the mitochondria by DPPD.

A similar experiment with Ethoxyquin showed it to also be inactive. In each case, when an antioxidant was added into the media, development of the TBA-reactant color was significantly decreased to the level of color development found in normal mitochondria (Table III).

When the TBA reaction was carried out in the absence of mitochondria, only a slight yellow color was found due to MLHP, a color which was much less pronounced than the red color produced by the combination of MLHP and mitochondria.

These findings indicate that lipid peroxidation is occurring due to the action of MLHP on the mitochondria. However, there does appear to be a direct relationship between the TBA reaction and the P/O ratio, and the present data appear to indicate a rather specific activity of α-tocopherol, in restoring the ability of oxidized mitochondria to carry out oxidative phosphorylation.

Two possibilities exist, (1) that the added peroxide is oxidizing the phospholipid which is required in the oxidative phosphorylation reaction (McCay and Caputto(8)), or (2) that the added peroxide is destroying some protein component of the oxidative phosphorylation pathway, in particular some component involved in the coupling of the phosphorylation with oxidation. To test the latter hypothesis, samples of pure MLHP (peroxide No. 5840) were sent to Dr. Racker for assay as to their effect on his protein coupling factors. His experiments indicate that coupling factor F2 is inhibited by this peroxide.

The decrease of P/O ratios sometimes observed in liver mitochondria from vitamin E-deficient animals (Zalkin and Tappel(12) and McCay and Caputto(8)) and the alleviating effects of α-tocopherol and of certain other synthetic antioxidants, such as DPPD and Ethoxyquin on vitamin E deficiency *in vivo* (Draper and Johnson(3)) and *in vitro* respiratory decline of vitamin E deficiency (Corwin and Schwarz(2)), at least suggest that this peroxide inhibition may be the type of reaction which occurs in vitamin E deficiency.

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Effect of Heparin and Sulfated Polysaccharides on *in vitro* Hepatic Phagocytosis.* (31187)

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Previous investigations of the influence of heparin on the phagocytic function of the reticuloendothelial system (RES) are contradictory. Knisely *et al*(1) reported that heparin transiently retarded the phagocytic engulfment of India ink, kaolin, and graphite particles by the sinusoidal macrophages of frog liver. Similarly, heparin hindered hepatic

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uptake of colloidal gold in mice and rats(2), and inhibited the intravascular removal of colloidal sodium-potassium polyphosphate in dogs and goats(3) and of colloidal gold in rabbits(4). In addition, Biozzi *et al*(5) observed that heparin facilitated RES depression by carbon in the rat. However, numerous investigators maintain that heparin does not appreciably alter the kinetics of inert particle clearance by the RES *in vivo*(6-9). In contrast to these observations on inert particles, heparin augmented the intravascular removal of *Salm. enteriditis* in mice(10), and increased leucocyte phagocytosis of *Strep. hemolyticus* in the rabbit and guinea pig(11).

In view of the recent observation that heparin facilitated the phagocytosis of colloidal carbon by the isolated perfused rat liver(12), the present studies were undertaken to evaluate further, by means of a recently developed *in vitro* procedure(13), the effect of heparin and other sulfated polysaccharides on Kupffer cell phagocytosis. A determinant role of heparin in the phagocytic function of hepatic macrophages is reported.

Methods. Phagocytic activity of rat liver slices was studied by a technique previously described(13). In brief, 300-400 mg slices of liver tissue from male Holtzman rats (300-330 g) were incubated in 3 ml of incubation media containing either 400 μg of colloidal radiogold (Abbott Laboratories, North Chicago, Ill.), or 1-5 μ human albumin aggregates labeled with radioiodine (E. R. Squibb and Sons, New Brunswick, N. J.). The incubation media employed were oxalated rat plasma, rat serum, or Krebs-Ringer phosphate buffer. After 30 minutes of incubation at 37.5°C, the liver samples were removed, washed to remove residual surface radioactivity, and weighed. The accumulated radioactivity was measured in a crystal scintillation system (Nuclear-Chicago, Chicago, Ill.) and the uptake expressed as a percent of the injected dose per 100 mg of liver tissue.

