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Triglycerides, cholesterol, and phospholipids in normal heart papillary muscle and in patients suffering from diabetes, cholelithiasis, hypertension, and coronary atheroma

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SYNOPSIS The triglyceride, cholesterol, and phospholipid contents of heart papillary muscle were measured in groups of obviously healthy and diseased females and males on whom either routine or forensic necropsies were performed. In healthy men the triglyceride content was 1·77 ± 1·30 mg/g of wet weight and in women 1·25 ± 0·48 mg/g wet weight. The corresponding values for cholesterol were 1·07 ± 0·24 mg/g and 1·21 ± 0·22 mg/g and those for phospholipids 17·70 ± 5·15 mg/g and 19·65 ± 10·21 mg/g. The differences between the sexes were not significant.

The hypertensive or cardiac hypertrophy group had about the same or slightly lower means for lipid content.

In the cholelithiasis group, women had significantly high triglyceride values (3·38 ± 2·36 mg/g). The cholesterol values were not significantly elevated in either men or women.

In the diabetic group, triglycerides were significantly increased both in men (mean 8·12 ± 0·54 mg/g) and in women (6·85 ± 5·66 mg/g). The cholesterol mean values were also high in both sexes, but the rise was not significant because of the great variation.

In the coronary atheroma group, both male and female hospital cases had high triglyceride contents (mean 4·48 ± 4·25 mg/g and 3·65 ± 3·94 mg/g) whereas the forensic cases had only slightly elevated or normal values. Cholesterol assays paralleled the triglyceride ones, but phospholipids showed an inverse trend.

The results showed that the lipid content of papillary muscle was increased in diseases where disturbances of lipid metabolism are evident, as in diabetes and cholelithiasis. In coronary atheroma only those cases with advanced obstruction of the arteries were associated with abnormal values of papillary lipids. No increase of the lipid content with age alone was found, nor was there any correlation with obesity.

It was found in previous investigations that Oil Red O-positive material accumulated in the cardiac papillary muscle and its arterioles to a greater extent in diseases associated with coronary atheroma such as diabetes mellitus and gallstones (Alavaikko, Hirvonen, and Räsänen, 1970). In further studies the lipid material was analysed both histochemically and by thin-layer chromatography. Histochemical staining revealed that triglycerides were present in the papillary muscle cells in cases of diabetes and coronary atheroma. Cholesterol was found in cholelithiasis cases in the chorda tendineae and arteriolar walls. No pathological accumulation of phospholipids was demonstrable by histochemical means (Alavaikko and Hirvonen, 1971).

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The histochemical findings prompted us to undertake quantitative analyses of the lipid content of papillary muscle. It was necessary to collect papillary muscle also from healthy persons, because knowledge of the amount of lipid in normal cardiac muscle is scanty. Some information was available on the normal content of phospholipids and cholesterol (Documenta Geigy, 1960) in the human heart, and two reports of triglycerides in the heart muscle were found (Wheeldon, Schument, and Turner, 1965; Lindlar and Zaki, 1966).

The object of the present investigation was to

determine whether there is an accumulation of lipids in cardiac papillary muscle in diseases known to be associated with disturbances in lipid metabolism. Triglycerides, cholesterol, and phospholipids were selected to represent the main lipid classes. The results were compared with a reference group consisting of obviously healthy persons who died violently.

Material

The material consisted of anterior papillary muscle collected from necropsies in the Departments of Pathology and Forensic Medicine. Patients who died in the hospital numbered 62 and the same number had suffered a violent death. In the last mentioned cases the agonal period was less than one hour. The corpses were stored in the cold (+4°C) and necropsy was performed within one to three days after death. None of the corpses showed autolysis. After excision the sample was quick frozen in liquid nitrogen and stored at −25°C until analysed.

