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ABSORPTION OF PROTEIN FROM THE PERITONEAL CAVITY

By F. C. COURTICE and A. W. STEINBECK*

From the Kanematsu Memorial Institute of Pathology, Sydney Hospital, Sydney, Australia

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In earlier experiments we investigated the absorption of an isotonic, protein-free solution, 0·9% NaCl, and of an isotonic, protein-rich solution, homologous plasma, from the peritoneal cavity of the cat, rabbit and guinea-pig (Courtice & Steinbeck 1950*a*, *b*). The results showed that a protein-rich solution such as plasma is absorbed rapidly, and more so than 0·9% NaCl. The proteins were shown to be absorbed by the lymphatics, mainly through the diaphragm. From the diaphragmatic lymph capillaries, the lymph passed through larger channels in the anterior mediastinum to lymph nodes adjacent to the thymus and thence generally to the right lymph duct. On an average in the cat about three-quarters of the absorbed protein passed to the right lymph duct and one-quarter to the thoracic duct.

This rapid removal of a protein-rich fluid from the peritoneal cavity is not in agreement with the general conception that such fluids are slowly removed, whereas crystalloid solutions are rapidly absorbed by the peritoneum (cf. Drinker & Yoffey, 1941). This conception has been based to some extent on early experiments concerning the removal of foreign substances, phenol-sulphonphthalein, indigo-carmine and potassium ferrocyanide, from anaesthetized animals, and on the belief that the lymphatics of the peritoneal cavity drained almost entirely into the thoracic duct (Starling & Tubby, 1894; Meltzer, 1897; Mendel, 1899; Dandy & Rowntree, 1914; Shipley & Cunningham, 1916). The appearance of these coloured substances in the blood, thoracic duct lymph or urine probably gives little indication of the rate and manner of removal of ascitic fluid or of normal peritoneal tissue fluid. These naturally occurring fluids resemble plasma in composition, but with varying protein concentrations (Maurer, Warren & Drinker, 1940; James, 1949).

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There have previously been few investigations on the absorption of proteins from the peritoneal cavity. In some experiments foreign proteins have been injected intraperitoneally. Kjöllerfeldt (1917) used milk, casein, peptones and amino-acids, and Watkins & Fulton (1938) determined the thoracic duct flow before and after the introduction of 300 ml. of horse serum into a dog. These experiments were interpreted in favour of a slow absorption of protein-rich fluid from the peritoneal cavity. Experiments with homologous plasma proteins, however, probably give a better indication of lymphatic absorption from the peritoneal cavity. Orlow (1895) injected varying quantities, on an average about 22 ml./kg., of normal dog serum into the peritoneal cavity of each of six dogs and determined the absorption in 7 hr. His results show that in three unanaesthetized dogs the average absorption was 64%, whereas in three under chloroform-ether the average absorption was 29%. In three other unanaesthetized dogs, 30% of a 0·8 or 1·0% solution of NaCl was absorbed in 3½ hr. although more was injected than in the case of the serum experiments. Bolton (1921) showed that cat ascitic fluid containing about 7·5% of solids could be fairly rapidly absorbed in the cat, probably by the diaphragmatic lymphatics; in one instance 30 ml. in 11 hr. These experiments, although few in number, at least do not suggest that there is a great difference between the rate of absorption of an isotonic NaCl solution and an isotonic, homologous protein-rich solution.

There seems, therefore, little evidence to support the conclusions of Cunningham (1926) and of Watkins & Fulton (1938) that the lymphatic removal of fluid from the peritoneal cavity is slow and that the actual removal of significant amounts of fluid from the peritoneal cavity by way of the lymphatics does not occur.

Our earlier observations on the rapid removal of plasma from the peritoneal cavity suggested that the absorption of more concentrated solutions of the individual proteins, especially albumin, was worthy of further investigation. In these experiments we proposed, for practical reasons, to use rats instead of rabbits and guinea-pigs. Individual rat plasma proteins were not available, so it was decided to use bovine albumin which was readily procurable in a crystalline form. In preliminary experiments which were performed to ascertain whether whole bovine plasma would be rapidly absorbed from the peritoneal cavity of the rat, it was found that absorption was initially slow, unlike the absorption of homologous plasma in the rabbit and guinea-pig. This finding led to the further investigation of the absorption of homologous and heterologous whole plasma, of dilutions of these fluids and of solutions of pure crystalline bovine albumin from the peritoneal cavity of the rat.

METHODS

The rats used were albino (Wistar strain) and black-hooded (Lister strain). The fluid was introduced into the peritoneal cavity under light ether anaesthesia. A small incision was made in the skin of the flank and the muscle exposed. The muscle was then lifted up with tissue forceps and the fluid injected through a fine, blunt needle into the peritoneal cavity. A tie was placed about the puncture, the skin was then sutured and the animal rapidly regained consciousness. By this procedure the intestines were rarely punctured, and leakage was prevented when the animal assumed its normal posture.

Groups of five or more rats were injected into the peritoneal cavity with 0·9% NaCl, rat, bovine, human, rabbit and guinea-pig heparinized plasma, dilutions of these plasmas, and solutions of crystalline bovine albumin in Ringer-Locke solution. In all cases the solution injected was isotonic or approximately so. In most of these experiments the protein was tagged with the blue dye T 1824 (Courtice & Simmonds, 1949*a*, *b*; Courtice & Steinbeck, 1950*a*, *b*). The quantity of fluid injected was 20 ml./kg., to which 4 mg./kg. of dye had been added.

At intervals of 1, 3, 5, 8, 16 and 24 hr. after the introduction of these fluids the animals were killed with coal gas. The abdomen was opened and the remaining peritoneal fluid was collected by Pasteur pipette and its volume measured. The pleural cavity was next opened and any fluid present collected and measured. A sample of cardiac blood was then withdrawn for the estimation of the dye concentration of the plasma.

The samples of peritoneal and pleural fluid were analysed for dye concentration, total protein and 'albumin' concentration and non-protein nitrogen. The dye concentrations were estimated with a Spekker absorptiometer after extraction with *n*-butyl alcohol as described by Harington, Pochin & Squire (1940). The total and non-protein nitrogen were determined also with the Spekker absorptiometer after micro-Kjeldahl digestion and Nesslerization. Albumin was determined after precipitation of the globulins with 21% Na_2SO_3 (Campbell & Hanna, 1937).

The percentage absorption of fluid, protein and dye was calculated for each rat, and the rates of absorption deduced from the results on the rats of each group. In some cases there was, at post-mortem, more fluid and protein in the peritoneal cavity than originally injected. For convenience, we have in the text expressed this movement of fluid and protein as a negative absorption. The calculation of the percentage of fluid absorbed from the peritoneal cavity is valid only if there is initially an insignificant volume of fluid present in this cavity. A number of normal rats were killed and the volume of fluid in the peritoneal and pleural cavities determined. An occasional rat had 0·1–0·2 ml. fluid in the peritoneal cavity and a trace in the pleural cavity, but the majority had no measurable volume of fluid in either cavity. The assumption that the fluid normally present in these cavities could form only a small percentage of that introduced in the experiment is, therefore, justified.

