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Peritoneal Fluid in the Rabbit: Permeability of the Mesothelium to Proteins, Lipoproteins and Acid Hydrolases

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Summary

The peritoneal fluid in rabbits fed a normal and a cholesterol added diet was analysed for a wide variety of macromolecules of different size, viz albumin, α-, β- and γ-globulins, high density lipoprotein and lipoproteins of S_f0-12, S_f12-20 and S_f>20 and three acid hydrolases, N-acetyl-β-d-glucosaminidase, acid phosphatase and β-glucuronidase. The composition of the lipoproteins and the concentrations of each substance were compared with corresponding values in plasma, hepatic lymph, thoracic duct lymph and leg lymph. The results indicate that the large lipoproteins of the thoracic duct lymph derived from the intestinal mucosa do not normally enter the peritoneal cavity probably because they do not mix with the subserous tissue fluid, that the macromolecular composition of peritoneal fluid resembles that of leg lymph, that the peritoneal mesothelium is freely permeable to these macromolecules and that the main plasma: peritoneal fluid barrier resides in the blood capillary membrane of the various subserous tissues.

Introduction

The peritoneal cavity usually contains a small amount of free fluid which has a protein concentration of about 2.5 g/100 ml (2, 35). The origin of this fluid is not certain, but it is probably derived from the several adjacent subserous tissue fluid pools. The mechanisms concerned in the passage of proteins through the mesothelial lining of the cavity are, however, not well understood. On the other hand, the return of protein from the peritoneal cavity to the blood stream by the lymphatic vessels of the diaphragm has been extensively studied (17, 51).

Since the movement of protein into the peritoneal cavity is normally small, the underlying mechanisms have been studied mainly in certain disorders, such as congestive heart failure and cirrhosis of the liver, where the increased intrahepatic portal pressure leads to a considerable formation and accumulation of peritoneal fluid. The altered haemodynamics in these disorders affect especially the tissue fluid pools of the liver and the gastrointestinal tract. The evidence suggests that the excess peritoneal fluid is derived mainly from the liver (19, 51).
Whereas this concept applies when the disorder is fairly acute, recent evidence suggests that in chronic conditions where the protein concentration of the peritoneal fluid is very low, the excess fluid is derived mainly from an extrahepatic pool, the gastrointestinal tract (45, 46, 47, 48, 49, 50).

In order to investigate further the function of the mesothelium of the peritoneal cavity as a barrier to protein, it was decided to study its permeability to macromolecules of varying size. Experiments were therefore devised to measure the concentrations of the plasma proteins and lipoproteins and the levels of activity of three acid hydrolases in the peritoneal fluid of the rabbit and to compare the values obtained with the corresponding levels in the blood plasma, in the adjacent tissue fluid pools of the liver and gastrointestinal tract, and in a remote tissue fluid pool, that of the leg.

Methods

General Procedure

Adult rabbits of various strains, of both sexes and of 2.5 to 3.5 kg body weight were used. For the collection of the various samples they were anaesthetized with intravenously admini-

stered pentobarbitone sodium (Nembutal^R, Abbott Laboratories, Sydney), anaesthesia being maintained by periodic injections of Nembutal through a polyethylene catheter introduced into the jugular vein. Coagulation of the samples was prevented by the addition of heparin (Pularin, Evans), 5 to 10 i.u./ml; excessive amounts of heparin were found to decrease the activity of acid phosphatase, one of the enzymes studied.

Peritoneal fluid was collected by pasteur pipette through a small mid-line abdominal incision, blood from a cannula in the carotid artery, hepatic lymph from the efferent vessel of the hepatic lymph node (12), thoracic duct lymph from the vessel at the base of the neck (53) and leg lymph from an afferent duct of the popliteal node (11).

The animals used were fed either their normal diet of pellets or this diet with added powdered cholesterol for 2 to 3 months. In the latter group the concentration of total cholesterol in the plasma was determined each week and the diet adjusted to keep the upper level below about 1500 mg/100 ml with a mean value of about 1,000 mg/100 ml.