Before the material was divided into the various groups the possible effect of obesity on the lipid content was checked. Thickness of abdominal subcutaneous fat had been measured at the necropsy. There was no correlation of obesity with the papillary lipid values either in forensic or hospital cases. Distribution of high and low values showed no trend in the group of cases with 1-2 cm thick fat layer and in that with 3-4 cm of fat or more.

DISEASE GROUPS

The hospital and forensic material was divided into the following disease groups in accordance with the earlier histochemical studies:

1 *Hypertension or cardiac hypertrophy*
Criteria of two kinds were used for this group; clinically diagnosed hypertension, and the presence of left cardiac hypertrophy at necropsy (heart weight over 400 g) without valvular lesions. None of these suffered from coronary sclerosis, cholelithiasis, or diabetes.

2 *Cholelithiasis*
Gallstones were found at necropsy.

3 *Diabetes mellitus*
Clinically diagnosed diabetes.

4 *Coronary atheroma*
Verified at necropsy and divided into two grades: (*a*) mild, soft endothelial plaques with no obstruction of the lumen, and (*b*) severe, calcified plaques with obstruction of the lumen.

Cases with more than one disease were at first studied separately. In the final analysis, however, these cases were classified according to the disease which seemed to affect the muscle lipid content. For instance, cases with both cholelithiasis and hypertension were finally placed in the cholelithiasis group, because hypertension alone had no significant effect on the papillary lipid values.

REFERENCE MATERIAL

The patients who had died from violence and had no disease served as a reference or control group. The causes of death included drowning, hanging, and severe crushing. As mentioned, cases with an agonal period of more than one hour were not accepted so that agonal metabolic disturbances which might affect the papillary lipids were excluded (see Alavaikko *et al*, 1970). Those with disease were the same as in the hospital series. The mean values for triglycerides, cholesterol, and phospholipids served as reference values with which the results from the disease groups were compared.

Methods

EXTRACTION OF LIPIDS

The papillary muscle sample was weighed and homogenized in a chloroform-methanol mixture (2:1) (Folch, Lees, and Stanley, 1957). The final ratio of tissue and solvent was adjusted to 1:20. The homogenate was filtered and washed through the filter with one fifth vol of 0·9% saline. The chloroform phase was separated and if turbid was cleared by adding a few drops of methanol. Thereafter the volume of chloroform was diluted to the original 1 g of tissue to 20 ml of solvent. The lipid assays were carried out on this chloroform extract.

TRIGLYCERIDES (TG)

Glycerides were first saponified and the glycerol formed was oxidized to formaldehyde which was treated with diacetyl-acetone and ammonia to form a fluorescent compound, 3·5-diacetyl-1·4-dihydrolutidine. This compound was assayed fluorometrically according to the method of Kessler and Lederer (1965).

CHOLESTEROL (CHOL)

The colorimetric method of Levine, Morgenstern, and Vlastelica (1967) based on the Liebermann reaction with acetic acid, acetic anhydride and sulphuric acid was used.

PHOSPHOLIPIDS (PL)

The Biochemica test combination package TC-PH, No 15920 TPAC was used. The organic phosphate

in the lipid extract was corrected by incineration into inorganic phosphate, which was assayed colorimetrically (Zilversmit and Davis, 1950).

STATISTICAL ANALYSIS

The results were treated with Mann-Whitney's U test, which is more suitable than the t test for data with a large standard deviation (Siegel, 1956).

Results

The results are given as the mean for this group ±1 SD.

