

Lipids in cadaver sera after fatal heart attacks

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SYNOPSIS Blood lipids have been measured in sera from 750 cadavers. All nine fractions measured show a remarkably consistent pattern of differences between the various gross morbid anatomical forms of ischaemic heart disease. The highest mean values are found when an obvious coronary thrombus is present and those in simple atheromatous occlusion are nearly as high. When there is recognizable necrosis the values are considerably more normal.

The serum lipids show relatively little change due to autolysis, about one half of 1% per hour or less.

Lipoprotein levels assayed with *Cl. welchii* alpha toxin correlate well with disease states.

Despite the precision of modern clinical diagnosis details of many morbid anatomical states can still only be ascertained at necropsy. Thus, in ischaemic heart disease, myocardial necrosis can confidently be predicted in a clinical attack but the presence or nature of arterial obstruction can only be inferred. In consequence, in most biochemical investigations, the label coronary thrombosis is used as a synonym but, whatever the frequency of minor mural thrombi, gross thrombi occluding the lumen are in fact relatively uncommon. This report describes consistent differences in the serum lipids between various morbid anatomical variants of the syndrome in a series of fatal cases. It also describes the effect of autolysis on the lipids, and contains data on the elegant method of Perrin (1959) for assessing lipoprotein with *Cl. welchii* alpha toxin lecithinase.

MATERIAL AND METHODS

About 750 cadaver arm vein sera have been examined over four years (Enticknap, 1960a and b). About 200 were rejected because of infection or haemolysis. Most of the subjects had died unexpectedly and they are grouped by causes of death in Table I. Atherosclerosis was assessed in six sites giving an index up to a maximum of 24. No special techniques were used to discover small thrombi, the cases being diagnosed as coronary thrombosis or atheromatous occlusion depending on whether the lumen was occluded by ante-mortem clot obvious to the naked eye after multiple incisions.

TOTAL SERUM CHOLESTEROL This was estimated by a Liebermann-Burchard colour development technique

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using the rather large amount of 0.2 ml. sulphuric acid. The acid ferric chloride methods give very high blanks with cadaver sera.

TOTAL SERUM ESTERIFIED FATTY ACIDS The triolein standard of Stern and Shapiro (1953) was used. The 95% range of 104 normal blood donors was 5.7-12.3-18.9 mEq./l. and there was no sex difference.

LIPOPROTEIN Perrin's (1959) technique was simplified: the change in opacity in 18 hours at 37°C. of 0.3 ml. serum in 3 ml. Sorenson's borate buffer pH 7.61 containing 0.025 M CaCl₂ and 0.8 Wellcome units of *Cl. Welchii* alpha toxin (2 mg. of preparation AGX 1552) in a 1 cm. cup at 650 m μ was directly recorded. Electrophoresis allowed visual assessment of the fractions (Dangerfield and Smith, 1955) and prebeta lipid was recorded in Smith (1957) units. Beta-lipoprotein was also recorded by a modification of the original polysaccharide flocculation phenomenon of Bunstein and Samaille (1956); the opacity developed by 0.2 ml. serum in 2.0 ml. of 0.06 M CaCl₂ on treatment with 0.04 ml. 1% calcium heparin was referred to Kunkel's (1947) barium sulphate standard, and assigned a value of 10 units with an Ilford 609 filter. The 95% range of 104 normal blood donors was 3.0 - 9.6 - 16.1 units and the original data suggest that one unit is about 0.4 g.

RESULTS

There are slight changes in some of the lipid values with time elapsing between death and necropsy (Table II). They show a diphasic pattern of accumulation in the serum around a skew rising mean with a declivity at around the end of the second day. In disease groups which were large enough to give

