hydroxy apatite crystals from otherwise stable solutions. In contrast to skin and tendon, bone removed calcium from all solutions, but all three tissues were equally efficient in crystal seeding.

1. Robison, R., *The Significance of Phosphoric Esters in Metabolism,* N. Y. Univ. Press, N. Y., 1932.
2. Logan, M. A., *Physiol. Revs.,* 1940, v20, 522.
3. Sendroy, J., Hastings, A. B., *J. Biol. Chem.,* 1926-27, v71, 783.
4. Greenwald, I., *ibid.,* 1942, v143, 711.
5. Strates, B. S., Neuman, W. F., Levinskas, G. J., *J. Phys. Chem.,* 1957, v61, 279.
6. Nordin, B. E. C., *J. Biol. Chem.,* 1957, v227, 551.
7. Neuman, W. F., *Trans. Josiah Macy, Jr. Conference, Metabolic Interrelations,* 1950, p41.
8. Neuman, W. F., Neuman, M. W., *Am. J. Med.,* 1957, v22, 123.
9. Glimcher, M. J., Hodge, A. J., Schmitt, F. O., *Proc. Nat. Acad. Sci.,* 1957, v43, 860.
10. Robinson, R. A., Cameron, D. A., *J. Biophys. and Biochem. Cytol.,* 1956, v2, 253.
11. Schmitt, F. O., Hall, C. E., Jakus, M. A., *J. Cellular Comp. Physiol.,* 1942, v20, 11.
12. Levinskas, G. J., Neuman, W. F., *J. Phys. Chem.,* 1955, v59, 164.
13. Sobel, A. E., Hanok, A., PROC. SOC. EXP. BIOL. AND MED., 1951, v77, 737.
14. Fiske, C. H., SubbaRow, Y., *J. Biol. Chem.,* 1925, v66, 375.
15. Levinskas, Doctoral Thesis, Univ. of Rochester, 1953.
16. Neuman, W. F., Neuman, M. W., *The Chemical Dynamics of Bone Mineral,* Univ. of Chicago Press, 1958.
17. Spector, W. S., *Handbook of Biological Data,* W. B. Saunders Co., Philadelphia, 1956.
18. Freudenburg, E., Györgi, P., *Biochem. Z.,* 1921, v118, 50.
19. Boyd, E. S., Neuman, W. F., *J. Biol. Chem.,* 1951, v193, 243.
20. Gutman, A. B., Warrick, F. B., Gutman, E. B., *Science,* 1942, v95, 461.
21. Sobel, A. E., *Trans. Josiah Macy, Jr. Conference, Metabolic Interrelations,* 1950, p113, cf. also, Rubin, P. S., Howard, J. E., *ibid.,* p155.
22. Cartier, P., Picard, J., *Bull. Soc. Chim. Biol.,* 1956, v38, 707.
23. DiStefano, V., Neuman, W. F., Rouser, G., *Arch. Biochem. Biophys.,* 1953, v47, 218.
24. Gutman, A. B., Yu, T. F., *Trans. Josiah Macy, Jr. Conference, Metabolic Interrelations,* 1949, 11; 1950, 167.

Received December 8, 1957. P.S.E.B.M., 1958, v97.

Effect of Heparin on Serum Lipids Following Intravenous Administration of Fat Emulsion in Dogs.* (23848)

H. C. MENG AND JOHN B. YOUMANS
Departments of Physiology and Medicine, Vanderbilt University School of Medicine, Nashville, Tenn.

The evidence that intravenous administration of heparin is capable of clearing alimentary lipemia *in vivo*(1,2,3), and that post-heparin plasma decreases turbidity of lipemic plasma(3,4) and of synthetic fat emulsion(5, 6) *in vitro* is well documented. Bragdon and Havel(7) and French and associates(8) have shown that administration of heparin increased rate of removal of intravenously injected lipoproteins and fatty chyle respectively. Meng and Youmans(9) and Grossman and Strub(10) found that heparin injected simultaneously with synthetic fat emulsion also accelerates the disappearance of the infused fat from the blood circulation. The present work was designed to study: (1) changes in serum lipids following intravenous administration of an olive oil emulsion, and (2) effect of heparin on removal of the infused fat and other serum lipids.