THE CONTROL GROUP OR HEALTHY CASES

It was found that the mean triglyceride concentration was higher in men than in women, the values being

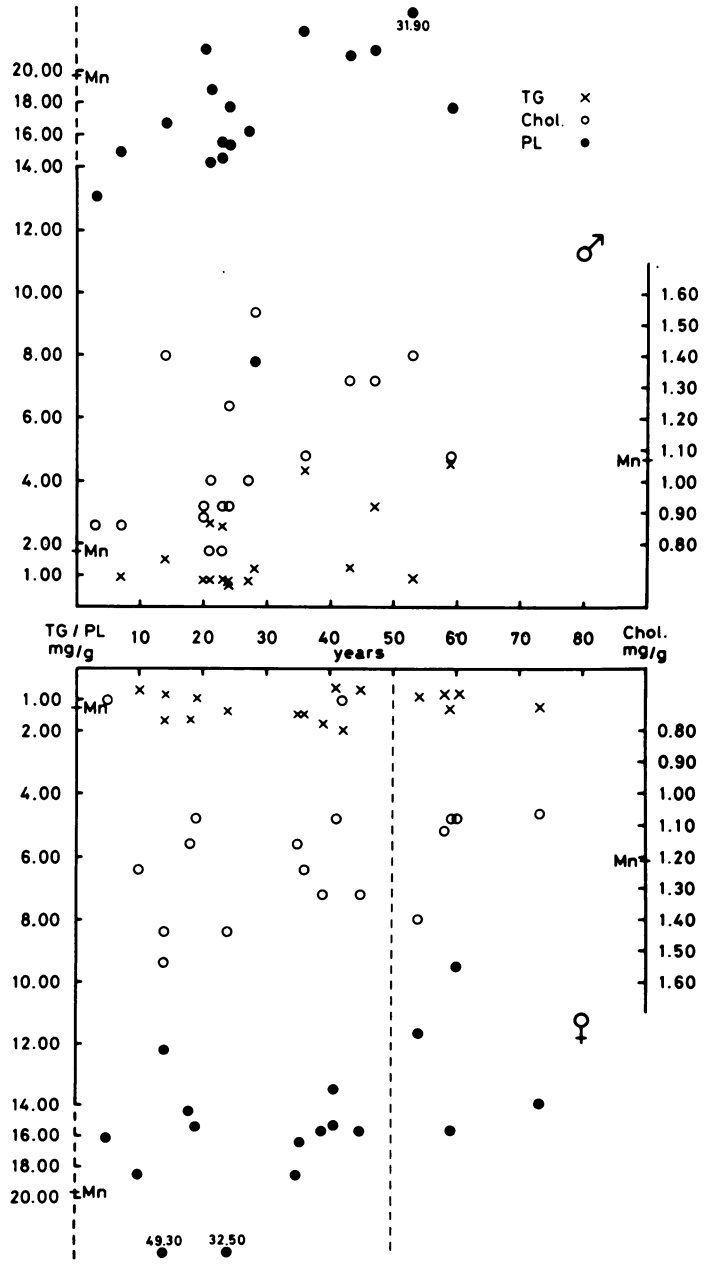

Fig 1 *Triglyceride* (TG), *cholesterol* (Chol), *and phospholipid* (PL) *values of papillary muscle in the normal or reference group. Every case is illustrated separately with three signs on one vertical line on the age abscissa. Mean values* (Mn) *are marked on the ordinate. The forensic groups of older women with mild coronary sclerosis or cardiac hypertrophy (right from the dashed line) have been added since no females aged more than 50 years without any disease could be found. No trend of the lipid values is demonstrable with age.*

