the isolated cell, is consistent with the observation (*11*) that brown adipose tissue incubated in vitro accumulates free fatty acids within the tissue fragment rather than in the incubation medium because of dense interstitial barriers to the diffusion and egress of fatty acids. Since accumulation of free fatty acid in intact tissue reduces glucose utilization (*12*) and since free fatty acids are causally implicated in the uncoupled oxidative metabolism frequently observed in slices and homogenates of brown adipose tissue (*13*), it is possible that of all the available preparations in vitro, the isolated cell system may best reflect the physiological properties of the parent tissue.

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Antigen-Associated Immunosuppressant: Effect of Serum on Immune Response

Abstract. *Serum from various animal species, including the test animals themselves, inhibits the antibody response of the rabbit to two bacterial antigens, provided that antigen and immunosuppressant interact prior to injection. The degree of immunosuppression is related to the length of incubation in vitro of antigen and serum. Serum does not hinder or destroy the antigenic determinant. Bacterial antibodies do not account for inhibition of the antibody response. Antigen-associated serum components, as yet unidentified, may affect the early events of the immune response.*

The immune response can be inhibited by two fundamentally different mechanisms, the one being specific and the other nonspecific. Specific interference can be accomplished by injection of antigen in suitable physical form and in adequate amounts, leading to immunologic unresponsiveness or tolerance, and by antibodies, particularly of the 7*S* variety, that interfere with antibody production. Nonspecific immunosuppression is effected by damage to the immunologic apparatus, which results from such procedures as thymectomy, x-irradiation, and administration of corticoids and antimetabolites. We now report on a novel type of immunosuppression in which an antigen-associated immunosuppressant, namely, normal serum, interferes with antibody production.

Our experiments followed investigations directed toward elucidation of the lack of immunogenicity of the common antigen (CA) of Enterobacteriaceae (with the exception of *Escherichia coli* O14), first described by Kunin *et al.* (*1*). The endotoxin (lipopolysaccharide) present in the bacteria and supernatants of cultures together with CA inhibits the antibody response to the latter (*2*). Presumably, CA and lipopolysaccharide form a complex that can be separated by ethanol, CA being soluble and lipopolysaccharide insoluble (*3*). Isolated CA proved highly immunogenic. Because various bacterial antigens readily form complexes with certain serum proteins and other substances as well (*4*), the effect of normal serum on immunogenicity of bacterial antigens was studied.

Groups of three albino rabbits, weighing between 2 and 3 kg, were immunized by three intravenous injections, 3 to 4 days apart, of CA from *Salmonella typhimurium* in aqueous solution (*3*) mixed with either normal rabbit serum or phosphate buffer for control purposes. One group was given the mixture of antigen and serum after the mixture was incubated in vitro for 30 minutes at 37°C, and the other group received the mixture prepared immediately prior to injection. The antibody response to CA was measured by means of hemagglutination with CA from *E. coli* O14 as indicator antigen, as described (*5*).

Serum almost completely prevented the antibody response, provided that antigen and serum were incubated prior to injection (Fig. 1). The effect of time for interaction of antigen and serum is evident from the results of an experiment in which the mixtures were incubated for 5, 15, or 30 minutes prior to injection, resulting in mean antibody titers of 1 : 480, 1 : 67, and <1 : 10, respectively. Additional experiments revealed that marked immunosuppressive effects take place when the serum was diluted 1 : 10, but not when used in a dilution of 1 : 100. Even autologous serum, obtained from the test animals before immunization, proved to be immunosuppressive. Inhibition of the antibody response was observed also with rabbit plasma, human serum obtained from healthy subjects, human cord serum, guinea pig serum (*6*), and calf serum (*7*, *8*). Fetal calf serum exerted immunosuppressive effects, although to a lesser extent than calf serum.

The possibility was considered that antibody, possibly by means of a feed-

Table 1. Effect of rabbit serum on antibody response to staphylococcal antigen. Rabbits were injected intravenously on days 0, 3, and 7 with 1 ml of mixtures of staphylococcal antigen and buffer or rabbit serum in various dilutions. Mixtures consisted of one part of culture supernatant and nine parts of serum or buffer. Antibody titers were measured by hemagglutination, with antigen from *Bacillus subtilis* as indicator.

