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Lipoprotein and Lecithin : Cholesterol Acyltransferase Changes in Galactosamine-Induced Rat Liver Injury

Abstract. *Abnormal lipoproteins and decreased lecithin : cholesterol acyltransferase activity are found in rat plasma following intraperitoneal injection of* D-*galactosamine. The changes observed, including absence of* α*-lipoprotein and the presence of lipoproteins rich in phospholipid and unesterified cholesterol but deficient in cholesteryl esters, are remarkably similar to changes found in human pathologic states of lecithin : cholesterol acyltransferase deficiency. When examined by electron microscopy, all of the major lipoprotein classes isolated by ultracentrifugation showed morphological abnormalities including the formation of rouleaus consisting of disk-shaped particles.*

Lecithin : cholesterol acyltransferase (LCAT), an enzyme found in the plasma of many animal species, catalyzes the transesterification of an unsaturated fatty acid from the 2-position of phosphatidyl choline (lecithin) to the 3-position of cholesterol. In man, nearly all of the esterified cholesterol in plasma is derived from this reaction (*1*). It has been postulated that this enzyme plays a vital role in the metabolism of plasma lipoproteins by facilitating the exchange of unesterified cholesterol and cholesteryl esters between plasma lipoproteins, since profound abnormalities in the composition, ultrastructure, and metabolism of lipoproteins have been demonstrated in familial LCAT deficiency (*2*). Abnormal lipoproteins have also been reported in other diseases in which plasma LCAT deficiency occurs, including liver disease (*3*), abetalipoproteinemia (*4*), and Tangier's disease (α-lipoprotein deficiency) (*5*). Despite these observations, the exact nature of the role of LCAT in cholesterol and lipoprotein metabolism is not clear nor has the relationship of deficiency in LCAT activity to specific abnormalities in lipoprotein composition and structure been determined.

In rats, intraperitoneal injection of D-galactosamine produces liver injury secondary to uridylate trapping in the form of uridine diphosphate hexosamines (*6*). Single injections of D-galactosamine (350 to 750 mg per kilogram of body weight), which result in minimal injury, cause accumulation of hepatic triglycerides without a decrease in plasma lipids as found with other inhibitors of protein synthesis that produce a fatty liver (*7*). The reported decrease in rat plasma LCAT activity following D-galactosamine injection (*8*) and the results of our own experiments (*7*) suggest that the D-galactosamine model can provide a unique means of investigating the regulation of cholesterol esterification by LCAT and the role of this enzyme in lipoprotein metabolism. In the studies reported here we demonstrate that D-galactosamine produces striking compositional and morphological changes in all classes of plasma lipoproteins associated with a rapid and reversible LCAT deficiency.

Female Sprague-Dawley rats, 180 to 200 g, received a single intraperitoneal injection of D-galactosamine hydrochloride (750 mg/kg) dissolved in isotonic saline. Blood samples were taken from the abdominal aorta of ether-anesthetized animals. Rats that were bled 24 hours after injection were fasted from the time of injection. Animals bled at other times were fasted at least 12 hours but no more than 24 hours before blood was drawn. Controls that were fasted for times equal to the treated animals were used for comparison. LCAT activity was defined as the incorporation of [^{14}C]cholesterol, equilibrated with plasma cholesterol, into cholesteryl ester (*9*). In some experiments LCAT activity was also measured as initial rate of disappearance of unesterified cholesterol as determined by gas chromatography. The results were essentially the same. Total cholesterol and unesterified cholesterol were determined by gas chromatography of extracts of saponified and nonsaponified samples (*10*).