		Triglycerides (mg/g)		*Cholesterol (mg/g)*		*Phospholipids (mg/g)*	
		Hospital Cases	*Forensic Cases*	*Hospital Cases*	*Forensic Cases*	*Hospital Cases*	*Forensic Cases*
Healthy cases or control group							
	M		1·77 ± 1·30 (16)		1·07 ± 0·24 (16)		17·70 ± 5·15 (17)
	F		1·25 ± 0·48 (12)		1·21 ± 0·22 (13)		19·65 ± 10·21 (13)
Hypertension or cardiac hypertrophy							
	M	1·39 ± 0·77 (12) NS		1·04 ± 0·28 (12)		15·96 ± 4·04 (12) NS	
	F	0·98 ± 0·25 (6) NS		1·25 ± 0·20 (6)		15·30 ± 3·34 (6) NS	
Cholelithiasis							
	M	1·13 ± 0·59 (4)		1·35 ± 0·35 (4) NS		14·05 ± 4·38 (4) NS	
	F	3·38 ± 2·36 (11) $P < 0·002$		1·32 ± 0·25 (11) NS		19·26 ± 4·79 (10)	
Diabetes							
	M	8·12 ± 0·54 (2) $P < 0·02$		1·50 ± 0·25 (2) NS		15·50 ± 8·48 (2) NS	
	F	6·85 ± 5·66 (9) $P < 0·02$		1·43 ± 0·23 (9) NS		15·21 ± 7·02 (9) NS	
Coronary atheroma Mild	M	1·31 ± 0·22 (4)	1·16 ± 0·60 (10)	1·09 ± 0·14 (4)	1·14 ± 0·15 (10)	15·67 ± 2·85 (4)	16·25 ± 4·47 (10)
	F	2·74 ± 1·01 (2)	1·03 (1)	1·08 ± 0·17 (2)	1·08 (1)	13·30 ± 2·26 (2)	15·80 (1)
Coronary atheroma Severe	M	4·48 ± 4·25 (10) $P < 0·05$	2·03 ± 2·43 (9)	1·35 ± 0·28 (10) $P < 0·05$	1·09 ± 0·33 (9)	16·10 ± 4·15 (8)	14·74 ± 2·11 (8) $P < 0·05$
	F	3·65 ± 3·94 (7) NS	1·03 ± 0·24 (2)	1·50 ± 0·28 (7) $P < 0·05$	1·08 ± 0·00 (2)	16·70 ± 2·90 (7)	11·70 ± 3·25 (2) NS

Table *Mean values and standard deviations of triglyceride (TG), cholesterol (Chol), and phospholipid (PL) values in the different groups*

The amount of lipid is expressed as mg/g of wet weight of tissue. The statistical significance of the differences between the disease group and the control group was calculated with the Mann-Whitney U test. The number of patients in each group is given in parentheses after the mean value. The significance of the change is given below the mean.

1·77 ± 1·30 mg/g and 1·25 ± 0·48 mg/g respectively. The cholesterol (1·07 ± 0·24 mg/g and 1·21 ± 0·22 mg/g) and phospholipid (17·70 ± 5·15 mg/g and 19·65 ± 10·21 mg/g) values were on average higher in women (see table). None of the sex differences, however, were statistically significant. The lipid concentration in men seemed generally to rise slightly with age, but not in the hypertension or mild coronary atheroma group, which showed patients aged 70-80 years with normal values. No trend of elevation with age was observed in women either (fig 1). A few very high values for phospholipids (over 30 mg/g) were encountered both in men and women (fig 1).

HYPERTENSION AND CARDIAC HYPERTROPHY GROUPS

A slight decrease (28%) of the phospholipid value was observable in women, but it was not significant by the U test. The cholesterol concentrations were similar to those in the control group (see table). The average triglyceride values were low in both sexes 1·39 ± 0·77 mg/g (= lower by 21% than in the controls) in females. However the differences from normal were not statistically significant.

CHOLELITHIASIS GROUP

The level of triglyceride was superficially increased in women ($P < 0·002$), the mean being 3·38 ± 2·36 mg/g, ie, higher by 170% than in the control group. The triglyceride content in men did not differ from that of the controls. The mean cholesterol values in men and women were slightly elevated, but the changes were not significant (see table). Phospholipids were about equal to the control values. The lipid contents did not vary significantly with age (fig 2).

