

Phagocytic and Opsonic Activities of Germfree Rats.* (32166)

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The phagocytic activity of the reticuloendothelial system (RES) is influenced by such factors as the nature(1), size(2), number (1,3), and surface charge(4) of the particles to be phagocytized. In addition, components exist in the plasma or serum which can markedly enhance the cellular uptake of particulate material(5-9). These plasma components originally called "opsonins" by Wright and Douglas(10) have been extensively shown to play a determinant role in phagocytosis (5-14).

Although the importance of opsonins in phagocytic activity has been amply demonstrated, little is known about the exact nature of the opsonic system. The possibility that opsonin may represent either natural antibody or some non-specific serum factor(s) has been entertained(15).

Previous studies from this laboratory have demonstrated that *in vitro* hepatic phagocytosis of a variety of particulate preparations can be markedly augmented by a serum or plasma opsonic system(8,9,16,17).

In an attempt to extend these observations and to determine whether opsonins or "recognition factors"(18) are in essence "natural antibodies"(18), an evaluation of the plasma opsonic and the hepatic phagocytic activity in the germfree rat was conducted.

Methods. Male germfree and "conventional" control rats were obtained from Manor Farms (Staatsburg, N. Y.) and maintained in their germfree isolators prior to experimental evaluation. Hepatic phagocytosis and opsonic activity were evaluated with the use of an *in vitro* liver slice technique(8), which consisted of the incubation of rat liver slices in 3 ml of heparinized incubation medium. Heparinized (100 USP units/ml) Krebs-Ringer phosphate adjusted to a pH of 7.4

or heparinized plasma obtained from either normal, *i.e.*, "conventional controls," or germfree rats was employed as the incubation media. Gelatin stabilized radioactive colloidal gold Au¹⁹⁸ (Abbott Laboratories, North Chicago, Ill.) was used as the test colloid in a dose of 400 µg per incubation flask.

Rats employed as liver donors were lightly anesthetized with ether and immediately de-sanguinated prior to liver extirpation. The livers were chilled in isotonic saline at 4°C and subsequently sliced with a Stadie-Riggs tissue slicer as previously described(8). Following addition of both the liver slice and colloidal gold to the incubation medium, all samples were gassed with 95% O₂ and 5% CO₂ prior to their 30-minute incubation in a Dubnoff metabolic shaker at 37°C.

Reticuloendothelial phagocytic function of control and germfree rats was evaluated by an analysis of the rate of *in vivo* vascular clearance of gelatin stabilized radio-iodinated "RE test lipid emulsion." The inability to give *in vivo* a "critical" test dose of colloidal gold to properly evaluate reticuloendothelial function necessitated the employment of another test particle such as this lipid emulsion whose clearance is an accurate index of RES activity. The emulsion was administered intravenously in a dose of 25 mg triglyceride/100 g body weight. The emulsion base consisted of glycerol, I¹³¹-triolein in peanut oil and alcohol soluble soya lecithin in a ratio of 10:10:1 by weight, respectively (19,20). Prior to its use, a 0.3% gelatin supplemented sterile dextrose (5%) and water solution was added to obtain a lipid emulsion with a final triglyceride concentration of 5%.

Radioactivity of both the colloidal gold (Au¹⁹⁸) and I¹³¹-triolein labeled "RE test lipid emulsion" was determined with a Nuclear-Chicago crystal scintillation system.

In vitro data are expressed as either the percent of injected dose phagocytized per 100 mg of wet weight of liver, or as the

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TABLE I. Intravascular Phagocytic Clearance of Gelatinized I¹²⁵ Labeled "RE Test Lipid Emulsion"* in Germfree and Conventional Rats.

Group evaluated	No. of animals	(t/2) Half-time in min
"Conventional" controls	5	33.0 ± 4.2
Germfree	5	33.2 ± 3.9

* "RE test lipid emulsion" was administered in a dose of 25 mg of triglyceride/100 g body wt. Half-time values are expressed as mean ± standard error of the mean in min.

μg of colloidal gold phagocytized per 100 mg of wet weight of liver. *In vivo* phagocytic activity is expressed as the half-time (t/2) in minutes determined for each experimental group by the analysis of a semi-logarithmic plot of the blood radioactivity as a function of time.

