

# The lipids of the myocardium, conducting bundle, and valves of beef heart \*

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## SUMMARY

The lipids of myocardium, conducting bundle, and valves of beef heart were investigated. The atrioventricular valves contained the highest content of total lipid, most of which was triglyceride. The lipids of myocardium and semilunar valves contained the highest amount of phosphatides and cholesterol. The plasmalogens were localized predominantly in the phosphatide fraction of the myocardium and conducting bundle. An analysis of the individual phosphatides showed that with respect to the total lipid phosphorus, the polyglycerolphosphatide was localized in the myocardium and conducting bundle, sphingomyelin occurred in highest amount in the conducting bundle, and lecithin occurred in highest amount in the valves.

Data on the lipids of heart tissues are given by Mallov and co-workers (1) and are summarized by Deuel (2). Little information is available on the amount of each specific lipid, in particular the phosphatides. In previous studies quantitative paper and column methods for phosphatide analysis were employed to study the individual phosphatides of pig heart muscle (3) and the cell fractions of pig heart (4).

In this paper the lipids of ventricular myocardium, conducting bundle, and valves of beef heart were investigated. The data show significant differences in the lipid distribution in the various heart tissues and emphasize the distinctive pattern of lipid localization in this organ.

## METHODS

Two beef hearts obtained from animals immediately after slaughter were maintained on ice until ready for extraction. Portions of myocardium were removed from the apex of the ventricles. The larger proximal portions of the conducting bundle were carefully dissected from surrounding muscle and connective tissue. The semilunar (pulmonary and aortic) valves and the atrioventricular (tricuspid and mitral) valves were removed and freed from muscle, extraneous connective tissue, and fat.

The myocardium, conducting bundle, and valves

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were minced and extracted with chloroform-methanol as described previously (4). Column and paper chromatography of the lipids were carried out by the method of Marinetti and co-workers (3, 4). Cholesterol analysis was performed on the nonphosphatide fraction obtained by column chromatography. The Liebermann-Burchard reaction was employed as described previously (5). Plasmalogens were detected by the Schiff test (3).

## RESULTS

The data on the lipid content of beef heart tissues are summarized in Tables 1 to 3. The experiments were repeated three times with essentially the same results. The data in Table 1 give the gross analytical picture of the percentage distribution of the major lipid groups on the basis of dry weight tissue. The high lipid content of the atrioventricular valves is noteworthy, particularly in view of the relatively low lipid content of the semilunar valves.

A more detailed analysis of the lipids of the heart tissues is given in Table 2. The content of the lipid components is expressed on a relative basis, namely, relative to the total dry weight of lipid, rather than to the dry weight of tissue. These data clearly show that the lipids of myocardium and semilunar valves are rich in phosphatides, whereas the lipids of the atrioventricular valves are predominantly nonphosphatides, in particular triglycerides. The difference in the relative cholesterol content of the lipids of the heart valves is also apparent.

The data in Table 2 demonstrate a qualitative difference in the plasmalogen<sup>1</sup> content of the heart tissues. The plasmalogens are present in the phosphatide fraction of all the tissues but they occur in higher amount in the myocardium and conducting bundle. The plasmalogen content of pig and beef heart has been published elsewhere (6, 7). A small amount of Schiff positive material was also found in the non-phosphatide fraction of myocardium and semilunar

TABLE 1. GROSS LIPID CONTENT OF BEEF HEART TISSUES

	Myo- cardium	Con- ducting Bundle	Valves *	
			A-V	S-V
Total dry weight tissue in mg.	1051.0	270.0	1088.0	590.0
Total lipid in mg.	128.0	31.0	248.0	22.0
Total lipid in % dry weight	12.1	11.5	22.8	3.7
Phosphatide † in % dry weight	6.6	2.7	1.2	1.8
Total cholesterol in % dry weight	0.8	0.45	0.38	0.65

\* A-V represents atrioventricular valves; S-V represents semilunar valves.

† The phosphatides were determined by column fractionation (see Table 2). This fraction also includes cerebroside.

valves. In part, some of this lipid material is believed to represent free aldehydes that are liberated as a result of hydrolysis of the native plasmalogens either during the lipid extraction or during column fractionation.

The phosphorus content of the phosphatide fraction of the heart tissues was low and variable. If this fraction consisted only of the typical phosphatides, the per cent phosphorus should be between 3.6 to 4.0. Hence, it is clear that this fraction which is eluted by chloroform-methanol and methanol from silicic acid columns contains some polar nonphosphatides. Part of this material is accounted for by cerebroside (3).

The analysis of the individual phosphatides is given in Table 3. The amount of each phosphatide is expressed as the per cent phosphorus which it comprises

<sup>1</sup> The plasmalogen test with the Schiff reagent may not be specific for these particular phosphatides; rather it measures total available aldehyde.

