THE INVESTIGATION OF LIPOPROTEINS IN LYMPH AND OTHER BODY FLUIDS BY PAPER ELECTROPHORESIS

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The protein and lipid electrophoresis patterns of lymph and various body fluids have been examined. Chylous fluids produce a characteristic lipid pattern with a dense deposit at the origin and a lipid trail. The value of centrifuging turbid fluids at high speed before electrophoresis is stressed.

During the course of work on the treatment and pathology of lymphatic disease a number of specimens of lymph were collected and examined by paper electrophoresis. The protein and lipid patterns obtained have been compared with those from various other body fluids, and a few additional simple investigations made to assist in the understanding of the lipid patterns.

As far as we are aware the lipid patterns of human lymph and body fluids have not been previously reported, although Courtice and Morris (1955) investigated the proteins and lipids of animals and their results are, in general, similar to ours. The protein contents and electrophoretic patterns of body fluids have been investigated by various workers, e.g., Crockett (1956) and Taylor, Kinmonth, and Dangerfield (1958).

Methods

Collection of Lymph.—Normal lymph was collected by needle aspiration from either the thoracic duct in the neck or one of the other major cervical lymph channels during the course of routine surgical operations.

Abnormal lymph containing chyle was obtained in a similar way during operation upon abnormally large trunks in the pelvis or groin of patients suffering from chylous reflux and lymphoedema. In these patients the retroperitoneal lymphatic trunks are congenitally dilated and incompetent and allow retrograde flow of lacteal fluid. With this abnormality lymphatic vesicles often occur on the skin of the thigh and perineum, and these leak a chylous fluid. Examination of fluid from such a vesicle is referred to below.

Laboratory Methods.—The specimens of lymph were taken to the laboratory in the syringe in which they were collected and paper electrophoresis started without delay employing the methods previously described (Dangerfield and Smith, 1955), using light green SF and sudan black B for staining protein and lipid respectively.

Towards the end of this investigation a high-speed centrifuge was acquired and a few specimens were centrifuged at speeds up to 12,000 r.p.m.; usually the specimens were layered under water and centrifuged, when the fat particles migrated to the top of the water layer and could be separated. Very small quantities of fat can be detected in this layer, particularly if a transparent centrifuge tube (glass or nylon) is used and the column of liquid examined in a powerful beam of light.

Assessment of Patterns.—The usual five protein bands were easily identified, but with the lipid patterns there is some difficulty in identification. We attempted to discern five lipid bands as follows:

- LDO —Lipid deposit at origin (due to chylomicrons)
- LTrail—Lipid "trail" extending from origin to β lipoprotein and sometimes beyond (probably due to smaller fat particles)
- β -LP — β lipoprotein, a narrow band level with β globulin
- α -LP — α lipoprotein level with α_1 globulin
- LA —"Lipalbumin" (probably due to "free" (unesterified) fatty acid adsorbed on albumin)

It is very difficult to decide whether staining in the β globulin region should be attributed to β lipoprotein or to the lipid "trail." In normal sera patterns the β lipoprotein is a sharp, well-defined band whereas the trail is a wide band seen most characteristically in lipaemic sera and chylous fluids. This difference does not provide a satisfactory method of distinction, because the sharpness and even the position of the bands vary slightly with unavoidable