RESULTS

The absorption of rat plasma and of isotonic NaCl solution from the peritoneal cavity of the rat. The plasma was obtained by bleeding out several rats through a cannula in the carotid artery, dry heparin being used as an anticoagulant. The mean rate of absorption of fluid from groups of rats was as follows: at 1 hr. plasma $57·1 \pm 5·5$ (S.E. of mean of seven observations), 0·9% NaCl $44·3 \pm 4·2$ (5); at 3 hr. plasma $91·7 \pm 1·9$ (8), 0·9% NaCl $71·2 \pm 5·5$ (5); at 5 hr. plasma $98·2 \pm 1·1$ (9), 0·9% NaCl $92·7 \pm 1·7$ (11). These results agree with our earlier experiments on rabbits and guinea-pigs in showing that homologous plasma is slightly more rapidly absorbed from the peritoneal cavity than is 0·9% NaCl, the 3 and 5 hr. differences being significant.

In other experiments rat plasma was diluted 1 in 4 and 1 in 6 with 0·9% NaCl, giving protein concentrations of 1·62 and 1·15% respectively. When 20 ml./kg. of these fluids was introduced into the peritoneal cavity of rats, the average absorption in 1 hr. was 64·9% for the former and 59·6% for the latter. It thus appears that dilution of plasma down to 1% of protein has little effect on its absorption.

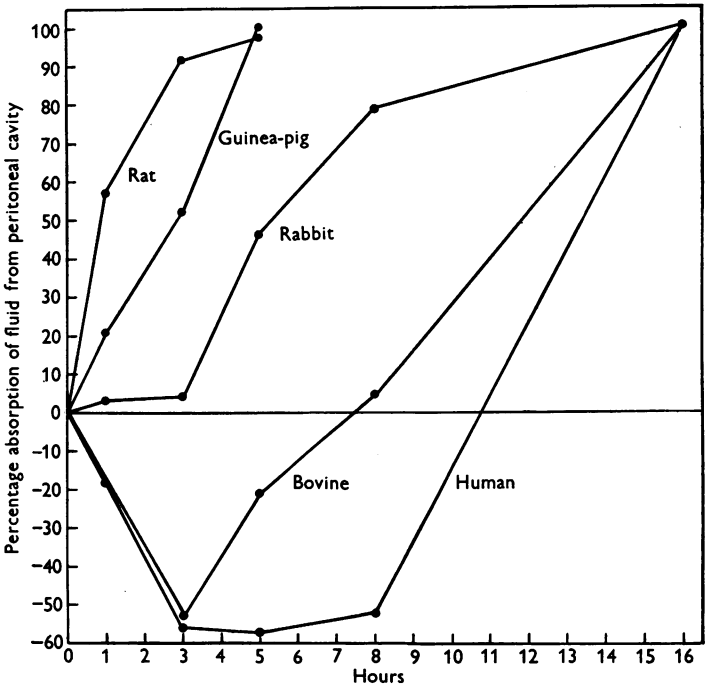

Fig. 1. The rate of removal of fluid from the peritoneal cavity of the rat after the intraperitoneal injection of 20 ml./kg. of bovine, human, rabbit, guinea-pig and rat plasma. Each point represents the average of results on five rats in the case of bovine, human and guinea-pig plasma, on seven to nineteen rats in the case of rabbit plasma and on seven to nine rats in the case of rat plasma.

The absorption of foreign plasma from the peritoneal cavity of the rat. The experiments described above show that the absorption of homologous plasma and of isotonic NaCl solution from the peritoneal cavity is fundamentally the same in the rat as in the rabbit and guinea-pig. Since, in preliminary experiments, bovine plasma was much more slowly absorbed than homologous plasma, the peritoneal absorption of bovine, rabbit, human and guinea-pig plasma has been investigated in the rat. Fresh bovine blood was obtained by puncture of the external jugular vein of a normal cow; human blood was taken from two donors and the plasma pooled, while in other experiments plasma was obtained from individual donors; in rabbits and guinea-pigs blood was collected by

cannulation of the carotid artery. In all cases the blood was taken aseptically and heparinized.

Using the standard procedure described above, the results set out in Fig. 1 were obtained. It is evident that the rate of removal of fluid from the peritoneal cavity was very different when heterologous plasma was used. With bovine and human plasma the volume of fluid in the peritoneal cavity increased considerably during the first 5–8 hr. Fluid must, therefore, have passed from the peritoneal tissue fluid into the peritoneal cavity. After 8 hr., however, absorption proceeded rapidly, so that by 16 hr. no trace of fluid remained. The graph for the absorption of rabbit plasma in Fig. 1 represents the average results for several batches of rabbit plasma. The absorption rates varied somewhat between samples of plasma, but in all cases was slow initially and then rapid.

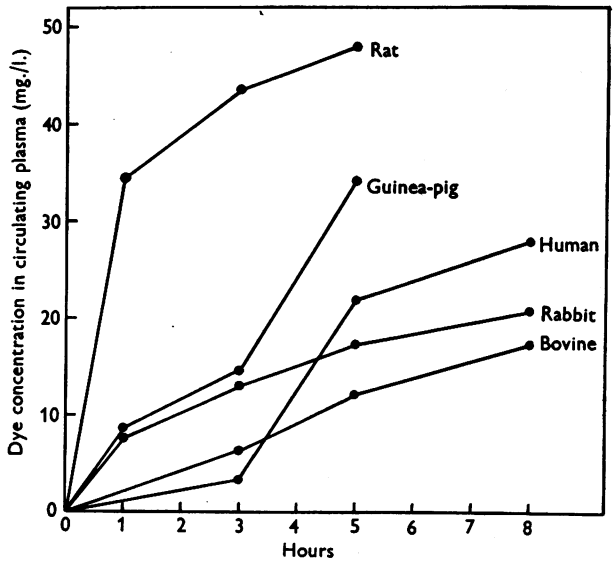

Fig. 2. The dye concentration in the circulating plasma of the rat after the intraperitoneal injection of 20 ml./kg. of plasma plus 4 mg./kg. dye. Average figures for groups as in Fig. 1.

Although with heterologous plasma there was usually an initial increase in the volume of fluid in the peritoneal cavity, some absorption was occurring at the same time. The concentration of dye in the circulating plasma of the rat gives a measure of the rate of absorption of the injected protein, since the dye-protein complex is absorbed unbroken (Courtice & Steinbeck, 1950*a*). The amount of dye in the plasma at any time will not accurately determine the quantity of protein absorbed, since dye-protein will gradually pass out of the circulation. The levels of dye in the circulating plasma, shown in Fig. 2, do, however, indicate that with heterologous plasma even though fluid was passing into the peritoneal cavity some protein was at the same time being absorbed.

