency of the lateral margins of the band to curve toward the antigen well (Fig. 1) indicated a protein in low concentration with a diffusion constant less than that of the reacting antibody. All of these observations seemed most consistent with serum IgM. Accordingly individual serums containing the antigen were separated on Sephadex G-200. The antigenic determinant of interest was located only in the first peak, that is, the high-molecular-weight area containing IgM (Fig. 2). Treatment of individual serums containing this antigenic specificity with 2-mercaptoethanol destroyed the capacity of these serums to form a precipitin band when they were reacted with the appropriate antiserums, but the a and b loci reactivities were not affected. Similarly mercaptoethanol also destroyed the reactivity of Msl-containing serums when they were reacted with antiserums to Msl. To define the macroglobulin on which the determinant was located immunoelectrophoresis was performed (Fig. 3). These analyses indicated that the allotype identified was indeed an allotypic determinant present on rabbit IgM.

To determine whether this IgM allotype represented a previously unreported determinant or was only a rediscovery of the previously reported IgM allotype Ms1, normal serums that were not Ab6 or Ab5 but contained the IgM allotype under investigation and

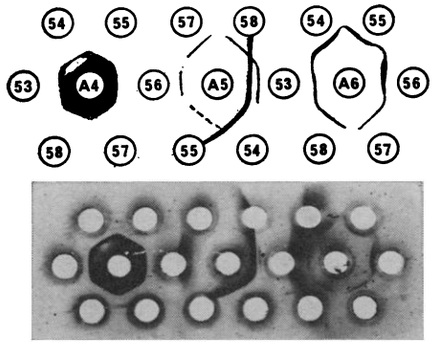

Fig. 1. Rabbit serums 53 through 58 with rabbit antiserums against allotypes Ab4 (A4), Ab5 (A5), and Ab6 (A6) tested by double diffusion in agar (dried and stained with Ponceau S). All serums react strongly with the antiserum to Ab4 (A4) and two serums, 53 and 54, also react strongly with the antiserum to Ab5 (A5). In addition, each serum also produces a weak precipitin band with the antiserums to Ab6 (A6), and all serums but 54 also produce weak bands with antiserum to Ab5. Faint bands produced with the antiserum to Ab4 (A4), and by the serums 53 and 58 actually cross the strong band, suggesting that a different molecular species is responsible. The weak lines are located near the antigen (serum) wells and tend to curve toward the antigen wells, suggesting that the size of the antigen is greater than the size of the antibody. Lines formed by reaction of the antiserum to Ab5 (A5) with Ab5 allotype present in the antiserum to Ab4 (A4), and by the reaction of the antiserum to Ab6 (A6) serum with the Ab6 allotype present in the antiserum to Ab5 (A5), are omitted from the line drawing.

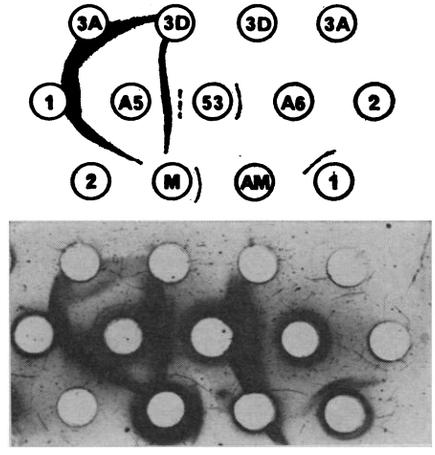

Fig. 2. Reactions (double diffusion in agar) of antiserums to Ab5 (A5) and to Ab6 (A6) with rabbit serum 53 (allotype 1, 3, 4, 5) and the first (1), the second (2), the ascending (3A), and the descending (3D) portions of the third peak obtained by fractionation on Sephadex G-200 of serum from rabbit 53. In addition, the reactions of A5 and an antiserum against the known IgM allotype Ms1 (AM) with a serum known to contain Ms1 (M) and with serum 53 are shown. Antiserum A5 reacts strongly with serum 53 and the second peak obtained after Sephadex fractionation of serum 53 because of allotype Ab5 in the IgG of serum 53. The reaction of A5 with the first peak obtained by Sephadex fractionation of serum 53 is most likely due to allotype Ab5 on IgM, whereas the reaction with 3A is most likely due to contamination with IgG. Antiserum A5 also produces a weak second line with serum 53, but does not react with serum M. Antiserum A6 only reacts with serum 53 and the first fraction obtained from the Sephadex fractionation of rabbit serum 53, indicating that this reaction is specific for a macroglobulin. The antiserum to Ms1 (AM) only reacts with serum M, and does not react with serum 53 or the first Sephadex fraction of serum 53. The precipitin line omitted from the line drawing is the result of reactions of the antiserum to Ab6 with Ab6 allotype present in the A5 serum and in serum M.

