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The Feed-Back Mechanism in Immunoglobulin Synthesis.* (31070)

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The amount of antibody formed in response to an antigen does not exceed a critical upper limit. It is therefore clear that some mechanisms must exist which do not allow the concentration of antibody to increase indefinitely. Two such mechanisms may operate independently of each other, having two different targets: the antigen and the antibody forming apparatus. The *first* of these mechanisms may operate by eliminating antigen molecules as a consequence of combination with antibody, thus depriving them of their immunogenicity. It has been known since 1901 that antigens lose their immunogenicity when injected as antigen-antibody complexes(1). Recently Uhr and Baumann(2) have described and analyzed this phenomenon. Their results may be interpreted as indicating that the loss of the immunogenicity is a consequence of the shielding of antigenic determinants by antibody molecules. Circulating antibody can, therefore, be regarded as a barrier, protecting the antibody forming cells from overexposure to antigens. If this barrier is broken by larger quantities of antigen, the antigen reaches the antibody synthesizing apparatus, which, as a response to this stimulation, increases its antibody output. With a rising concentration of antibody a *second* regulatory mechanism my become noticeable. This mechanism, in which the concentration of the product of the reaction (in our case

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the concentration of antibody) controls the rate of the reaction is designated as a *feed-back* or *homeostatic* mechanism. In the case of antibody synthesis, the *feed-back* control may operate by affecting either the cellular transformation or the synthetic activity of the antibody forming cells. If such a *feed-back* mechanism controls antibody synthesis, administration of large quantities of antibody would inhibit active antibody synthesis. Rowley and Fitch(3) have tested this prediction and found that antibody synthesis can indeed be inhibited by passive immunization with specific antiserum, given before injection of the antigen. Although this inhibition could be attributed to a *feed-back* effect, the contribution of the first mechanism cannot be completely ruled out.

The present study is concerned with the role of allotypic specificities A4 and A5 in the feed-back regulation of immunoglobulin synthesis in the rabbit.

Materials and methods. Experimental animals. Rabbits homozygous with respect to the A^4 or A^5 allotypic genes were obtained from High Oak Ranch Ltd., Richmond Hill, Ont. Animals which were heterozygous with respect to their A^4/A^5 allotypic genes were obtained by mating of homozygous animals:

(1) ♂ $A^4/A^4 \times$ ♀ A^5/A^5
(2) ♂ $A^5/A^5 \times$ ♀ A^4/A^4

Sera. Normal rabbit sera were obtained from homozygous A^4/A^4 or A^5/A^5 rabbits. Immune sera containing antibodies to A4 or A5 allotypic specificities were obtained from rabbits immunized with *Proteus* bacteria agglutinated with rabbit anti-bacterial serum, according to Dubiski *et al*(4). The anti-A4 serum used for neonatal injections contained 162 μg antibody N/ml, anti-A5 serum contained 125 μg antibody N/ml.

Allotypic phenotype of normal rabbits was determined by double diffusion in agar against anti-allotype sera of known specificities(4,5).

The quantity of antibody nitrogen in the immune sera used for neonatal injections was determined by absorption on cellulose conjugated with Cohn fraction II of rabbit serum (Hyland Laboratories run 11). This preparation was found to contain both A4 and A5 allotypic specificities and therefore, after conjugation with cellulose, had the capacity to absorb antibodies of both anti-A4 and anti-A5 specificities. Cellulose was prepared and conjugated with Cohn fraction II of rabbit serum according to Gurvich(6). For determination of antibody nitrogen a quantity of immunoabsorbent (conjugated cellulose) was mixed with a volume of an immune serum, incubated for 1 hour at 37°C and then kept at +4°C for 4 days. Finally the suspension of immunoabsorbent was washed and its nitrogen content determined by micro-Kjeldahl technique. The antibody nitrogen content of the antiserum was the difference between nitrogen values of the immunoabsorbent before and after absorption.

Relative concentrations of allotypic immunoglobulins† in the sera of the experimental rabbits were measured using the single diffusion method (Oudin 7) in a previously described modification(8). Single diffusion tubes were partly filled with a mixture of antiserum and agar, the agar allowed to set, and sera to be assayed layered above it. The tubes were then sealed with plasticine and stored in a vertical position at 20.5°C ± 0.2°C. After incubation for 7 days, the precipitation bands were photographed at the high magnification of the Cordis Immunodiffusion Camera. The distance between the interphase and the leading edge of the precipitation band was measured with a precision of ± 0.1 mm. The measurements so obtained were evaluated by comparison with a calibration curve, in which the distance between interphase and leading edge of the precipitation band was plotted against logarithm of various concentrations of a standard serum.