Methods. Healthy adult male dogs weighing from 9 to 15 kg were used. All animals were fasted for 24-36 hours prior to use and experiments were carried out under sodium pentobarbital (Nembutal Sodium) anesthesia. One gram of fat per kilo was infused intravenously as a 10% olive oil emulsion which was stabilized with purified soybean phos-

* This work was supported by Office of Surgeon General, Department of Army.

phatide,† 0.5%; Demal-14,† 0.5%, Span 20,† 0.25%; sodium cholate, 0.1% and dextrose, 5%. The infusion was given by drip and completed in 30 minutes. Heparin sodium,‡ 0.5 mg/kg was injected intravenously 5 minutes prior to administration of fat emulsion; an additional amount (1 mg/kg) was mixed with the emulsion and was given by intravenous drip. Eight dogs received fat emulsion + heparin and 10 dogs received only the emulsion. Blood samples for serum lipids, including total fatty acids, total cholesterol and lipid phosphorus were drawn before, and at intervals during and after infusion of fat. Serum lipids were determined as follows: Total fatty acids by the method of Smith and Kik(11), total cholesterol according to Hoffman(12), and the lipid phosphorus according to Youngberg and Youngberg(13). The hematocrit value was obtained by centrifuging Wintrobe tubes which contained oxalated blood for 30 minutes at a rate of 2,500 rpm.

Results. Fig. 1 shows the changes in serum lipids following intravenous administration of olive oil emulsion in both heparinized and non-heparinized dogs. The results are expressed in terms of differences from initial levels. The vertical lines through each point on each curve represent the magnitude of the standard error of the mean. It can be noted that the serum total fatty acids were increased in both groups of animals during the 30-minute infusion period. However, the serum total fatty acids returned to the pre-infusion level within one and one-half hours after fat in the heparin-treated group ($p = 0.280$), while it remained significantly at a high level even 3 hours after completion of fat infusion in the animals receiving no heparin ($p = 0.037$).

A very gradual and progressive increase in

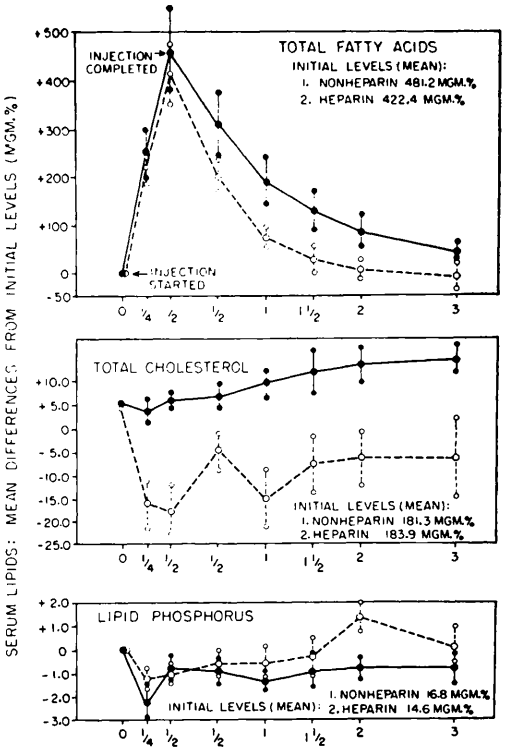

FIG. 1. Changes in serum lipids: Mean differences from initial levels. ●——●, non-heparinized dogs (10 dogs); ○---○, heparinized dogs (8 dogs). Vertical lines represent stand. error of mean.

serum total cholesterol was observed in dogs receiving no heparin (Fig. 1). The increase was significantly higher 2 and 3 hours after completion of fat infusion than that of the preinfusion level ($p = 0.050$ and 0.004 respectively). Contrary to that of the non-heparinized animals the serum total cholesterol was consistently decreased in the heparinized dogs (Fig. 1). The decrease was statistically significant at 15 and 30 minutes during ($p = 0.006$ and 0.005 respectively) and 30 and 60 minutes after infusion ($p = 0.058$ and 0.015 respectively). The differences in average serum total cholesterol between the heparin- and non-heparin-treated animals are highly significant at all points ($p =$ from 0.001 to 0.026).

The changes in serum lipid phosphorus following intravenous administration of fat emulsion were not consistent in either the heparin-treated or non-heparin-treated animals (Fig. 1).

† The authors wish to acknowledge the courtesy of Dr. J. Eichberg, American Lecithin Co., Woodside, L. I.; Dr. C. F. Fuchs, Emulsol Corp., Chicago, Ill.; and Mr. C. D. Pratt, Atlas Powder Co., Wilmington, Del. in furnishing purified soybean phosphatides; Demal-14, A polyglycerol ester; Span 20, sorbitan monolaurate; respectively.

‡ Heparin sodium was kindly supplied by Dr. L. L. Coleman, Upjohn Co., Kalamazoo, Mich.