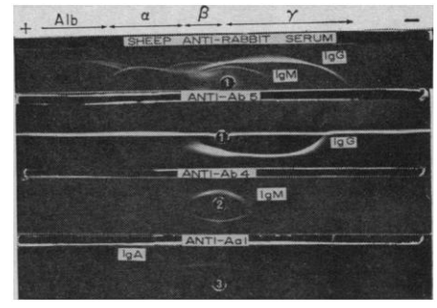

Fig. 3. Composite immunoelectrophoretic patterns demonstrating that rabbit IgM is the antigen responsible for the weak reaction with the antiserum to Ab5. The wells were filled with: (1) serum from rabbit 58; (2) the concentrated first Sephadex peak of rabbit serum 58; and (3) rabbit IgA isolated from the colostrum of an As1,4 rabbit. The top pattern is the result of the reaction of a polyvalent sheep antiserum to rabbit whole serum and illustrates the position of rabbit serum proteins after electrophoresis. The antiserum to Ab4 reacts with the serum IgG and IgM owing to the presence of Ab4 specificity on these proteins. The antiserum to Ab5 does not react with the IgG of serum 58 because rabbit 58 is allotype 1,4. However the antibody to Ab5 does react with IgM from serum 58 because of determinants present on IgM that are different from the "a" and "b" locus allotypic determinants. The antiserum to Aa1 reacts with the serum IgM and colostral IgA due to the presence of "a" locus determinants (H-chain) on these immunoglobulins. The horizontal lines in the two middle patterns are due to reactions of the antiserums to allotype with allotypic determinants present on the serums placed in the opposite trough.

normal serum containing Ms1 were tested with the appropriate antiserum to Ab5 or Ab6 and with an antiserum directed against Ms1 (*5*), and a representative pattern is shown in Fig. 2. The results indicate that the IgM allotype under investigation is antigenically distinct from the known IgM allotype Ms1. In accordance with previous terminology this allotype was named Ms2.

The serums of 114 rabbits were tested for Ms1 and Ms2 determinants. Only one serum, obtained from Professor P. G. H. Gell and known to have Ms1 specificity, reacted with the antiserum to Ms1. This serum did not contain Ms2 specificity. Of the other 113 rabbit serums tested, 19 serums reacted with antiserums to Ms2. Breeding experiments are now planned to determine the genetic relationship between Ms1 and Ms2; and the relationship, if any, between these IgM determinants and the "a" and "b" locus determinants (*6*). Most of the rabbit serums tested did not apparently contain either Ms1 or Ms2. It is possible (i) that there are other alleles at the Ms locus, (ii) that more than one locus may be operative, or (iii) that false negative results may be obtained when rabbits are tested for IgM determinants because of the low concentration of IgM in rabbit serum. The third possibility may be especially pertinent in the case of heterozygote rabbits since less than 50 percent of the IgM might be expected to be of one IgM allotype and would explain the absence of any Ms1,2 rabbits in the population tested.

The identification of an antibody reacting specifically with IgM in antiserums originally prepared to react with an allotype of IgG is explained by the method of immunization. To obtain an antiserum specific for Ab6 an allotype 1,6 rabbit is immunized with killed *Proteus vulgaris*. When a good titer of antiserum to *Proteus* is obtained, a second rabbit allotype 1,4,5 is immunized with complexes of the allotype 1,6 antibody and *P. vulgaris*. Although the antibody in such immunizing complexes is mostly IgG, it is not unlikely that some of the antibody is IgM. If there are differences in the allotypic determinants of IgM between the donor of the antiserum to *Proteus* and the recipient of the *Proteus*-antibody complexes that are not also present on IgG, then antibodies specific for this IgM determinant could be produced.

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6. It is also possible that the colostral IgA of rabbits having IgM specificity Ms1 or Ms2 may also carry these determinants. Accordingly, when does with a known IgM specificity are bred the colostral IgA of these rabbits will be tested for the presence of Ms1 or Ms2 determinants.
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Hemopoietic Colony-Forming Units in Regenerating Mouse Liver: Suppression by Anticoagulants

Abstract. *After hepatic injury induced by carbon tetrachloride, mitotically active hematopoietic cells of nonhepatic origin localize in the liver as judged by an increase in colony-forming nodules in the spleens of lethally irradiated recipient mice on intravenous injection of cells from these livers. The administration of warfarin suppresses the localization of colony-forming units in the regenerating liver by inhibiting the coagulation mechanism of the donor animals.*

While fibrin formation in hemostatic and inflammatory processes has been investigated for decades, the significance of fibrin formation in the growth of primary tumors and in the fixation and extension of metastases has only recently become apparent. Hiramoto (*1*) has shown that extravascular fibrin deposits are found in various spontaneous human tumors. O'Meara (*2*) showed that fibrin forms lattice work on which tumor cells grow and spread. Bale, Spar, and Goodland (*3*) used antibody to fibrin to localize I^{131} in tumor tissue, producing permanent regression of a transplantable rat rumor, and demonstrating the localization of I^{131}-labeled antibody to fibrinogen in spontaneous human tumors (*4*).