<sup>2</sup> This lipid is believed to be the same as cardiolipin (4). In heart muscle and most other tissues it consists of two to four components having similar structure and properties.

of the total lipid phosphorus. It can be seen that the polyglycerolphosphatide<sup>2</sup> occurs predominantly in the myocardium and in the conducting bundle. We have previously shown that this phosphatide is localized in mitochondria (4) and, moreover, in the rat occurs in highest amount in heart muscle (8).

The lipids of the conducting bundle were found to contain less phosphatidylethanolamine than the lipids of myocardium. In the case of the heart valves phosphatidylethanolamine and phosphatidylserine were not sufficiently resolved on paper chromatograms to analyze them separately. However, phosphatidylethanolamine occurred in higher amount in both cases.

The component having the chromatographic mobility and staining properties of inositol phosphatide was not completely separated from sphingomyelin in the case of myocardium. Hence, these two phosphatides were analyzed together. Sphingomyelin was, however, the major component.

Although precautions were taken during the extraction of the lipids to prevent their degradation, the myocardium lipids were found to contain an appreciable amount of lysolecithin. Since the nonphosphatide

TABLE 2. COLUMN CHROMATOGRAPHY OF BEEF HEART TISSUE LIPIDS \*

	Myo- cardium	Con- ducting Bundle	Valves	
			A-V	S-V
I. Total lipid put on column in mg.	22.0	30.0	39.0	17.0
II. Nonphosphatides † in mg.	8.0	22.0	36.0	8.0
III. Phosphatides ‡ in mg.	12.0	7.0	2.0	8.0
Phosphatide content of I in %	54.5	23.3	5.1	47.2
Cholesterol content of I in %	6.6	3.9	1.7	17.4
Cholesterol content of II in %	18.0	5.3	1.8	37.0
Phosphorus content of III in %	2.6	1.3	1.4	1.0
Schiff test on II	++	neg.	neg.	+
Schiff test on III	+++++	+++	+	+

\* Column fractionation was carried out on silicic acid (10 g.) as described previously (3).

† Represents the lipid fraction eluted with chloroform. This fraction has no phosphorus and contains mainly cholesterol, cholesterol esters, and triglycerides. These lipids were identified by paper chromatography (4).

‡ Represents the lipid fraction eluted with chloroform-methanol 1:1 and by methanol. This fraction also contains cerebroside (3).

fraction of myocardium (Table 2) contained Schiff positive material, it was concluded that the lysolecithin arose as a result of hydrolysis of the cholineplasmalogens. The relatively low content of lecithin in this sample supports this conclusion. Subsequent extractions of other beef hearts have shown that under carefully controlled conditions the amount of lysolecithin in myocardium is much smaller (less than 5 per cent of the total lipid phosphorus).

TABLE 3. PHOSPHATIDE COMPOSITION OF BEEF HEART TISSUES \*

Phosphatide	Percentage of Total Lipid Phosphorus †			
	Myo- cardium	Con- ducting Bundle	Valves	
			A-V	S-V
Polyglycerolphosphatide	8	4	trace	trace
Phosphatidylethanolamine ‡	20	11	} 20	18
Phosphatidylserine	6	8		
Lecithin ‡	28	39	46	47
Sphingomyelin	} 24	26	23	20
Inositol phosphatide		5	7	8
Lysolecithin	13	trace	trace	trace
Others §	1	7	4	7

\* Quantitative paper chromatography was carried out as described previously (3).

† The total lipid phosphorus is the sum of the phosphorus in all the phosphatide spots, including the origin material.

‡ These phosphatides contain the plasmalogens indicated in Table 2 as the Schiff positive material. The actual content of the plasmalogens of beef heart myocardium have been published elsewhere (7).

§ Trace amounts of other phosphatides were observed on the chromatograms. One of these is believed to be lysophosphatidylethanolamine. This fraction also includes 1 to 2 per cent material at the origin.

#### DISCUSSION

These experiments demonstrate the distinctive distribution of lipids in various tissues of beef heart. The lipid content of atrioventricular valves was high and consisted mainly of triglycerides. On the other hand, the lipids of semilunar valves consisted mainly of phosphatide and cholesterol. The response of these valves to pathogenic organisms may in part be attributed to this marked difference in lipid composition.

The plasmalogens were found in higher concentration in the myocardium and conducting bundle than in the valves. In addition, the polyglycerolphosphatides occurred nearly exclusively in the myocardium and conducting bundle. Since this lipid fraction is localized in mitochondria (4) and occurs in highest concentration in the heart (8), it may play a special role in heart metabolism.

The high content of sphingomyelin in the conducting bundle is noteworthy. In this respect this tissue appears to resemble nerve tissue, yet its morphology is similar to that of myocardium. It would be of interest if the conducting properties of the bundle of His were due to specific lipids.

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