Role of Vitamins in Lipid Metabolism

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THE data presented herein have already been reported in part by Dr. Ling.[1] However, some unpublished work by Dr. Hsu[2] and myself is also included.

Ling[3] previously showed that the lipid content of a vitamin B_{12}-deficient rat is 4.4 per cent, and that of the vitamin B_{12}-treated rat 16.8 per cent. The difference in the fat content of these two groups of animals may be attributed to vitamin B_{12} deficiencies or to a more general phenomenon, such as inanition or poor appetite. For example, it should be pointed out that the mean body weight of the deficient animals was approximately 84 gm. and that of the treated animals, 200 gm. The food consumption of the two groups was different.

In subsequent experiments, two groups of rats were pair-fed. One can pair-feed these animals in two ways, that is, limit the food intake of the vitamin B_{12} treated group to the amount of food taken by the vitamin B_{12}-deficient one; or, feed *ad libitum* the amount of food and calories taken by the vitamin B_{12}-treated group and force-feed the vitamin B_{12}-deficient group to the same extent.

When these animals were pair-fed according to the amount taken in by the vitamin B_{12}-deficient group, then we found that vitamin B_{12} had no effect, and the weight gain of the two groups was essentially the same.

On the other hand, when we pair-fed according to the amount consumed by the vitamin B_{12}-treated animals—and to achieve this goal we had to use a liquid diet consisting of oil, emulsified fat, sucrose and soybean protein—we found that there was a marked difference again in the lipid composition of the carcass, and most of the fat in the food consumed by the vitamin B_{12}-deficient group was excreted in the feces.

As a further control on the effect of body weight, a third group of rats was used. It consisted of vitamin B_{12}-treated rats that had been starved so that their weight had been considerably reduced. Upon feeding with sufficient calories they gained much weight and the fat content was the same as that of the vitamin B_{12}-treated group. This type of control study is essential if we are to be reasonably certain that the effect is due to vitamin deficiency.

Ling[1] demonstrated that vitamin B_{12}-deficient animals show a small amount of carcass lipid and phospholipids. Hsu[2] recently found that the serum cholesterol content in plasma and adrenals of vitamin B_{12}-deficient animals was elevated almost twofold. When these animals were treated with vitamin B_{12}, the serum cholesterol level was brought down to almost normal. Addition of an excessive amount of pyridoxine had no effect.

Of interest is that the administration of vitamin B_{12}, 10 μg. per day orally or 10 μg. once weekly subcutaneously, results in a definite decrease in plasma cholesterol.

The most effective way of correcting hypercholesteremia in rats caused by a deficiency of vitamin B_{12} is by the subcutaneous administration of vitamin B_{12}, 10 μg. per day, plus the oral administration of 10 μg. of vitamin B_{12} with 100 mg. of sorbitol. Sorbitol[4] has been shown to be an agent which will enhance absorption of vitamin B_{12} under some experimental conditions.

When vitamin B_{12} is given orally in small doses, only a certain portion of it is absorbed. The absorbed portion of this vitamin, unlike

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other ones, is not distributed evenly to all organs. The vitamin can be transported[5] to certain target organs such as liver, adrenals and pancreas, but it is retained in these depots so tenaciously that it is not available for redistribution to other organs. When vitamin B_{12} is given as an injection even in large quantities, most of it is excreted rapidly and certain target organs do not have a chance to retain it.

The addition of sorbitol has been shown to enhance the absorption of vitamin B_{12}, not only does it raise the serum level, but vitamin B_{12} can also go into certain organs (eyes and brain) which cannot be reached even by the injection of large doses of vitamin B_{12}.

We were interested in determining whether the administration of vitamin B_{12} would have any effect on lowering the high serum cholesterol levels of old people. In a study conducted by other workers, it was found that a certain mixture of lipotropic agents (Liptril[6]) possessed the property of lowering serum cholesterol levels. In view of our findings, we thought it might be possible to decrease the cholesterol levels in these older subjects by injections of vitamin B_{12}. Our first attempt to do so was unsuccessful.

In view of these results, we considered the possibility of lowering the serum cholesterol levels by administering vitamin B_{12} with sorbitol orally. It was found[7] that the administration of a mixture of sorbitol and vitamin B_{12} resulted in an increase in vitamin B_{12} serum level and a concomitant decrease in the cholesterol level.

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DISCUSSION

Dr. George F. Wilgram (*Toronto, Canada*): Dr. Friedman, you said you believed that the hypercholesteremia under the conditions of your experiment is caused by, may we use the word, "secretion" of cholesterol into the blood from sources other than the liver. Is this correct?