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TABLE I
SUMMARY OF RESULTS

Fluid	No. of Samples	Approximate Protein Content (%)	Main Protein Characteristics	Main Lipid Characteristics	Fluid	No. of Samples	Approximate Protein Content (%)	Main Protein Characteristics	Main Lipid Characteristics
<i>Lymph</i> Cervical ..	4		Similar to serum but weak α_2 globulin	Usually a weak β lipoprotein. Contaminated with blood	(g) Spermatocoe fluid	2	$\frac{1}{2}$	Albumin and tr. β globulin	Nil
Thoracic duct	7		" "	Dense deposit at origin and trail; β lipoprotein difficult to assess	(h) Amniotic fluid	2		" "	"
Chylous inguinal	4		" "	As thoracic duct lymph	(i) Bladder fluid	1	$2\frac{1}{2}$	Ill-defined pattern. Mainly albumin	"
<i>Clear fluids</i> (a) Oedema fluid Heart failure	4	$\frac{1}{2}$ -1	Weak albumin, v. faint β and γ globulin	Nil	(j) Hernial sac fluid	1	$\frac{1}{2}$	Weak albumin and tr. β globulin	"
Lymph-oedema	12	$\frac{1}{2}$ -3	Stronger bands than with heart failure fluids; α_2 globulin relatively weak	Nil or trace	(k) Branchial cyst fluid	1	—	Strong albumin and deposit at origin; some β globulin. Ill-defined pattern	Intense lipid deposit at origin, rich in cholesterol
(b) Hydrocele fluid	6	4-6	Similar to serum but α_2 globulin low	Usually some lipalbumin and occasionally α lipoprotein. No β lipoprotein	(l) Parotid fluid	1	—	Albumin and deposit at origin. Faint globulin bands	Nil
(c) Pleural fluid Heart failure	6	$1\frac{1}{2}$ -3	Very weak serum pattern	Traces of lipid	(m) Vitreous humour	1	—	Faint deposit at origin only	"
Carcinoma	7	4-5	Similar to serum	Similar to serum	<i>Turbid fluids</i> (a) Chylous ascitic fluid	7		Like weak serum	Dense deposit at origin; trail extends to α_2 globulin front. β lipoprotein difficult to assess
Other malignant disease	3	2-5	" "	" "	(b) Chylous pleural fluid	1		As above	As above
(d) Ascitic fluid Heart failure	4	2-4 $\frac{1}{2}$	Weak serum pattern, α_2 globulin low	Like dilute serum	(c) Chylous blister fluid	1		Like serum	" "
Cirrhosis ..	9	$\frac{1}{2}$ -2 $\frac{1}{2}$	Weak albumin and weak diffuse γ globulin, only trace of α_2 and β globulin	Traces only	(d) Chylous urine	3		Albumin and faint β globulin and deposit at origin	Very dense deposit at origin; trail very short or absent; no β lipoprotein
Carcinoma	5	4-5	Like serum	Like serum	(e) Ovarian cyst fluid	1		Ill-defined pattern, fairly high protein content	Strong deposit at origin, some lipalbumin
(e) Bursa fluid	4	—	" "	Nil "					
(f) Urine (nephrotic)	3	—	Varied protein patterns but α_2 globulin very low compared with patient's serum						

variations in electrophoretic technique, e.g., density of application and age of specimens. High-speed centrifugation helps in distinguishing these lipids, for the trail lipids are of low density and can be partly spun up into an overlying layer of water, whereas the β lipoprotein remains in the lower layer.

The separation of α lipoprotein and "lipalbumin" is also not entirely satisfactory. In spite of these limitations useful information can be gained from the lipid patterns, and their value grows as technique improves and as more patterns accumulate for comparison.

Results

An examination was made of 15 samples of lymph, four from the cervical lymph ducts, seven from the thoracic duct of patients with a normal lymphatic system, and four from the inguinal lymphatics of patients with chylous reflux. These have been compared with 72 clear body fluids and 13 turbid fluids. The clear fluids were mainly oedema, ascitic and pleural fluids, and the turbid ones were mainly chylous ascitic fluid and urine. The findings are summarized in Table I.

FIG. 1.—Electrophoretic strips of lymph and chylous fluids. (a) Cervical lymph, (b) thoracic duct lymph, (c) chylous lymph, (d) chylous lymph, (e) chylous ascitic fluid, (f) chylous urine.

Most of the fluids showed protein patterns related to that of serum and dependent on the total protein content, but the lipid patterns were less closely related to serum and dependent on the turbidity of the fluid as well as its total protein content.

The main classes of fluid examined will be considered briefly here.

Lymph.—Clear lymph from the cervical lymphatics gave protein patterns resembling those of serum, but the lipid patterns were either almost blank or showed a weak β lipoprotein band,

perhaps partly due to contamination of the specimen with blood (Fig. 1a).

