Kuntzman, R., Brodie, B. B., *J. Pharm. and Exp. Ther.*, 1957, v119, 188.

7. Krayer, O., Alper, M. H., Paasonen, M. K., *ibid.*, 1962, v135, 164.

8. Goodman, H. M., Knobil, E., PROC. SOC. EXP. BIOL. AND MED., 1959, v102, 493.

9. Levy, A. C., Ramey, E. R., *ibid.*, 1958, v99, 637.

10. Wertheimer, Shafrir, E., *Rec. Progress Hormone Research*, 1960, v16, 467.

Received June 7, 1962. P.S.E.B.M., 1962, v110.

Comparison of Cholesterol Uptake in Normal and Atherosclerotic Individuals. (27645)

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It is widely accepted that an elevated concentration of cholesterol in serum is related to an accentuation of the process of atherosclerosis(1). However, the mechanism by which cholesterol separates from serum lipoproteins and accumulates in plaques in vessel walls remains unclear(2).

Since atherosclerosis is frequently associated with an elevated concentration of serum cholesterol, the question arises as to what extent the capacity of serum lipoproteins to bind and transport cholesterol is normally utilized, *i.e.*, in normal persons, is the capacity of lipoproteins to bind cholesterol saturated, or is there a reserve cholesterol binding capacity? If a reserve cholesterol binding capacity exists, is it increased, decreased, or unchanged in persons with atherosclerosis?

To investigate these questions, we measured the concentration of serum cholesterol before and after incubation of the serum with solid cholesterol. Serum was obtained from putatively normal young men and from patients hospitalized for some form of arteriosclerotic cardiovascular disease such as angina pectoris, myocardial infarction, or hypertension. Preliminary results confirmed that human serum can dissolve additional cholesterol and revealed that serum from the group of patients dissolved as much cholesterol as did serum from healthy young men.

Some of the data reported herein were the basis of a previous preliminary report(3).

Materials and methods. Cholesterol was USP grade, recrystallized twice from 95% ethanol. To prepare batches of crystals of similar size, the recrystallized product was dissolved in the minimum volume of boiling 95% ethanol required to effect solution and precipitated by rapid addition of 3 volumes of distilled water at 20°C. After cooling to 15°C, the crystals were filtered off, washed with copious volumes of ethanol-water (1:3, v/v), and sucked dry on the filter. The product was then air dried for 24 hours and stored in a vacuum desiccator.

The procedure for incubation of serum with solid cholesterol was somewhat different from that used by the French workers(4,5,6) or by Avigan(7). After investigating the results of incubating serum for various periods with various amounts of cholesterol(8), the following procedure was adopted:

1. Pipette a suitable volume (*e.g.*, 4 ml) of serum into each of six 16 × 125 mm screw-cap test tubes.

2. Add sufficient cholesterol, recrystallized as described above, to provide 40, 80, 120, 160, and 200 mg cholesterol/ml serum in tubes 1 through 5, respectively. Tube 6 is a control; no cholesterol is added to it.

3. Incubate all tubes at 37°C for 4 hours with gentle inversion of all tubes at 15-minute intervals.

4. Filter the contents of all tubes through Whatman No. 41 filter paper. Use only gravity flow.

5. Determine cholesterol concentration in the filtrate. Tube 6 is an incubated control. As an additional control, determine concen-

tration of cholesterol in the original unincubated serum sample. Cholesterol concentration in these 2 controls should be the same within the limits of experimental error. The difference between cholesterol concentration in the controls and highest concentration of cholesterol in the filtrate from any of the other tubes is taken as the "cholesterol uptake."

Concentration of cholesterol in the filtrate was measured by the method of Robinson and Pugh(9). Phospholipid concentration was measured by a modification(10) of the method of Youngsburg and Youngsburg(11) using the colorimetric determination of Fiske and Subbarow(12).

Serum used for measurement of cholesterol uptake was obtained in the usual manner after venipuncture. The subjects from whom blood was obtained were a group of 34 normal young men and a group of 14 hospital patients with clinical evidence of atherosclerosis (hypertensive vascular disease, myocardial infarct, or angina).

Results and discussion. Fig. 1 illustrates the results obtained when various amounts of cholesterol are incubated with serum in this experimental procedure. These data were obtained with a sample of human serum incubated 4 hours with the quantity of cholesterol added as indicated on the abscissa.

To achieve saturation of the serum 160 mg of cholesterol was added per ml of serum, yet cholesterol uptake was only approximately 23 mg per 100 ml of serum, or 0.23 mg per ml of serum.

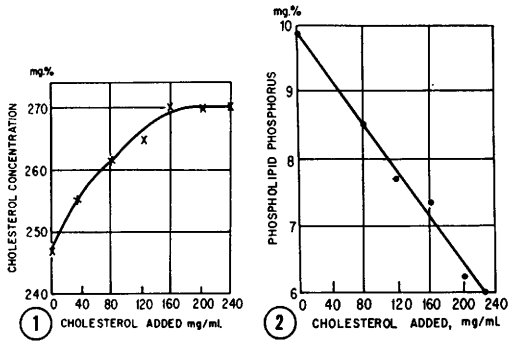

FIG. 1. Cholesterol concentration in filtrate after 4-hr incubation of serum with various amounts of cholesterol.

