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A Homeostatic Mechanism for the Removal of Antigen from the Portal Circulation*

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Summary. Immunogenic molecules are being absorbed continually from the alimentary tract and a substantial proportion of normal people have detectable levels of circulating antibody against soluble food proteins and intestinal bacteria.

In our view antibodies to soluble proteins form part of a physiological mechanism for preventing these proteins from entering the systemic circulation. In the absence of antibody the liver failed to remove significant quantities of soluble protein antigen from the portal circulation, but in the animal with sufficient circulating antibody to combine with antigen in optimal proportions, the liver retained 80 per cent of the injected protein. The liver became progressively less efficient for the removal of injected complexes which had been prepared *in vitro* and contained increasing amounts of antigen in excess of optimal proportions.

The consequences of antigen excess, as they relate to inflammatory bowel disease, are discussed. The theoretical consequences of antibody excess are also discussed.

INTRODUCTION

There is substantial evidence that immunogenic molecules are absorbed from the alimentary tract of adult animals (Alpers and Isselbacher, 1967; Bernstein and Ovary, 1968; Chisiu, 1963; Cornell, Walker and Isselbacher, 1971; Straus, 1969). These molecules probably contribute one of the major antigenic challenges to the body from birth onwards.

The intestinally derived antigen enters both the mesenteric lymphatics and mesenteric capillaries (Warshaw, Walker, Cornell and Isselbacher, 1971). Antigen entering the portal circulation passes through the liver before entering the caval circulation and one of the liver's functions may be to remove this antigen and to eliminate it as a stimulus for antibody production. Indeed, in cirrhotic patients with impaired function of Kupffer cells (Thomas, McSween and White, 1973) the level of all immunoglobulins (Björneboe, 1971) and the level of agglutinins to intestinal bacteria (Björneboe, Prytz and Ørskov, 1972; Triger, Alp and Wright, 1972) are higher than normal.

Both normal people and patients with inflammatory bowel disease have detectable quantities of serum antibody to food antigens (Jewell and Truelove, 1972) and intestinal bacterial antigens (Sell and Fahey, 1964). Whether these antibodies are involved in the pathogenesis of the diseases is uncertain and the observation that normal people

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have significant quantities of these antibodies suggests that they are part of a physiological mechanism rather than a pathological one.

In this study, we have measured the ability of the liver to remove soluble and particulate antigen from the portal circulation; we have also studied the effect of immunization on this process. These observations led to further studies on the ability of the liver to remove from the portal circulation immune complexes of varying composition.

MATERIALS AND METHODS

Female Charles River rats aged 2–3 months were used in this investigation. They were fed on a diet free of bovine serum albumin (BSA) or cross-reacting antigens. The mesenteric vein was exposed by laparotomy under ether anaesthesia and antigen was injected into a tributary of this vein. Animals injected via the femoral vein were subjected to a sham abdominal procedure. In all experiments antigen was infused at a constant rate, using a peristaltic pump. Each infusion lasted 5 minutes. The animals were killed by exsanguination 1 minute after cessation of the infusion. The liver, spleen and kidneys were then excised, weighed and the antigen content measured as counts due to ^{125}I, ^{131}I or ^{51}Cr in a gamma-radiation counter (Nuclear Chicago). Autoradiography was used to demonstrate the cellular localization of the antigen.

The antigen used in the first series of experiments was BSA (Cohn Fraction V: Armour Pharmaceutical Co., Ltd). It was trace-labelled with carrier-free (^{125}I) or (^{131}I) iodide (Amersham, Bucks.) according to the method of MacConahey and Dixon (1966). The specific activities ranged from 80–100 μCi/mg of protein. [^{131}I]Iodide was used except when autoradiography was required. The labelled protein was separated from free iodide by passage through a Sephadex G-25 column.

A group of rats was immunized to BSA by intramuscular injection of 2 mg of BSA in Freund's complete adjuvant (1 mg/animal of heat-killed human strain C *Mycobacterium tuberculosis*). Four weeks after the first injection the animals were boosted with 1 mg of BSA intraperitoneally.

The antibody response was measured by the Farr technique (Farr, 1958) and the results expressed as the antigen-binding capacity at 30 per cent binding (ABC_{30}) using an antigen concentration of 1 μg/ml.

Immune complexes were prepared from ^{131}I-labelled BSA and rabbit IgG class antibody against BSA. Antigen was reacted with antibody in optimum proportions and with twice and five times this amount of antigen. The reaction mixtures were left at 4° overnight. These complexes were infused using the same procedure described for infusion of antigen. The animals were killed 1 minute after cessation of the infusion.