Heparin was supplied in 5,000 U.S.P. units/ml vials in which 100 U.S.P. units approximate 1 mg of heparin (Upjohn, Kalamazoo, Mich.). The other sulfated polysaccharides used were: mepesulfate (Hoffmann-La-Roche, Inc., Nutley, N. J.); sulfopolyglucin

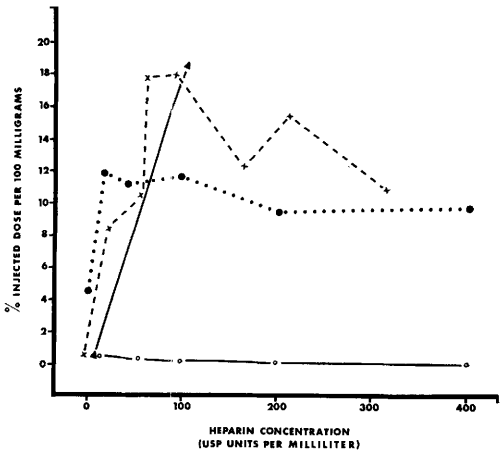

FIG. 1. Effect of heparin on rat liver slice uptake of colloidal gold and human albumin aggregates. O——O = colloidal gold in Krebs-Ringer phosphate buffer. ▲——▲ = colloidal gold in serum. X- - - - X = colloidal gold in oxalated plasma. ● .... ● = albumin aggregates in oxalated plasma. Each point represents the mean of 6 determinations. Average standard error of the determination is 8-10% of the mean.

(Riker Laboratories, Inc., Northridge, Calif.); dextran sulfates of average molecular weights: 16,200, 60-90,000, 200-300,000, 500,000, and 2,000,000 (Sigma Chemical Co., St. Louis, Mo.); chondroitin sulfate and zymosan (Nutritional Biochemicals Corp., Cleveland, Ohio).

Data were statistically analyzed for significance by Student's 't' test employing a minimum 95% level of confidence.

Results. Effect of heparin on uptake of colloidal gold and albumin aggregates by rat liver slices. To test the influence of heparin on phagocytosis in the liver slice system, a series of experiments were performed in which increasing doses of heparin were added to oxalated plasma prior to addition of the test particles. As shown in Fig. 1, the uptake of colloidal gold was slight in non-heparinized oxalated plasma or serum and approximated the uptake of 0.56% of the injected dose per 100 mg of liver which occurred in Krebs-Ringer phosphate buffer. In contrast, albumin aggregates were removed to a moderate extent in nonheparinized oxalated plasma which however was 8-fold greater than colloidal gold uptake in oxalated plasma.

As the heparin concentration of the incu-

TABLE I. Effect of Heparin and Other Sulfated Polysaccharides on Colloidal Gold Uptake by Rat Liver Slices.

Incubation media	Agent* added	No. of livers	No. of samples	% I.D./100 mg liver† (mean ± S.E.)
Krebs-Ringer phosphate buffer	None	4	10	.56 ± .08
Idem	Heparin	3	10	.28 ± .08‡
Serum	None	4	12	.41 ± .07
"	Heparin	8	23	17.98 ±1.39‡
Oxalated plasma	None	9	24	.52 ± .05
" "	Heparin	4	12	17.98 ± .70‡
" "	Mepesulfate	2	6	3.50 ± .53‡
" "	Sulfopolyglucin	2	6	.40 ± .06
" "	Chondroitin sulfate	2	6	.58 ± .14
" "	Zymosan	2	6	.43 ± .13
Serum	Dextran sulfate (2,000,000 mol wt)	2	9	.79 ± .13

* Data cited are for dose of 1.0 mg/ml; however, all agents other than heparin had no significantly different effect at doses of 0.5, 2.0, 4.0, and 8.0 mg/ml.

† % I.D. is per cent of injected or added radioactivity recovered from liver slices after 30 min incubation at 37.5°C.