Results and discussion. Intravascular phagocytic clearance rates (t/2) of the gelatinized "RE test lipid emulsion" were similar for both the germfree and "conventional" control rats (Table I). These findings are in basic agreement with those made by Doll (21), as well as Thorbecke and Benacerraf (22), who have shown that germfree and "conventional" control mice was minimal, being 0.77 and 0.61% of the added radioactivity per 100 mg, respectively, when Krebs-Ringer phosphate buffer was employed as the incubation medium (Table II).

In marked contrast to these observations, the incubation of liver slices derived from either germfree or conventional rats in normal plasma resulted in an approximate nine-fold enhancement in uptake of colloidal gold.

In essential agreement with the *in vivo* observations, no differences were observed in the ability of germfree rat liver tissue to phagocytize colloidal gold when incubated in the presence of germfree plasma as the uptake was comparable to control values.

The results indicate that the opsonic system which has been shown to enhance *in vitro* Kupffer cell phagocytosis of colloidal gold is functionally developed in the germfree animal. These observations essentially confirm and extend previous observations(23), concerning the existence of opsonins for goat erythrocytes in the circulation of germfree chicks. Furthermore, hepatic tissue obtained from both the germfree and "conventional" control rats manifested similar phagocytic capacities, in that both tissue preparations manifested a comparable increase in phagocytosis in the presence of plasma. In this regard, Bauer *et al*(24) have shown that lymph node and splenic tissue obtained from germfree and normal mice display similar macrophage structure and development. The results support the concept that the opsonic system may be similar to "natural antibody" (18) since it can be amply demonstrated to exist in the germfree animal who would have a minimal exposure to antigenic stimulation.

Summary. Phagocytic activity was comparable in germfree and "conventional" control rats as evaluated by the intravascular clearance of a gelatinized radioiodinated "RE test lipid emulsion." Both germfree and conventional plasma manifested similar opsonic activity as evaluated by their ability to enhance Kupffer cell phagocytosis of colloidal

TABLE II. *In vitro* Kupffer Cell Phagocytosis of Colloidal Gold in Krebs-Ringer Phosphate, "Conventional" Control Plasma, and Germfree Plasma.

Source of liver slice*	Incubation medium	No. of incubation samples	Phagocytic uptake	
			% ID†/100 mg	μg gold/100 mg
Germfree	Krebs-Ringer phosphate	8	5.89 ± 1.02	23.6
Control	Krebs-Ringer phosphate	9	.61 ± .10	2.4
	Control plasma	8	5.89 ± 1.02	23.6
	Germfree plasma	9	7.42 ± .78	29.7
Germfree	Krebs-Ringer phosphate	9	.77 ± .16	3.1
	Control plasma	8	6.70 ± 1.19	26.8
	Germfree plasma	8	9.82 ± .88	39.3

* Liver tissue was obtained from 3 rats in each group evaluated.

† % ID is percent of radioactivity recovered per 100 mg of wet wt of liver. Values are expressed as means ± standard error.

gold *in vitro*. Opsonin is suspected to be a "natural antibody" in view of the phagocytic stimulatory activity of plasma from the germ-free animals.

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Replication of Rubella Virus in a Continuous Line of African Green Monkey Kidney Cells (Vero).* (32167)

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Rubella virus has been cultivated in a variety of continuous mammalian cell cultures which develop cytopathic changes (CPE) as a consequence of virus infection(1-8). Such virus host cell systems have been tested in many laboratories engaged in the study of rubella virus. Thus far no single tissue culture system has been applied with uniform ease and advantage from one laboratory to another. Among the factors which probably contribute to this variability are: 1) CPE may be subtle and slow in developing, thus making its recognition and differentiation

from non-specific changes difficult for the observer having little or no experience with a particular system. 2) the appearance and development of CPE is dependent on the composition of the nutrient medium used; for example, the rabbit kidney line LLC-RK-1 cells will develop CPE with certain lots of Medium 199 and not with others(9). Exactly how the lots differ from one another is not known. Leerhøy(10) has clearly shown the variations in morphology of uninfected SIRC cell cultures as well as the variation in appearance of CPE with different nutrient media.

The overall usefulness of the host cell systems in which CPE develops is further

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