FIG. 2. Decrease in serum phospholipid concentration after 4-hr incubation with various amounts of cholesterol.

TABLE I. Comparison of Cholesterol Uptake in Control and Hospitalized Groups.

	No. in group	Age	Serum cholesterol*	Cholesterol uptake*
Control	34	17-32	198 ± 44	37.6 ± 13.6
Hospitalized	14	37-54	292 ± 70	53.0 ± 22.3

* Mean ± S.D.

Fig. 2 shows the phospholipid phosphorus content of the filtrate in the various incubation tubes. Concentration of phospholipid phosphorus in the filtrate decreased with addition of increasing amounts of cholesterol. Comparison with Fig. 1 reveals that phospholipid concentration continued to fall after the serum had been saturated with cholesterol, suggesting that the drop in phospholipid concentration probably is proportional to the amount of cholesterol added to the incubation tube rather than to cholesterol uptake. This decrease in phospholipid phosphorus with increasing amounts of cholesterol in the incubation tube was routinely observed with the serum samples used in this study. The cause of this relationship and the nature of phospholipid removed are being studied.

Cholesterol uptakes measured in both the normal and the patient groups are recorded in Table I. The mean serum cholesterol level is significantly lower in the controls than in the hospitalized group, as is to be expected since the individuals in the latter group were older, with clinical symptoms of cardiovascular disease. The mean cholesterol uptake for the hospitalized group is higher than for the normal group. The best interpretation of the data at this point is that serum of the hospitalized patients dissolved fully as much cholesterol as did serum from the normal group.

Examination of data on cholesterol uptake showed no correlation with initial cholesterol concentration.

Although the cholesterol uptakes measured vary widely within the groups, there were no instances of failure of a serum sample to dissolve cholesterol. This demonstrates that serum does have the capacity to bind additional cholesterol and suggests that additional cholesterol could be transported by the blood

without a concomitant increase in concentration of lipoprotein carrier. However, it was noted that concentration of phospholipid falls during the measurement of cholesterol uptake. Obviously, then, the conditions under which this serum dissolves additional cholesterol are rather unphysiologic. Therefore, the physiological implication of the findings must be accepted with great reservation. However, the data obtained imply that the capacity of serum to dissolve cholesterol is not fully saturated and that there is some reserve cholesterol transport capacity.

These data also have a bearing upon the question of whether the reserve cholesterol transport capacity is altered in persons with atherosclerosis. If atherosclerosis were associated with a cholesterol concentration that exceeds the capacity of the blood to maintain cholesterol in a stable soluble form, it should follow that in atherosclerotic individuals cholesterol transport capacity would be saturated, or more nearly saturated, than in normal persons. With the method of measuring cholesterol uptake employed here, no such difference was found between serum from "normal" and atherosclerotic individuals. The data show that the serum of the atherosclerosis patients displayed at least as much cholesterol uptake as did the serum of the normal young men.

While it would be significant that in even a few cases of atherosclerosis the cholesterol transport capacity of blood is not saturated, it is questionable whether these data document this. First, the patients with cardiovascular disease have been subjected to various therapeutic regimens having unknown effects upon blood cholesterol transport capacity measure as described above. Second, the extent of day-to-day variation in this mechanism is unknown, as are the limitations of this experimental method. Third, the hospitalized group were considerably older than the control group, and the effects of age upon the reserve cholesterol transport capacity are unknown. Experiments are therefore being continued to extend these observations.

Summary. 1. A method is described for measuring "cholesterol uptake" of serum incubated with solid cholesterol. 2. The method has been used to measure cholesterol uptake of serum from normal young men and from older men hospitalized with cardiovascular disease. 3. All sera examined were found to dissolve additional cholesterol. 4. Cholesterol uptake by serum from the hospitalized group was equal to or greater than uptake by serum from the normal group. 5. The data indicate that the capacity of serum lipoproteins to bind cholesterol is not fully saturated in either the normal young men or in the patients with atherosclerosis.

1. Lown, B., Stare, F. J., *Circ.*, 1959, v20, 161.
2. Van Itallie, T. B., and Felch, W. C., *New Eng. J. Med.*, 1960, v263, 1125, 1179, 1243.
3. Clark, D. A., Franks, J. J., *Fed. Poc.*, 1960, v19, 235.
4. Loeper, M. A., Lemaire, A., Tonnet, J., *Compt. Rend. Soc. Biol.*, 1928, v98, 101.
5. Eck, M., Desorbes, J., *ibid.*, 1934, v117, 428.
6. Kermorgant, Y., *Bull. Acad. Nat. de Med.*, 1955, v139, 180.
7. Avigan, J., *J. Biol. Chem.*, 1959, v234, 787.
8. Clark, D. A., Haven, C. B., Franks, J. J., *School of Aerospace Med. Report No. 61-1*, 1960.
9. Robinson, L. G., Pugh, E. R., *U. S. Armed Forces Med. J.*, 1958, v9, 501.
10. Clark, D. A., Haven, C. B., Franks, J. J., *School of Aerospace Med Report No. 62-50*, 1962.
11. Youngsburg, G. E., Youngsburg, M. V., *J. Lab. Clin. Med.*, 1930, v16, 158.
12. Fiske, C. H., Subbarow, Y., *J. Biol. Chem.*, 1925, v66, 375.

Received June 11, 1962. P.S.E.B.M., 1962, v110.