Grossi, Agostino, and Cliffton (*5*) have shown that activation of the fibrinolytic system reduced the pulmonary metastases resulting from intravenously administered tumor cells in rats. Anticoagulant therapy as well as fibrinolysis reduces the incidence of pulmonary metastases resulting from intravenous injection of tumor cells or from massaged intact tumors (*6*). Conversely, agents which increase clotting or inhibit fibrinolysis increase metastases (*7*). Alexander and Altemeier (*8*) noted a striking increase in the number of metastases to injured tissues, which Agostino and Cliffton (*9*) attributed to fibrin formation resulting from the inflammatory reaction. These metastases could be greatly reduced by anticoagulant therapy or fibrinolysin. Metastases were produced in liver tissue after carbon tetrachloride feeding, while intravenous injection of tumor cells resulted only in pulmonary metastases in control animals (*10*).

It is our hypothesis that fibrin formed in injured tissues traps normal mitotically active cells which usually are not localized in uninjured tissue and apparently do not play a role in the inflammatory response or subsequent repair processes. One such cell type is the hemopoietic colony-forming cell.

Till and McCulloch (*11*) devised a test system in which cells from bone marrow with the characteristics of stem cells are injected intravenously into lethally irradiated host animals. Gross nodules of proliferating cells, which can be counted macroscopically, are produced in the spleens of the host animals. The spleen colony technique appears to satisfy the requirements of an assay for stem cells, since it detects a cell type which has the capacity to proliferate extensively and to give rise to progeny containing differentiated cells (*12*). Single cells, which have been given the operational name of colony-forming cells (*13*), produce colonies which contain, within a single colony, erythrocytic, granulocytic, and megakaryocytic cells (*14*). The number of colonies in the spleens of irradiated recipients is, over a wide range, proportional to the number of cells or colony-forming units intravenously injected (*15*).

Our work shows that (i) after carbon tetrachloride–induced hepatic injury, colony-forming units, presumably circulating peripheral blood leukocytes, localize in the liver and are trapped in the network of fibrin. Hemopoietic stem-cell elements are found in peripheral blood leukocytes with a frequency 1/30 to 1/50 that in bone marrow cells (*16*). Karyotype studies (*17*) have shown that mitotically active myeloid cells, presumably of bone marrow origin, are present in livers after injection of carbon tetrachloride. (ii) Anticoagulant therapy with warfarin of sufficient dosage to increase the normal prothrombin time 3.4 to 4.5 times inhibits the localization of colony-forming units in the regenerating liver. (iii) Warfarin has no direct effect on colony-forming units; it affects only their localization in the regenerating liver by inhibiting the coagulation mechanism. This inhibition can be reversed by the administration of vitamin K. When bone marrow cells from warfarin-treated mice are intravenously injected into lethally irradiated host mice, spleen nodule counts are the same as those of recipients injected with bone marrow cells from control mice.

The donor and recipient mice used were 12- to 16-week-old genetically homogeneous female F_1 hybrids $(C57L \times A/He)F_1$. All warfarin-treated mice were injected intraperitoneally with the following dosage of warfarin sodium: day 1, 4.8 mg; day 2, 3.36 mg; day 3, 1.92 mg; day 4, 1.20 mg.

All carbon tetrachloride–treated mice were injected subcutaneously on day 2 with 0.2 ml of 40 percent CCl_4 in olive oil. All mice treated with vitamin K (Mephyton) were injected intraperitoneally at the same times as mice treated with warfarin. The dosage (milligrams) of Mephyton was the same as for warfarin. All donor mice were killed on day 5, liver slices were removed, weighed, and homogenized by hand in a glass homogenizer, and the homogenates were suspended in cold TC-199 culture medium to a cell dilution of 10 percent by volume. Recipient mice, which had received 900 rad of whole-body x-irradiation, 250 kv (peak), within the previous 3 hours, were injected intravenously in the tail vein with 0.20 ml of suspended cells containing 20 mg of liver tissue. Approximately 15 minutes before the intravenous injection of liver cells, 10 mg of heparin was injected intraperitoneally in the recipient mice to reduce the mortality from intravascular coagulation produced by the liver-