DIABETIC GROUP

Triglycerides were significantly ($P < 0·02$) increased both in men (mean 8·12 ± 0·54 mg/g) and in women (6·85 ± 5·66 mg/g) (see table). The cholesterol mean values were also elevated by 43% (1·50 ± 0·25 mg/g) in men and by 18% (1·43 ± 0·23 mg/g) in women, but the changes were not significant because of the great variation. In women, phospholipids were lower by 23% (15·21 ± 7·02 mg/g) than the reference

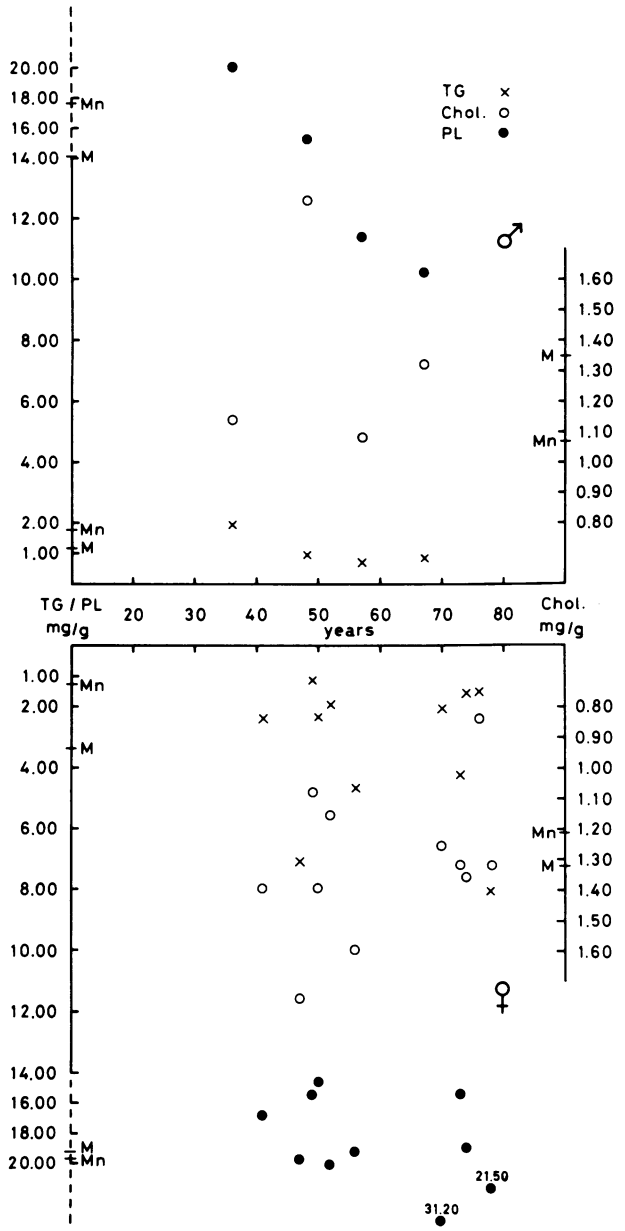

Fig 2 *Lipid values of the papillary muscle in cholelithiasis cases. Every case has three signs on the same vertical line. The mean* (M) *and normal mean* (Mn) *are marked on the ordinate. Cholesterol values are high in many cases, but the mean value does not differ significantly from the control. Triglycerides were significantly elevated in women* (*U test*, $P < 0{\cdot}002$).

value, but the difference was not significant. The lipid contents did not show any general trend attributable to age (fig 3).

MILD CORONARY SCLEROSIS

Lipid concentrations were about the same as in the control material. The only group showing possible deviation was that of the female hospital cases: triglycerides were higher by 119% (2·74 ± 1·01 mg/g) and phospholipids lower by 32% (13·30 ± 2·26 mg/g) than the reference values. The number of cases in this group was only two, but the trend is the same as that seen more definitely in the severe coronary atheroma cases (see also fig 1, females).