Results. 1. *Regulation of immunoglobulin synthesis in normal newborn rabbits.* Although both parents participate equally in determining the allotypic genotype of their offspring, only the immunoglobuin of maternal type can be detected in the serum of a newborn rabbit. This maternal immunoglobulin is present in a relatively high concen-

† Immunoglobulins characterized by certain allotypic specificity are referred as "allotypic immunoglobulins".

tration as a consequence of transfer of γG-globulins during pregnancy(9). It seemed therefore possible that the presence of maternal immunoglobulin might inhibit or delay active immunoglobulin synthesis by the young rabbit through a *feed-back* mechanism. This was, however, not easily demonstrable, as the following experiment showed.

Heterozygous (A^4/A^5) rabbits born as a result of matings: ♂ A^5/A^5 × ♀ A^4/A^4 were divided into 2 groups. The first group of 7 rabbits was untreated; the second group of 2 animals was injected with normal rabbit serum obtained from homozygous A^4/A^4 animals. Each rabbit was given 11 intraperitoneal injections during the first 25 days of life, the total volume injected being 140 ml. Changes in concentration of immunoglobulins of maternal and paternal types in both groups were compared and it was concluded that the rates of synthesis of immunoglobulin of maternal type were virtually the same in both groups. These rates were also indistinguishable from the rate of synthesis of immunoglobulin of the paternal type in untreated animals, *i.e.*, with the rate which could not have been affected by *feed-back* inhibition. Furthermore the time at which a relatively stable concentration was reached was identical in all 3 situations.

Thus we had no evidence that the presence of maternal immunoglobulin had affected the synthesis of immunoglobulin of maternal type. Nevertheless, the presence of maternal immunoglobulin may have an effect on the onset of synthesis of immunoglobulin by the young rabbit, but this effect might have been obscured by the high concentration of circulating maternal immunoglobulin. To overcome this difficulty, another experimental system was adopted.

2. *Regulation of synthesis of immunoglobulin in animals whose allotypic phenotype has been changed by neonatal injection of anti-allotype serum.* It has been shown that immunization of female rabbits with allotypic specificity of their mating partners, brings about changes in allotypic phenotype of the progeny obtained from such matings. In rabbits born from such mothers, the synthesis of paternal type allotypic specificity was reduced to various degrees and for varying periods of time(10,11).

In our preliminary experiments we found that this type of suppression can be induced by injection of antiserum shortly after birth. Injection of 1 mg of antibody N resulted in suppression‡ of synthesis of the paternal allotype for a period of at least 80 days. Thereafter, the immunoglobulin of paternal type appeared in the serum, but in reduced concentration, and remained at this low level for more than one year.

Such animals, in which synthesis of immunoglobulin of the paternal type was reduced, were used to investigate the *feed-back* inhibition of immunoglobulin synthesis.

Rabbits born as a result of mating ♂ A^4/A^4 × ♀ A^5/A^5 were injected with anti-A4 serum. Each rabbit was given 2 intraperitoneal injections during the first 36 hours after birth (0.5 mg antibody N/per injection). Whereas the sera of untreated animals contained measurable levels of immunoglobulin of paternal type on the 14th day and had reached a fairly stable concentration on the 80th day, the sera of treated animals contained no detectable immunoglobulin of paternal type during the first 71 days. Immunoglobulin of paternal type was first demonstrable on the 78th day (Fig. 1). At the 93rd day of life, some of the pretreated rabbits (3/5), were injected intravenously with 10 ml of normal rabbit serum obtained from homozygous A^4/A^4 (paternal type) rabbits. The two remaining littermates served as controls and were left without further injections. The concentration of A4 in all the rabbits was then followed for more than 100 days.

The injection of A4 serum resulted in an immediate rise in A4 concentration, but after the animals eliminated the injected immunoglobulin, this concentration fell below that in control animals. The concentration of A4 in the animals injected with normal serum remained lower than that of the controls for 55 days.

‡ An immunoglobulin was regarded as "suppressed" when its presence could no longer be detected by a single diffusion test, *i.e.*, when its concentration was less than 0.0033 units (for definition of a unit, see Materials and Methods).

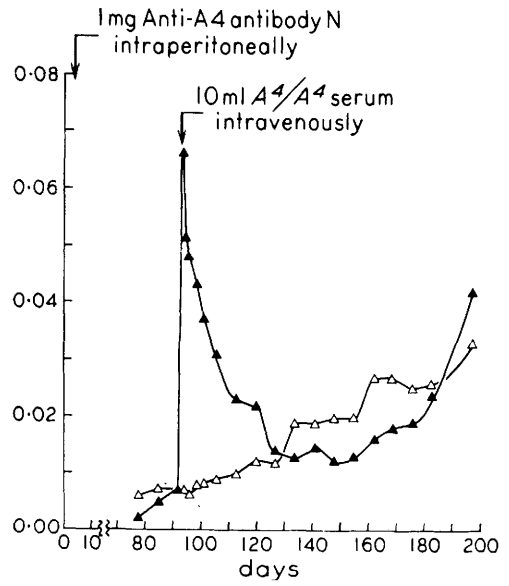

FIG. 1. Relative concentrations of A4 allotypic immunoglobulin (paternal type) in heterozygous A^4/A^5 offspring of a mating $\delta\ A^4/A^4 \times \varphi\ A^5/A^5$, All rabbits were injected at birth with anti-A4 serum (1 mg antibody N). Then, at the 93rd day of life one group was injected intravenously with 10 ml of normal rabbit serum from A^4/A^4 homozygotes. —▲—Average A4 concentrations in 3 rabbits injected with normal rabbit serum. —△— Average A4 concentrations in 2 control rabbits.