This absorption of heterologous plasma protein was not nearly as rapid as was the absorption of homologous plasma protein.

The absorption of crystalline bovine albumin solutions from the peritoneal cavity of the rat. Although freshly obtained whole bovine plasma was initially slowly absorbed from the peritoneal cavity, solutions of crystalline bovine albumin did not behave in the same way. The crystalline bovine albumin was obtained from Armours in London and made up in Ringer-Locke solution. The pH of the Ringer-Locke solution was 7·25 and of 9% albumin in Ringer-Locke solution 5·55. Bovine albumin was used in various concentrations—3, 5, 9, 15 and 25%.

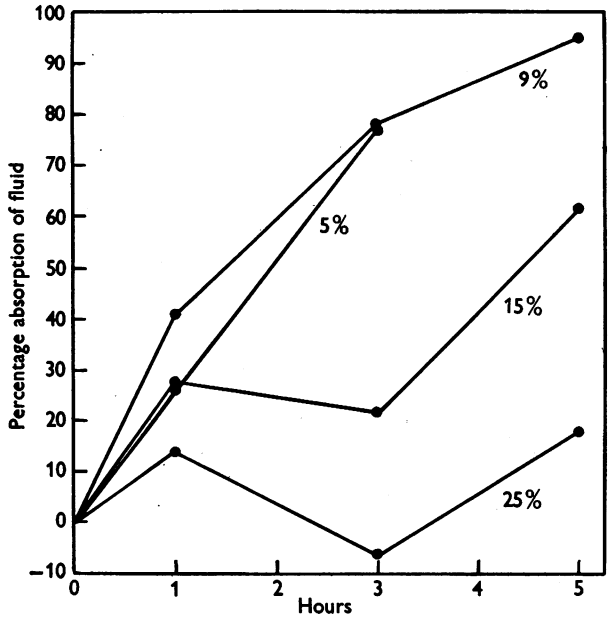

Fig. 3. The rate of removal of fluid from the peritoneal cavity of the rat after the intraperitoneal injection of 20 ml./kg. of solutions of crystalline bovine albumin in Ringer-Locke solution. Each point represents the average of results on four rats.

As before 20 ml./kg. was injected. The rate of absorption of these fluids is represented in Fig. 3. With concentrations of albumin up to 9% the absorption of fluid was rapid and there was no indication of a considerable outpouring of fluid into the peritoneal cavity. Even from the 15 and 25% solutions of albumin the rate of removal of fluid was much more rapid than from whole bovine plasma.

Protein exchange between peritoneal tissue fluid and free peritoneal fluid. When hypertonic or hypotonic solutions of innoxious crystalloids are injected intraperitoneally, there is a rapid exchange of ions and small molecules across the peritoneal membrane (Orlow, 1895; Clark, 1921; Putman, 1923). Determinations of the protein content of the peritoneal fluid at various intervals

after the introduction of fluid into the peritoneal cavity showed that the concentration of protein also tended to reach an equilibrium, presumably with the proteins in the tissue fluid of the peritoneum. In Fig. 4 are depicted the changes which occurred in the protein level of the peritoneal fluid when 20 ml./kg. of 0·9% NaCl, rat, bovine and human plasma, dilutions of these plasmas with 0·9% NaCl and solutions of crystalline bovine albumin in Ringer-Locke solution were injected intraperitoneally. It is evident that if the protein concentration of the introduced fluid was high, the level fell, whereas if the concentration was low, the level rose. Before protein equilibrium was reached,

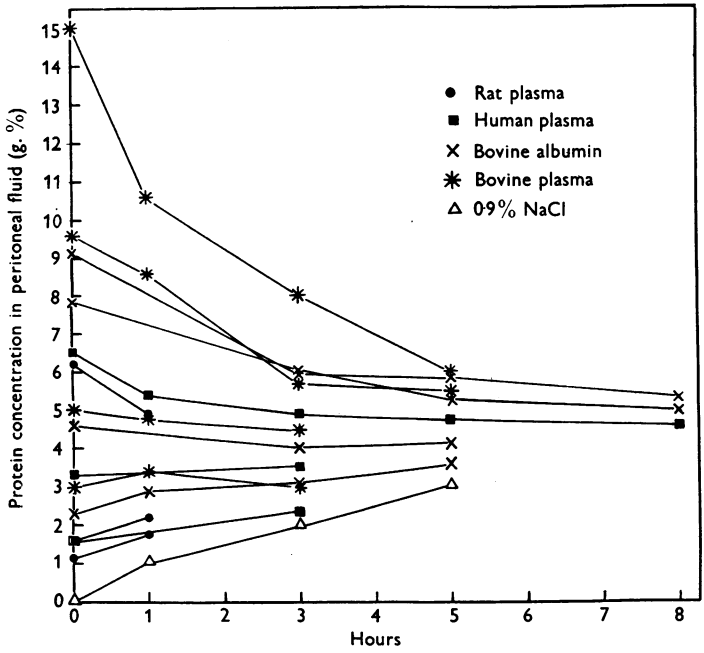

Fig. 4. The changes in protein concentration in the peritoneal fluid at intervals after the introduction of 20 ml./kg. of various fluids into the peritoneal cavity of the rat.

however, all the peritoneal fluid had been absorbed. As far as the proteins are concerned, there are two processes going on at the same time—the slow movement of protein molecules across the peritoneal membrane in order to establish equilibrium and the rapid absorption of proteins through the diaphragmatic peritoneum into the subjacent lymphatic capillaries.

The changes that occurred in the protein concentration in the peritoneal fluid were attained by different means for the various fluids used. When isotonic NaCl (0·9%) was injected, protein was slowly added to the fluid. The smaller ions and molecules, on the other hand, rapidly attained equilibrium with the tissue fluid. In groups of rats at 1 hr. the NaCl level of the fluid had fallen to 660 mg.% while the N.P.N. had risen to 33 mg.%, that is approximately the

same levels as in the blood plasma. When whole plasma or pure albumin in high concentrations was injected, the protein level in the peritoneal fluid fell. This was attained by dilution with a fluid, the protein content of which varied with the type of fluid introduced and not with the concentration of protein in the injected fluid. By determining the absorption of dye-protein and of total protein, any addition of protein could be detected. The amount of dye absorbed from the peritoneal cavity gives a measure of the removal of injected protein. If this is the same as the amount of protein absorbed calculated from the determination of protein, then no protein has been added. If, however, the