Dr. Meyer Friedman (*San Francisco, California*): Yes.

Dr. Wilgram: Could you elucidate on what you believe to be the role of the liver in the contribution of cholesterol to the blood under these conditions?

Dr. Friedman: This is about what we believe at the present stage of our work: if the animal is pre-fed a high cholesterol diet, a decline in liver cholesterol occurs after infusion of phosphatide. This is accompanied by marked hypercholesteremia. At the same time, there is also a marked decline in adrenal cholesterol. When the animals receive no cholesterol in the diet, the cholesterol content of the liver is increased and that of the adrenal decreased during the period of the phosphatide-induced hypercholesteremia. We also found a similar hypercholesteremia after phosphatide infusion in the liverless animal which leads us to believe that it is not the normal function of the liver to supply the blood with cholesterol.

Dr. Sachs will also discuss this question, since he has too been working with a phosphatide and getting a rise of cholesterol in two or three minutes.

I do not know exactly where the cholesterol is coming from; however when we feed cholesterol it is obtainable from the liver and the adrenal.

Dr. Wilgram: How then does this tie in with the clinically well known statement of Thannhauser about thirty years ago that in severe liver disease the first real sign that can be detected in the blood is a decrease of the cholesterol esters? I think most people would agree that there is some correlation between the disease and the drop in the cholesterol esters, presuming that the cholesterol has been esterified and released from the liver.

Dr. Friedman: I think we went to long lengths to show that the liver was the chief site of cholesterol esterification but that does not mean that quantitatively it is supplying an excess cholesterol for the blood. I think there is an easy transfer of cholesterol back and forth.

I believe the liver cell has a certain amount of cholesterol which it is not going to give up to the plasma, irrespective of what you do. I think it will give up phosphatides and triglycerides.

I am not prepared now to discuss this because it really does not make any difference to me whether or not the liver produces a lot of cholesterol. As a matter of fact, half of those in my lab do not believe it does.

DR. WILGRAM: I think Dr. Lucas' work and also Dr. Olson's corroborate the fact that in the choline-deficient animal, when choline is fed, the cholesterol increases in the blood. But where does this come from, if not the liver? It decreases in the liver at the same time it increases in the blood.

DR. FRIEDMAN: Dr. Worthason has shown that the liver is the only organ in the body that has a net increase in cholesterol. Never has anyone found an excess, showing that the liver could manufacture cholesterol in excess. It is just short of turnover.

Now I do not know, and I do not like to discredit my own early work either, because we thought we were pretty good in showing that the liver was the chief source of cholesterol in the blood. However I do not care. I would like you to show me that this statement is wrong.

DR. W. STANLEY HARTROFT (*St. Louis, Missouri*): I think this view of yours is very stimulating because it makes us look in other directions.

Dr. Lucas, would you tell us more about this aspect? Would you also tell us where the cholesterol goes in a choline-deficient animal when the serum cholesterol falls; and where it comes from when choline is restored to the diet and the serum cholesterol rises; and where it is delivered?

DR. C. C. LUCAS (*Toronto, Canada*): I wish I knew the answers. The actual fall, even the blood level was about 200 and fell back to 80, is not many milligrams compared to the amount of cholesterol in the rest of the body. You would have to be a pretty tricky analyst unless you were going to use tracer technics in order to pick that up. We have not applied tracer technics so I could not tell you where it goes.

DR. HARTROFT: If it goes into the aorta we would be very interested.

DR. W. E. CORNATZER (*Grand Forks, North Dakota*): In the effect of the rise in cholesterol caused by giving choline to the choline-deficient animal, I would like to point out that in the choline-deficient animal given either a dose of choline, a single dose of oil or a single dose of oil plus choline, the greatest stimulation of phospholipid synthesis occurs in the intestinal mucosa and in the liver.

(This is our initial work with Dr. Artom.) We obtain not only a rise in serum cholesterol following a given dose of choline or choline plus food containing some oil, but also a rise in phospholipids above that which is obtained when giving oil alone, choline alone, or oil plus choline.

Now if this occurs in the liver, I am sure it occurs in the plasma, although we did not study the plasma at that time because from all other work the plasma simply reflects that which is taking place in the liver.

DR. HARTROFT: What do you think the increase in phospholipid synthesis in the intestines means and why shoud it occur there?