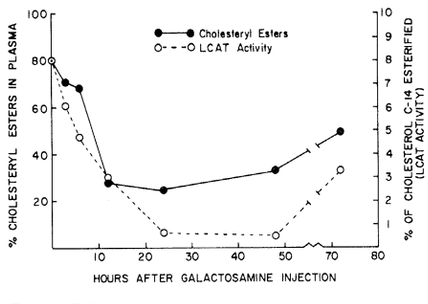

Fig. 1. Effect of a single intraperitoneal injection of D-galactosamine hydrochloride (750 mg/kg) on cholesteryl esters and lecithin : cholesterol acyltransferase (LCAT) in rat plasma. Cholesteryl esters are represented as percentage of total plasma cholesterol.

The effect of a single dose of D-galactosamine on plasma LCAT activity and on the proportion of total cholesterol present in the esterified form is shown in Fig. 1. Within 3 hours there is a 25 percent decrease in LCAT activity, and this decrease continues until 24 hours, when LCAT activity is less than 10 percent of control levels. During this same period the total plasma cholesterol is essentially constant (61 mg/dl), which means that the decrease in cholesteryl esters is accompanied by an increase in unesterified cholesterol. The LCAT activity remains at this level at 48 hours but has begun to increase again by 72 hours. Parallel to the decreased LCAT activity there is a concomitant decrease in the percentage of esterified cholesterol in plasma, which reaches a minimal value by 12 hours and begins to recover after 48 hours. This effect of D-galactosamine on LCAT activity is not a direct effect on the LCAT reaction, since the direct addition to normal rat plasma of more than five times the theoretically possible plasma concentration of D-galactosamine that could result from the dose has no effect on LCAT activity.

The plasma lipoproteins from these animals were separated by agarose electrophoresis and stained with Fat Red 7B (*11*). Within 3 hours the α-lipoprotein is decreased and is completely absent at 24 and 48 hours (Fig. 2). After 3 hours the pre-β-lipoprotein [very low density lipoprotein (VLDL)] has disappeared and the β-lipoprotein has a greater mobility. These changes progress until the only remaining lipoprotein at 24 hours is a band that stretches from the point of application (origin) to approximately the pre-β position and by 48 hours this single band is even further diminished. By 72 hours all of the lipoproteins have reappeared; however, the β-lipoprotein band, which is normally low in fasted rats, is increased, and the α-lipoprotein band is still decreased compared to the control.

Lipoproteins from a pool of plasma obtained from rats 24 hours after injection of

D-galactosamine were separated by sequential ultracentrifugation (*12*). The density classes separated were chylomicrons, VLDL ($d < 1.006$), low density lipoprotein 1 (LDL) ($1.006 < d < 1.05$), LDL_2 ($1.05 < d < 1.07$), and high density lipoprotein (HDL) ($1.07 < d < 1.225$). Chylomicrons as used here is an operational definition referring to the lipoprotein fraction obtained by centrifugation for 30 minutes at 30,000*g* at plasma density and does not imply an intestinal origin.

In Fig. 3 the ultrastructure of the lipoprotein fractions from control rats is shown on the left and the corresponding fractions from D-galactosamine-treated rats on the right. Few if any morphologically normal lipoproteins are present in the plasma from treated rats. The chylomicron-like particles were poorly visualized by negative staining techniques, in which they appeared as very large ill-defined lipid droplets. The VLDL from treated rats are larger particles (Fig. 3B) than normal VLDL (Fig. 3A) and are variable in shape, sometimes appearing to form flattened, bilamellar structures. The LDL_1 from treated rats (Fig. 3D) appear similar to the VLDL particles and are much larger (52 nm) than normal LDL_1 (30 nm). The LDL_2 particles (Fig. 3, E and F) and HDL particles (Fig. 3, G and H) from the treated rats demonstrate the most striking differences from the control animals. Each of these fractions (Fig. 3, F and H) consists primarily of stacked, bilamellar disks. Although these fractions appear morphologically much the same, there is a difference in the size of the disks, those in the LDL_2 being larger (55 nm) than in the HDL (15 nm). This size difference reflects that in control rats, in which the LDL_2 particles are larger than HDL.