All seven specimens of thoracic duct lymph showed protein patterns resembling serum, but the lipid patterns were dominated by dense deposits of lipid at the origin and in the trail, which in some cases extended forwards as far as the α_2 globulin (Fig. 1b). In five samples a β lipoprotein band was present, but in four of these there was evidence of contamination with blood, so it is uncertain how much β lipoprotein was present in the uncontaminated lymph.

Chylous lymph from dilated inguinal and iliac lymphatics gave patterns (Fig. 1c and 1d) similar to those from thoracic duct lymph with a very dense lipid deposit at the origin and in two cases a moderately dense trail. In one sample some fat particles were so large that they separated as a cream at the top of the milky specimen when it was stored overnight in a refrigerator.

Clear Body Fluids.—The clear fluids (and a few turbid ones that cleared on ordinary centrifuging) showed patterns consistently related to the total protein content, which ranged from 0.4% to 4.9% (Fig. 2). In general the patterns fell into three groups:

(a) Specimens with a low protein content (oedema fluids, spermatocoele and amniotic fluid) which, unless previously concentrated, showed only a definite albumin band and perhaps one or two faint globulin bands, β globulin usually being the most apparent; no lipid bands were visible (Fig. 2a).

(b) Fluids with an intermediate protein content (generally 2–4.5%) which showed the usual four or five protein bands but in altered proportions, and characteristically the α_2 globulin band was relatively weak; traces of lipid were sometimes visible but no definite β lipoprotein band (Fig. 2b).

(c) Fluids with a high protein content (4% or more) which gave protein and lipid patterns practically indistinguishable from those of serum (Fig. 2e, f, g).

Some of these clear fluids were centrifuged under water at 12,000 r.p.m. for one hour and examined in a focused beam of light. The lower layer scattered light strongly throughout its height, but the upper layer showed only very faint scattering indicating that only minute quantities of fat particles were present. A few of these fluids were treated with small quantities of dextran sulphate and calcium chloride in the manner of Burstein and Samaille (1958), when the pleural and ascitic fluids gave a precipitate of β lipoprotein but not the hydrocele fluids.

FIG. 2.—Electrophoretic strips of clear fluids. (a) Oedema fluid, (b) hydrocele fluid, (c) urine (nephrosis), (d) serum, (e) pleural fluid, (f) ascitic fluid, (g) bursa fluid.

One rare specimen that came into this survey was the fluid which had accumulated in a bladder that had been isolated surgically on account of a congenital obstruction of the bladder neck. This fluid contained 2.6 g.% protein, and although it was not viscous it produced a pattern with ill-defined protein bands and no lipid bands.

Nephrotic urines, although rich in protein, contained no lipid (Fig. 2c); the protein patterns generally showed little α_2 globulin, in striking contrast to the serum, where it was markedly increased. Two amniotic fluids showed only albumin, a faint β globulin, and no lipid bands.

Turbid Body Fluids.—Most of these were chylous fluids taken from patients with lymphoedema. Seven specimens of chylous ascitic fluid (Fig. 1e) and one each of chylous pleural fluid and chylous blister fluid produced patterns similar to those given by thoracic duct lymph and chylous inguinal lymph. All showed a dense deposit at the origin, but there was considerable variation in the intensity and extent of the lipid trail. It was extremely difficult to tell from these patterns whether any β lipoprotein was present. Unfortunately these fluids were aspirated before

the centrifuge had been acquired. Two specimens of chylous ascitic fluid that had been kept refrigerated or frozen for some months were centrifuged, when most of the turbidity rapidly migrated to the top of an overlying water layer. The lipid separated in this way was washed with water and analysed and found to be mainly neutral fat with relatively small proportions of cholesterol (4% and 7%) and phospholipid (13% and 18%).

Five specimens of chylous urine were examined, including three from one patient taken during the last month of pregnancy. (This patient produced markedly chylous urine during the last few weeks of her third, fourth, and fifth pregnancies; the condition disappeared gradually during the fortnight following delivery.) In all specimens albumin and β globulin predominated in the protein pattern, and there was a dense deposit at the origin in the lipid pattern (Fig. 1f). A very short trail was seen with two specimens, but in no case was a β lipoprotein band visible. Two specimens were layered under water and centrifuged, when the lipid particles rapidly migrated to the top of the water. Separation and analysis of this lipid showed that it was mainly neutral fat with small proportions of cholesterol (3% and 1%) and phospholipid (1.5% and 7%).