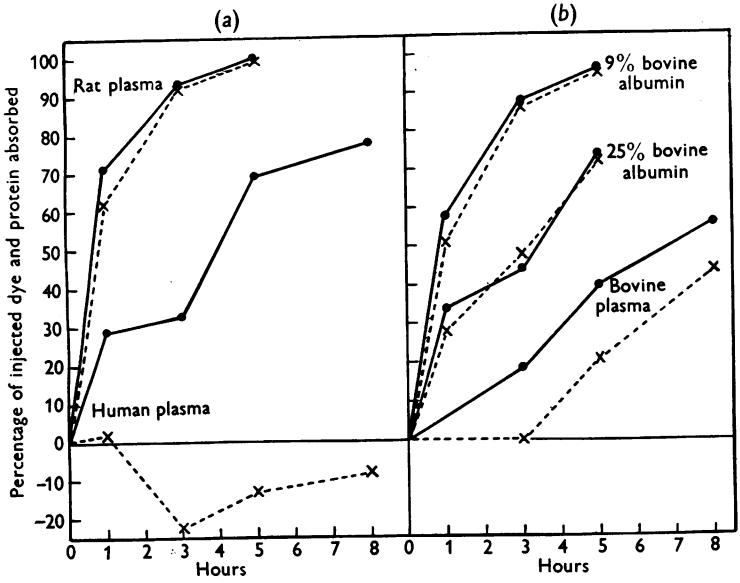

Fig. 5. The absorption of dye and of protein from the peritoneal cavity of the rat after the intraperitoneal injection of 20 ml./kg. of fluid plus 4 mg./kg. of dye. ●——●, dye; ×----×, protein. Each point represents the average of results on five rats in the case of human, bovine and rat plasma and on four rats in the case of bovine albumin.

absorption of dye runs at a greater rate than the absorption of protein, then protein must be added to the peritoneal fluid from the circulation. Fig. 5 shows typical results. In Fig. 5(*a*) it is evident that when rat plasma was injected into the peritoneal cavity of the rat, the dye and protein were absorbed at about the same rate whereas with human plasma much protein accompanied the fluid which passed into the peritoneal cavity. The protein content of the injected rat plasma was 6·19% while that of the human plasma was 6·54%. The difference in behaviour of these two fluids could not, therefore, be due to a difference in their protein content. Although the human dye-protein was not absorbed as rapidly as rat dye protein, nevertheless in 8 hr. as much as 77% of the injected dye of the human dye-protein had left the peritoneal cavity. In

spite of this, there was at this time more protein (and considerably more fluid, Fig. 1) remaining in the peritoneal cavity than was injected. During this time, therefore, a considerable amount of protein as well as fluid must have been added.

In Fig. 5(*b*), the absorption rates of dye and protein after the intraperitoneal injection of 9 and 25% bovine albumin and of whole bovine plasma containing 9% plasma proteins are shown. With pure bovine albumin, the protein content was considerably diluted as shown in Fig. 4, but it is evident that the diluting fluid contained very little protein. A little globulin did appear in the injected fluid, but the amount passing in the short time taken for the absorption of all the albumin was barely measurable. Even when 25% bovine albumin solution was introduced the dilution was considerable, but the diluting fluid contained practically no protein. When bovine plasma was used, however, considerable dilution occurred, but the absorption of the dye-protein was much more rapid than the absorption of total protein. This means that the diluting fluid contained protein.

TABLE 1. The absorption of dye and of protein from the peritoneal cavity of the rat after the intraperitoneal injection of 20 ml./kg. of diluted plasma plus 4 mg./kg. of dye. The plasma was diluted with 0·9% NaCl. Average values for groups of five rats

	Protein in peritoneal fluid (g. %)		Time (hr.)	(%) Absorption	
	Injected	Final		Dye	Protein*
Rat plasma	1·15	1·77	1	63·9	41·6
	1·62	2·19	1	61·2	53·7
Bovine plasma	2·30	2·91	1	35·6	−11·1
	2·30	3·12	3	69·9	36·1
	2·30	3·61	5	93·3	78·6
Human plasma	1·65	2·40	3	53·5	−13·6
	3·36	3·55	3	40·9	− 4·5

* These values represent changes in total protein in the peritoneal fluid, positive values a decrease and hence an apparent absorption, negative values a gain in protein.

When diluted plasma of a low protein content was introduced into the peritoneal cavity, the protein level of the fluid rose. This might be due to the addition of protein or the absorption of protein-free fluid, or to both processes going on simultaneously. Owing to the slower diffusion of protein molecules, it would be expected that the more rapid diffusion of the water, ions and small molecules would be the main factor in reaching a protein equilibrium. Table 1 shows the amounts of dye-protein and of total protein absorbed when diluted rat, bovine and human plasmas were injected intraperitoneally. In all cases there was more dye-protein absorbed than total protein which means that protein was added to the peritoneal fluid. With bovine and human plasma, however, there was a very considerable difference indicating that much more protein was added than with diluted homologous plasma.

The movement of protein into the peritoneal fluid can also be seen when the blue dye T 1824 is injected intravenously and undyed fluid is introduced into the peritoneal cavity. The dye combines with the circulating plasma albumin so that if this protein passes into the peritoneal fluid dye will also be added. In these experiments 20 ml./kg. of an undyed fluid was introduced intraperitoneally and 4 mg./kg. of dye intravenously, which gave a terminal dye concentration in the circulating plasma, after 1 or 3 hr., of approximately 70 mg./l. The results of these experiments are given in Table 2. With whole or half diluted homologous

TABLE 2. The dye concentration in the peritoneal fluid at intervals after the intraperitoneal injection of 20 ml./kg. of undyed fluid and the intravenous injection of 4 mg./kg. of the dye T 1824 in rats. Average values for groups of four rats in 1 hr. experiments and two rats in 3 hr. experiments

Fluid injected	Protein content of peritoneal fluid (g. %) Initial	Final	Time (hr.)	Dye conc. in remaining peritoneal fluid (mg./l.)
Rat plasma	6·25	4·61	1	0
One-half diluted rat plasma	3·13	3·12	1	0
One-quarter diluted rat plasma	1·56	2·18	1	1·0
Bovine albumin in Ringer-Locke	25·00	18·10	1	0
	25·00	13·60	3	2·9
Fresh rabbit plasma	6·32	5·24	1	7·2
	6·19	4·95	3	12·3
One-half diluted rabbit plasma	3·09	3·35	1	8·0
Fresh human plasma	7·53	6·65	1	8·7
	7·53	5·52	3	35·0
0·9% NaCl	0	0·62	1	1·5
	0	0·91	3	7·5

plasma none of the dye-protein was found in the peritoneal fluid, but with plasma diluted 1 in 4 containing 1·56% protein, some dye-protein was found and the protein content of the peritoneal fluid rose. With a high concentration of bovine albumin, the protein was diluted because here also fluid was added, but only after 3 hr. did a small amount of dye appear. These results are in contrast to those obtained with fresh rabbit or human plasma where a considerable amount of blood plasma dye-protein passed into the fluid in the peritoneal cavity. When 0·9% NaCl was injected, the dye concentration rose as the protein content of the peritoneal fluid rose. These experiments, therefore, confirm the previous findings concerning the protein exchange between peritoneal tissue fluid and the free fluid in the peritoneal cavity.

