Lipoprotein Content of Human Pathological Serous Fluids.* (26548)

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The enigma of lipid transport in the aqueous system of the body has been partially clarified during the past 25 years by the demonstration in plasma of lipid-protein complexes that behave as soluble proteins(1,2,3). There is abundant evidence that body lipids are more active participants in metabolism than has been supposed(4) and that these lipoproteins play an active role in fat metabolism(5). It becomes of some importance to consider the mechanism of transport of these lipids across the body membranes.

Complexes of lipids and proteins were demonstrated in serous fluids 3 decades ago(6,7, 9). Lipoprotein transport from plasma to lymph has been investigated in animals by several investigators(9,10,11,12,13) and both labeled albumin and cholesterol have been found in the aortic wall of the dog(14). The present study is an extension of this problem to measurement of the lipid and protein composition of serous fluids obtained from diseased patients with special reference to lipoproteins which have not been previously described in such fluids.

Materials and methods. Thirty-three fluids were collected from patients at the time of diagnostic or therapeutic taps. They were analyzed after a preliminary low speed centrifugation at 800 g for 30 minutes to remove fibrin and cells. The chemical methods were applied to a 1:20 Bloor's extract of the fluids. Total lipid was determined according to the method of Bragdon(15) and expressed as milligrams of dichromate reduced per 100 ml. Phospholipid was measured by the method of Youngburg, Fiske, and SubbaRow as described in Hawk, Oser, and Summerson(16). Total and free cholesterol were measured by the method of Sperry(17). Triglyceride was derived by correcting total dichromate reduced by cholesterol and phospholipid and introducing Bragdon's factor (17.7 mg of reduced dichromate equivalent to 1 mg of triglyceride). Nitrogen was determined by wet digestion and Nesslerization as described in Hawk, Oser, and Summerson (18). Albumin was separated by the method of Pillemer and Hutchinson(19). Low density lipoproteins were measured by the ultracentrifugation method of DeLalla and Gofman(20). The high density lipoproteins were separated by the method of Green, Lewis, and Page(21). For lipoprotein analyses aliquots of fluid were taken for preparatory centrifugation and the supernate was concentrated 8- or 10-fold. The second milliliter at the top of the preparative tube was routinely taken for a final density measurement in a one milliliter pycnometer constructed from a volumetric pipette. Migration rates were then corrected to densities 1.0630 and 1.2100 for the low and high density lipoproteins respectively. In practice, these corrections were small. The concentrations of lipoproteins were derived from the deflection areas on the analytical ultracentrifuge film. The analytical centrifuge was calibrated with the Spinco reference cell.

Results. The data are arranged in Table I according to source of fluids and clinical diagnosis. Those fluids which appeared to result from an increase in pressure gradient across the vascular membrane, either hydrostatic or osmotic, are separated from those fluids which appeared to result from an irritation to the capillary membrane, either inflammatory or neoplastic. This distinction is readily apparent in the peritoneal fluids, but it is uncertain in several of the pleural fluids. These indeterminant fluids are termed "mixed" because of the probable combination of pressure and irritative factors and are described in Table II along with a miscellaneous group of fluids from other serous cavities.

There were readily measurable quantities of both low and high density lipoproteins in

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TABLE I.

Type	Clinical diagnosis	Total protein	Albumin	Globulin	Total lipid‡	Total chol.	Lipid phos.	Triglyceride	Lipoproteins Low density	Lipoproteins High density
Peritoneal										
(A)										
"Pressure"	Laennec's cirrhosis (4)†	2,336 ±	1,381 ±	956 ±	2,217 ±	45.2 ±	1.79 ±	19.8 ±	65.3 ±	66.5 ±
	Mitral stenosis									
n = 6*	(2)	894	926	520	826	19.1	.68	18	27.	42.
(B)										
"Irritative"	Carcinomatosis (5)	4,016 ±	2,434 ±	1,909 ±	4,632 ±	83.6 ±	3.08 ±	80.6 ±	144 ±	60.8 ±
n = 5		694	761	570	1,680	14.0	.29	127	16	57.
(B) - (A)	p§	<.02	<.1	<.01	<.01	<.01	<.01	<.05	<.01	
Pleural										
(C)										
"Pressure"	Congestive heart failure (4)	906 ±	525 ±	381 ±	2,556 ±	9.6 ±	.88 ±	160 ±	13.2 ±	3.5 ±
n = 4		161	215	99	1,069	2.8	.44	32.4	7.9	4.2
(D)										
"Irritative"	Tuberculosis (2) Lymphosarc. (1)	3,714 ±	1,849 ±	1,866 ±	3,914 ±	68.6 ±	2.40 ±	58.4 ±	136.4 ±	25.0 ±
n = 5	Pneumonia (2)	1,541	439	1,252	2,326		1.32	84.4	66.1	20
(D) - (C)	p§	<.01	<.01	<.01	.05–.01	<.01	<.01	<.01‖	<.01	<.02

See text for method of classification. All results are expressed as mean milligrams % ± one stand. dev.

* n represents No. of fluids.

† No. of cases in each diagnosis is in parentheses.

‡ Expressed as mg dichromate reduced per 100 ml fluid.

§ Probability for observed value of "t."

‖ C > D.

all of the specimens except the edema fluid obtained by Southey tube. The high density lipoprotein determination could not be made on the pericardial fluid because of insufficient quantity. The ultracentrifugal patterns of the lipoproteins in these body fluids are similar to those of human plasma (Fig. 1). The low density material migrates principally as Sf 8-11, but it is often not homogenous (Fig. 1c).