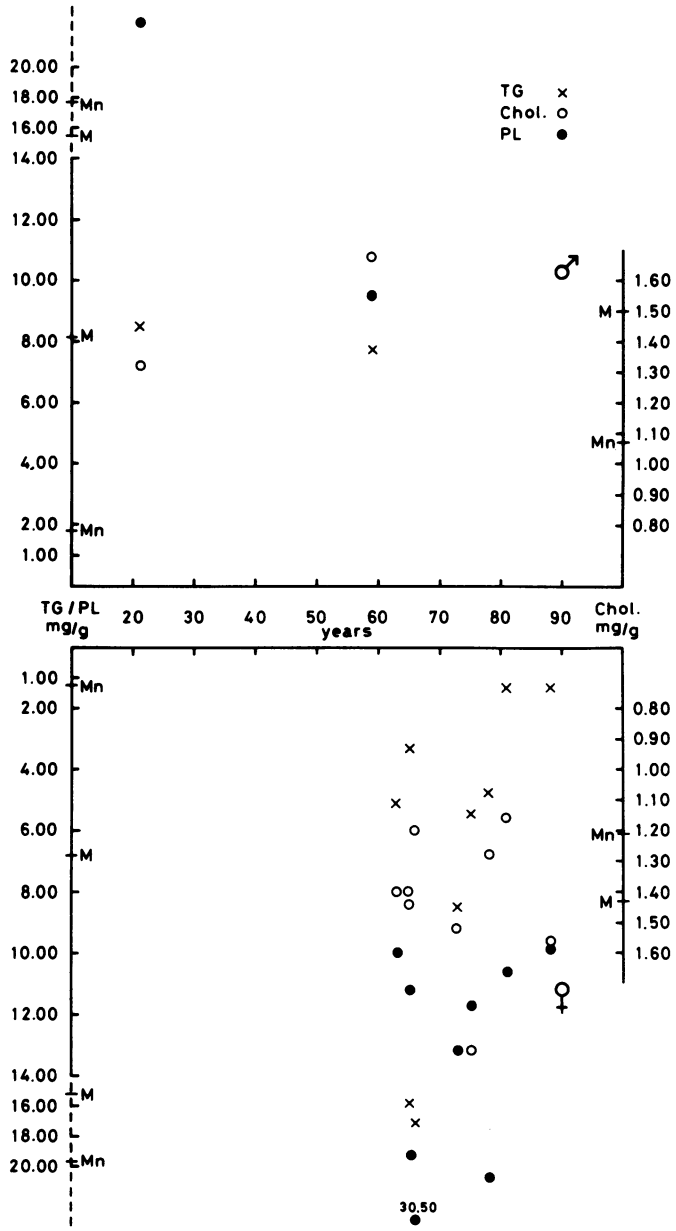

Fig 3 *Lipid values of the papillary muscle in diabetic patients. The mean* (M) *and normal mean* (Mn) *are marked on the ordinate. Note the significantly increased mean value for triglycerides in both sexes* ($P < 0.02$). *Elevation of cholesterol mean values was not significant. The trend of low phospholipid content is also noticeable in individual cases.*

SEVERE CORONARY ATHEROMA

Several abnormal lipid values were found in this group (see table). The mean triglyceride concentration of the male hospital cases was 4·48 ± 4·25 mg/g, an increase of 153%, which was statistically almost significant ($P < 0.05$). There were two female hospital cases with very high triglyceride values causing the mean value to be higher by 192% (3·65 ± 3·94 mg/g) than the reference, but because of the large variation in the group the change was not significant (fig 4). Among the forensic cases only one man had an increased triglycerides value which was as high as 8 mg/g.

