Phosphatides in Experimental Atheroma

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PLASMA phospholipid has been found elevated in almost all types of endogenous hypercholesteremia and it has always been thought that the phospholipid rise was concomitant with that of cholesterol or possibly compensatory for the rise of cholesterol. Etiologically speaking, phospholipid is possibly discharged into the blood to keep the excess cholesterol soluble in the serum.

It is easier to carry out a cholesterol analysis than a phospholipid analysis, so perhaps cholesterol elevations took preference in our thinking. However, we decided to study whether it was possible to determine if a hypercholesteremic state was due to a primary rise of plasma phospholipid or triglyceride, or both.

We thought this might be possible first, because phospholipid is a more mobile substance as far as blood plasma is concerned, and second, any attempt to find a change in the rate of synthesis of cholesterol in hypercholesteremic states in both the human being and the experimental animal has resulted in the findings that there is no increase. There is, on the contrary, a slight decrease or no change.

If a primary phospholipid rise caused hypercholesteremia it should be possible to cause the plasma phospholipid to rise in a normal animal and thus obtain a hypercholesteremic state. We found that when the normal rat was infused with phospholipid (2 per cent solution) at the rate of 0.5 cc. per hour, within a few hours the plasma cholesterol rose, and as the plasma phospholipid became higher the cholesterol followed suit.

In our studies, the infusion of phospholipid had no effect on either the plasma cholic acid or the plasma triglyceride. It elevated only the plasma cholesterol, and this elevation was only apparent after two or three hours.

The next problem was to determine whether or not a primary rise in plasma triglyceride would also elevate plasma cholesterol. There are some hypercholesteremic states, such as occur in nephrosis and after the injection of triton (trinitrotoluene) and in diabetic acidosis, in which hyperlipemia and a hypercholesteremia result.

We subjected the normal rat to an infusion of fat at a high level. Within a short time both the plasma cholesterol and the phospholipid rose. We found an initial induced rise of triglyceride will cause a rise of both of these other lipids. But if one elevates the plasma phospholipid first, a rise in cholesterol, but not in plasma triglyceride, is observed.

We believe that all forms of endogenous hypercholesteremia are secondary phenomena. There is nothing initially wrong with the cholesterol metabolism *per se*, at least in the beginning, but it represents the response of the body to a prior rise of either phospholipid or triglyceride, or both.

Another consideration is biliary obstruction. An accumulation of bile acid in plasma is the first manifestation of biliary obstruction. Within fifteen minutes after the bile duct of a rat is ligated the plasma cholic acid rises and it precedes by hours any rise of cholesterol, and by minutes any accumulation of excess phospholipid. In a short time (by a presently unknown mechanism) the bile acid effects an accumulation of phospholipid.

In other words, if a normal animal is infused with bile acid even without biliary obstruction and the bile acid is elevated in plasma by giving bile acid so rapidly that the liver cannot excrete sufficient bile acid to prevent its rise in the

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plasma, the phospholipid rises, followed within a short time by a rise in plasma cholesterol.

The elevation in plasma cholesterol apparently stems from other tissue sources besides the liver. We were misled by our own previous work to believe that the liver was the only source of plasma cholesterol in hypercholesteremia, and other investigators also came to that conclusion.

We do not believe now that only the liver is capable of supplying large amounts of cholesterol to the plasma. Removal of the liver removes the source of the blood's phospholipid and since a cholesterol rise depends on a preceding rise in phospholipid, removal of the liver does lead to a failure of cholesterol to increase in the blood after supposedly hypercholesteremic procedures. This is not due to the necessity of cholesterol being furnished to the blood only by the liver. It is probably occasioned by the failure of the animal without a liver to supply phospholipid to its blood.

After injection of triton, a failure of the clearing mechanism is seen. We believe, as did the original workers, that this happens because administration of triton interferes with the acceptance by plasma albumin of the fatty acids resulting from hydrolysis. Whether this is due to the sequestration of the triglycerides by triton or the physiologic sequestration of albumin by triton is not known at the present time. However, we do know that whenever there is an accumulation of triglycerides occurring after administration of triton (which occurs within the first few hours), it is this triglyceride rise which then mobilizes the tissue cholesterol everywhere in the body to effect a hypercholesteremia, whether or not the liver has been removed.

Various workers thought that the presence at the liver was necessary for the occurrence of hypercholesteremia after injection of triton. However, if one injects one dose of triglyceride and also gives triton to a hepatectomized animal, the animal becomes and remains hyperlipemic and, as a consequence, becomes hypercholesteremic in six hours. On the other hand, if one gives triglyceride without triton to an animal whose liver has been removed, it rapidly rids its serum of the fat, but in the presence of triton it cannot dispose of the triglyceride. As a result the animal becomes hypercholesteremic within six to eight hours.

It is our opinion that in nephrosis, when the albumin falls below 1 gm. per 100 ml., there is no receptor for the fatty acids coming from the hydrolysis of fats. Therefore, the triglyceride remains in the plasma and when it accumulates it becomes a cholesterol receptor and retainer.