Pleural fluid during peritoneal absorption. In normal animals there is usually only a trace of free fluid in the pleural cavities. We have previously shown that when homologous plasma was introduced into the peritoneal cavity of rabbits and guinea-pigs some of the absorbed fluid passed out of the lymphatics into the mediastinal tissues and the pleural cavities. We also described a few experiments in which rabbit plasma was introduced into the peritoneal cavity

of the rat, in which case a considerable amount of the injected plasma passed from the lymphatics into the pleural cavities (Courtice & Steinbeck, 1950*b*).

When rat plasma was introduced into the peritoneal cavity of the rat, there was generally no mediastinal oedema and only a trace of fluid could be found in the pleural cavities. Even when 50 ml./kg. of rat plasma was given, 98·5% was absorbed in 5 hr. and only a trace of fluid appeared in the pleural cavities. With guinea-pig plasma, which was also rapidly absorbed in the rat, only a small volume of fluid appeared in the pleural cavities even when 50 or 100 ml./kg. was introduced. In these cases where the lymphatic absorption was rapid, there was little or no leakage from the lymphatics during their course through the thorax.

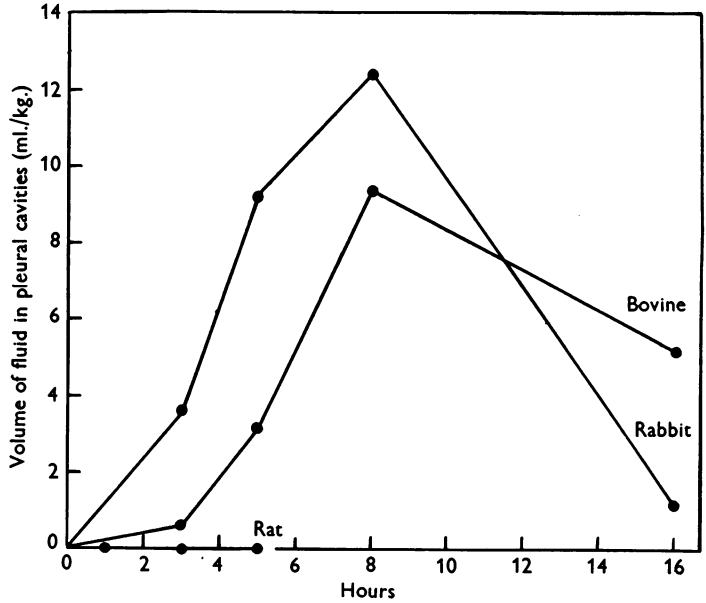

Fig. 6. The volume of free fluid found in the pleural cavities of rats at intervals after the intraperitoneal injection of 20 ml./kg. of bovine, rabbit and rat plasma. Each point represents average of results on five rats.

With rabbit or bovine plasma, however, the picture was entirely different. Fig. 6 shows the volume of fluid in the pleural cavities after the intraperitoneal administration of 20 ml./kg. of fresh rabbit and bovine plasma. The volume of fluid in the pleural cavities reached its peak usually at about 8 hr. After this time the pleural fluid gradually decreased. By 16 hr. there was no fluid left in the peritoneal cavity but the pleural fluid was then more slowly absorbed and it was not until about 48 hr. that the pleural cavities were once again clear of excess fluid. This pleural fluid had a high protein and dye concentration, approximately the same as in the peritoneal fluid. Table 3 gives typical results.

The fact that the dye concentrations in the pleural and peritoneal fluid were much higher than in the circulating plasma indicates that the fluid was escaping from the lymphatics. Besides this large volume of fluid in the pleural cavities there was also a difference in the appearance of the retrosternal tissues where the draining lymphatics lie. In the early stages the dye-protein could be seen to diffuse into the tissues along the line of the para-mammary lymph channels. Later the retrosternal tissues became very oedematous and free fluid appeared in the pleural cavity. It is clear, therefore, that not only was the removal of rabbit and bovine plasma from the peritoneal cavity slow initially, but a great deal of what was absorbed into the lymphatics leaked out into the thoracic cavity. Considered as part of the total fluid absorption the volume of pleural fluid further emphasized the slow initial absorption when rabbit and bovine plasmas were used.

TABLE 3. The concentration of dye and of protein in the pleural and peritoneal fluid of rats at intervals after the intraperitoneal injection of 20 ml./kg. fresh rabbit or bovine plasma plus 4 mg./kg. T1824. Average values from groups of five rats

Time (hr.)	Protein in peritoneal fluid (g. %)		Pleural fluid		Dye concentration (mg./l.)		
	Injected	Final	Volume (ml./kg.)	Protein (g. %)	Peritoneal fluid	Pleural fluid	Circulating plasma
			Rabbit plasma				
1	6·45	5·24	1·0	3·32	148	—	6·7
3	7·22	5·39	5·5	4·56	78	60	9·8
5	7·22	4·99	9·1	4·61	62	71	14·1
8	5·62	4·61	4·7	—	70	65	19·5
			Bovine plasma				
3	7·84	6·03	3·1	4·45	92	61	10·6
5	7·84	5·26	7·6	5·04	72	65	15·6
8	7·84	5·02	8·3	4·66	68	78	18·5

While the absorption of human plasma followed the same pattern (see Fig. 1) only a trace of excess pleural fluid was found and this contained only a trace of dye.

The effect of storage on the absorption of bovine, rabbit and human plasma from the peritoneal cavity of the rat. It was decided to test the absorption of bovine plasma which had been kept in the refrigerator for 26 days. The plasma was clear and sterile, and the protein and N.P.N. content were found to be the same as they were originally. To our surprise the absorption rate was entirely different from that of fresh plasma (see Fig. 1). There was no initial increase in volume of the fluid in the peritoneal cavity, and absorption was quite rapid. After storage for 70 days, the rate of absorption was greater still, nearly as rapid as that of homologous plasma; moreover there was practically no diffusion into the mediastinal tissues and practically no free fluid in the pleural cavity. The results are given in Table 4. It can be seen that the difference in the rate of absorption of both fluid and of protein is greatly altered by storage of the bovine plasma. Not only is the rate altered, but the exchange of fluid is modified.