The above experiments were repeated using formalinized *Salmonella adelaide*. The organisms were grown in nutrient broth, treated with 0·3 per cent formaldehyde, washed and resuspended in saline. They were then labelled with [^{131}I]iodide by the technique of MacConahey and Dixon (1966). Before use the organisms were washed and then made into a suspension containing 1×10^8 microorganisms/ml.

Non-immune and immune animals were infused with labelled *Salmonella* under the same conditions used in the previous studies. The animals were immunized by injecting 1×10^9 micro-organisms intraperitoneally. Antibody activity was assessed by tube agglutination 3 weeks after immunization. The animals were then used for antigen infusion.

RESULTS

The first experiment was designed to investigate the fate of albumin in the portal circulation. BSA was labelled with [^{131}I]iodide and then passed through a Sephadex G-100 column. The fractions containing monomeric albumin were kept. Higher polymers which were contained in the exclusion volume were discarded. ^{131}I-labelled BSA (50 μg in 1 ml of phosphate-buffered saline) was infused at a constant rate over a period of 5 minutes, into either the mesenteric vein or the femoral vein. One minute after stopping the infusion only 4 per cent of the injected dose was retained in the liver after infusion by both mesenteric and femoral veins.

This experiment was repeated using rats immunized with BSA in Freund's complete adjuvant. The antibody response was measured using the Farr technique with an antigen concentration of 1 μg/ml; the antigen-binding capacity (ABC_{30}) of these sera ranged from 60–140 μg/ml. These animals retained up to 30 per cent of the injected dose of ^{131}I-labelled BSA in their livers. The group receiving ^{131}I-labelled BSA via the femoral vein showed a significantly lower hepatic uptake (24 per cent) than the group receiving antigen via the mesenteric vein (31 per cent). This difference was statistically significant ($P = 0{\cdot}02$). There was no significant difference in the level of immunization of these two groups.

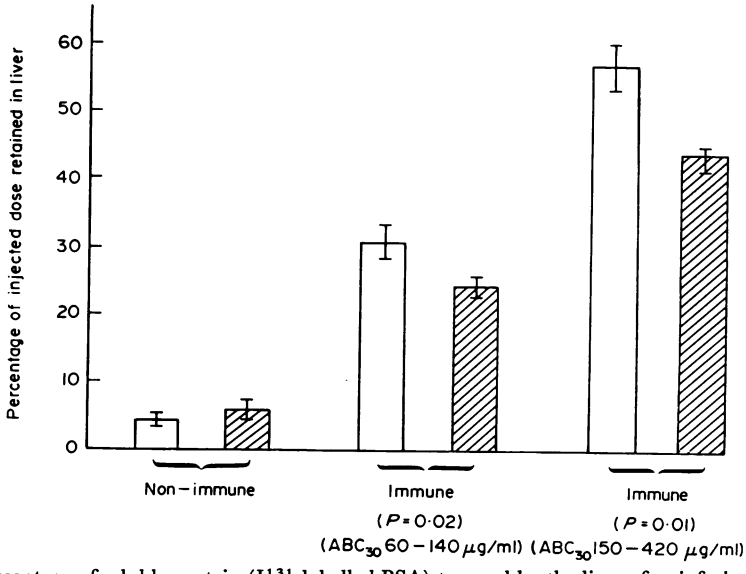

FIG. 1. Percentage of soluble protein (I^{131}-labelled BSA) trapped by the liver after infusion of 50 μg of ^{131}I-labelled BSA into either (□) the mesenteric vein (MV) or (▨) the femoral vein (FV) of unimmunized and immunized rats. The values shown are means $\pm$ 1 standard error. *P* values refer to comparison of antigen trapping after mesenteric vein infusion and after femoral vein infusion.

A further group of animals was immunized by the same technique and had serum antigen binding capacities ranging from 150–420 μg/ml. These animals were subjected to the same procedure as the previous group of immune animals. At this higher antibody level the liver trapped 56 per cent of the injected dose of antigen. This is significantly more than the group of animals with lower antigen binding capacities ($P = 0{\cdot}05$). These results are summarized in Fig. 1.

The difference in hepatic uptake of antigen between the group injected via the mesenteric vein and the group injected via the femoral vein, is a measure of the amount of antigen removed by the liver in the first passage from the portal vein to the systemic circulation. The difference in hepatic uptake between animals receiving BSA into the femoral as opposed to the mesenteric vein was only demonstrated in immune animals with circulating antibody, and was largest in the animals with the highest serum antigen binding capacities.

The site of formation of antigen–antibody complexes is presumably within the portal blood and we therefore explored the ability of the liver to remove complexes which were injected into the portal vein.

