additional work, particularly in delimiting the areas in which brain manipulations evoke C_1 lay, is needed before these notions can be accepted.

Summary. Piqure of the hen's brain evoked premature lay of the C_1 and C_t eggs of a 2-egg sequence during restricted periods which stood in the same relationship to each other as the periods of normal lay for the respective eggs. C_t lay was advanced by piqure of any of several brain sites, but C_1 lay was advanced only by piqure of the preoptic brain. The frequency of premature lay in relation to time of preoptic piqure differed markedly for the two eggs.

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Comparative Evaluation of the Influence of Opsonins on Hepatic, Splenic and Pulmonary Phagocytosis.* (32165)

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The ability of the reticuloendothelial system (RES) to phagocytize circulating foreign particulate material is well established(1,2). However, the diffuse anatomical nature of the RES, as well as the multiplicity of factors(3-5) which can independently or collectively alter RE activity have enabled this system to elude complete quantitative analysis. Quantification of RE phagocytosis has been most successfully accomplished by determining the intravascular clearance rate of previously injected particulate material (6-8). In conjunction with such studies, tissue distribution of the injected colloids(9) has provided some insight as to relative participation of various organs. Studies of this nature have demonstrated that the greatest phagocytic activity resides in the cell populations comprising the fixed macrophages of the liver, spleen, and lung which collectively represent the major segment of the reticuloendothelial system.

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That phagocytic activity can be enhanced by non-specific serum factors called "opsonins" has been well established(10-15). Indeed, the interaction of a specific serum component(s) with foreign particulate material appears to be a prerequisite for cellular recognition and subsequent phagocytosis of foreign macromolecules(16,17). Early experiments by Manwaring and Coe(18) as well as Manwaring and Fritschen(19) have demonstrated that serum can augment hepatic phagocytosis of bacteria. Filkins and Smith (12) have shown that opsonic activity will enhance hepatic phagocytosis of colloidal carbon. In addition, recent studies from our laboratory(14,15) have established the existence of an opsonic system which can stimulate *in vitro* Kupffer cell phagocytosis. In contrast to these observations on liver, very little is known concerning the role of serum factors or "opsonins" in the phagocytic activity of fixed macrophages localized in the lung and spleen. In view of this apparent lack of information, the present studies were conducted to evaluate the influence of op-

sonic activity on splenic and aveolar phagocytosis of particulate materials.

Experimental methods. Male Holtzman rats (250-300 g) maintained on Purina Laboratory Chow and tap water *ad libitum*, were used in all experiments. Rats employed as tissue donors were anesthetized with ether and rapidly desanguinated prior to liver, lung, and spleen removal. All organs were rapidly chilled in cold isotonic saline and sliced with a Stadie-Riggs tissue slicer according to previously described procedures(14). All slices were washed and briefly maintained in cold isotonic saline prior to incubation.

The incubation media consisted of heparinized rat serum or heparinized Krebs-Ringer phosphate adjusted to a pH of 7.4. Heparin (Upjohn, Kalamazoo, Mich.) was employed at a concentration of 50 USP units per ml of incubation medium. Serum used as the incubation medium was obtained from normal, non-fasted rats by cardiac puncture. When necessary, serum dilution was accomplished with the Krebs-Ringer phosphate buffer.

Radio-iodinated triolein labeled "Re Test Lipid Emulsion"(15) stabilized in a 0.1% gelatin (Nutritional Biochem. Corp., Cleveland, Ohio) supplemented sterile dextrose (5%) and water solution was employed as the test particle. The emulsion base consisted of glycerol, I^{131}-triolein (Trioleotope, Squibb Laboratories), and alcohol soluble soybean lecithin in a ratio of 10:10:1 by weight, respectively(15). A dose of 2000 μg of I^{131}-labeled triglyceride emulsion was added to each incubation vial.