TABLE I

CLASSIFICATION BY CAUSES OF DEATH AND DISTRIBUTION OF SERUM LIPIDS

	<i>Ischaemic Heart Disease</i>					<i>Other Forms of Heart Disease</i>	<i>Other Forms of Arterial Disease</i>	<i>Control Cases</i>
	<i>Coronary Occlusion by Atheroma</i>	<i>Myocardial Fibrosis</i>	<i>Myocardial Necrosis</i>	<i>Coronary Thrombosis</i>	<i>All Cases</i>			
Average age in years	62	74	69	68	66	68	69	62
Proportion of males to females	1.7	0.8	0.9	1.1	1.2	1.9	0.5	0.9
Index of atherosclerosis	12	12	14	13	13	8	10	6
<i>Total serum cholesterol</i>								
Mean value (mg./100 ml.)	262	251	205	306	260	197	231	175
Standard deviation	83	65	78	85	83	78	90	71
Number of observations	17	15	15	7	54	11	14	46
<i>Total serum fatty acids</i>								
Mean value (mEq./l.)	19.3	13.7	15.1	15.5	15.7	11.9	15.9	12.9
Standard deviation	8.4	3.9	5.7	5.0	7.1	4.3	9.2	5.0
Number of observations	71	13	41	27	152	25	55	85
<i>Total lipoprotein</i>								
Mean values (α toxin dE _{1cm.} ⁶⁵⁰)	0.53	0.45	0.35	0.54	0.47	0.28	0.45	0.30
Standard deviation	0.27	0.40	0.32	0.24	0.35	0.21	0.33	0.18
Number of observations	39	7	21	18	85	20	33	43
<i>Beta lipoprotein</i>								
Mean value (heparin units)	16.9	14.1	14.1	22.7	16.1	11.8	15.3	11.0
Standard deviation	6.0	4.5	6.9	2.5	6.5	7.0	7.5	5.7
Number of observations	12	7	8	3	30	8	7	35
Mean value (electrophoretic units)	1.4	1.0	1.4	1.6	1.4	1.0	1.0	1.1
Standard deviation	0.48	1.0	0.73	0.6	0.74	0.85	0.68	0.77
Number of observations	71	11	38	23	143	17	27	92
Mean value (Smith units of pre-beta lipid)	2.0	1.6	2.3	3.1	2.2	1.4	1.5	1.2
Standard deviation	1.61	1.82	1.64	1.94	2.24	1.58	1.41	1.55
<i>Alpha/beta ratio</i>								
Mean value (normal = 1.0)	0.41	0.54	0.65	0.35	0.47	0.81	0.64	0.67
<i>Alpha lipoprotein</i>								
Mean value in electrophoretic units	0.7	0.5	0.8	0.7	0.7	0.7	0.8	0.7
Mean opacity of serum diluted 1 : 10 in borate buffer (E ₆₅₀)	0.23	0.19	0.19	0.18	0.21	0.20	0.19	0.16

TABLE II

CHANGES IN BLOOD LIPIDS WITH INCREASING INTERVALS BETWEEN DEATH AND NECROPSY

	<i>0-12 Hours</i>	<i>13-24 Hours</i>	<i>25-36 Hours</i>	<i>37-48 Hours</i>	<i>49-60 Hours</i>	<i>More than 60 Hours</i>	<i>Mean Hourly Increment</i>
Cholesterol (mg. per 100 ml.)							
Mean value for all sera tested	195	207	213	232	200	215	0.5
Total fatty acids (mEq./l.)	11.8	13.2	13.4	15.8	11.2	13.6	0.04
<i>Beta lipoprotein</i>							
(a) heparin units	8.3	11.3	13.5	8.5	15.0	14.9	0.1
(b) Sudan black units after electrophoresis	0.9	1.2	1.4	1.2	1.4	1.4	0.01
(c) Prebeta lipid in Smith units	1.1	1.8	2.0	1.8	2.1	2.1	0.01
Lipoprotein (Perrin) (dE ₆₅₀)	0.49	0.35	0.37	0.50	0.35	0.40	—
Alpha/beta lipoprotein ratio (normal = 1)	0.8	0.5	0.8	0.5	0.5	0.7	—
Alpha lipoprotein	0.6	0.7	0.8	0.7	0.8	0.7	—
Gamma globulin	1.2	1.2	1.3	1.2	1.2	1.3	—
Serum opacity	0.05	0.18	0.30	0.18	0.16	0.12	—

informative means the pattern was similar. A mean hourly increment was calculated from lines of best fit. The disease groups were all of comparable mean

age and the necropsies were performed at fairly uniform mean time intervals.

