Effect of Soybean Phosphatides on Serum Lipids and Lipoproteins

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OUR STUDIES on phospholipids and serum lipids were initiated following the observation that incubation of human serum with an emulsion of fats containing soybean phosphatides caused an increase in the migration velocity of serum lipoproteins. It appeared that the phosphatide used as an emulsifier was the active ingredient.

I plan to show that the soybean phosphatide complex produces this "lipoprotein shift" and that the inositol phosphatide fraction is responsible for this phenomenon.[1] The effect of the intravenous administration of this fraction on serum lipids and lipoproteins will also be demonstrated.

An extract of soybean phosphatide was dissolved in normal human serum. Aliquots of the mixture were streaked on filter paper strips for electrophoretic analysis immediately after mixing and after incubation at 37°C. for twenty-four hours. The strips showed a definite lipoprotein shift or increase in the migration velocity of the beta lipoprotein to the alpha. Addition of the soybean phosphatide to hyperlipemic serum produced a similar effect.

Whole soybean phosphatide complex consists of approximately equal quantities of lecithin, cephalin and inositol phosphatide, or lipositol. Fractionation with alcohol produces an alcohol-soluble lecithin fraction and an alcohol-insoluble lipositol fraction, both of which contain one-third cephalin. The alcohol-soluble fraction contains two-thirds lecithin and the alcohol-insoluble fraction contains two thirds lipositol.

Using these fractions and the same technic with normolipemic sera, we found that the alcohol-insoluble lipositol fraction had the same effect as the whole phosphatide complex, and the alcohol-soluble lecithin fraction had no effect on the migration velocity of the beta lipoprotein. Essentially the same results were obtained using the sera of patients with hyperlipemia.

The increased migration velocity of serum lipoprotein is similar to that seen after administration of heparin *in vivo*. The heparin effect is associated with the liberation of free fatty acids.[2] Incubation of either phosphatide fraction with human serum was associated with increases in free fatty acid. However, no difference between the two fractions was noted. This suggests that the lipoprotein shift is not due simply to the liberation of free fatty acids. Heating to 56°C. did not inhibit the reaction, but addition of adequate amounts of protamine *in vitro* did.

The *in vitro* studies with the lipositol fraction were repeated *in vivo* in twenty-one normal rabbits,[3] and an effect similar to that with heparin was obtained. In a half-hour there was a marked increase in migration velocity, which was still abnormal at the end of four hours; at the end of twenty-four hours it had returned to basically the normal or control value.

The serum lipid analysis, after intravenous administration of a 5 per cent emulsion of this alcohol-insoluble fraction, showed a marked increase in the total phospholipids at the end of one hour averaging 372 per cent, an increase in neutral fat of 124 per cent and in cholesterol of 93 per cent. To be more specific, there is a marked increase in phospholipids, a mobiliza-

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tion of neutral fat and a lesser but significant elevation of cholesterol values in the normolipemic rabbit within three minutes after the phosphatide infusion. These values return to normal or almost normal after four hours and are slightly elevated after twenty-four hours. By the seventh day there is still a mild elevation in cholesterol and at the end of two weeks initial values are reached.

In the rabbits with hyperlipemia, following the phosphatide infusion, there is an increase in the phospholipid, neutral fat and cholesterol of the same magnitude as in the normal animal. However, the increase of neutral fat and cholesterol is delayed and the greatest mobilization of these lipids appears twenty-four hours after administration of the phosphatide. In experiments on rabbits with hypercholesteremia, using dextrose as the control, an unexplained fall in all lipids of similar magnitude is noted.

The soybean phosphatide is administered as an emulsion in 5 per cent dextrose. We believe that in the rabbits with hyperlipemia, the changes in lipids represent a summation effect of phosphatide and of dextrose.

These findings are of interest when evaluated with investigations of others. Lever and Waddell[4] noted falls in levels of serum cholesterol in hyperlipemic subjects following the infusions of lipid. These infusions were in 5 per cent dextrose, and the results obtained might be accounted for by the effects of dextrose alone. Gordon[5] and Dole[6] have shown that the rise in non-esterified fatty acids following fasting can be reversed following a glucose meal. Albrink et al.[7] recently demonstrated that the rise in triglycerides and nonesterified fatty acids following a fatty meal can be abolished by the administration of glucose. We are now using saline in addition to dextrose for a control.

To help explain the differences between the alcohol-soluble and the alcohol insoluble fractions, we have isolated lipositol from the soybean phosphatide and demonstrated that lipositol is responsible for the increase in the migration velocity of the serum lipoproteins.

We have given the lipositol fraction orally to ten patients with disorders of lipid metabolism. We noted no increased migration velocity of serum lipoproteins suggesting some alteration during digestion. Half of the patients showed significant falls in serum cholesterol levels.

SUMMARY

In vitro studies have shown that the alcohol insoluble inositol phosphatide (lipositol) fraction of soybean phosphatide complex produces an increase in the electrophoretic migration velocity of serum lipoproteins, while the alcohol soluble lecithin fraction is without effect. Lipositol isolated from the alcohol insoluble fraction was found to be the compound responsible for the observed effects. The *in vitro* effects were confirmed *in vivo* by the intravenous administration of lipositol fraction to normal rabbits. In the normal rabbit, the "lipoprotein shift" is accompanied by a prompt and profound mobilization of neutral fat and cholesterol to the blood stream. Oral administration of this fraction to patients failed to result in similar changes in serum lipoproteins but in half of those studied, significant falls in serum cholesterol levels were observed.

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DISCUSSION

Dr. Meyer Friedman (*San Francisco, California*): I am glad to hear that Dr. Sachs is obtaining a hypercholesteremic effect with a phosphatide infusion. We

first started with either a glycerine preparation containing cephalin and the alcohol-soluble fraction, or normal saline. We now alternate these preparations with 5 per cent glucose or normal saline.

We were not able to induce hypertriglyceremia when using phosphatide during our experiments on rats. I am not sure that would be true in the rabbit which has a peculiar lipid metabolism and in which cholesterol, when elevated, remains high for a long period of time. In the rabbit hypertriglyceremia is not present after infusion of the type of phosphatides we used in our original study.

According to the experiments of Lever on human beings, the patients had a lower cholesterol level for several days after the infusion of lipid but when he used triglyceride or the phosphatide alone, he was unable to obtain the same results.

It is our belief that by perfusing the blood stream of the animal with triglycerides we are actually washing out surplus cholesterol from the various depots that are easily accessible to the blood. For a few days the animal will have a lower blood cholesterol level because he is not synthesizing it. If you use an animal with atherosclerosis which has its own deposits of triglyceride in the blood and introduce another triglyceride, i.e., sesame oil (which is very rapidly removed from the blood), you are competing in the blood for two areas of solubility. The cholesterol already present will go into the liver. Cholesterol can be removed from the animal with atherosclerosis by giving him fat, because this fat goes to the liver and is broken down into cholic acid. The cholesterol is removed from the blood by the liver, and the plasma cholesterol level, instead of being 800 mg. per cent for 24 to 48 hours will be 300 to 400 mg. per cent. In other words, it is practically an *in vitro* phase phenomenon These results may also be obtained with dextrose. When we perfuse the blood stream of the rat with dextrose there is, in general, a lower lipid level as determined by tissue analysis of cholesterol.

When we analyze the skin, liver or intestines six hours after an infusion of dextrose, there will be less, or about the same amount, of cholesterol in the tissues. A rise in the cholesterol level is never observed.