Complexes with varying antigen and antibody composition were infused into either the mesenteric or femoral veins in the same way as before. The results are illustrated in Fig. 2. Immune complexes were trapped more efficiently by the liver than soluble protein.

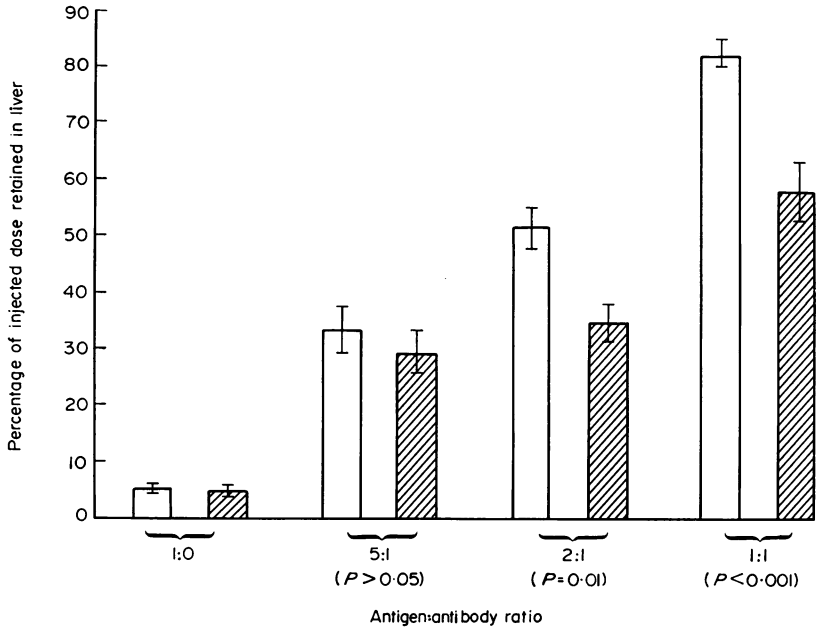

FIG. 2. Percentage of complexes trapped by the liver after infusion of 50 μg of ^{131}I-labelled BSA complexed with varying amounts of antibody into either (□) the mesenteric vein (MV) or (▨) the femoral vein (FV). A 1:1 Ag:Ab ratio represents optimal proportions.

The complexes with antigen–antibody composition near to equivalence were more efficiently phagocytosed by the liver than the complexes formed in antigen excess. When complexes were infused via the femoral vein the liver retained less of the total injected dose but once again the hepatic uptake of complexes with antigen and antibody in optimum proportions was greater than the uptake of complexes formed in antigen excess. The difference in hepatic uptake of immune complexes between the group injected via the mesentric vein and the group injected via the femoral vein increased as the composition of the complexes was changed from antigen excess to equivalence.

The localization of antigen and immune complexes to hepatic Kupffer cells was confirmed by autoradiography.

Splenic and renal uptake of complexes was also measured; these results are presented in Fig. 3. A smaller quantity of complexes reached the spleen and kidneys after injection into the mesenteric vein than after injection into the femoral vein. This was presumably due to the greater hepatic uptake of complexes when the injection was made into the mesenteric vein.

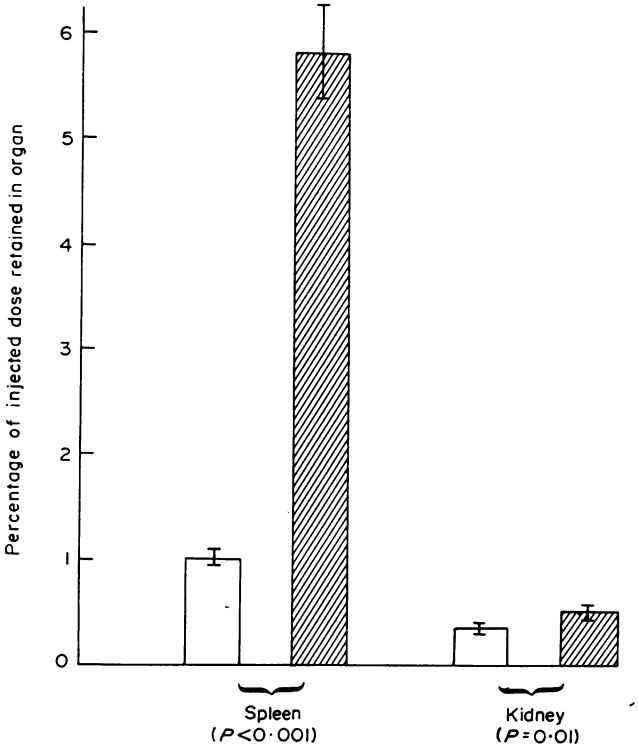

FIG. 3. Percentage of complexes (50 μg of ^{131}I-labelled BSA complexed with IgG anti-BSA in optimal proportions) trapped by spleen and kidney after infusion into either (□) mesenteric (MV) or (▩) femoral vein (FV).

