Technical Papers

Susceptibility to Experimental Atherosclerosis and the Methylation of Ethanolamine-1,2-C^{14} to Phosphatidyl Choline*

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There are indications in the literature that there may be a relationship between the susceptibility of certain species to atherosclerosis and their phospholipid metabolism. It has been demonstrated that the incidence and severity of cholesterol-induced atherosclerosis in rabbits is decreased if the blood phospholipid is elevated concomitantly with the cholesterol (*1*). In the human being, the ratio of total cholesterol to phospholipids was appreciably increased in coronary disease as compared with normal control groups (*2*). The poorer clearing of the alimentary lipemia observed in atherosclerotic males as compared with the clearance rate in normal males (*3*) may also bear a relationship to the metabolism of the phospholipids in that the enzymic removal of lecithin from serums by the α-toxin of *Cl. welchii* will produce or increase lipemia (*4*). This enzymic degradation has been shown to be specific for lecithin, with a slow degradation also occurring for sphingomyelin (*5, 6*). Cephalin and phosphatidyl serine are unaffected.

Collectively these reports suggest that part of the metabolic block in the metabolism of the lipids that occurs in the atherosclerotic is a lack of formation of lecithin (phosphatidyl choline) or an increased abnormal degradation thereof. The possibility exists that it may be necessary for the ethanolamine base to be methylated and to form lecithin before clearing action becomes evident.

In order to gain further information on the possible relationship of the biosynthesis of lecithin to atherosclerosis, the formation of lecithin from free ethanolamine was investigated in liver slices of rats, which to date have been demonstrated to be immune to experimental gross atherosclerosis (*4, 8, 9, 10*) and in liver slices of guinea pigs and chicks, species that are susceptible (*10, 11*).

Table 1 demonstrates a striking difference between the ability of the rat to form lecithin from free ethanolamine and the ability of the chick or the guinea pig to accomplish this process. The chick and the guinea pig possess a total liver capacity equal to 3.8 and 3.1 percent, respectively, of the capacity of the rat. Comparing the ratios of lecithin formation of the livers to body weight, the chick possesses a capacity equal to 2.2 percent and the guinea pig 1.2 percent of the capacity of the rat. These differences are also supported by the data for the conversion per unit weight of liver tissue.

The results obtained with ethanolamine-1,2-C^{14} could be construed as indicating that, instead of a greater capacity for synthesis, the rat liver possessed a larger pool of active methionine for the methylation of ethanolamine than did the chick or the guinea pig, or that the rat liver diluted the labeled ethanolamine less than did the liver tissue of the other two species, with the consequence that a greater yield of labeled lecithin was produced. That this is not correct is proved by the much higher radioactivity of the phosphatidylcholine of rat liver obtained when $C^{14}H_3$-methionine was used as the methyl donor for unlabeled ethanolamine.

Present knowledge points to a correlation between the state of the chemical metabolism of cholesterol and the susceptibility to atherosclerosis. Data on total cholesterol/phospholipid ratios and their change with atherosclerosis (*1, 2*) suggest that at least part of the metabolism of cholesterol is related to the phospholipids. The mechanism has not been clarified. Examination of the published observations of changes in the total cholesterol/phospholipid ratios that are correlated with atherosclerosis reveals that in all cases total phospholipid phosphorus or total phospholipids were measured. It would appear, therefore, that at least part of the present confusion on whether the phospholipids deter atherogenesis (*10*) could be due to the analytic and interpretative treatment of the phospholipids as a homogeneous group of substances in relation to the cholesterol/phospholipid ratio.

Table 1. Comparison of phosphatidyl choline formation from ethanolamine by the rat, guinea pig, and chick. The relative ability of nonfasted Long-Evans strain rats, English Shorthair guinea pigs, and White Leghorn chicks to convert ethanolamine-1,2-C^{14} to phosphatidyl choline: Liver slices, totaling 500 mg, were incubated aerobically for 2 hr at 37°C in 5 ml of Krebs-Ringer phosphate solution containing 0.0016*M* $CaCl_2$ at a *p*H of 7.4. The isolation of phosphatidyl choline was carried out as reported previously (*12*) with the exception that the acetone-precipitation step was eliminated and that choline was counted as the Reinecke salt derivative.

Animal species	No.	Total counts in phosphatidyl choline per 100 sec		
		Per 500 mg of liver tissue	Per total liver mass	Total liver count/wt. (g) of animal
Rat	10*	1,180	16,520	94.5
	2†	32,000		
Guinea pig	4*	9	515	1.1
Chick	4*	40	640	2.1
	2†	768		

Substrates: * Ethanolamine-1,2-C^{14} ($3.8 \times 10^{-4}M$ and 239,000 counts/100 sec); Methionine ($7.5 \times 10^{-4}M$). † $C^{14}H_3$-Methionine ($3.0 \times 10^{-4}M$ and 311,000 counts/100 sec); Ethanolamine ($3.8 \times 10^{-4}M$).