TABLE 4. Alterations in the absorption of bovine plasma with storage. 20 ml./kg. plus 4 mg./kg. dye injected intraperitoneally into rats of bovine plasma containing 7·84% protein. Each determination is the average of seven results obtained with a group of five rats

	1st day		26th day		70th day		
	3 hr.	5 hr.	1 hr.	2 hr.	1 hr.	3 hr.	5 hr.
'Absorption' of fluid (%)	−33·7	29·3	17·6	40·2	39·7	74·6	100·0
'Absorption' of protein (%)	−4·0	54·5	32·5	56·4	—	—	—
Pleural fluid (ml./kg.)	3·1	7·6	0·6	0·9	0	0	0
Dye in circulating plasma (mg./l.)	10·6	15·6	13·5	24·1	15·9	44·5	51·1

Storage of rabbit and of human plasma modified their absorption in the same way. Results of these experiments are depicted in Fig. 7. Rabbit plasma, which when fresh was slowly absorbed and during absorption leaked to a large

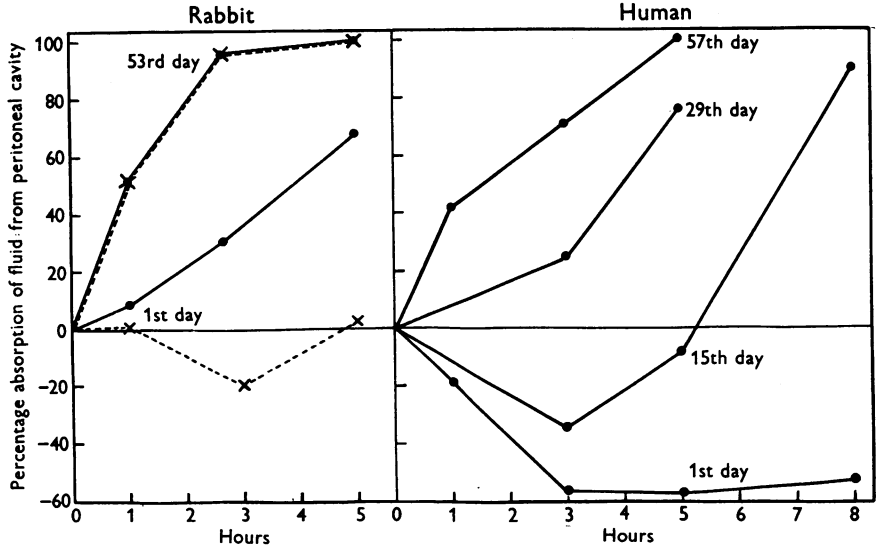

Fig. 7. The absorption of fluid from the peritoneal cavity of the rat after the intraperitoneal injection of 20 ml./kg. of rabbit and human plasma which had been kept in a refrigerator for varying periods. ●——●, absorption of fluid from peritoneal cavity; ×----×, net absorption of fluid after allowing for the fluid in the pleural cavities. Each point represents average of results on five rats.

extent into the pleural cavities, after 53 days in the refrigerator was absorbed almost as readily as homologous plasma. Human plasma absorption showed the gradual change depicted. After 57 days' storage its absorption was nearly comparable with homologous plasma. When undyed stored human or rabbit plasma was introduced intraperitoneally and the dye was injected intravenously, only a trace of dye appeared in the peritoneal fluid. This further showed that

storage abolished the effect of the foreign plasma on the capillary vessels of the peritoneum. Storage of rat plasma had no significant effect on its absorption rate.

The absorption of protein when solutions of crystalline bovine albumin are injected intraperitoneally. It has been seen that solutions of crystalline bovine albumin, even in concentrations up to 25%, were rapidly absorbed from the peritoneal cavity of the rat. In high concentrations the albumin exerts an osmotic effect and an almost non-protein fluid was added to the peritoneal cavity with a resultant dilution of the albumin. At the same time, however, lympathic absorption of a protein-rich fluid proceeded through the diaphragmatic lymphatics.

The amount of bovine albumin which can be absorbed from the peritoneal cavity of the rat is remarkable. Assuming a plasma volume of 40 ml./kg. and an average plasma protein concentration of 6·8% with a plasma albumin level of 4·0%, the total amount of circulating plasma protein in the rat will be 2·7 g./kg. and of albumin 1·6 g./kg. When 20 ml./kg. of solutions of bovine albumin were introduced into the peritoneal cavity, the amount of albumin absorbed in from 1 to 5 hr. is shown in Table 5. By using high concentrations

TABLE 5. The amount of protein absorbed from the peritoneal cavity of rats after the intraperitoneal injection of 20 ml./kg. of solutions of crystalline bovine albumin in various concentrations. Each determination represents the average of results obtained with a group of four rats

Albumin in injected fluid		Protein absorbed (g./kg. of rat)		
g. %	g./kg. of rat	1 hr.	3 hr.	5 hr.
3·02	0·60	0·14	0·26	—
5·15	1·03	0·30	0·78	—
8·73	1·75	0·89	1·51	1·68
15·00	3·00	1·46	1·74	2·51
25·00	5·00	1·38	2·41	3·62

of albumin, an amount of protein equal to, or even greater than, the total circulating albumin could be absorbed from the peritoneal cavity. With 25% albumin, more than twice the normal circulating albumin could be absorbed within 5 hr.

The general post-mortem appearance. After an intraperitoneal injection of homologous plasma the post-mortem appearance in the rat was similar to that in the cat, rabbit and guinea-pig. In the rat, however, the lymphatic drainage of the diaphragm included both a ventral and dorsal pathway. This latter was a paired lymphatic of variable calibre, at times appearing larger than the parasternal vessel to which it seemed subsidiary, lying in the paravertebral position. In addition, the paravertebral lymphatics were transversely or obliquely anastomotic across the immediate anterior surface of the vertebral pillar. At the intercostal levels there were irregular dilatations continuous with the corresponding intercostal lymphatics. Ultimately the paravertebral

lymphatics passed to the lymph nodes into which the lymphatics from the anterior mediastinum—the paramammary vessels or ventral drainage of the diaphragm—also passed.

During absorption of the homologous plasma, the dye-protein showed little diffusion from the paramammary lymphatics into the retrosternal tissues and only minimal diffusion about the apical lymph nodes into the mediastinum. So, after 5 hr., when absorption was complete, there was almost no evidence that the dye-plasma had been introduced into the peritoneal cavity.

With stored heterologous plasma when the absorption was rapid, and with solutions of pure crystalline bovine albumin, the macroscopic post-mortem appearance was similar to that observed with homologous plasma, except that human plasma on storage diffused slightly both into the retrosternal and mediastinal tissues.