Discussion. It is well established that the clearing of lipemic plasma or of synthetic triglyceride emulsion by post-heparin plasma *in vitro* is lipolytic in nature. Clearing of alimentary lipemia *in vivo* following intravenous administration of heparin is most likely of the same mechanism, namely hydrolysis of the triglyceride component of plasma chylomicrons and lipoproteins. As demonstrated by Meng, *et al.*(14) synthetic triglyceride emulsion particles, as were used in the present study, are probably readily "coated" by plasma proteins forming chylomicrons and lipoproteins similar to those of alimentary lipemia. Intravenous injection of heparin in some way produces clearing enzyme which hydrolyzes the triglycerides releasing unesterified fatty acids. As reported by Havel and Fredrickson(15) the unesterified fatty acids can leave the blood stream very rapidly. It is not surprising that intravenous injection of heparin increased the rate of removal of the intravenously infused fat as was observed in the present investigation.

Lever and Waddell(16) found that intravenous infusion of cottonseed oil emulsion into patients with hypercholesteremia lowered the serum cholesterol. However, results of our study showed a gradual and progressive increase in serum total cholesterol. This may be due to (1) the differences of oils used (cottonseed *vs.* olive oil) and (2) the dogs used in the present work were not hypercholesteremic.

We are unable to explain why heparin was capable of lowering serum cholesterol in the dogs receiving fat emulsion. It is known that cholesterol esters are not hydrolyzed by the lipemia clearing enzyme. However, Basu and Stewart(17), Constantinides and coworkers(18), and Friedman and Byers(19), have found that heparin decreases serum cholesterol levels in hypercholesteremic patients, cholesterol-fed rabbits and Triton WR-1339 (oxyethylated tertiary-Octyphenol-formaldehyde) injected rats respectively. According to Basu and Stewart(17) the decrease occurs in both free and esterified cholesterol.

Summary. Intravenous infusion of olive oil emulsion produced elevation of serum total fatty acids and possibly total cholesterol, but without consistent changes in lipid phosphorus. Heparin injection simultaneously with fat infusion increased the rate of removal of the intravenously infused fat and lowered serum cholesterol, but produced no significant changes in lipid phosphorus.

The authors wish to express their appreciation to Dr. Margaret P. Martin and Mr. C. W. Hayes, Jr., Department of Preventive Medicine, Vanderbilt University School of Medicine, for statistical analysis of data.

1. Hahn, P. F., *Science,* 1943, v98, 19.
2. Weld, C. B., *Canad. Med. Assn. J.,* 1944, v51, 578.
3. Anderson, N. G., Fawcett, B., PROC. SOC. EXP. BIOL. AND MED., 1950, v74, 768.
4. Anfinsen, C. B., Boyle, E., Brown, R. K., *Science,* 1952, v115, 583.
5. Meng, H. C., Hollett, C., Cole, W. E., *Am. J. Physiol.,* 1954, v179, 314.
6. Brown, R. K., Boyle, E., Anfinsen, C. B., *J. Biol. Chem.,* 1953, v204, 423.
7. Bragdon, J. H., Havel, R. J., *Am. J. Physiol.,* 1954, v177, 128.
8. French, J. E., Morris, B., Robinson, D. S., *Proc. 3rd Internat. Conf. on Bioch. Problems of Lipids, Brussels,* 1956, page 323.
9. Meng, H. C., Youmans, J. B., *Fed. Proc.,* 1953, v12, 424.
10. Grossman, M. I., Strub, I. H., PROC. SOC. EXP. BIOL. AND MED., 1954, v85, 356.
11. Smith, K. E., Kik, N. C., *J. Biol. Chem.,* 1933, v103, 391.
12. Hoffman, W. S., *Photelometric Clinical Chemistry,* William Morrow, 1941, 119.
13. Youngberg, G. E., Youngberg, M. V., *J. Lab. Clin. Med.,* 1930, v16, 158.
14. Meng, H. C., Youmgn, J. A., Shoulders, H. H., Jr., Youmans, J. B., *Fed. Proc.,* 1957, v16, 392.
15. Havel, R. J., Fredrickson, D. S., *J. Clin. Invest.,* 1956, v35, 1025.
16. Lever, W. F., Waddell, W. R., *J. Invest. Dermat.,* 1955, v25, 233.
17. Basu, D. P., Stewart, C. P., *Edinburgh Med. J.,* 1950, v57, 596.
18. Constantinides, P., Szasz, G., Harder, F., *A. M. A. Arch. Path.,* 1953, v56, 36.
19. Friedman, M., Byers, S. O., *J. Exp. Med.,* 1953, v97, 117.

Received January 6, 1958. P.S.E.B.M., 1958, v97.