Since some of the samples were small, three separate series of experiments were carried out. In the first series, concentrations of total protein, albumin, α-, β- and γ-globulins, total cholesterol and phospholipid were determined in blood plasma, peritoneal fluid and hepatic lymph of 6 normal and 18 cholesterol fed animals. In the second series the lipoproteins in blood plasma, peritoneal fluid, hepatic lymph and thoracic duct lymph of cholesterol fed rabbits were separated by ultracentrifugation into four fractions, and the protein and lipid composition of each fraction determined. This series consisted of two groups, one fed the diet with added cholesterol for 2 to 3 months and the other fed the same diet for the same time except that for the last week of the experiment 4 g corn oil was added to the diet each day. In the third series of experiments the concentration of protein and the levels of activity of the acid hydrolases, acid phosphatase, β-glucuronidase and N-acetyl-β-D-glucosaminidase, were determined in the blood plasma, peritoneal fluid, hepatic lymph, thoracic duct lymph and leg lymph of normal and cholesterol fed animals.

Analytical Procedures

In the first series of experiments the concentrations of total protein, albumin and globulin were determined by microkjeldahl digestion and paper electrophoresis (13), of total cholesterol by the method of *Abell* et al. (1) and of phospholipid by the method of *Zilversmit* and *Davis* (54).

In the second series of experiments the lipoproteins were separated into 4 density classes by a discontinuous density gradient ultracentrifugal procedure (39). The samples (4 ml) were centrifuged for 24 h at 20°C in an SW41 rotor in a model L3-50 Beckman ultracentrifuge (Beckman Inc. Palo Alto, California 94034) at 41,000 r.p.m. (286,000 g; R_{max} = 15.23 cm). Each of the four fractions of lipoproteins — d < 1.006 (S_f > 20); d 1.006-1.019 (S_f 12-20); d 1.019-1.063 (S_f 0-12) and d 1.063-1.210 (high density lipoprotein, HDL) — was removed into tubes containing ethanol : diethyl ether (3 : 1 v/v 40 ml). The tubes were stoppered and warmed in a boiling water bath for approximately 10 sec to denature the protein. After cooling and maintaining at 4°C for 2 hr the protein was precipitated by centrifugation (3,000 g, 20 min) and the lipid-containing supernatant aspirated into flasks. The protein precipitate was washed twice with diethyl ether (20 ml) and the solvent evaporated from the pooled extract under vacuum on a rotary evaporator. Non-lipid contaminants were removed from the extract by the method of *Folch* et al. (24) and neutral lipids separated from phospholipids by the method of *Zilversmit* (52) on columns of silicic acid: kieselgur. Lipid phosphorus was estimated by the method of *Eibl* and *Lands* (20) and triglyceride by the method of *Zilversmit* (52).
Cholesterylester was hydrolysed and extracted by the method of *Mann* (34). Cholesterol in the petroleum ether extract was measured using the o-phthaldialdehyde colour reagent described by *Zlatkis* and *Zak* (55). The protein precipitate was dissolved in 1 N sodium hydroxide

and an aliquot taken for estimation of protein nitrogen by direct nesslerization of kjeldahl digests (36).

In the third series of experiments the concentration of total protein was determined by the microkjeldahl method and the levels of activity of the three acid hydrolases, acid phosphatase, β-glucuronidase and N-acetyl-β-D-glucosaminidase as follows:

Acid phosphatase (orthophosphoric acid monoester phosphohydrolase, EC 3.1.3.2): was determined by using the method of *Bessey* et al. (5). Activity was assessed by the amount of p-nitrophenol released from the disodium salt of p-nitrophenol phosphate at pH 5.0 in 0.05 M citrate buffer. Samples, sample blanks and reagent blanks were incubated for 30 min at 37°C, the hydrolysis was stopped and the chromagen was developed on the addition of alkali. The amount of p-nitrophenol released was determined from a standard curve. Activity was expressed as the amount of p-nitrophenol (μg) released per hr, per ml of sample.

β-D-glucuronidase (β-D-glucuronide glucuronohydrolase EC 3.2.1.3.1) was determined by a modification of the method of *Talalay* et al. (43), using phenolphthalein glucuronide as substrate at a final concentration of 2×10^{-4}M. Samples were diluted with 0.1 M acetate buffer, pH 4.6, and samples and blanks were incubated for 16 hr at 37°C. Phenolphthalein released by the enzyme was developed by the addition of alkaline glycine buffer pH 11.2, the removal of proteins by TCA was found not to be necessary.
The amount released was determined from a standard curve of phenolphthalein. Activity was expressed as the amount of phenolphthalein (μg) released per hr of incubation per ml of sample.