In a second experimental series heterozygous offspring from mating $\delta\ A^5/A^5 \times \varphi\ A^4/A^4$ were injected with anti-A5 serum. On the 85th day of life 3 of these rabbits were injected with 10 ml of A5 normal serum (obtained from A^5/A^5 homozygous rabbits). Three littermates of these rabbits were left untreated as control. In this experiment the *feed-back* effect, resulting from administration of allotypically defined immunoglobulin, was even more pronounced and lasted for about 150 days (Fig. 2).

Discussion. In our experiments a 10-fold increase in concentration of an immunoglobulin of a given allotypic specificity resulted in prolonged inhibition of synthesis of the immunoglobulin of this specificity. This could be explained as a result of *feed-back* inhibition. Whereas in earlier experiments(2,3) the apparent inhibition of antibody synthesis by administration of antibody might have been attributed to the shielding of antigenic determinants by the administered antibody, this possibility can be excluded in the interpretation of the foregoing results.

For *feed-back* inhibition to be effective, it has to be specific and the control has to depend on certain features of the antibody molecule. Some investigators have proposed that control depends on the specificity of the antibody combining site. Our results show that the control can be mediated through the allotypic specificity of immunoglobulin. Thus the quantity of immunoglobulin can be controlled through structural features (a) directly concerned with combination with antigen, and (b) through structures which may only indirectly be connected with the antibody combining site(12-18). Clearly the second type of inhibition may affect antibodies adapted to many different determinants, which means that this type of regulation is less specific than the one operating through antibody combining sites. However, it seems probable that the structural features recognized as allotypic specificities are not the

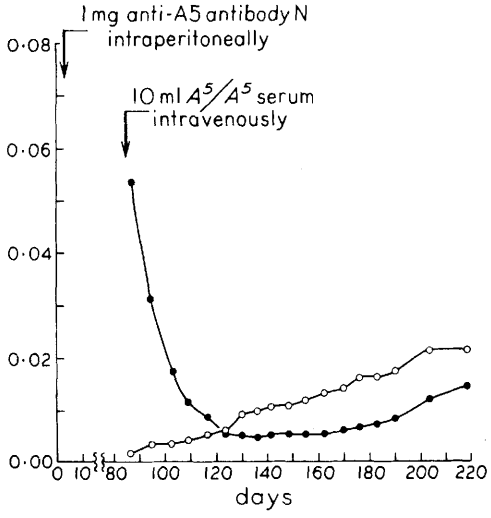

FIG. 2. Relative concentrations of A5 allotypic immunoglobulin (paternal type) in heterozygous A^4/A^5 offspring of a mating $\delta\ A^5/A^5 \times \varphi\ A^4/A^4$. All rabbits were injected at birth with anti-A5 serum (1 mg antibody N). Then, at the 85th day of life one group was injected with 10 ml of normal rabbit serum from A^5/A^5 homozygotes. —●— Average A5 concentrations in 3 rabbits injected with normal rabbit serum. —○—Average A5 concentrations in 3 control rabbits.

only ones through which *feed-back* control of immunoglobulin synthesis can operate. Therefore, under physiological conditions, the combined effect on regulation of antibody synthesis of all these structural features, might still be sufficiently specific without involving the antibody combining sites.

Summary. Regulation of immunoglobulin synthesis by *feed-back* inhibition was investigated so as to test whether the specificity of *feed-back* inhibition depends on recognition of allotypic specificities. This inhibition was observed in rabbits which were pretreated by neonatal injections with antiserum directed against their paternal allotypic specificity. As a result of this pretreatment, synthesis of immunoglobulin of paternal type was reduced (suppressed) for 3-4 months. After this time, some of the pretreated rabbits were injected with normal rabbit serum, containing immunoglobulin of the suppressed type. This resulted in an inhibition of the synthesis of this allotypic immunoglobulin lasting from 50 to 150 days. It was concluded that *feed-back* inhibition of immunoglobulin synthesis may operate through recognition of allotypic specificities and possibly through recognition of other structural features of immunoglobulins as well.

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Further Studies on the Incubation of Tyrosine-UL-C^{14} with Beef Thyroid Tissue Slices.* (31071)

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Phenylalanine is considered to be one of the essential amino acids, although tyrosine, its para-hydroxylated derivative, can substitute for it. The major site at which phenylalanine is converted to tyrosine is the liver. Subsequent utilization follows several pathways, one of which is believed to be the synthesis by the thyroid of thyroxine and its precursor analogs. The order of magnitude of

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