We have made initial studies of the lipid content of whole plasma and of the ultracentrifugal fractions derived from control and treated animals. In whole plasma, the most striking effect is the decrease in cholesteryl esters (Fig. 1). Unesterified cholesterol increased from 8 mg/dl to 32 mg/dl, and triglycerides increased from 16 mg/dl to 42 mg/dl. Neither the phospholipid level nor the total of these four classes of lipids (not including unesterified fatty acids) changed significantly. The increased triglyceride content of the plasma from treated animals could be accounted for almost entirely by an increase in the chylomicron fraction. The decreased cholesteryl ester content was most marked in the LDL_1, LDL_2, and HDL fractions, all of which contained less than 20 percent cholesteryl ester. The relative amount of unesterified cholesterol was more than five times greater in the LDL_2 and HDL from the treated animals than from the controls.

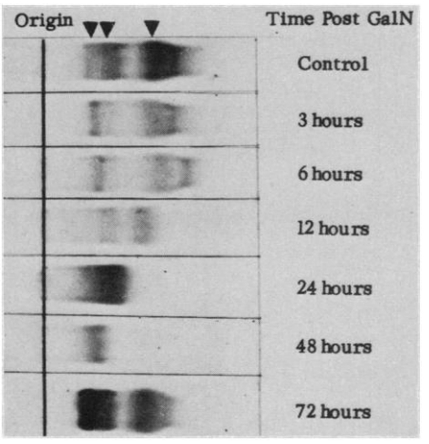

Fig. 2. Agarose electrophoresis patterns of plasma lipoproteins from control and D-galactosamine-treated rats at various times after a single injection. Samples were applied at the origin line. Markers indicate β-, pre-β-, and α-lipoproteins, respectively, left to right. Anode is to the right; *GalN*, D-galactosamine.

Phospholipid accounted for approximately two-thirds of the total lipid in the LDL_1, LDL_2, and HDL fractions. In control plasma, 79 percent of the total lipid, excluding unesterified fatty acids, was in the HDL fraction compared to only 10 percent in this fraction following D-galactosamine treatment. Thus, the net effect of the induced LCAT deficiency on the plasma lipoproteins is an accumulation of very large, chylomicron-like particles and phospholipid-rich, cholesteryl ester poor higher density lipoproteins, primarily at the expense of normal HDL.

We have also examined the apoprotein composition of the ultracentrifugally isolated lipoprotein fractions from D-galactosamine-treated rats by electrophoresis on polyacrylamide gels containing sodium dodecyl sulfate (*13*). All of the apoproteins found in normal lipoproteins are present; however, there are quantitative differences evidenced primarily as greatly reduced levels of apolipoproteins of the A family.

Although no α-lipoprotein was detectable in the plasma of treated animals, we were able to isolate an HDL fraction by ultracentrifugation. This fraction is neither quantitatively nor qualitatively equal to normal HDL nor detectable by electrophoresis even when ten times as much sample was applied. Since α-lipoprotein (HDL), which is postulated to be the substrate for LCAT, contains both activators and inhibitors of LCAT (*14*), it is conceivable that the LCAT deficiency is a result of a primary deficiency of HDL. On the other hand, the metabolism of VLDL in plasma, leading to the formation of LDL and involving interchange of unesterified cholesterol, cholesteryl esters, and certain apoproteins with HDL, requires the participa-