‡ $P < .05$ as compared to preceding value.

bate was increased, the uptakes of both gold and albumin aggregates from oxalated plasma were markedly enhanced with increases of 31-fold and 2.7-fold, respectively. In contrast, gold uptake from Krebs-Ringer phosphate buffer, albeit slight, was significantly retarded by heparin addition (Table I). As also shown in Fig. 1, gold removal from serum was insignificant without heparin but approximated maximal plasma removal in the presence of 100 U.S.P. units/ml heparin.

Effect of other sulfated polysaccharides on colloidal gold phagocytosis by rat liver slices. Since heparin produced such a striking increase in gold uptake, experiments were performed with other sulfated polysaccharides to test the specificity of the phagocytosis-promoting action of heparin. As shown in Table I, no other polysaccharide tested produced an effect of comparable magnitude to heparin. Indeed, only mepesulfate produced a slight but significant increase in gold uptake. It should be noted that dextran sulfate was tested in serum since it caused the precipitation of fibrinogen in oxalated plasma. In addition, other molecular weight species of dextran sulfate were ineffective. The yeast cell wall polysaccharide complex zymosan, which is a potent stimulator of phagocytic function *in vivo*(14), produced no effect on gold phagocytosis when added to oxalated plasma.

Discussion. The ability of heparin to influence markedly the uptake of colloidal gold as well as human albumin aggregates is clearly demonstrated in these studies; less clear, however, is the mechanism of its action. Early reports of an interaction of heparin in phagocytic systems were complicated by test materials which promoted fibrin depositions(12). Thus, the retardation of phagocytosis by heparin was attributed to prevention of a fibrin coating on the particle which, if present, facilitated phagocytosis. Since in our system heparin produced similar effects in serum as in plasma, the probable importance of fibrin is minimal.

Recently, heparin has been shown to increase the pinocytic activity of fibroblasts (15,16). It is possible, therefore, that heparin might affect hepatic macrophages in a similar fashion and thereby promote phagocytosis by a direct cellular effect. The inhibitive effect of heparin on colloidal gold uptake from Krebs-Ringer phosphate buffer does not support this notion; however, heparin might require a plasma factor to initiate alterations in membrane activity. In addition, heparin stimulation of phagocytosis may reflect its surface-acting properties since amphipathic

agents markedly influence Kupffer cell phagocytosis(17).

Another possible mechanism for the heparin effect is related to its interaction with an opsonic system involving gelatin stabilized colloids(18-20). Thus, heparin may react with gelatin directly or promote the interaction of plasma opsonin with the gelatin stabilized colloid. Since heparin is essential for the agglutination of colloid gold *in vitro*(19), it would seem that a direct effect of heparin on the opsonin-particle interaction is plausible.

The finding that other sulfated polysaccharides do not mimic the action of heparin on phagocytosis suggests the mechanism of heparin action involves some degree of specificity and is not merely due to the high density charge of a sulfated polysaccharide. Since heparin is an ubiquitous constituent of connective tissue elements, it is probably available in the phagocytic environment and may, indeed, serve an important role in the particle preparatory phase of phagocytosis due to its potential ability to promote opsonization.

Summary. Colloidal gold phagocytosis by rat liver slices was slight in oxalated rat plasma or serum; however, heparin addition markedly increased the uptake. Heparin also augmented the hepatic phagocytosis of human serum albumin aggregates from oxalated plasma. Other sulfated polysaccharides either had no effect, or only slightly enhanced, gold uptake. The mechanism of heparin action in promoting phagocytosis may be related to facilitation of opsonin-particle interaction.

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Anti-Hypercholesterolemic Action of Scleroglucan and Pectin in Chickens.* (31188)

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Pectin has been shown to reduce hypercholesterolemia in cholesterol-fed rats(1), and in chickens, rabbits, and man(2). Although a number of other roughages or bulk-forming materials tested by Wells and Ershoff showed no such activity(1), they later described inhibition of hypercholesterolemia by feeding certain gums and carrageenan(3).

The present report describes the strong anti-hypercholesterolemic activity of sclero-

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