When fresh heterologous plasma was injected intraperitoneally the lymphatic pathways of its uptake were the same but the general appearance differed in many respects. With rabbit and bovine plasma peritoneal staining was more than with homologous plasma. Extra-peritoneal collections of dyed fluid occurred in the greater omentum and some areas of mesentery. Retroperitoneal collection of a similar fluid also occurred at the base of the greater mesentery and at the costal insertions of the diaphragm. There was also a diffusion of dye-protein into the diaphragm, retrosternal tissues and mediastinum. The retrosternal tissues were at times very oedematous. With extensive oedema the mediastinum was waterlogged, but even though the oedema extended to the hila of the lungs the hilar lymph nodes were not stained. Free fluid was present in the pleural cavities as already described. In addition, an excess of pericardial fluid was present in some cases with the free pleural fluid. This pericardial fluid had a high dye concentration but less than that of the pleural fluid. With human plasma, peritoneal or subperitoneal petechiae in the omentum, mesentery and visceral peritoneum of the stomach and intestines were observed. This haemorrhagic response was maximal at 5–8 hr. and was lost with prolonged storage. On the other hand, although the peritoneal reaction was more severe with human than with rabbit or bovine plasma, retrosternal and mediastinal oedema was not found nor was there any significant accumulation of fluid in the pleural cavities. Diffusion of dye-protein with the absorption of human plasma appeared to a variable degree with storage.

Experiments with an intravenous injection of T 1824 and an intraperitoneal injection of fresh undyed foreign plasma suggested that dyed protein lost from the blood vessels appeared about the gastro-intestinal tract and associated mesentery.

The cellular response within the peritoneal fluid was studied. All plasmas, homologous and foreign, both fresh and stored, produced finally a neutrophil polymorphonuclear leucocyte response. There was a relative variation with the

other leucocytes and tissue cells. Foreign plasmas produced a greater response. The established response with bovine albumin fraction was, however, small mononuclear leucocytes.

The apical parasternal lymph nodes through which the lymph passes were also studied histologically. In all cases during absorption the peripheral sinus was dilated and the medullary sinuses were distended with plasma. With saline and with plasma, except human plasma, the sinuses were filled with polymorphonuclear leucocytes. Foreign plasma, especially bovine, produced a greater packing than rat plasma. With storage the packing seemed less. Bovine albumin absorption resulted in a small mononuclear leucocyte lodgment within the nodes. On the absorption of fresh human plasma the dilated sinuses contained red blood cells, some of which at 8 hr. were engulfed by macrophages, and only a small number of neutrophil polymorphonuclear leucocytes. The histological changes in terms of a gross pattern had resolved within 48 hr. though irritation phenomena persisted.

DISCUSSION

The purpose of this investigation was to study the absorption of plasma protein from the peritoneal cavity. In the rat as in the rabbit and guinea-pig the lymphatic absorption of homologous plasma through the diaphragm is rapid—20 ml./kg. of plasma being absorbed in 3—5 hr. and 50 ml./kg. in 5 hr. The removal of the plasma or plasma diluted to resemble ascitic fluid is no doubt aided by the rhythmic contractions of the diaphragm. It is possible, therefore, that absorption in a small animal like the rat may be relatively greater than from a larger animal. In the rat, rabbit and guinea-pig homologous plasma is absorbed fairly rapidly in all; 20 ml./kg. is absorbed, in the rat within 5 hr., in the rabbit within 8 hr., and in the guinea-pig within 16–24 hr. Why the absorption of 0·9% NaCl and blood as well as plasma is so much slower in the guinea-pig than in the rat and rabbit, we do not know. In larger animals Orlow's early experiments in dogs and Bolton's in cats suggest that the lymphatic absorption of homologous plasma proteins from the peritoneal cavity is not slow. How rapid absorption would be in man can at present only be a matter of conjecture. Since, however, whole blood can be relatively quickly removed from the peritoneal cavity of children (Cole & Montgomery, 1929; Clausen, 1940) and since in the rabbit, guinea-pig and rat whole blood is much more slowly removed than is plasma (Courtice & Steinbeck, unpublished), it is probable that in man a protein-rich fluid such as plasma or a solution of albumin will be absorbed quite rapidly.

The experiments with heterologous plasma demonstrate how easily erroneous ideas of lymphatic absorption of proteins may be obtained. If, for example, only fresh rabbit, bovine or human plasma had been injected into the rat for a short-term experiment, it would have been concluded that plasma proteins

were only very slowly absorbed whereas an electrolyte solution such as 0·9% NaCl was rapidly absorbed. A foreign plasma or serum has often been used in the study of the part played by the lymphatics in removing protein from a certain region. If foreign plasma exerts a similar effect in other regions of the body and in other animals, it would be difficult to interpret the results of some experiments on the physiology of lymphatic absorption.

Fresh foreign plasma appears to affect the permeability of the capillaries of the peritoneum temporarily. It has been shown that the introduction of this plasma into the peritoneal cavity causes an outflow of fluid containing some protein during the first few hours. This is not an osmotic effect because it does not occur with homologous plasma of the same protein concentration, nor to any degree with the same foreign plasma after it has remained in the refrigerator for 2 weeks or more. After the initial effect on the peritoneal capillaries which lasts from 3 to 8 hr. with different foreign plasmas, the fluid in the peritoneal cavity is rapidly absorbed. Although absorption through the diaphragmatic lymphatics, assessed by the rise of dye-protein in the circulating plasma, occurs in the early stages, the rate may not be as great as the formation of more peritoneal fluid. At 16 hr. or earlier, however, all the fluid has left the peritoneal cavity and there is usually little indication to show that fluid has been introduced.

Whatever the factor in fresh heterologous plasma may be, it becomes progressively less active on storage and it is not present in solutions of crystalline bovine albumin. All the foreign plasmas used, including human plasma of group AB, agglutinated rats' red cells *in vitro*. In all the experiments with bovine or rabbit plasma, there was no haemolysis of the blood samples taken from the rat at the various times after injection. With human plasma there was sometimes a slight degree of haemolysis which was not observed with stored human plasma. The fresh human plasma also caused petechial haemorrhages in the peritoneum whereas stored plasma did not. All the stored foreign plasmas, however, also caused rapid agglutination of rats' red cells *in vitro*, but the titres were not determined. When foreign plasma, fresh or stored, was injected intravenously into the rat in a dose of 10 ml./kg. there was no haemolysis except a slight degree with fresh human plasma. It seems, therefore, that with human plasma of group A or AB (the plasma used in these experiments) the factor which causes agglutination of rats' red cells *in vitro* and *in vivo* decreases in strength with storage. We have, however, no proof whatever that such a factor could affect the permeability of the peritoneal vessels.

Once this factor in heterologous plasma has in some way been altered by storage, bovine, human and rabbit plasma are all absorbed at approximately the same rate as fresh rat plasma. It would seem, therefore, that but for this one factor in plasma, the plasma proteins would all be absorbed at the same rate by the lymphatics irrespective of species. The experiments with pure bovine albumin are, therefore, justified. Although we have not done any

experiments with pure rat albumin it seems probable that solutions of both bovine and rat albumin will be absorbed at the same rate. If, therefore, the peritoneal absorption of proteins in man is rapid, even if not as rapid as in the rat, a considerable quantity of albumin may be absorbed into the circulation by this route after the introduction of solutions of pure human albumin.