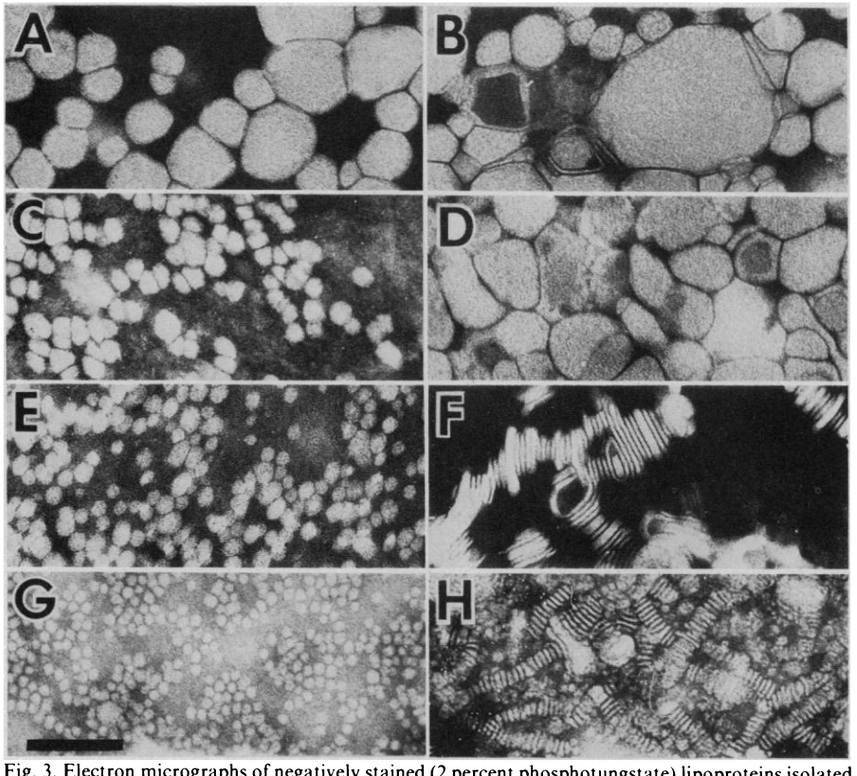

Fig. 3. Electron micrographs of negatively stained (2 percent phosphotungstate) lipoproteins isolated by ultracentrifugation. Micrographs at left are from control rats; those at right are from D-galactosamine-treated rats 24 hours after injection; A and B, VLDL; C and D, LDL_1; E and F, LDL_2; G and H, HDL; scale, 100 nm (×130,000).

tion of LCAT (*2*). Thus, all of the lipoprotein abnormalities observed could be due to LCAT deficiency. There is evidence that lipoproteins secreted by the liver are different from those normally found in plasma and that the secreted forms are rapidly metabolized and transformed in the blood (*15*). The abnormal lipoproteins observed in LCAT deficiency might more nearly represent the secretory forms of the lipoproteins.

Lipoprotein classes have been studied in great detail in human familial LCAT deficiency (*2*). In these studies, VLDL, LDL, and HDL have all been shown to differ from normal both in lipid composition and in morphology. In this disease the LDL and HDL contain high concentrations of phospholipids and low concentrations of esterified cholesterol. It has been suggested that cholesteryl esters are necessary in order to maintain the normal, spherical structure of these lipoproteins and that, in their absence, bilamellar discoidal structures similar to phospholipid bilayers are formed.

The lipoproteins from the plasma of rats made LCAT-deficient by treatment with D-galactosamine exhibit an ultrastructural appearance essentially identical to that observed in familial LCAT deficiency. Furthermore, both the absence of electrophoretically demonstrable α-lipoprotein and the changes in lipid composition are similar to those observed in the human condition. Similar bilamellar stacked disks have also been described in liver disease. D-Galactosamine provides an experimental model with which to study the relationship of LCAT deficiency to lipoprotein abnormalities in rats. All of the changes are reversible and can be followed as they develop over a relatively short time period.

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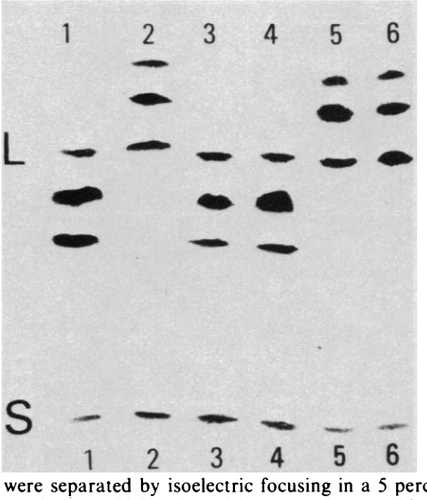
Fraction 1 Protein and the Origin of Polyploid Wheats

