glucan-induced RE organ hypertrophy is of a reversible nature. Indeed, RE organ hypertrophy induced by zymosan and *Myocobacterium phlei* Halpern, both excellent RE-stimulatory agents, has been shown to be reversible (*11*).

The exact role of the RES in antibody formation has not yet been clearly defined. It has been suggested that RE stimulation enhanced the period of antigen recognition (*12*) and presumably hastens antibody formation. However, studies have indicated that the induction period of antibody formation is not reduced by RE stimulation (*2, 3*).

Stimulation of the RES markedly increases the phagocytosis of inert colloidal material and enhances the intracellular metabolism of I^{131}-labeled denatured serum albumin (*13*). Since particulate antigenic material is known to be phagocytized, it is likely that RE stimulation increases the intravascular clearance of the antigen and the subsequent intracellular digestion of the phagocytized antigen.

It is well established that the spleen is a major site of antibody formation. The marked hypertrophy of the spleen induced by RE stimulation is the result of proliferation of RE cellular elements which are derived from undifferentiated mesoderm (*14*). Among these newly formed reticular cells may be a number of cells which can mature into plasmocytic cells or cells with antibody-forming potential, and under these conditions more cells are available to react with the antigen (*15*). Possibly these newly formed, relatively undifferentiated cells can develop either as cells with phagocytic or antibody-forming capacity or both and only in the presence of antigen do these cells develop as plasmocytic cells. Thus RE stimulation increases antibody formation by enhancing the solubility of the antigen and by increasing the population of potential antibody-forming cells.

The degree of depression of the phagocytic activity of the RES induced by methyl palmitate is comparable to that reported for other simple lipid complexes (*6*). However, such depression, induced by ethyl stearate, was associated with destruction of hepatic and splenic RE cellular elements (*16*). Histological observations of our mice treated with methyl palmitate have indicated a selective depression of phagocytosis by a resulting impairment in the phagocytic activity of hepatic RE cells with no alteration in the uptake of colloidal carbon by splenic or pulmonary RE cellular elements (*7*). No losses of hepatic and splenic RE cellular elements were observed in mice treated with methyl palmitate.

The liver, which is not directly involved in the formation of antibody, participates in the phagocytosis and subsequent intracellular digestion of particulate antigenic material (*17*). The solubilized antigen is then made available to the antibody-forming cells (*18*). If the process of phagocytosis and intracellular solubilization is reduced by methyl palmitate, little or no solubilized antigen is available to react with antibody-producing cells and the hemolysin titers would be correspondingly reduced.

Although the underlying mechanisms of the effects of glucan and methyl palmitate are as yet unknown, there is a unique relation between the functional state of the RES and the immune response to a particulate antigen. A decisive factor in the early acceptance or rejection of marrow grafts in lethally irradiated mice is the functional state of the RES of the host (*19*). Moreover, from the results reported here this rejection of allogenic and xenogenic bone marrow in RE hyperfunctional mice could be due to hyperphagocytosis, a hyperimmune response, or a combination of both factors.

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Gamma-Globulins: Quantitative Relationships in Human Serum and Nonvascular Fluids

Abstract. *Three types of γ-globulins, γ_2, γ_{1A}, and γ_{1M}, are present in certain body fluids and secretions in proportions significantly different from those of normal human serum. Although γ_{1A}-globulin is present in only small amounts in serum, it represents a major fraction of the gamma globulin of tears, bile, saliva, colostrum, and fluid of the small intestine.*

Three types of gamma globulin, designated γ_2, γ_{1A}, and γ_{1M}, are present in normal human serum (*1*) in the approximate proportions of 85:10:5. While the majority of serum antibodies are γ_2 and γ_{1M}, certain antibodies have been identified as γ_{1A}-globulins (*2*). Studies by immunoelectrophoretic and gel-diffusion techniques with antiserums to gamma globulin and to whole human serums have shown that gamma globulin is present in many fluids and secretions (colostrum, aqueous humor, bile, parotid, synovial, bronchial, nasal, amniotic, and cerebrospinal fluids). While γ_2 and γ_{1M} were demonstrated in many of these fluids, γ_{1A} has not been clearly distinguished from the other globulins and the relative amounts of the various globulins present have not been measured quantitatively.

Recently, Tomasi and Zigelbaum (*3*) noted that γ_{1A} was the predominant gamma globulin of parotid saliva. They postulated a specific and highly selective transport mechanism in the passage of γ_{1A} from serum to secretions or, alternately, the local synthesis of γ_{1A}.

Fluids and secretions free from contamination with blood were collected from a variety of noninfected sources. The types of gamma globulin present were measured by a two-dimensional gel-diffusion technique (*3*). The test fluids diffuse from a well into an agar gel containing an antiserum specific for the component to be measured. The concentration of gamma globulin is

proportional to the diameter of the ring of immune precipitate formed, and is estimated by reference to a standard curve relating ring diameters to known concentrations of the purified globulin. The antiserums were prepared by immunization of rabbits with purified antigens isolated from normal human serum. The antiserums were made specific by absorption with the slow fragment of a papain digest of fraction II gamma globulin. The preparation of the purified antigens for standardization and of the antiserums has been previously described (*3*). The values obtained for normal serum per 100 ml (γ_2, 1335 mg S.D. 267; γ_{1A}, 178 mg S.D. 60; and γ_{1M}, 103 mg S.D. 22) are slightly higher than those reported by Heremans (*4*).

