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Relative Rates of Absorption of Fluid and Protein from the Peritoneal Cavity in Cats

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Summary

The relative rates of fluid and protein absorption from the peritoneal cavity of anesthetized cats were measured over 6 hours at an intraperitoneal pressure of 15 mm Hg and with intraperitoneal protein concentrations from 1–8 g%. The fractional absorption rates of fluid and protein did not change significantly over the 6 hours and were not significantly different from each other within each one hour period. In addition both fractional absorption rates were unaffected by the protein concentration of the fluid within the peritoneal cavity. Although the absolute rate of absorption is greatly increased by elevation of the intraperitoneal pressure, these data indicate that the process remains iso-oncotic as would be expected for lymphatic rather than transcapillary absorption.

Introduction

When the intraperitoneal pressure is low in the absence of ascites, there is evidence that fluid is absorbed from the peritoneal cavity via the diaphragmatic lymph vessels (1, 2, 3, 4). However, recent work (5, 6) has shown that the absorption rate is greatly increased when the intraperitoneal pressure is elevated and it is possible that the capillary circulation of the peritoneal membranes participates in the absorption process under these conditions. The present experiments have examined the relative importance of lymphatic and transcapillary absorption processes by monitoring the rates of fluid and protein removal when the pressure was artifically elevated within the peritoneal cavity of cats.

Methods

Eighteen cats (1.9–4.5 kg) were anesthetized by intraperitoneal injection of sodium pentobarbital (30 mg/kg) and supplementary doses (2 mg/kg) were administered intravenously as required. The trachea was cannulated and the bladder catheterized. Mean arterial pressure was recorded from the right femoral artery and abdominal venous pressure from a catheter in the inferior vena cava. Pressures were recorded relative to the right atrium (P23 transducers, Statham Instruments) and all recordings were displayed on a Beckman Type RM dynograph.

As previously described (5), the rate of intraperitoneal fluid absorption was measured with a servo-controlled plethysmograph designed to record volume changes without compliance artefacts caused by variations of the hydrostatic pressure (7). A length of silastic tubing, connected to the plethysmograph, was inserted into the peritoneal cavity and the abdominal wall was closed to form an air-tight seal. The abdomen was then enclosed with a plaster cast so that the peritoneal cavity functioned as a rigid plethysmographic chamber. In the present study two additional silastic tubes were also present in the abdomen to allow continuous circulation of the intraperitoneal fluid through the flow cell of a gamma spectrometer (Intertechnique CG30). This allowed the protein reabsorption rate to be monitored using radioactive labelled albumin.

At the start of each experiment the intraperitoneal pressure was raised to 15 mm Hg by filling the peritoneal cavity with a known volume of Ringer-Locke solution containing from 1.4–8.0 g% bovine serum albumin (Grade V, Sigma Chemical Co.). The initial protein concentration was varied randomly in different experiments and the plethysmograph fluid which entered the peritoneal cavity to replace the absorbed fluid was always protein-free resulting in a progressive decrease in intraperitoneal protein concentration during the

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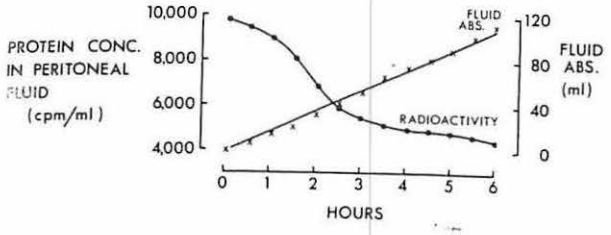

Fig. 1 Cumulative fluid absorption and change in intraperitoneal protein concentration as shown by the decline in radioactivity over 6 hours of a typical experiment.

experiment. In 12 cats the absorption of protein from the peritoneal cavity was studied by adding electrolytically labelled ^{125}I-bovine serum albumin (8) to the intraperitoneal fluid so that the radioactive content averaged 15000 CPM/ml. In the other 6 animals, the movement of protein into the peritoneal cavity was studied by injecting an aliquot of labelled protein intravenously to give a plasma radioactive content of about 60000 CPM/ml. In each experiment one hour was allowed for equilibration after opening the plethysmograph, and subsequently, the rate of fluid removal and protein removal or entry were followed for 6 hours. Samples of the plasma and intraperitoneal fluid were obtained at hourly intervals and these were analysed for total protein content using the assay of Lowry et al (9).