DR. CORNATZER: Probably increased metabolism, because we do not think it is involved in absorption.

DR. C. ARTOM (*Winston-Salem, North Carolina*): We are not sure.

DR. CORNATZER: That is so. The pendulum has swung away from such an explanation and may be back again if you interpret Reiser's experiments, so it depends on what the exact mechanism is. Dr. Artom, I am sure, can answer this question.

DR. ARTOM: It is an old question. It was discussed twenty or twenty-five years ago with Dr. Verta, who was at that time very enthusiastic about the participation of phospholipids in the absorption of fat. I think if we try to calculate the increase in the synthesis of these phospholipids, certainly the increase will not justify that all the fatty acids pass through the stage of phospholipids during absorption. That is part of it.

DR. LUCAS: There appears to be quite a large discrepancy in the calculation.

DR. ARTOM: Yes, it is too literal. In other words, an increase in the rate of synthesis would not be high enough to justify the absorption of fats which occurs normally, so certainly they are not all absorbed as phospholipids.

It is certainly only a secondary pathway but the secondary pathway may become important when you have some impairment in the major pathways. That might be part of the process in the absorption but I think we have to leave the question as it is, in other words, still somewhat unsolved.

DR. LUCAS: I think it is possible that the observed phospholipid turnover may not be an obligatory part of the system, but a parallel process, something that is going on simultaneously that is important.

DR. ARTOM: It could perhaps be just the expression of an increased metabolism.

DR. HARTROFT: Would some one comment on the phenomenon that enables ethanolamine to increase phospholipid turnover in the liver just as choline does, without being lipotropic.

DR. ARTOM: I do not know exactly, but I would say that there is one point perhaps that can reconcile these two differences. In the liver we have lecithins, and cephalin, including ethanolamine; in the plasma we have only the lecithins or very little phospholipid ethanolamine. It might well be that when the one is reflected in the phospholipids and the other is not, it is because it remains in the liver.

What the real mechanism of this increased synthesis is, I do not know. Perhaps it is just a mass effect of overflowing phospholipid from the liver, with the compound that we give. We have so many compounds that increase phospholipid synthesis and none of them

certainly is directly or easily explained as a lipotropic substance.

DR. LUCAS: In Dr. Cornatzer's discussion of the lipotropic effect of methionine, he noted that at the 6 per cent level a marked change in the level of lipids occurred. I do not think I would call that a lipotropic effect of methionine. In some of our work (using quite a few different basal diets), it we added much over 1 per cent of methionine to the diet we began to get a cutback on food consumption and a decrease in weight gain, until finally we got a weight loss, just as Dr. Cornatzer showed. I do not think you can get a fatty liver unless enough calories are given to supply the body needs, and a little excess.

I believe 5 gm. a day loss of weight does not show a lipotropic effect. I would call it a toxic effect.

DR. CORNATZER: We interpret this as an antilipotropic effect, showing the loss in weight and decreased food consumption.

DR. LUCAS: You could not call it an antilipotropic effect in one sense because the liver fat is falling, but it is falling because of diminished fat intake.

DR. CORNATZER: That is right.

DR. HARTROFT: You could not describe that as an unique action of methionine?

DR. CORNATZER: No.

DR. HARTROFT: Because if you can do anything to the animal that will cause a decrease in food intake, the amount of fat will decrease.

DR. WILGRAM: Dr. Friedman, I was very much impressed by your closing remarks when you described the implantation of a segment of the aorta into the anterior chamber of the eye by the Higginbotham technic and observed *in vivo*, so to speak, the atherogenic process. Normally, does the aorta graft on the iris or not? That is, does the adventitia adhere to the iris?

DR. FRIEDMAN: Yes. It sticks and it does not die; that is, it does not form a necrotic mass for weeks and months, up to even six months. I have not done it for six months but Dr. Higginbotham has and rarely does he find a normal aortic plaque calcified like the one I showed you today.

DR. WILGRAM: And you believe that the cholesterol deposition under those circumstances occurs through the capillaries?

DR. FRIEDMAN: Of the iris, yes. And from the adventitious side, not the intimal side.

DR. WILGRAM: You do not believe that any cholesterol transgresses the blood barrier and goes into the fluid? That would be unlikely, would it not?

DR. FRIEDMAN: It surely would. We have never noticed any opacity or turbidity of that fluid and we do not notice the intimal side ever acquiring a deposit unless the iris tissue comes around and covers the intimal side.

DR. HARTROFT: I think this technic is certainly one that more people will find interesting.