In the group of hospital cases, the mean values for

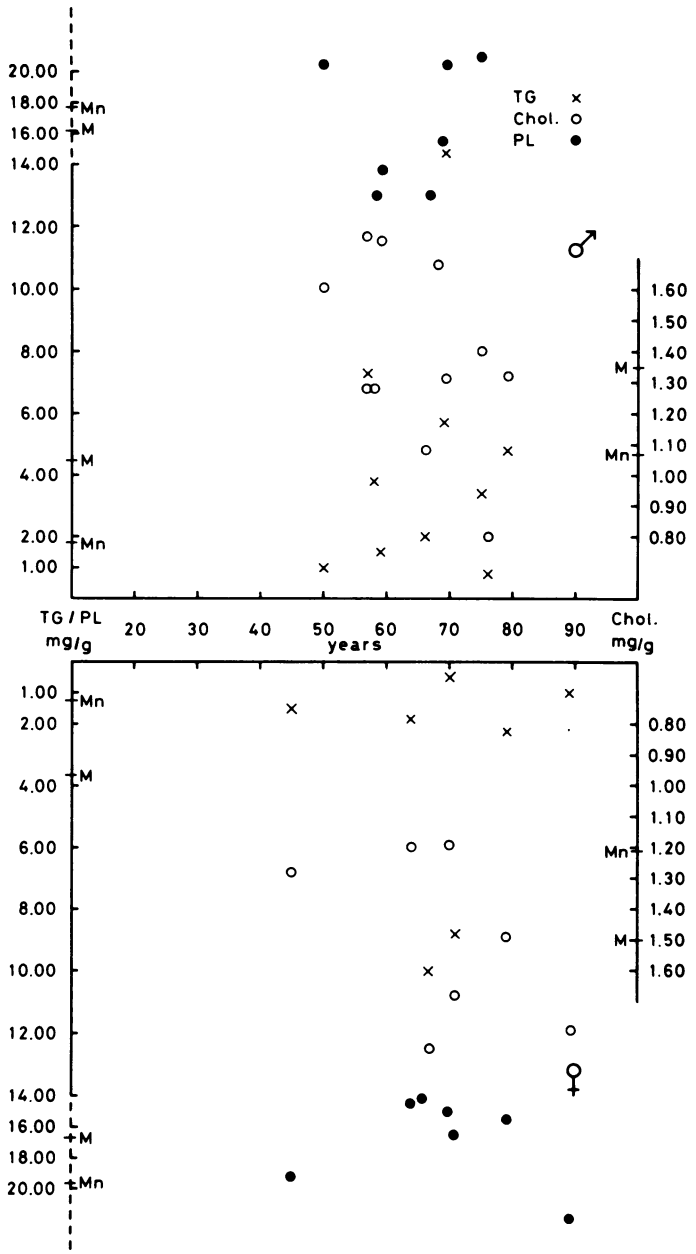

Fig 4 *Lipid values of papillary muscle in hospital cases with severe coronary atheroma. The mean* (M) *and normal mean* (Mn) *are marked on the ordinate. High content of triglyceride (increase almost significant in males,* $P < 0.05$) *and cholesterol (almost significant in females,* $P < 0.05$) *is observed in many cases.*

cholesterol were 1·35 ± 0·28 mg/g in men (an increase of 26%) and 1·50 ± 0·28 mg/g in women (an increase of 24%). The changes were almost significant ($P < 0.05$). In the forensic series, cholesterol was within the normal limits. The means for phospholipid were slightly lower in the forensic group both in men (14·74 ± 2·11 mg/g) and in women (11·70 ± 3·25, two cases). The decrease of 17% in men was almost significant ($P < 0.05$).

Discussion

Lipid analysis of postmortem samples involves several factors which cause difficulties in the com-

parison of results obtained by different investigators. Comprehensive reviews of the clinical, postmortem, and technical variables are given in the articles of Khan, Cox, and Asdel (1963) and Lindlar and Zaki (1966). The amounts of lipid in cardiac papillary muscle obtained in the present study are within the same range as those reported in most previous studies (Khan *et al*, 1963; Lindlar and Zaki, 1966; Nieminen, 1967). As to the amount of triglycerides in the heart, Wheeldon *et al* (1965) reported triglyceride values as high as 7·8 mg/g (converted from 8·9 mmol/kg) in the ox heart. Their sample was a whole heart ventricle which probably contained more adipose tissue on average than does the papillary muscle. Cholesterol and phospholipid values were within the same range as in the present analysis. Autolysis might have affected the present results of triglyceride assays, although this is unlikely, since little lipid breakdown occurs in the myocardium for the first two days after death (Lindlar and Zaki, 1966). Variation of water content might affect the results, because the amount of water in tissues depends on the agonal factors.