A specimen that was turbid but not chylous was taken from a pseudomucinous cyst. This dark, viscous fluid cleared on centrifugation at 12,000 r.p.m. for two hours, the turbid material settling to form a brown deposit. The clear brown supernatant fluid gave a strip with ill-defined protein bands; there was a dense deposit of lipid at the origin and a considerable lipid staining level with the albumin.

Discussion

Traditionally, extravascular collections of fluid are divided into two classes: exudates with high protein content and transudate with low protein. Our collection of fluids showed no clear dividing line, and in fact continuous ranges of protein concentrations and patterns were found.

The lipid patterns fell into three groups:

(a) Almost blank patterns given by fluids containing little or no lipid, such as cervical lymph and most oedema fluids.

(b) Patterns similar to serum, usually showing a sharp β -lipoprotein band, given by clear fluids with a high protein content; these fluids were generally of neoplastic or inflammatory origin.

(c) "Chylous" patterns showing a dense deposit at the origin and a moderately dense "trail" and sometimes a lipalbumin; these were given by

chylous fluids, thoracic duct lymph, and hyperlipaemic sera.

The lipid trail of chylous fluids is of particular interest; it is very probably due to a "spectrum" of particles migrating at diverse speeds, and probably of diverse sizes. They are presumably largely composed of neutral fat, and could be removed by centrifugation under water as with hyperlipaemic sera. When such a serum is centrifuged under water or saline much of the lipid responsible for the trail and deposit at origin in the original serum pattern migrates into the upper layer, and this layer on electrophoresis gives a very dense deposit at origin and usually only a very short trail (although with a few patients the trail is quite extensive).

The front of the lipid trail in patterns from chylous fluids is usually level with the α_2 globulin, but it is often ill defined and may vary in position with the details of electrophoresis or the amount of fluid applied. A similar "advanced front" to the β -lipid complex is seen in the serum patterns of patients with essential hyperlipaemia and in the lipaemia of uncontrolled diabetes (Wolff and Salt, 1958). Another condition in which the serum pattern shows an advanced front to the β -lipid complex is nephrosis, where a definite band can sometimes be discerned, a "pre- β lipid," and this band remains largely undissolved when a portion of the strip is extracted with ethyl alcohol before staining. In the case of chylous fluids and lipaemic sera the whole of the β lipid complex is extracted in alcohol, even its front edge. (An advanced β -lipid front is also given by stale sera.)

Smith (1957) has correlated for normal serum the paper electrophoretic components and the ultracentrifugal Sf groups of Gofman (de Lalla and Gofman, 1954). The deposit at the origin corresponds to particles with Sf values greater than 400 and the trail corresponds roughly to the Sf range 100–400; a lipid band, a "pre- β lipid," which in serum migrates between β globulin and the front of α_2 globulin, corresponds approximately with the range Sf 20–100. This correlation may not apply to lymph although there is no obvious reason why it should not do so. The presence of the origin and trail (presumably Sf 400 and Sf 100–400) lipoprotein groups in thoracic duct lymph suggests that this is a possible source of these groups in plasma and chylous fluids.

The conclusions that can be drawn from examination of paper electrophoresis strips must always be guarded, particularly where lipids are concerned. Although it is simple to produce strips giving a fair representation of the various proteins, considerably more skill and experience

are required to obtain a satisfactory comparative picture of the lipids. Nevertheless, carefully made strips give a more complete picture of the lipids than any other single method that can be carried out in the ordinary hospital laboratory. The difficulty in distinguishing between β lipoprotein and trail in chylous fluids may sometimes be overcome by high-speed centrifugation. Further, quantities of β lipoprotein too small to give a well-defined lipid band may sometimes be detected by dextran sulphate precipitation. Such a combination of methods offers a valuable means of lipid investigation.

Although the range of fluids examined here is quite limited, the following conclusions would seem permissible.

(1) A sharp β lipoprotein band will only be seen if the fluid has a high protein content and is clear.

(2) A chylous fluid gives a dense deposit at the origin and usually a lipid trail.

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