Abstract. *The nature of the interspecific hybridizations giving rise to the polyploid wheats has been determined from an analysis of the polypeptide compositions of fraction 1 proteins isolated from diploid, tetraploid, and hexaploid wheat species. In the origins of the tetraploid wheat, the B-genome donor provided the chloroplast genome and was therefore the maternal parent in the cross with* Triticum monococcum. *Subsequently, the tetraploid wheat,* T. dicoccum, *was the maternal parent in the cross with* Aegilops squarrosa, *giving rise to the hexaploid wheat,* T. aestivum. *Of the small number of diploid species examined, only* Ae. speltoides *has a chloroplast genome similar to that of the polyploid wheats.*

The polypeptide composition of fraction 1 protein, as revealed by isoelectric focusing in the presence of 8*M* urea (*1*), provides excellent phenotypic markers for both the nuclear and chloroplast genomes of higher plants, because the genetic information for the primary structure of the small subunit is located in the nuclear genome (*2*), whereas the information for the large subunit is located in the chloroplast genome (*3*). In F_1 interspecific hybrids in the genus *Nicotiana*, fraction 1 protein is composed of large subunit polypeptides inherited solely from the maternal parent species and small subunit polypeptides inherited from both parent species (*4*). Doubling the chromosome number of such F_1 interspecific hybrids, giving rise to new self-fertile amphiploid species, does not affect the polypeptide composition of the fraction 1 protein (*5*). Therefore, examination of the polypeptide patterns of fraction 1 proteins isolated from amphiploid species and from their putative progenitor species provides a method for determining the origins of amphiploid species. This method has been used successfully to determine the identity of the interspecific hybrid which gave rise to the commercial tobacco plant, *N. tabacum* (*6*). To demonstrate the potential of



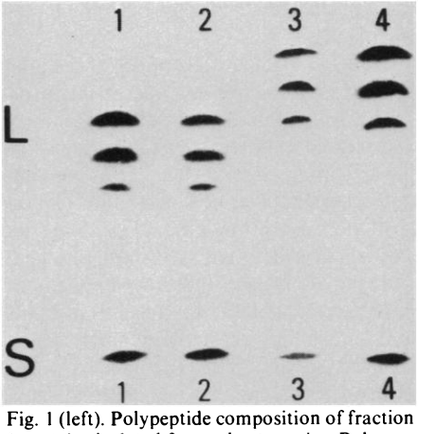


Fig. 1 (left). Polypeptide composition of fraction 1 proteins isolated from wheat species. Polypeptides of *S*-carboxymethylated fraction 1 proteins were separated by isoelectric focusing in a 5 percent polyacrylamide gel slab containing 1 percent ampholine, *p*H 5 to 7, and 8*M* urea. Polypeptide bands were stained with bromophenol blue. Samples (20 μg of protein) from left to right: (1) *Ae. squarrosa*; (2) *Ae. speltoides*; (3) *T. urartu*; (4) *T. monococcum*; (5) *T. dicoccum*; (6) *T. aestivum*. *L*, large subunit polypeptides, *p*H 5.8 to 6.1; *S*, small subunit polypeptides, *p*H 5.5. Fig. 2 (right). Polypeptide composition of fraction 1 protein from reciprocal, interspecific F_1 hybrids of *T. boeoticum* and *T. dicoccoides*. Polypeptides were separated as described in the legend to Fig. 1. Samples (20 μg of protein) from left to right: (1) *T. boeoticum*; (2) *T. boeoticum* ♀ × *T. dicoccoides* ♂; (3) *T. dicoccoides* ♀ × *T. boeoticum* ♂; (4) *T. dicoccoides*. *L*, large subunit polypeptides; *S*, small subunit polypeptides.