The mean values of the serum lipids have been

grouped (Table I) to show the changes attributable to ischaemic heart disease, its four morbid anatomical variants of simple atheromatous coronary occlusion, myocardial fibrosis, myocardial necrosis, and coronary thrombosis, and to enable the effects of arterial disease not primarily affecting the heart (systemic atherosclerosis) and heart disease not due to vascular occlusion to be assessed. All other causes of death formed the control group, with the exception of cancer which caused uniformly low lipid values and so was excluded. This control group was analysed into six components but no consistent differences were seen.

There was a close correlation between the serum fatty acid content and lipoprotein measured by lecithinase with lipaemia assessed both with the naked eye and photometrically (Fig. 1). Eight out of 10 milky sera occurred in ischaemic heart disease. Numerous calculations show that haemolysis does not regularly affect the various lipid fractions of

cadaver sera nor do they correlate with the index of atherosclerosis at all closely. There was no consistent relationship with the state of the stomach contents and, compared with the controls, there was no significantly greater frequency of recent ingestion in the subjects with ischaemic heart disease. There is a significant, but not close, correlation between the individual values of fatty acids and alpha-toxin units of lipoprotein in 200 sera ($r = 0.33$; s.e. 0.071).

DISCUSSION

These four semantic variants of ischaemic heart disease are commonly used on death certificates despite the overlap between their morbid anatomical counterparts. Their proportions of approximately 43, 10, 25, and 15% respectively are quite characteristic of the more sudden seizures. This investigation was begun to elucidate those cases, here referred to as coronary occlusion, in which death was undoubtedly due to acute cardiac ischaemia but in which careful *post-mortem* dissection does not reveal any recent gross changes in myocardium or arteries. As they show no obvious thrombotic occlusion and, indeed, unless special techniques are used, no thrombi at all, the mechanism of death is obscure. Enzyme assays are consistent with the absence of necrosis in these cases (Enticknap, 1960b). This investigation shows abnormalities of their serum lipids which present rather more clear differences than those found when necrosis is established. Further, it demonstrates that while the most abnormal lipids are found in those cases with an obvious thrombus, the cases of occlusion resemble them fairly closely.

Autolytic changes must be considered. Laves (1960) has shown that the ultra-violet absorption spectrum of the blood is changing even within the moments before death. Other substances, particularly serum enzymes, also change with extreme rapidity (Enticknap, 1960b) and rise to very high levels. Serum lipids, on the other hand, although showing qualitatively similar changes, are quantitatively much less altered, and corrections based on the increments in Table II do not affect the argument. In 1936, Landé and Sperry, at Schonheimer's suggestion (Sperry, 1957), made *post-mortem* cholesterol analyses and Faber (1946) considered that any differences he encountered in serial estimations were within the limits of experimental error. More recently Paterson and Dyer (1959) have measured *ante-* and *post-mortem* cholesterol analyses in a closed population and concluded that they were comparable, provided that death was sudden, and Glanville (1960) has reported on hospital subjects. Schlang and Davis (1958), Schleyer (1954), and Spain, Bradess, and Greenblatt (1954) have all reported satisfactory

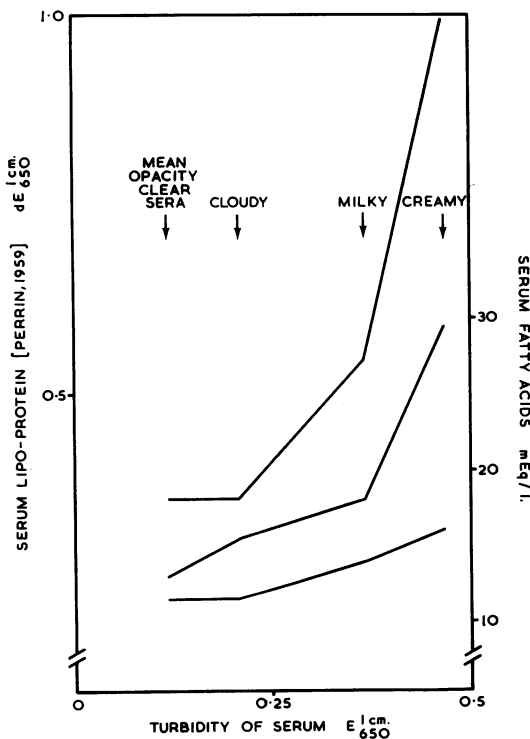


FIG. 1. The relationship between serum turbidity and its content of lipoprotein and fatty acids. The four points represent groups of sera considered visually to be comparable. In the top line the mean opacity of groups of cadaver sera is plotted against lipoprotein content (Perrin, 1959), in the second line against the mean fatty acid content of these same groups, and in the third line against the mean fatty acid content of groups of blood donor sera.

separations of cadaver serum proteins. It appears legitimate, therefore, directly to correlate lipid analyses on cadaver sera with disease states.

