No doubt, future experiments will elucidate the relationship of this phenomenon to the suppression of antibody formation in the primary response by means of passive administration of the specific homologous antibody^{5,6}.

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Sensitization of Mice with Human IgA

It is well known that an increased vascular permeability produced by various agents can be visualized by the leakage of intravenously administered dye through the affected vessels. The vascularity and high histamine and serotonin content of mouse ears¹ suggested that these organs might be useful in the visualization of *in vivo* antigen-antibody reactions. While investigating the ability of intravenously administered pollen extracts to induce blueing of the ears of mice², Kind *et al.* also found that mice sensitized with bovine serum albumin (BSA) developed blueing of the ears when later challenged intravenously with BSA and Evans blue dye. When blueing of the ears was produced in normal mice by the intravenous injection of BSA, rabbit anti-BSA and dye, no latent period was found to be necessary for the 'fixation' of antigen or antibody³. Indeed, in mice, maximal incidence of anaphylaxis with rabbit antibody can be obtained by injecting antibody and antigen intravenously in rapid succession⁴. The apparent absence of a requirement for tissue fixation in mice suggested that blueing of the ears in anaphylactic mice might be useful for detecting such proteins as normal human IgA or myeloma IgA, which will not sensitize guinea-pig skin for reverse passive cutaneous anaphylaxis probably because of the lack of fixation sites on such molecules⁵.

Swiss-Webster female mice weighing 17–20 g were inoculated intravenously with 0.05 ml. of various dilutions of normal human serum (NHS). One hour later the animals were again injected intravenously with 0.1 ml. of goat anti-human IgA (Hyland Laboratories, Los Angeles, California) and 0.2 ml. of 0.5 per cent Evans blue dye. The anti-IgA serum was found by immunoelectrophoresis to develop a single, strong precipitin line with whole human serum. The data in Table 1 indicate that as little as 0.05 ml. of a 1:20 dilution of NHS was able to sensitize mice. The concentration of IgA in normal human serum is 150 to 400 µg/ml. (ref. 6). Since no pure IgA was available and since an anti-serum to IgA, although absorbed to make it specific, might contain small amounts of antibodies to other serum components, it was necessary to demonstrate by additional experiments that the observed blueing of the ears was actually due to IgA-anti-IgA interaction. The data in Table 1 indicate that an IgA myeloma serum which contained about ten times more IgA than did NHS could sensitize mice for blueing of the ears at a dilution of 1:200 in contrast to NHS, which could not be diluted more than 1:20 and still retain its ability to sensitize. In gel-diffusion investigations this IgA myeloma serum produced a single precipitin band with anti-IgA and this band fused (reaction of identity) with the band formed by NHS and anti-IgA. The data in Table 2 indicate that cord

Table 1. SENSITIZATION OF MICE WITH A NORMAL HUMAN SERUM AND WITH AN IgA MYELOMA SERUM

Mice sensitized intravenously with: 0.05 ml. normal human serum (NHS)	Mice challenged intravenously 60 min later with: 0.1 ml. goat anti-IgA plus 0.2 ml. of 0.5 per cent Evans blue dye	No. of mice reacting (blue ears) Total No. challenged 10/16
NHS 1:10		7/11
NHS 1:20	"	2/6
NHS 1:50	"	0/5
Saline	"	1/20
0.05 ml. IgA myeloma serum (diluted 1:200)	"	8/14
0.05 ml. IgA myeloma serum (diluted 1:500)	"	0/5
0.5 ml. IgA myeloma serum (diluted 1:200)	Dye plus saline	0/5
0.05 ml. NHS	"	0/5

Table 2. SENSITIZATION OF MICE WITH CORD AND MATERNAL SERA

Mice sensitized with:	No. of mice reacting (BE)* No. of mice tested	IgA present in serum (by immunoelectrophoresis)
maternal serum†	4/8	+
cord serum	0/6	—
maternal serum	2/3	+
cord serum	0/5	—
maternal serum	4/5	+
cord serum	1/5	—
maternal serum	3/4	+
cord serum	1/5	—

* Mice injected with 0.05 ml. of cord or maternal serum were challenged 60 min later with 0.1 ml. of goat anti-IgA and 0.2 ml. of 0.5 per cent Evans blue dye. Control mice injected with antiserum plus dye or antigen plus dye produced no blueing of the ears (BE).

† Four different pairs of cord and maternal sera were tested.

sera which contain no IgA detectable by immunoelectrophoresis (as previously demonstrated by Allansmith *et al.*⁷) rarely sensitized mice for blueing of the ears with goat anti-IgA, whereas maternal sera shown to contain IgA by immunoelectrophoresis were effective sensitizers. Mice failing to give blueing of the ears after injection of cord serum, goat anti-IgA, and dye were later challenged intraperitoneally with goat anti-IgG. Blueing of the ears occurred in all animals tested³. Although no pure IgA was available for these investigations, the results obtained with an IgA myeloma serum (greatly increased sensitization) and with cord sera (greatly reduced sensitization) strongly suggest that human IgA can sensitize mice. Since it has been shown that human IgM, like human IgA, will not sensitize guinea-pig tissues⁸, the method described here may also be useful for quick detection of human IgM proteins or their fragments.

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Electrophoretic Behaviour of H and L Chains of Human Serum and Colostrum Gamma-globulin

DISSOCIATION of gamma-globulin molecules by reduction in urea with such thiol reagents as mercapto-ethanol and thioglycolate^{1,2}, or in neutral aqueous solution³, or by S-sulphonation^{4,5}, allows the identification of two types of polypeptide chain, called H (A) and L (B), which may be separated by gel filtration.