We also believe that a certain amount of phospholipid or triglyceride, or both, can hold only a certain amount of cholesterol in plasma, and if a nephrotic animal whose triglyceride component is saturated with cholesterol is given another injection of cholesterol, it will dispose of that excess cholesterol as easily as a normal rat. The cholesterol goes directly to the liver of the animal for removal because, seemingly, the animal has no way of retaining the cholesterol when its triglyceride component is saturated. The mechanism is unknown.

This occurs also after injection of triton. Thus, if an animal is given triton followed immediately by administration of a soluble form of cholesterol, the animal will not receive any cholesterol from its tissues but instead will retain the administered excess cholesterol in its plasma. In twenty-four hours, the same rise in blood cholesterol is observed in an animal given an initial injection of cholesterol plus triton as in an animal given triton alone.

Studies in our laboratory show that almost all lecithins were capable of inducing a cholesteremic effect, except lecithin from the duck's egg. Cephalin as well as inositol and synthetic lecithins provided a hypercholesteremic effect. The animal whose liver has been removed also becomes hypercholesteremic after infusion of phospholipids.

We have found that there are two tissues with a reduced cholesterol content after phosphatide infusion: (1) the liver of an animal that is fed cholesterol in excess; and (2) the adrenal gland of a starved animal.

If cholesterol could be removed from various depots by phosphatide infusion, the possibility existed that we might be able to remove cholesterol from an atherosclerotic plaque. Therefore, using fourteen rabbits, seven were infused

twice a week (eight to eleven infusions before autopsy) with a crude extract of brain lecithin, and seven others served as control subjects. The extract (a very potent cholesterol mobilizer), containing various types of lecithins and cephalins was not toxic in this particular group of animals although it did contain a high content of lysolecithin. During each infusion the plasma phospholipid would be artificially elevated and the plasma cholesterol would usually rise from 50 to 250 mg. per 100 ml. in six hours in the rabbit. (If phosphatides are administered too frequently, e.g., three times a week, a chronic hypercholesteremia results which will continue as long as infusions of phosphatides are given.)

Results of phospholipid infusions in our group of fourteen animals after three months were as follows: The degree of aortic atherosclerosis was 4.9 in the control rabbits, and only 2.7 in the rabbits infused with phosphatide. The actual cholesterol and fat analyses of the aortas indicated a 51 per cent difference in the aortic cholesterol and a 37 per cent difference in the total lipid.

These apparently therapeutic results obtained with infusion of a crude phosphatide suggested that perhaps a means was at hand that might reverse the atherosclerotic process. However, we were handicapped by the tediousness of preparing the atherosclerotic animal. Cholesterol must be fed for two to three months and then cholesterol feeding must be stopped for another two months for the animal to become normocholesteremic. Therefore, we adopted the Higginbotham technic. This consists of removing an atherosclerotic aorta from a hypercholesteremic animal and cutting it into small segements. Every other segment is given to a control animal and the animal to be infused is given the alternate segment. Such segments are placed in the interior chamber of the eye. One can easily perform fifteen such corneal implants in one day.

Our experiences do not agree with Dr. Higginbotham's statement that the intima of even an atherosclerotic plaque in the cornea will be vascularized. We do not believe there is any vascularization of the intima when we place the adventitial side of the aorta directly upon the iris, which is richly supplied with blood.

One week after implantation, we infused half of the animals with a phosphatide suspension (5 per cent) for approximately six to eight hours, twice a week for four to six weeks. The corneal implants then were removed, a section taken of each and stained with Sudan III. The remainder of each corneal implant was then analyzed for cholesterol. Similar procedures were carried out on the control rabbits, on which corneal implant had not been performed.

The rabbits withstood the infusions very well and little or no toxicity was observed. During infusions, the plasma phospholipid is elevated six to ten times above the normal value, effecting a four- to sixfold rise in the cholesterol level. Between infusions, both lipid levels returned to normal.

This work has just begun and it is not certain whether or not phosphatide infusions hasten the disappearance of cholesterol from such implants. Unlike aortic atherosclerosis, these implants naturally lose their cholesterol very quickly in a period of four to eight weeks. It remains to be seen whether or not phosphatide therapy will alter this natural rate of disappearance.

DISCUSSION

DR. W. STANLEY HARTROFT (*St. Louis, Missouri*): These are exciting results that Dr. Friedman has just given us. It is interesting that treated plaques with the fat removed contain only calcium and the calcium is all in the media. That is the picture of Mönckeberg's sclerosis. Have the plaques progressed from atheroma to Mönckeberg's sclerosis? If so, it has tremendous implications.

DR. FRIEDMAN: There is sometimes calcification of the intima but it is mostly in the media.

DR. HARTROFT: You demonstrated mainly medial calcifications; therefore you have transformed an atheromatous plaque into Mönckeberg's sclerosis. If this is accepted on its face value it will greatly affect our thinking in pathologic study.