N-acetyl-β-D-glucosaminidase (β-2-acetamido-2-deoxy-D-glucoside acetamido-deoxyglucohydrolase EC 3.2.2.30) was determined by a procedure based on the method developed by *Leaback* and *Walker* (31) and *Aronson* and *De Duve* (3). Activity measurements were based on hydrolysis of p-nitrophenyl-A-acetyl-β-D-glucosaminide at a final concentration of 8×10^{-4}M in 0.1 M citrate buffer, pH 5.0. Blood plasma and lymph samples were diluted with 0.1 M citrate buffer and incubated with the substrate for 2 hr at 37°C. The hydrolysis was stopped by the addition of glycine-carbonate buffer, pH 10.7 when the colour of the released aglycone was developed. The amount of p-nitrophenol released was determined from a standard curve. Glucosaminidase activity was expressed as the amount of p-nitrophenol (μg) released per hr, per ml of plasma or lymph.

Results

The amount of fluid in the peritoneal cavity varied within a range of 1 to 7 ml with a mean of about 2 ml from animals fed their normal diet, and within a range of 1 to 12 ml with a mean of about 5 ml from animals fed the cholesterol diet. In some animals, however, no free peritoneal fluid could be collected.

Protein and lipid levels in plasma, peritoneal fluid and hepatic lymph (First Series)
The mean values obtained in this series of experiments are given in Table 1. The results show no statistically significant difference between the concentrations of the various proteins in the plasma, hepatic lymph and peritoneal fluid in the animals fed a normal diet and the corresponding levels in the cholesterol fed group. An increase in the concentrations of total cholesterol and phospholipid in the plasma of the cholesterol-fed animals led to an increase in the corresponding levels in hepatic lymph and peritoneal fluid.

The mean ratios of the concentrations in hepatic lymph (HL:P) and in peritoneal fluid (PF:P) to the concentration in the plasma are expressed graphically in Fig. 1. In this figure mean values obtained in similar experiments for leg lymph (14) have been included for comparison. In all three fluids the lymph : plasma ratio for albumin is statistically higher than that for α-, β- and γ-globulins. These results also show that the corresponding ratios for peritoneal fluid and leg lymph are somewhat similar, and much lower than the values for hepatic lymph.

Table 1 The concentrations of total protein, albumin, α-, β- and γ- globulins, total cholesterol (TC) and phospholipid (PL) in plasma, hepatic lymph and peritoneal fluid of rabbits fed a normal diet or the same diet with added cholesterol for 2 to 3 months. Values are the means and S.E.

	Total protein g/100 ml	Albumin g/100 ml	α-glob g/100 ml	β-glob g/100 ml	γ-glob g/100 ml	TC mg/ 100 ml	PL mg/ 100 ml
Normal diet (6)							
Plasma	6.76±0.17	3.70±0.15	0.72±0.05	0.97±0.05	1.36±0.20	149±21	143±16
Hepatic lymph	5.72±0.20	3.51±014	0.56±0.04	0.72±0.05	0.89±0.16	92±13	112± 8
Peritoneal fluid	2.70±0.39	1.63±0.24	0.25±0.03	0.38±0.05	0.45±0.09	34± 6	44± 6
Cholesterol diet (18)							
Plasma	6.84±0.14	3.70±0.12	0.76±0.04	0.94±0.07	1.45±0.14	1033±98	463±32
Hepatic lymph	5.63±0.21	3.37±0.12	0.58±0.04	0.63±0.05	1.05±0.12	378±41	231±20
Peritoneal fluid	3.15±0.12	2.04±0.08	0.30±0.04	0.35±0.04	0.49±0.06	181±19	110± 9

In this series of experiments the total amounts of lipoprotein were not determined. Instead, the sum of cholesterol (TC) and phospholipid (PL) was taken as a measure of total lipoprotein. The mean ratios of the concentrations of TC + PL in lymph and peritoneal fluid were in each case much lower than the corresponding values for albumin and globulin, being 0.41 for hepatic lymph, 0.20 for peritoneal fluid and 0.15 for leg lymph. With a mean plasma cholesterol concentration of about 1,000 mg/100 ml, most of the lipid would be in lipoproteins larger in size than albumin and globulin (27).