Incubation samples which consisted of 3 ml of heparinized incubation medium, gelatin supplemented I^{131}-"RE Test Lipid Emulsion," and either liver, lung, or spleen tissue slices were gassed with 95% O_2 and 5% CO_2 prior to incubation in a Dubnoff metabolic shaker at 37°C for 30 minutes. Following the incubation procedure all tissues were removed, washed, and analyzed for total tissue radioactivity. Accumulated hepatic, splenic, and pulmonary tissue radioactivity was determined with a Nuclear-Chicago crystal scintillation system. Data are expressed as the per cent of added radioactivity (% I.D.) phagocytized per 100 mg of wet weight of tissue or the μg of triglyceride phagocytized per 100 mg of tissue.

TABLE I. Hepatic* Phagocytosis of Gelatinized "RE Test Lipid Emulsion" as a Function of Serum† Concentration.

% Serum in incubation medium	No. of incubated samples	% ID‡/100 mg tissue (mean ± SE mean)	% Maximum uptake§
.0	11	.74 ± .16	4.7
5.0	4	1.87 ± .19	11.9
10.0	4	2.70 ± .25	17.2
16.7	11	9.65 ± 1.41	61.6
25.0	4	11.65 ± .49	74.4
33.3	8	15.72 ± 2.18	100.4
66.7	7	15.11 ± 1.82	96.5
100.0	7	15.66 ± 3.31	100.0

* Liver tissue was obtained from 6 rats.
† Serum was pooled from 16 rats.
‡ Injected dose (ID) equaled 2000 μg of I^{131}-labeled triolein lipid emulsion.
§ Maximum uptake was equivalent to that observed in 100% serum medium.

Results. In an attempt to demonstrate the effect of opsonization on *in vitro* phagocytosis, hepatic uptake of the gelatinized I^{131}-"RE Test Lipid Emulsion" is presented as a function of the serum concentration in the incubation medium (Table I). Kupffer cell phagocytosis of the lipid particles in the heparinized Krebs-Ringer phosphate medium without serum was 0.74% of the injected dose per 100 mg of hepatic tissue. In marked contrast to such minimal uptake, the addition of serum to the incubation medium produced a significant enhancement of phagocytosis with a maximal phagocytic uptake equivalent to 15.72% of the injected dose being observed when serum was used in a concentration of 33.3% (Table I). Further increases in serum concentration, *i.e.*, up to 66.66 and 100% had no effect on the degree of hepatic phagocytosis.

The comparative evaluation of hepatic, splenic and pulmonary phagocytic activity is presented in Table II. In agreement with previously reported observations(15), as well as those presented in Table I, hepatic phagocytosis was minimal in Krebs-Ringer phosphate and markedly enhanced by the presence of heparinized serum. The use of heparinized serum as the incubation medium resulted in a comparable enhancement in splenic phagocytosis as indicated by the 38-

TABLE II. Comparative Tissue Phagocytic Uptake* of Gelatinized "RE Test Lipid Emulsion" in Krebs-Ringer Phosphate and Serum.

Tissue	Incubation medium	No. liver donors	No. of incubated samples	Phagocytic uptake: % ID†/100 mg (mean ± SE mean)	Phagocytic uptake: μg emulsion phagocytized/100 mg
Liver	Krebs-Ringer phosphate	8	12	.54 ± .05	10.8
"	Serum	8	12	18.35 ± 1.78	367.0
Spleen	Krebs-Ringer phosphate	8	12	.51 ± .09	10.2
"	Serum	8	11	19.54 ± 1.98	390.8
Lung	Krebs-Ringer phosphate	8	12	.87 ± .19	17.4
"	Serum	8	11	9.17 ± 1.08	183.4

* Incubations were conducted in 3 ml of heparinized medium for a 30 min duration.
† Injected dose (ID) equaled 2000 μg of I^{131}-labeled triolein lipid emulsion.

fold increment in uptake relative to that observed in the absence of serum. Although the phagocytosis demonstrated by lung tissue was enhanced 11-fold by serum (Table II), the increase observed was significantly less dramatic than that manifested by the hepatic and splenic tissue in response to the presence of similar serum opsonic activity.