In the next group of experiments we investigated the ability of the liver to remove particulate antigen from the portal circulation. *Salmonella adelaide*, labelled *in vitro* with ^{131}I, were infused into the mesenteric vein of unimmunized and immunized rats. Each animal received 1×10^8 organisms in 1 ml of saline. The results are illustrated in Fig. 4. In the non-immune animal the liver retained 20 per cent of the injected dose of bacteria but in the immune animal the percentage retained increased to 76 per cent. These experiments were repeated, but this time the infusion was given via the femoral vein. After femoral vein infusion in both non-immune and immune animals hepatic uptake was less than the uptake observed after mesenteric vein infusion. This demonstrates that even in the unimmunized animal, the liver removes significant numbers of organisms from the portal blood but that immunization improves the process. The splenic uptake of antigen was smaller after mesenteric vein injection than after femoral vein injection, but this difference reached statistically significant levels only in immune animals ($P = 0{\cdot}001$).

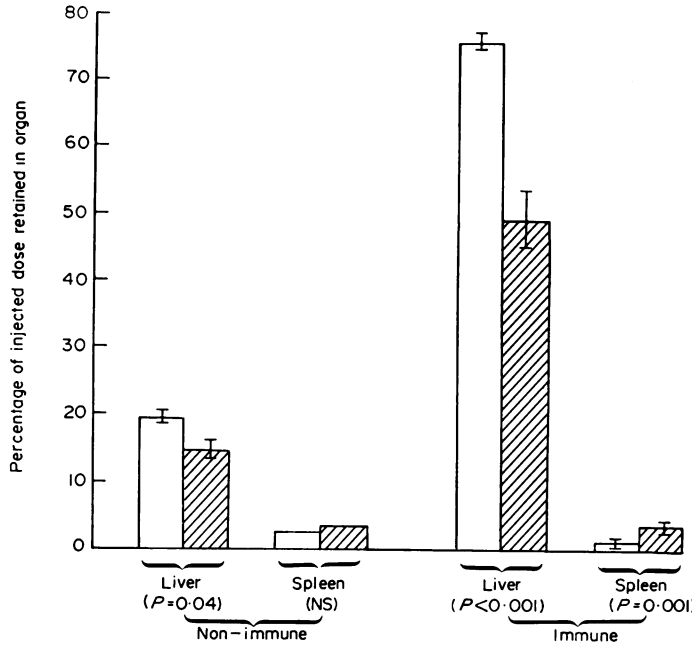

FIG. 4. Percentage of particulate antigen (^{131}I-formalinized *S. adelaide*) trapped by the liver and spleen after infusion of 1×10^8 labelled bacteria into either (□) the mesenteric vein (MV) or (▨) the femoral vein (FV).

DISCUSSION

In this study we have demonstrated that in the unimmunized animal significant quantities of particulate material are removed from the portal blood by the liver but virtually no soluble protein is removed. Immunization improved the ability of the liver to deal with both particulate and soluble antigens. At optimal levels of circulating antibody the percentage hepatic uptake of soluble antigen was equal to that of particulate antigen in the immunized animal.

It is known that after intravenous injection of a particulate antigen, 80 per cent of the antibody is produced in the spleen and this antibody enters the portal circulation via the splenic vein (Rowley, 1950a and b). Absorption of antigen from the gastro-intestinal tract is functionally equivalent though not quantitatively comparable to an intravenous injection of antigen and thus the antibody produced in response to intestinal antigens is produced predominantly in the spleen and is delivered in the highest possible concentration to the site of entry of the antigen. If the intestine is a major site of entry of soluble protein antigens, and the liver is unable to remove these in the absence of antibody, then the spleen is very well sited to supply antibody for the formation of immune complexes with this intestinally derived antigen. It is therefore likely that the antibodies to soluble food proteins found in normal people are part of a physiological mechanism aiding the phagocytic cells of the liver to remove foreign proteins before they enter the systemic circulation (Fig. 5).