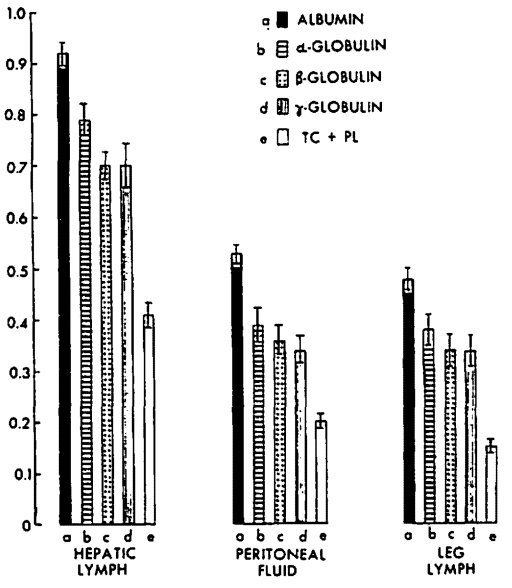

Fig. 1 The mean ratios with standard error in 24 animals of the concentrations of albumin, α-globulin, β-globulin, γ-globulin and total cholesterol (TC) + phospholipid (PL) in hepatic lymph, peritoneal fluid and leg lymph relative to the corresponding values in plasma. (Values for leg lymph from *Courtice*, 1961).

Lipoproteins in plasma, peritoneal fluid, hepatic lymph and thoracic duct lymph

The measurements of the various lipoproteins in these fluids were made in the second series of experiments. Fig. 2 depicts the compositions of the four lipoprotein fractions in plasma, hepatic lymph, peritoneal fluid and thoracic duct lymph in two groups of animals, those fed the cholesterol diet only and those fed an additional supplement of corn-oil during the last week of the cholesterol diet. The additional corn-oil increases the amount of triglyceride in the thoracic duct lymph mainly in the $S_f > 20$ fraction (25).

These results show that the composition of each of the four lipoproteins is essentially the same in plasma, hepatic lymph and peritoneal fluid. The total amounts of lipoprotein in each lipoprotein fraction in these three fluids are given in Table 2. At the levels of hypercholesterolaemia present in these experiments, the greatest amount in all fluids was in the $S_f > 20$ fraction and the levels decreased with increasing density of the lipoprotein. The ratios of the concentrations of lipoprotein in each fraction for hepatic lymph (HL:P) and peritoneal fluid (PF:P) relative to the corre-

Table 2 Amount of lipoprotein, mg/100 ml, in the fractions $S_f > 20$, $S_f 12\text{-}20$, $S_f 0\text{-}12$ and HDL in plasma, peritoneal fluid and hepatic lymph of cholesterol-fed rabbits. The figures in brackets represent the number of animals in each group.

	$S_f > 20$	S_f 12-20	S_f 0-12	HDL
Plasma	1143 ± 256	570 ± 76	201 ± 35	89 ± 5
Peritoneal fluid (13)	135 ± 330	82 ± 13	38 ± 5	27 ± 4
Plasma	1328 ± 363	683 ± 94	183 ± 39	91 ± 7
Hepatic lymph (7)	431 ± 96	249 ± 29	75 ± 6	61 ± 6

sponding concentrations in plasma are given in Fig. 3. Since there was no statistical difference between the ratios for $S_f > 20$ and S_f 12-20, these two fractions have been combined for comparison with previous results on leg lymph (16) which are included in this figure. In these earlier results for leg lymph only the total cholesterol was determined in each of three fractions, HDL, S_f 0-12 and $S_f > 12$. The present experiments show that the ratio, leg lymph : plasma, for concentrations of cholesterol is statistically the same as that for total lipoprotein in each fraction, so the values given for leg lymph : plasma ratios are valid. These results show that in each fluid the concentra-