The data in Table 1, obtained by methods developed (*12*) to distinguish between such constituents as phosphatidyl ethanolamine, serine, choline, and sphingomyelin may provide a clue leading to the nature of the metabolic block that gives rise to prolonged alimentary lipemia in the atherosclerotic and to atherosclerosis. A fraction of this metabolic block may be the relative lack of ability of certain animal species to form lecithin. This conclusion is reinforced by the observations of Ahrens and Kunkel on the necessity of lecithin for clearing action (*4*) and by the correlation of the results reported in this paper with the species difference in the susceptibility to experimental atherosclerosis induced by cholesterol and other means. The data suggest that *certain* phospholipids are necessary for normal cholesterol breakdown or metabolism.

References and Notes

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Similarities in Histochemical Differentiation of Insect Cuticle and the Walls of Parasitic Fungi

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Incidental to a study of the pathology induced by the parasitic fungus *Herpomyces stylopygae* Spegazzini growing on the cuticle of oriental cockroaches (*Blatta orientalis* Linné) it was noticed that Mallory's triple stain gave a similar staining picture for the fungal walls and the insect cuticle. In both, the developing membrane first stains blue; later, parts of it change and stain red; still later, some parts become brown or black and refractory to staining. In insects this well-known sequence of changes occurs in areas called sclerites and the process is known as sclerotization (*1*). In this fungus all the walls remain undifferentiated (that is, stain blue) except the peculiar basal "shield" growing from the secondary receptacle. This shield undergoes changes paralleling those of cuticle undergoing sclerotization, as is shown in Fig. 1.

The genus *Herpomyces* belongs to the Laboulbeniales of the Ascomyceteae. Like ascomycetes in general,

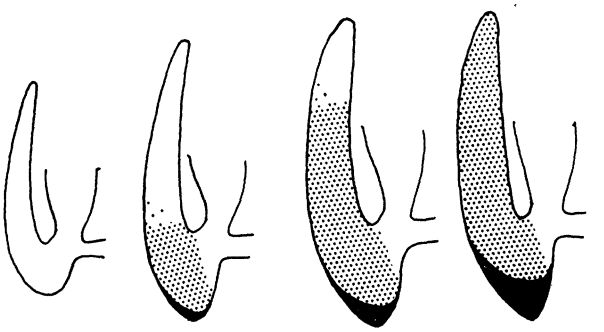

Fig. 1. Diagrammatic representation of color changes shown with Mallory's triple stain during differentiation of the basal "shield" of *H. stylopygae*. Sketched as seen in longitudinal section with "shield" on left and connection to secondary receptacle and rhizoid on right; cell protoplasts ignored. *Unshaded outline*, blue staining walls; *stippled areas*, red staining walls; *black areas*, amber to black walls refractory to staining.

its walls contain chitin instead of cellulose. Von Wettstein (*2*) failed to obtain a positive test for chitin in a species of *Laboulbenia* and tentatively stated that chitin was absent from this group. Ulrich (*3*) accepted this report as fact despite von Wettstein's clear reservation on such a conclusion because he had inadequate material for testing. The chitosan test for chitin (*1*) is positive with *Herpomyces*, although one has to be relatively gentle in applying the test or the walls will disintegrate and be lost (the same is true for the wing scales of certain moths). The walls are also positive for protein (Millon and ninhydrin tests).

Serial sections of the fungus show that the wall is composed of a relatively thick chitin-protein layer giving the afore-mentioned reactions and an extremely thin ($< 1\,\mu$ thick) surface layer which stains red or purplish with Mallory's stain.

These similarities suggested that some of the other color tests currently used in studies on insect cuticle be performed. The data may be summarized by saying that insect cuticle and *Herpomyces* cell walls are similar in these respects:

1) They have a thin surface layer staining red with Mallory's stain.

2) They have a thicker underlying layer containing chitin and protein.

3) They have the thicker layer showing the illustrated staining sequence during development of certain areas.

4) They have the brown or black color removed by treatment with hot NaOH or KOH solutions and by Diaphanol.

5) They are positive to the argentaffin test (ammoniacal $AgNO_3$), which in insect cuticle has been shown to be indicative of orthodihydroxyphenols involved in the sclerotization process.

6) They give the argentaffin reaction primarily in the