The results of the quantitative analysis of the gamma globulins in various fluids are shown in Table 1. Three types of fluid were found. In one, represented by parotid saliva, colostrum, and lacrimal secretions, there is little or no γ_2-globulin and the gamma globulin is predominantly γ_{1A}. In a second type represented by small intestinal fluid and bile, γ_2-globulin is the predominant gamma globulin, but relatively large amounts of γ_{1A} are present and the ratio γ_2/γ_{1A} is significantly lower than that of serum. Similar results have been found with human bronchial secretions (*5*). In a third type, represented by vaginal and prostatic secretions, the ratio of γ_2/γ_{1A} did not differ significantly from that of serum. However, both of these fluids showed variation in the proportions of the gamma globulins. Moreover, as a result of very low protein content in several vaginal fluids, neither γ_2 nor γ_{1A}-globulin was detected, thus precluding determination of a mean γ_2/γ_{1A} ratio. Although studies on more samples are necessary, the results suggest that amniotic fluid represents a fourth type of fluid with a high γ_2/γ_{1A} ratio compared with serum.

Table 1. Gamma globulins and mean γ_2/γ_{1A} ratios in normal human serum and various fluids. The number of samples for each fluid is given in parentheses.

Mean of globulin fraction (mg/100 ml)		Mean γ_2/γ_{1A}	P*
γ_2	γ_{1A}		
	Serum (14)		
1335	178	8	
	Parotid fluid (12)		
0†	28	> 1	
	Colostrum (5)		
0†	151	> 1	
	Lacrinal fluid (8)		
0†	7	> 1	
	Intestinal (small) fluid‡ (6)		
153	74	3.8	.01
	Bile ‡ (8)		
143	53	2.6	.025
	Prostatic fluid (17)		
157	26	10	.5
	Vaginal fluid (16)		
37	6.3		.7
	Amniotic fluid (4)		
21	1.6	15	
	Cerebrospinal (4)		
7.9	1.8	5	

* P reported for comparison of γ_2/γ_{1A} ratios of serum and fluids by t-test (intestinal fluid) and analysis of median test with the chi-square method (bile, prostatic, and vaginal fluids). † The γ_2-globulin was detected in some of these fluids by the double diffusion precipitin test (Ouchterlony) but the amounts were too small for quantitative measurement. ‡ Values for the concentration of γ_2- and γ_{1A}-globulins of these fluids are on concentrated samples.

Four cerebrospinal fluids from patients with cerebrovascular disease contained slightly higher ratios (mean γ_2/γ_{1A} ratio 5/1) than normal serum. The γ_2/γ_{1A} ratio of cerebrospinal fluids obtained from two patients with disseminated sclerosis was 176/1 indicating that the increase in total cerebrospinal fluid gamma globulin in these two cases is almost entirely the result of an increase in γ_2-globulin.

The γ_{1M}-globulin was detected in trace amounts in many fluids but was present in measurable quantities only in colostrum. This indicates that the relative γ_{1M} concentration of the fluids (excepting colostrum) is not greater, and probably lower, than that of serum.

The data suggest that the gamma globulins of these fluids are not derived from serum by simple transudation. This is indicated not only by the relatively high γ_{1A} content of these fluids, but by the low to absent γ_2-globulin, particularly in proportion to its high concentration in serum. However, changes in the original gamma globulin content of the fluids may have resulted from a differential susceptibility of the various types of gamma globulin to the proteolytic enzymes in these fluids. After the incubation of γ_2-globulin with concentrated parotid saliva no evidence of proteolysis could be detected. Further work is necessary, however, to exclude this possibility in other secretions such as bile and intestinal fluid.

The observation of Brambell *et al.* (*6*) that fraction III of papain-digested rabbit gamma globulin passes the placental barrier much more readily than fractions I and II suggests the presence of an "active transport site" on the protein molecule and supports the concept that gamma globulins are selectively transported from serum to certain secretions. The occurrence of a higher specific antibody titer in colostrum, feces, and vaginal mucous than in serum (*7*) also suggests that active transport is involved in the secretion of gamma globulins into these fluids.

During acute infections at mucous surfaces the antibodies in the fluid bathing the infected area are derived largely from lymph nodes in the immediate vicinity (*7*). The gamma globulins in these fluids may therefore reflect gamma globulin production of adjacent lymph nodes rather than serum gamma globulins.

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Color Polymorphism in Pacific Tree Frogs

Abstract. *Crosses between green and non-green* Hyla regilla *suggest that green color is determined by genes at two loci; each loci must have a dominant gene. Red is the result of a recessive gene and brown a dominant gene. The frequency of frogs with each color varies in different populations.*

The background colors of *Hyla regilla* may be classified into four main groups: red, green, grey, and brown. An individual frog may have one or more of these colors. When more than one color is present, the colors are discrete. The animal may get very pale or very dark; the colors maintain their integrity. The intensity of the color varies seasonally and, to some extent, with age. The frequencies of the colors vary geographically and from year to year at a single locality. In the experi-