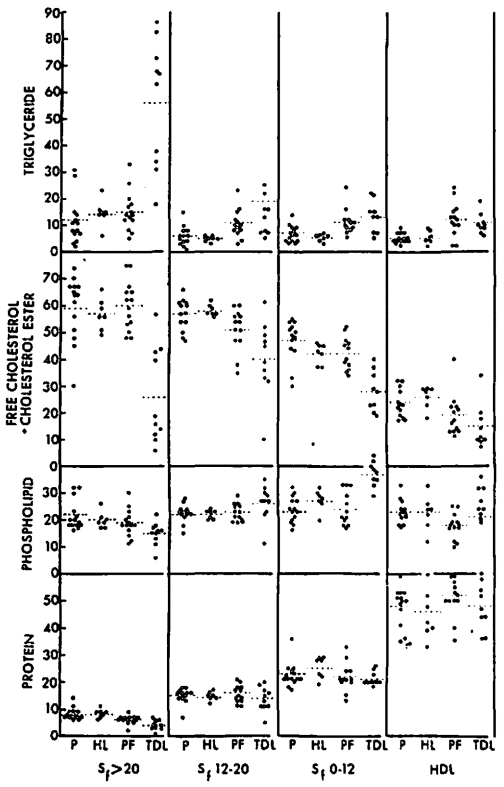

Fig. 2 The protein, phospholipid, cholesterol and triglyceride expressed as percentages of the total lipoprotein in the $S_f > 20$, S_f 12-20, S_f 0-12 and HDL fractions in plasma (P), hepatic lymph (HL), peritoneal fluid (PF) and thoracic duct lymph (TDL) in rabbits. Open circles represent animals on a cholesterol diet for 2 to 3 months; closed circles animals with 4 g corn oil added each day for the last week of the cholesterol diet.

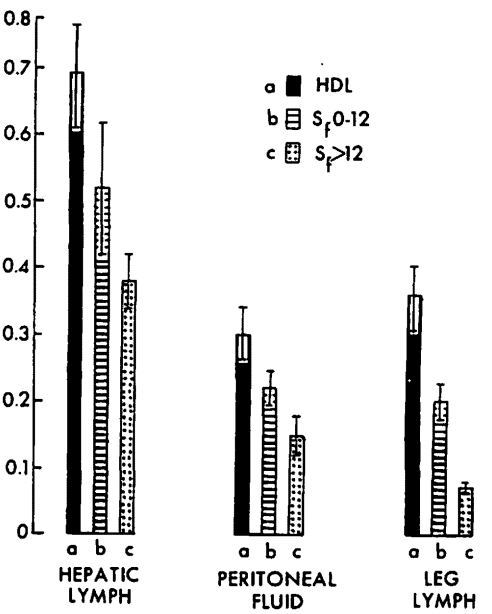

Fig. 3 The mean ratios with standard error of the concentrations of high density lipoprotein (HDL), S_f 0-12 and $S_f > 12$ in hepatic lymph, peritoneal fluid and leg lymph relative to the corresponding values in plasma. (Values for leg lymph from *Courtice* and *Garlick* 1962).

Table 3 The levels of activity of N-acetyl-β-D-glucosaminidase, acid phosphatase, β-glucuronidase and the concentrations of total protein in blood plasma, hepatic lymph, thoracic duct lymph, leg lymph and peritoneal fluid in rabbits. Values are expressed as means and S.E. Figures in brackets are the number of animals in each group.

	Protein g/100 ml	N-acetyl-β-D-glucosaminidase Units	Acid-phosphatase Units	β-glucuronidase Units
Plasma	6.68 ± 0.21	86.0 ± 7.6	833 ± 150	33.8 ± 4.6
Hepatic lymph (11)	5.42 ± 0.25	68.0 ± 8.1	496 ± 75	19.8 ± 2.5
Plasma	6.91 ± 0.21	103.6 ± 8.1	938 ± 109	25.7 ± 3.4
Thoracic duct lymph (13)	4.95 ± 0.26	63.7 ± 4.3	448 ± 61	8.6 ± 1.2
Plasma	6.44 ± 0.10	83.7 ± 8.7	841 ± 82	19.0 ± 1.6
Peritoneal fluid (20)	2.90 ± 0.09	23.8 ± 2.2	251 ± 14	3.7 ± 0.5
Plasma	6.46 ± 0.15	107.0 ± 8.1	910 ± 95	20.8 ± 1.7
Leg lymph (21)	2.35 ± 0.09	40.0 ± 2.2	224 ± 14	1.2 ± 0.1

tion of each lipoprotein relative to the corresponding value in plasma varices inversely as the size of the lipoprotein complex, that the values for peritoneal fluid and leg lymph are somewhat similar and much lower than the corresponding values for hepatic lymph.