Discussion. Although previous studies strongly support the concept that serum or plasma factors are intimately involved in leucocyte(20,21) and hepatic(12,14,15,18,19) phagocytic activity, little definitive information exists on the role of opsonization in the phagocytic activities of fixed macrophages localized in lung and spleen. Recent evidence has demonstrated that *in vitro* hepatic Kupffer cell phagocytosis of non-viable particles can be enhanced by opsonic activity(14,15,22,23). The data presented confirm these previous observations on the ability of heparinized serum to enhance *in vitro* Kupffer cell phagocytosis.

The fact that hepatic phagocytosis was apparently maximal in a medium consisting of serum at a concentration of 33.3% can be contrasted to previous observations(14) which demonstrated that hepatic phagocytosis of colloid gold was maximal only in a 100% plasma medium. The possibility exists that the lipid particles which are larger than the gold particles require less opsonin molecules per mass of material due to a concomitant reduction in particle number. It is conceivable that particle size may regulate opsonin utilization or opsonin-particle affinity and in this manner become a factor associated with the rapid intravascular clearance of large particles(3).

The *in vitro* Kupffer cell phagocytosis of the gelatinized "Re Test Lipid Emulsion" particle employed in these studies is highly dependent on the interaction of a heparin dependent opsonic system(15). We have previously postulated(15) that heparin may either activate the opsonic system operative in Kupffer cell phagocytosis, or indeed, that it may be an important segment of an active opsonin-heparin complex whose interaction with foreign particulate material is essential for cellular recognition of foreign macromolecules. The specificity of the interaction of heparin and opsonin is clearly illustrated by studies of Filkins and Di Luzio who have found that other sulfated polysaccharide compounds do not possess the ability to promote phagocytosis(23).

Evaluation of pulmonary phagocytic activity as influenced by opsonization demonstrated that lung macrophages will indeed manifest increased phagocytosis of particles in the presence of opsonins. These observations are in basic agreement with those of Ouchi *et al*(24) who have shown that rabbit alveolar macrophages will display maximal bacterial phagocytosis only in the presence of serum. In the present studies, the responsiveness of the lung tissue to serum was significantly less than that observed with the hepatic or splenic tissue preparations. Whether this reflects a physical cellular saturation of the pulmonary macrophages, variable functional activity, or indeed variable population sizes, has not been determined. The latter

possibility is suggested by the observations of Russell and Roser(25) that pulmonary alveolar macrophages which were injected intravenously and localized in the liver were undistinguishable both morphologically and functionally from indigenous Kupffer cells.

In contrast to the observations of Biozzi and Stiffel(26) on the apparent lack of dependence of spleen phagocytosis on serum opsonins, the present studies demonstrate that the splenic phagocytosis can be markedly augmented by serum factors. In accordance with these observations, Loewenthal and Micseh(27) observed that spleen macrophages maintained in culture will increase their phagocytosis of pneumococci due to the opsonic effect of type specific antiserum.

The data presented indicate that the phagocytic activity of the hepatic, splenic and pulmonary macrophages which collectively represent the major segments of the reticuloendothelial system (RES) can be markedly influenced by serum factors. Therefore, the possibility exists that the membrane sites involved in cellular recognition and adsorption of opsonized particles are basically similar for liver, lung, and spleen tissue macrophages, since in these studies, all 3 cell populations responded with increased activity to the same type of opsonized particle. Since such opsonic activity can augment phagocytosis by liver, lung, and spleen, it is apparent that *in vivo* opsonic activity can be a major factor in regulating reticuloendothelial function.

Summary. A comparative evaluation of *in vitro* hepatic, splenic, and pulmonary macrophage phagocytosis has demonstrated that serum opsonin can significantly augment the phagocytic activity manifested by all 3 macrophage populations. Furthermore, liver and spleen phagocytosis of a gelatinized "Re test lipid emulsion" is more markedly influenced by opsonization than is lung phagocytosis. Opsonin activity represents a major factor in regulating RE phagocytic function in view of its ability to markedly enhance hepatic, splenic and pulmonary phagocytosis.

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