Materials for immunization	Mean hemagglutinin titers (reciprocal) at day				
	0	7	10	14	21
Antigen + buffer	<10	907	3627	3520	3467
Antigen + 1:1 serum	<10	13	13	13	10
Antigen + 1:10 serum	<10	120	400	960	320
Antigen + 1:100 serum	<10	120	1920	3200	800

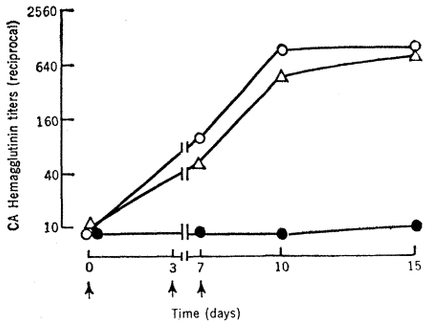

Fig. 1. The effect of normal rabbit serum on hemagglutinin response to common enterobacterial antigen. Groups of rabbits were injected intravenously on days 0, 3, and 7 with 1 ml of ethanol-soluble CA from *Salmonella typhimurium* in aqueous solution (1 part) mixed with either rabbit serum (1:10) or buffer (9 parts). Group 1 (●——●) received the mixture of antigen and serum incubated at 37°C for 30 minutes before injection; group 2 (△——△) received the same mixture without prior incubation; group 3 (○——○), serving as control, received antigen alone.

back mechanism, may be responsible for inhibition of the immune response. The following observations suggest that this is not the case. Calf serum obtained before and after colostrum feeding (*8*) were equally effective as immunosuppressants. Calf serum devoid of γ-globulin (*7*) inhibited the antibody response as well as whole calf serum did. Removal of the bacterial antibodies by absorption of normal rabbit serum with erythrocytes modified by antigen did not abolish its immunosuppressive effect. Finally, antiserum against CA obtained from rabbits, after dilution to antibody concentrations present in normal serum, did not inhibit the antibody response under identical conditions. Thus, serum of several animal species has immunosuppressive properties provided that the common enterobacterial antigen and serum interact first in vitro prior to immunization of rabbits.

Immunosuppression by normal serum was supported by experiments with another antigen shared by gram-positive bacteria and first described by Rantz *et al.* (*9*). The methods of antigen preparation and hemagglutinin titration have been described (*10*). The results of a representative experiment with this antigen (in a dilution of 1 : 10) obtained from *Staphylococcus pyogenes* and normal rabbit serum, incubated before immunization for 30 minutes at 37°C, are summarized in Table 1. Undiluted serum almost completely prevented the antibody response, and dilution of 1 : 10 was moderately effective. We conclude, then, that normal serum associated with either of two bacterial antigens inhibits their immunogenicity.

Studies directed toward elucidation of the mode of action of normal serum as immunosuppressant indicate that the serum does not hinder, bind, or destroy the antigenic determinant. This conclusion is based on the observation that antigen, either in the presence or absence of normal serum used in identical proportions as employed for immunization, neutralizes antibodies equally well. In these experiments, the materials were mixed with the appropriate antiserum, and the mixtures were tested for antibody by means of the passive hemolysis test (*5*). Nor do antibodies account for the immunosuppressive effects of normal serum. The possibility may be considered that serum alters enzymatically the antigen carrier. This is unlikely in view of the fact that other substances, such as lipopolysaccharide, lipid A, and cardiolipin (*2*, *10*, *11*) inhibit the immune response under identical conditions. Rather, it is postulated that antigen and inhibitor interact and form a complex and that the antigen-associated immunosuppressant affects early events of the immune response, such as antigen uptake or processing. Experiments are needed to determine the identity of the immunosuppressant component of normal serum. Immunosuppression by serum, and particularly by alpha proteins, has been reported (*12*), although some of the results could not be fully confirmed (*13*). At any rate, in these studies immunosuppression did not depend upon the injection of antigen-inhibitor mixtures. Our experiments then, extend previous observations of immunosuppression by antigen-associated inhibitors, to include normal serum, and suggest the possibility of other substances being immunosuppressants that are ineffective or less effective when given independently of the antigen.

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Sclerospora graminicola
Axenic Culture

Abstract. Sclerospora graminicola *(Sacc.) Schroet., the obligate pathogen causing downy mildew (green-ear) of pearl millet (*Pennisetum typhoides *Stapf. and Hubb.) has been successfully cultured for the first time on a known semisynthetic medium with no evident loss of fructifications.* Sclerospora graminicola *was first grown on host callus tissue and subsequently on a modification of White's basal medium that contained casein hydrolyzate (Oxoid), 2,4-dichlorophenoxyacetic acid, and kinetin.*

Plant pathologists have attempted without much success to culture viruses, downy mildews, powdery mildews, and rusts on nutrient media (*1*). Morel cultivated *Plasmopara viticola* (Sew.) Burr. on grape stem callus tissues in culture (*2*). Several other obligate parasites have been grown recently on tissue cultures of their respective hosts (*3*). We have grown *Sclerospora graminicola* (Sacc.) Schroet. on the callus tissues of pearl millet (*Pennisetum typhoides* Stapf. and Hubb.) and have maintained it for 4 years (*4*).

Axenic culture of *Gymnosporangium juniperi-virginianae* Schw. on a relatively simple medium containing mineral salts, a carbon source, ascorbic acid, and yeast extract represented a major