In contrast to the results with hepatic lymph and peritoneal fluid, the composition of the lipoproteins in the thoracic duct lymph, which reflects the absorption of fat from the gastrointestinal tract, differs considerably from that of plasma, especially in the cholesterol and triglyceride components of the $S_f > 20$ and S_f 12-20 fractions. In the $S_f > 20$ fraction, the percentages of protein and phospholipid are lower in thoracic duct lymph than in plasma suggesting that the complexes are larger in the lymph (25). Adding corn oil to the diet accentuated these differences as would be expected. When these lipoproteins reach the blood stream the triglyceride is rapidly removed by lipoprotein lipase. Since the composition of the lipoproteins in thoracic duct lymph differs from that of the corresponding fractions in plasma, the lymph : plasma ratio cannot be interpreted in the same way as the hepatic lymph : plasma, peritoneal fluid : plasma and leg lymph : plasma ratios.

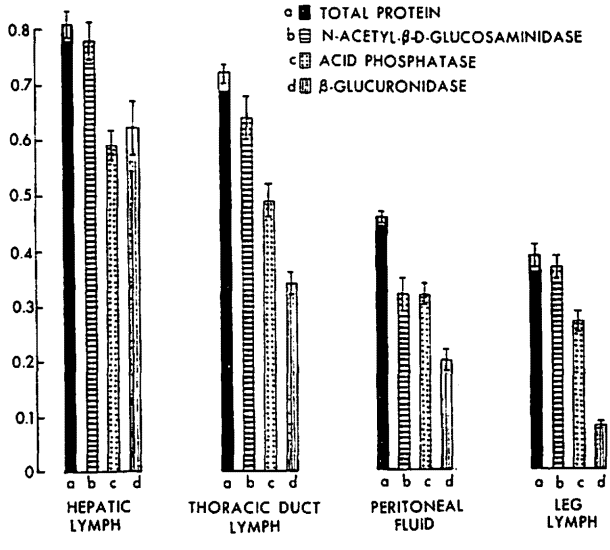

Fig. 4 The mean ratios with standard error of the concentration of protein and and of the levels of activity of N-acetyl-β-D-glucosaminidase, acid phosphatase and β-glucuronidase in hepatic lymph, thoracic duct lymph, peritoneal fluid and leg lymph relative to the corresponding values in plasma.

Acid hydrolases in plasma, peritoneal fluid, hepatic lymph and thoracic duct lymph
In the third series of experiments the levels of activity of the three acid hydrolases, acid phosphatase (M.W. 100,000-120,000), N-acetyl-β-D-glucosaminidase (M.W. 100,000–150,000) and β-glucuronidase (M.W. 160,000–290,000) were determined in the plasma, peritoneal fluid, hepatic lymph, thoracic duct lymph and leg lymph of normal and cholesterol fed rabbits. Since there was no statistically significant difference between the two groups, the results are for the series as a whole.

The levels of activity together with the protein concentration are given in Table 3. These results show that the level of activity of each enzyme in each of the extravascular tissue fluid pools is lower than the corresponding value for plasma. The ratios of the levels in hepatic lymph, thoracic duct lymph, peritoneal fluid and leg lymph relative to the corresponding levels of activity in plasma are shown in Fig. 4. These results show that these ratios for each enzyme are highest for hepatic lymph, intermediate for thoracic duct lymph and lowest for peritoneal fluid and leg lymph.

Discussion

The present experiments show that peritoneal fluid contains a wide spectrum of macromolecules similar to those present in plasma. The concentration of each of these macromolecules is in all cases less than the corresponding level in plasma and in general the peritoneal fluid : plasma concentration ratio varies inversely with the size of the molecule. A similar phenomenon is seen in the formation of lymph where macromolecules pass from the plasma to the tissue fluid to the lymph. The evidence suggests that the wall of the blood capillary is the main blood : lymph barrier where molecular sieving occurs and that the wall of the lymphatic capillary offers little or no resistance to macromolecules, so the composition of lymph reflects that of the tissue fluid from which it is derived (15, 42). To what extent the mesothelium acts as a barrier to the passage of macromolecules in the formation of peritoneal fluid from the adjacent tissue fluid pools has not been determined, but it is well known that the portion covering the diaphragm is freely permeable to macromolecules during absorption of fluid from the peritoneal cavity (51).

The structure of the mesothelium is somewhat similar to the endothelium lining the blood and lymph vessels. The flattened mesothelial cells, with their numerous microvilli, are closely opposed, the tortuous junctions being mainly of the adherens type (22) although a pentalaminar structure (tight junction) is observed in some regions. The cells also contain vesicles which vary in prominence in different regions, being more numerous for example in the omentum than in the parietal peritoneum. The mesothelial cells are supported by a basement membrane and a layer of connective tissue beneath which are blood and lymphatic capillaries (4, 8, 9, 10, 21, 23, 26, 30, 37, 38, 40, 44).