Earlier histochemical reports have made it clear that the fatty change of the myocardium is due to accumulation of neutral lipids (*eg*, Lindlar and Zaki, 1966). In a histochemical analysis of a part of the present material it was found that the papillary muscle arteries and chorda tendineae mainly take up cholesterol esters (Alavaikko and Hirvonen, 1971; Alavaikko and Hirvonen, 1973). Fatty changes in these tissue components did not correlate with the grade of extramural coronary atheroma, but factors predisposing to coronary atheroma—diabetes, cholelithiasis, and the climacteric—seemed to be of more importance (Alavaikko *et al*, 1970).

The increased amount of triglyceride in our diabetes group is consistent with our histochemical observations and is probably due to diabetic lipaemia. A distinct increase of triglycerides was also found in hospital cases with severe coronary atheroma, but such a rise was not observed in the forensic cases of equal severity. Possibly, coronary sclerosis must be so far advanced that cardiac dysfunction follows before accumulation of lipids begins in the muscle. The coronary atheroma patients who died in hospital were patients who had this disease as the primary cause of death, whereas the forensic cases were able to live normal lives indicating good cardiac function. Utilization of exogenous triglycerides by the myocardium is suppressed in hypoxaemic conditions (Yasuda, 1968), and this fact partly explains the increased accumulation of triglyceride in severe coronary atheroma. The finding that papillary triglycerides were also high in female cholelithiasis patients agrees well with the earlier histochemical observations. Accumulation of lipids generally (Alavaikko *et al*, 1970), and of triglycerides more specifically (Alavaikko and Hirvonen, 1973) was noticed in the myocardial parenchyma and arteriolar walls. High content of triglyceride in the myocardium of cholelithiasis cases may be attributed to hyperlipaemia resulting from a lipid metabolic error and/or dietary factors. Obesity as such was found to have no effect on the lipid values in this material.

Low levels of triglyceride in our present hypertension or cardiac hypertrophy group were somewhat inconsistent with our earlier histochemical studies which revealed moderate fatty change in hypertonic cases with Oil Red O staining (Alavaikko *et al*, 1970). The material of the two studies was derived from different cases and the methods are not comparable, which may explain the discrepancy. The low level of triglycerides may be due to the increased demands of fuelling the strained myocardium in hypertension.

It was concluded from our histochemical studies that an abundance of cholesterol esters accumulates in the chorda tendineae and arterioles of patients with diabetes or gallstones. Cholesterol and phospholipids are normal structural lipids in the myocardial cells and do not participate in myocardial fatty change (Lindlar and Zaki, 1966; Alavaikko and Hirvonen, 1973). The variation in cholesterol and phospholipid values noted in the present study can be partly due to variation in water content. Another factor is also likely to have affected the results, namely, disintegration of muscle cells and increase of scar tissue in the papillary muscle. Degenerative changes are particularly common in the papillary muscle (Brand, Brown, and Berge, 1969). The observation that phospholipid tended to be low in the disease groups, especially in the ones with pathologically high triglyceride values, can be explained by disintegration of muscle cells. Cholesterol values showed an opposite trend. Slightly elevated concentrations were found in diabetes, cholelithiasis, and the severe coronary atheroma of hospital cases. One of our histochemical findings was that cholesterol esters accumulate in the chordae and arterioles of patients suffering from these illnesses. The increase of cholesterol values was not statistically significant in the present study, apparently because the pathological or non-structural cholesterol represents only a small proportion of the total papillary cholesterol.

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