While the level of total esterified fatty acids appears to be the most useful test in confirming a necropsy diagnosis of simple coronary occlusion as a cause of death, the other fractions all appear to be of about equal value. Perrin's technique for total lipoprotein yields results with a fairly wide spread of differences between the syndromes and also gives very high values in the puzzling cases of occlusion, but the discrimination between the various groups is insufficiently good to justify the routine use of this rather complex method. He shows that it correlates with phospholipids in his Fig. 1 but no coefficient is given. In this series a loose relationship with total esterified fatty acids is shown but the technique clearly does not simply measure this parameter.

The lipids are most raised in the ischaemic heart disease group and moderately raised in the systemic atherosclerosis group, and many of the differences attain conventional significance ($p < 0.05$). Thus ischaemic heart disease has a significantly higher mean cholesterol level than heart disease not due to vascular occlusion (standard error of difference 25 mg./100 ml.) and than the control group (s.e. diff: 15), but not than systemic atherosclerosis (s.e. diff: 27). Heart disease not due to vascular occlusion, on the other hand, closely resembles the controls, there being in fact no significant differences (s.e. diff: for pre-beta lipid: 0.34). Beta-lipoprotein and pre-beta lipid also differ significantly in the ischaemic heart disease and systemic atherosclerosis groups (s.e. diff: 0.147 and 0.103). Data from 13 of the cases of Landé and Sperry (1936) and from 28 of Faber's (1946) cases can also be arranged to compare these groups and show a similar small mean cholesterol difference to that reported here (31, 28, and 29 mg. per 100 ml. respectively).

The differences between the various forms of ischaemic heart disease are, however, more interesting and cannot be determined in clinical cases. The lipids in myocardial fibrosis, in which the heart failure is less sudden, are among the lowest recorded in fatal atherosclerosis and closely resemble those of non-cardiac arterial disease, there being no significant differences in this series. The mean fatty acid level is significantly lower than in coronary occlusion (s.e. diff: 1.66) and most of the other values differ markedly from those found when a thrombus is present.

Coronary thrombosis, on the other hand, is associated with the most abnormal pattern. Beta-lipoprotein is significantly greater than in any other group (s.e. diff. from coronary occlusion; 2.25) and so is pre-beta lipid (s.e. diff. from myocardial

necrosis 0.22). This is in fact the biggest mean increase of any fraction, being more than 2.5 times that of the control group mean. The mean cholesterol level (s.e. difference 38), total lipoprotein (s.e. diff. 0.04), and the alpha-beta ratio all differ significantly from those of myocardial necrosis although not from those of the rest of the group. Thus the cases of myocardial necrosis have a significantly lower cholesterol, total lipoprotein, beta lipoprotein, and pre-beta lipid, and higher alpha-beta ratio than cases in which a thrombus was present.

The cases of coronary occlusion by uncomplicated atheroma share the highest mean levels with the thrombotic cases. Significant differences are seen only in beta lipoprotein and in pre-beta lipid but the serum fatty acids are higher in this class than in any other. Apart from this all the other most abnormally raised mean values occurred in the group of cases of coronary thrombosis (italics in Table I).

Two conclusions emerge. First, when cases of fatal ischaemic heart disease are grouped by conventional naked-eye necropsy criteria consistent differences are observed in the levels of the serum lipids. This fact is consistent with the hypothesis that the causes of death certified may have different mechanisms. Secondly, stoppage of the heart when the myocardium is apparently normal or when necrosis is minimal occurs when the blood lipids are most markedly abnormal. The more abnormal they are the more likely is a large thrombus to be present; established necrosis, on the other hand, is found when the lipids are much less abnormal. In any event the similarity between the findings in simple and thrombotic occlusion is confirmatory evidence that the former may indeed cause death when there is no evidence of gross recent thrombotic or necrotic change.

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