This general structure is modified in specific areas. Over the diaphragm there are stomata between the mesothelial cells, the basement membrane is poorly developed and the connective tissue layer consists only of a lattice-work of collagen fibres. Lymphatic capillaries in the form of lacunae therefore come into close contact with the mesothelium. This modification of the mesothelium together with the rhythmic contraction and relaxation of the diaphragm aids the rapid absorption of macromolecules and particles from the peritoneal cavity into the diaphragmatic lymphatic vessels (51). Even when the lymphatic trunks draining the diaphragm are ligated, lymph from the diaphragm does not spill over into the peritoneal cavity; instead, fluid in the peritoneal cavity is absorbed into the lymphatics but then spills over into the pleural cavity (18). It is therefore unlikely that the tissue fluid of the diaphragm would normally contribute to the peritoneal fluid, even though the mesothelium in this region is freely permeable. In the parietal region other than the diaphragm, the mesothelium is supported by a relatively dense layer of connective tissue. The composition of the tissue fluid in this region is not known, but it seems possible that it would resemble that of the tissue fluid pool of the leg.

The visceral mesothelium covers two sets of viscera — the gastrointestinal tract including the mesentery and omentum, spleen and liver which are supplied by the portal circulation, and other organs such as the kidneys and uterus. In the latter group the subserous tissue fluid and lymph contain proteins in concentrations of the same order of magnitude as observed for the peritoneal fluid in the present series of experiments (51).

In the mesothelium covering the liver (Glisson's capsule) there are numerous lymphatic vessels just beneath the collagen layer (7). Since the concentrations of the macromolecules in hepatic lymph are much greater than those in peritoneal fluid, it is likely that only a small percentage of the peritoneal fluid is normally derived from the liver unless the mesothelium acts as a partial barrier. However, when the tissue fluid formation in the liver is greatly increased by an increased intrahepatic portal pressure, tissue fluid similar in composition to hepatic lymph passes though the mesothelium to enter the peritoneal cavity and the protein concentration of the peritoneal fluid rises considerably (45, 47). This suggests that the mesothelium over the liver does not act as an effective barrier to macromolecules.

In the small intestine there are tissue fluid pools drained by plexuses of lymphatic vessels in the mucosa and in the muscular and subserous regions (33). The composition of the tissue fluid and lymph in the mucosa is greatly affected by the absorption of fluid and fat from the intestinal lumen. The composition of lymph from this tissue fluid pool will, therefore, be very different from that of lymph derived from the muscular and subserous pools. The lymph from these two pools ultimately enters the intestinal duct. What proportions of intestinal lymph are derived from each of these pools is not known, but it seems likely that in normal circumstances the greater amount would come from the mucosa. With a chronic portal hypertension, however, it is likely that the lymph flow from the muscular and subserous tissue fluid pools would greatly increase and the protein concentration decrease as in the uterus during pregnancy (41). A low concentration of protein would therefore be present in ascitic fluid even though the mesothelium did not act as a barrier.

The present experiments show that the lipoproteins in the mucosal tissue fluid pool and lymph do not normally enter the peritoneal fluid. The presence of valves in the lymphatic vessels prevent the lymph in the lacteals from mixing with the subserous tissue fluid. When there is a complete blockage of intestinal lymph flow, however, lymph from the lacteals of the villi containing absorbed fat does enter the peritoneal cavity to form chylous ascites. Here the lymphatic valves would become incompetent and the lymph from the lacteals would enter the subserous tissue fluid and pass through the mesothelium to enter the peritoneal cavity. It seems that in these circumstances lipoprotein complexes of the size of chylomicrons ($S_f > 400$) pass though the intact mesothelium, (6, 28, 29, 32).

Normally most of the macromolecules that escape from the blood-stream into the tissue fluid enter the lymphatic capillaries. In those tissue fluid pools adjacent to the peritoneal mesothelium, however, some will pass through this membrane to enter the peritoneal cavity. The results of the present experiments are in agreement with the concept that the peritoneal mesothelium is freely permeable to the movement of macromolecules from the plasma. The plasma: peritoneal fluid barrier to macromolecules therefore resides mainly at the blood capillary membrane.

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