The phenomenon of mediastinal oedema and free pleural fluid during absorption from the peritoneal cavity has been previously described for the rabbit and guinea-pig (Courtice & Steinbeck, 1950*b*). The excess fluid was shown to come from the lymphatics during absorption and not from the circulating blood. In the experiments on the rat, the occurrence of free mediastinal and pleural fluid was not commonly observed when homologous plasma, stored heterologous plasma and solutions of crystalline bovine albumin were injected intraperitoneally. Absorption was so rapid that fluid rarely leaked into the thorax. With fresh bovine and rabbit plasma a considerable quantity of free fluid appeared in the thoracic cavity. From its dye concentration and from the post-mortem appearance at all stages it seems that the fluid came from the lymphatics during absorption. There are at least two possible causes of this leakage. The first is a partial blockage of the lymph nodes through which the lymph passes. We have shown that if a ligature is passed around the main paramammary lymph trunks causing a complete occlusion of these lymphatics, homologous plasma in the peritoneal cavity is absorbed into the lymphatics and then leaks out giving rise to massive mediastinal oedema and a considerable amount of free pleural fluid (Courtice & Steinbeck, unpublished). A partial block, therefore, could probably cause an increased lymphatic pressure with leakage into the mediastinum. Histological investigations in the rat have shown that with homologous as well as with bovine and rabbit plasma and with 0·9% NaCl, a large number of neutrophil leucocytes appear in the peritoneal fluid and in the lymph nodes through which the lymph passes. With bovine and rabbit plasma the sinuses of the lymph nodes become closely packed with these neutrophils, probably more so than with homologous plasma. Whether these neutrophils could temporarily cause a partial block is not known, but it is a possibility. With human plasma, the peritoneal reaction is even more pronounced than with bovine or rabbit plasma, but there are few neutrophils in the parasternal lymph nodes. Also, in these experiments there is little or no free fluid in the pleural cavity.

The second possible cause of the leakage is the factor which affects the peritoneal blood capillaries. It is possible that this may also affect the lymph vessels and allow protein to escape. If this is so, it is difficult to understand why pleural fluid is *not* produced after the intraperitoneal injection of human plasma in rats and why pleural fluid *is* produced after the intraperitoneal injection of homologous plasma in rabbits and guinea-pigs, and sometimes with 0·9% NaCl in all animals.

SUMMARY

1. The removal of 0·9% NaCl, rat, bovine, rabbit, human and guinea-pig plasma, dilutions of these plasmas with 0·9% NaCl and solutions of pure crystalline bovine albumin from the peritoneal cavity of the rat has been investigated. The quantity introduced was 20 ml./kg.

2. Homologous plasma was absorbed slightly more rapidly than 0·9% NaCl, 20 ml./kg. of plasma being absorbed in 5 hr. and of 0·9% NaCl in 5–8 hr.

3. Fresh heterologous plasma produced an initial reaction causing an increase in the volume of fluid in the peritoneal cavity for the first few hours. This was brought about by an increased permeability of the peritoneal blood capillaries. After this initial period of 3–8 hr., the rate of absorption overcame the rate of formation of fluid and by 16 hr. all the peritoneal fluid was removed.

4. When any of these approximately isotonic fluids is introduced into the peritoneal cavity there is a rapid exchange of ions and the small readily diffusible molecules and a slower exchange of the large protein molecules between the blood and tissue fluid on the one hand and the free peritoneal fluid on the other. The protein concentration falls if initially high and rises if initially low.

5. When heterologous whole plasma has been allowed to remain in the refrigerator for 2–8 weeks, the initial reaction decreases, until there is no reaction at all and the heterologous plasma is absorbed as rapidly as homologous plasma.

6. During absorption of the fluid through the mediastinal lymphatics, there is little leakage of lymph into the mediastinum and pleural cavities when homologous or stored heterologous plasma is used, but a considerable leakage occurs with fresh bovine or rabbit plasma. Possible causes of this are discussed.

7. Solutions of crystalline bovine albumin are rapidly absorbed from the peritoneal cavity of the rat. When 25% albumin is introduced an amount more than twice that of the circulating plasma albumin can be absorbed in 5 hr.; correspondingly smaller quantities are absorbed when lower concentrations of albumin are used.

8. The probable rapidity and significance of the absorption of whole plasma or of solutions of albumin from the peritoneal cavity of man are discussed.

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REFERENCES

Bolton, C. (1921). *J. Path. Bact.* **24**, 429.
Campbell, W. R. & Hanna, M. I. (1937). *J. biol. Chem.* **119**, 15.
Clark, A. J. (1921). *J. Pharmacol.* **16**, 516.
Clausen, J. (1940). *Acta paediatr., Stockh.*, **27**, 24.
Cole, W. C. C. & Montgomery, J. C. (1929). *Amer. J. Dis. Child.* **37**, 497.
Courtice, F. C. & Simmonds, W. J. (1949*a*). *J. Physiol.* **109**, 103.

Courtice, F. C. & Simmonds, W. J. (1949*b*). *J. Physiol.* **109**, 117.
Courtice, F. C. & Steinbeck, A. W. (1950*a*). *Aust. J. exp. Biol. med. Sci.* **28**, 161.
Courtice, F. C. & Steinbeck, A. W. (1950*b*). *Aust. J. exp. Biol. med. Sci.* **28**, 171.
Cunningham, R. S. (1926). *Physiol. Rev.* **6**, 242.
Dandy, W. E. & Rowntree, L. G. (1914). *Ann. Surg.* **59**, 587.
Drinker, C. K. & Yoffey, J. M. (1941). *Lymphatics, Lymph and Lymphoid Tissue.* Harvard University Press.
Harington, C. R., Pochin, E. E. & Squire, J. R. (1940). *Clin. Sci.* **4**, 311.
James, A. H. (1949). *Clin. Sci.* **8**, 291.
Kjöllerfeldt, M. (1917). *Biochem. Z.* **82**, 188.
Maurer, F. W., Warren, M. F. & Drinker, C. K. (1940). *Amer. J. Physiol.* **129**, 635.
Meltzer, S. J. (1897). *J. Physiol.* **22**, 198.
Mendel, L. B. (1899). *Amer. J. Physiol.* **2**, 342.
Orlow, W. N. (1895). *Pflüg. Arch. ges. Physiol.* **59**, 170.
Putnam, T. J. (1923). *Amer. J. Physiol.* **63**, 548.
Shipley, P. G. & Cunningham, R. S. (1916). *Anat. Rec.* **11**, 181.
Starling, E. H. & Tubby, A. H. (1894). *J. Physiol.* **16**. 140.
Watkins, A. L. & Fulton, M. N. (1938). *Amer. J. Physiol.* **122**, 281.