The irritative fluids from both peritoneal and pleural cavities show significantly higher levels of low density lipoproteins, total cholesterol, and globulins ($p<0.01$) than do the pressure fluids (Table I). Concentrations of high density lipoproteins are similar. The mixed pleural fluids appear to have characteristics between those of the pressure and irritative groups (Table II). The peritoneal pressure fluids are more similar to the mixed group of pleural fluids than to the pressure group except for the low amount of triglyceride in the former. The 2 chylous fluids show large amount of triglyceride and low density lipoproteins migrating at rates of Sf 400 and faster.

Discussion. The large complexes demonstrated in these fluids were either derived from cellular debris or plasma lipoproteins. The differences in content between fluids may be related to differences in passage of molecules out of the body cavity rather than into the body cavity. The data of Oncley, Scatchard, and Brown(22) indicate that the minimum diameter of the high density lipoprotein molecule (mol. wt. 200,000) is 50 Å and that of the low density lipoprotein molecule (mol. wt. 1,200,000) is 185 Å. Although the pore theory of capillary permeability(22) limits the free diffusion of particles to those having diameters less than 20-45 Å, there is some evidence for the existence of a larger pore

TABLE II.

Type	Clinical diagnosis	Total protein	Albumin	Globulin	Total lipid*	Total chol.	Lipid phos.	Triglyceride	Low density	High density
Pleural "mixed" (see below)		1,742 ±	1,104 ±	638 ±	4,435 ±	32.2 ±	1.83 ±	140 ±	72.2 ±	27.2 ±
n = 5		327	212	327	1,720	8.4	.33	108	20.8	16.6
Joint fluids	Rheumatoid arthritis	3,902	2,794	1,112			4.92		168	110.
		4,575	2,300	2,275	3,650	91.5	2.32	17	184	59.0
Hydrocele fluids	Prostatic stricture	4,225	3,062	1,163	1,400	23.5	1.63	88	12.5	31.0
	Hernia	1,869	1,306	563	680	11.9	.92	0	0	
Southey fluid	Diabetes mellitus Congestive heart failure	1,462	1,312	150	3,220	15.4	1.04	137	0	0
Pericardial	Laennec's cirrhosis (autopsy)	2,662					2.72		14.3	
Chylous pleural fluids	Lymphoma	3,993	3,150	843	17,600	110.	4.00	750	98	S_f 35-100: 45.1 S_f 100-400:133.5
		2,869	1,893	776	17,450	64.7	4.88	785	34	S_f 35-100: 45.0 S_f 100-400:101.0

See Table I and text for explanation. "Mixed" fluids appeared to have both "pressure" and "irritative" elements in their pathogenesis, *e.g.* congestive heart failure complicated by pulmonary infarction, neoplasia or pneumonia.

* Expressed as mg dichromate reduced per 100 ml fluid.

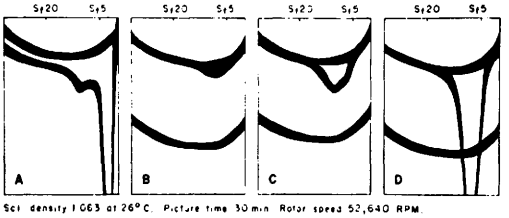

FIG. 1. Comparison of high density ultracentrifugation patterns. A represents a serum pattern from a 51-year-old man who had experienced a myocardial infarction. The serum was concentrated 4 times. B, C, and D represent patterns from pleural "pressure," "mixed," and "irritative" groups respectively, concentrated 8 times. The lower patterns in frames B, C, and D represent the second one-half milliliter from the preparative centrifugation tube. These samples were run simultaneously in a wedge window cell. The base lines are superimposed.

(24). It is also possible that the pathological process enlarges the diameter of the pores. There are data for such a phenomenon in dogs(13). Electronmicroscopic observations (25) suggest that lipoproteins pass through intima by a mechanism similar to phagocytosis.

The peritoneal fluids have higher solute concentrations than the pleural fluids of the same presumptive pathogenesis. This is consistent with studies of infused dextran in dogs (24) and may reflect the fact that peritoneal pressure fluid traverses principally the liver capsule and not the portal capillary bed(26). Despite the similar total lipid concentrations in peritoneal and pleural pressure fluids, concentration of triglycerides was low in the former whereas concentration of cholesterol and lipid phosphorous was low in the latter. The high amount of triglyceride in the pleural pressure fluids was not manifest as free floating lipid.

The miscellaneous joint, hydrocele, pericardial, and edema fluids show small amounts of lipoproteins resembling those of plasma. The absence of demonstrable lipoprotein in the edema fluid obtained by Southey tube despite a significant protein content sheds no light on the debate concerning the existence of lipoprotein in normal extravascular fluid (10,27,28).

The chylous fluids described in Table II must be considered separately since they were each a consequence of lymphoma which may have caused a rupture or a congestion of the thoracic duct leading to a mechanical transfer

of thoracic duct lymph into the pleural space. The composition of these chylous fluids is similar to that of cannulated thoracic duct lymph as in fat fed dogs(9) and that found by Peters and Man(29). The low density components of such fluids resemble those of post-prandial serum and the lipid content is comparable.

Summary. Lipids and proteins exist in peritoneal, pleural, joint, hydrocele, and pericardial effusions in large complexes having migrational characteristics in the ultracentrifuge similar to plasma lipoproteins. Concentration ranges from 5 to 50% of that in plasma. Classification of fluids according to traditional concepts of pressure and irritation reveals that irritative fluids tend to have higher concentrations of low density lipoproteins, total cholesterol, and globulin than do pressure fluids. The origin of these large lipoprotein aggregates is uncertain. The various possibilities are discussed.

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