Our experiments show that the removal of soluble protein antigen is most efficient when antigen and antibody mix in optimal proportions. Immune complexes formed in

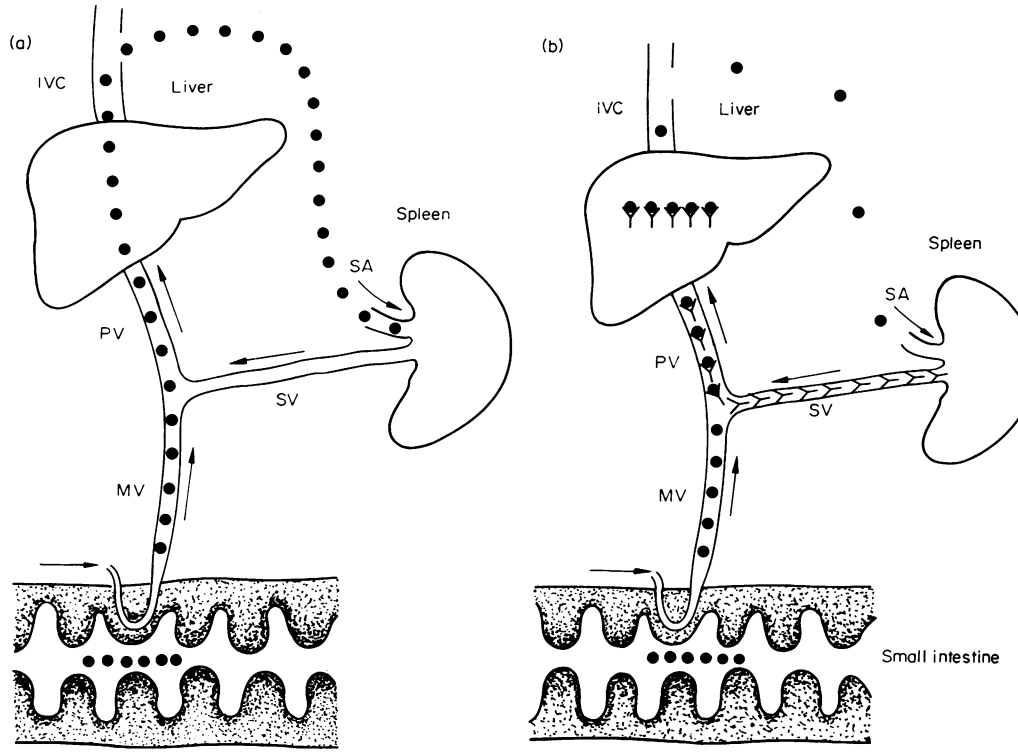

FIG. 5. (a) In the non-immune animal intestinally derived soluble protein antigens pass through the liver to stimulate antibody production by the spleen. (●) Intestinally derived antigen. (b) This splenic antibody enters the portal circulation, complexes are formed and these are removed by the liver, thus removing the antigenic stimulus and completing a negative feedback loop. (●) Intestinally derived antigen. (Y) Antibody. (SA = splenic artery; SV = splenic vein; MV = mesenteric vein; PV = portal vein; IVC = inferior vena cava).

antigen excess are less efficiently removed than complexes formed at equivalence; therefore, when insufficient antibody is produced to combine with intestinal antigen in optimal proportions, soluble complexes will be formed and some of these will pass through the liver to enter the systemic circulation. This situation occurs in inflammatory bowel diseases such as ulcerative colitis, coeliac disease and dermatitis herpetiformis. Antigen is absorbed in increased amounts through the damaged mucosa and the antibody titre is insufficient to combine with the antigen in optimum proportions. Soluble immune complexes are formed and although the liver may remove a substantial amount of these complexes, significant quantities reach the systemic circulation. This is substantiated by the observation that patients with inflammatory bowel disease do have increased levels of immune complexes when examined by the C1q precipitation technique (Doe, Booth and Brown, 1973; Mowbray, Hoffbrand, Holborow, Seah and Fry, 1973). These complexes may be the cause of lesions such as uveitis, arthritis and dermal vasculitis when they are seen in association with inflammatory bowel disease.

In the normal animal, the inflow of antigen from one day to another would be reasonably constant and antibody would be used in the formation of complexes as soon as it

was produced. One would not, therefore, expect to find high levels of antibody to either food protein or intestinal bacterial antigens while the antigen absorption was continuing. Jewell and Truelove (1972) have shown that these antibodies belong to IgG and IgA classes of immunoglobulin and the former may be expected to fix complement. It is therefore important that the titre of circulating antibody should remain low because of the danger of soluble protein reacting with complement-fixing antibody in the wall of the gut resulting in an Arthus reaction.

It is therefore evident that antigen or antibody excess in the above system may result in disease and only when antibody is produced in the right amount is the above system physiological.

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