Calculations

Fig. 1 shows the rates of intraperitoneal fluid absorption and the radioactivity changes observed during 6 hours of a typical experiment. Both parameters were recorded on a minute-to-minute basis but the data were averaged for one hour periods. Since the intraperitoneal fluid volume and radioactive content were known, the rates of fluid and protein absorption were calculated according to their fractional rates of removal:

$$\text{Fractional fluid absorption rate} = \frac{\text{absorption rate (ml/min)}}{\text{intraperitoneal volume (ml)}}$$

$$\text{Fractional protein absorption rate} = \frac{\text{radioactivity removal rate (CPM/min)}}{\text{intraperitoneal radioactivity (CPM)}}$$

When the radioactive protein was present in the plasma (6 experiments), the rate of protein entry to the peritoneal cavity was calculated in μg/min on the basis of the plasma and intraperitoneal radioactive content and the plasma protein level as determined by the *Lowry* assay.

Pooled data were analysed by random design one–way analysis of variance in conjunction with *Duncan*'s test of multiple comparisons. Other statistical tests are mentioned where appropriate in the results.

Results

In the 18 experiments mean arterial pressure was 128 ± 5 mm Hg (mean ± S. E.) at the start and 110 ± 6 mm Hg at the end of the 6 hour recording period. Mean intraperitoneal pressure was 15 ± 0.4 mm Hg and mean inferior vena cava pressure was 16 ± 0.4 mm Hg. These pressures did not change significantly during the course of the experiments. The mean volume of intraperitoneal fluid was 126 ± 10 ml and the mean rate of its absorption was 0.27 ± 0.02 ml/min. Mean plasma protein concentration was 8.7 ± 0.45 g%.

In the 12 experiments in which fluid and protein absorption from the peritoneal cavity were studied, the fractional fluid absorption rate averaged $2.09 \times 10^{-3} \pm 0.08 \times 10^{-3}$. As shown in Fig. 2, there were no significant differences of the rates which were calculated for each of the six 1 hour periods. Since the intraperitoneal protein concentration was variable at the plethysmograph throughout the absorption period, it could also be shown that the rate of fluid absorption from the peritoneal cavity was unaffected by the protein concentration of the intraperitoneal fluid.

In the same 12 cats, the fractional protein absorption rates were calculated for each of

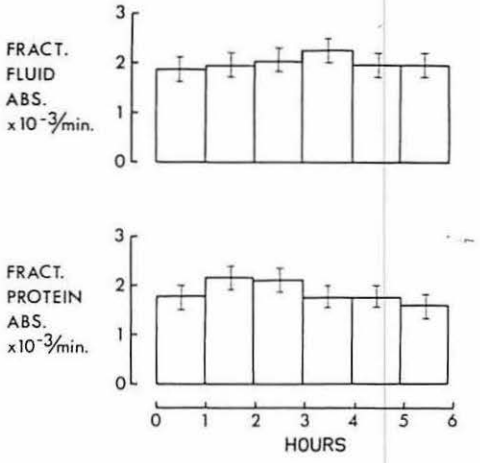

Fig. 2 Fractional absorption rates for fluid and protein for the six 1 hour recording periods.

the six 1 hour periods. As with the fluid absorption data, the protein rates did not differ significantly from each other, indicating that the fractional protein absorption remained constant throughout the experiment. Moreover, this rate averaged $1.86 \times 10^{-3} \pm 0.12 \times 10^{-3}$ which was not significantly different from the mean fractional fluid absorption. This equivalence of the fractional rates of fluid and protein absorption was examined more closely by a paired comparison of the two rates calculated for twelve to thirty minute periods in each experiment ($n = 144$). This analysis also showed no significant difference of the protein and fluid absorption rates ($p > 0.05$).

In Fig. 3 the fractional absorption of protein is shown as a function of the intraperitoneal protein concentration as calculated from the intraperitoneal fluid radioactivity and confirmed by direct analyses of total protein concentrations. There were no significant differences of protein absorption for intraperitoneal protein concentrations that ranged from 1 to 8 g%. This indicates that the fractional absorption of protein is independent of the concentration of protein in the intraperitoneal fluid.

For each experiment the actual rates of protein absorption were also calculated in μg/min on the basis of the known protein concentration of the intraperitoneal fluid and the fractional absorption rate. A correlation analysis of these data, based on 30 minute recording periods, is shown in Fig. 4. The correlation coefficient of 0.59 ($p < 0.001$) indicates that there is a significant relationship of the protein absorption and the intraperitoneal protein concentration. For the 6 experiments which examined the transfer of protein in the opposite direction, there was no significant correlation of the rate of protein entry and the intraperitoneal protein concentration ($r = 0.22$, $p > 0.05$). More importantly, the rate of entry of protein was very low in relation to the rate of protein absorption (Fig. 4). The mean rate of protein entry was 0.98 ± 0.08 mg/min which is equivalent to a total entry of only 350 mg protein throughout the 6 hour period of the experiments (range 100–460 mg).

Discussion

The intention of this study was to examine the nature of intraperitoneal fluid and protein

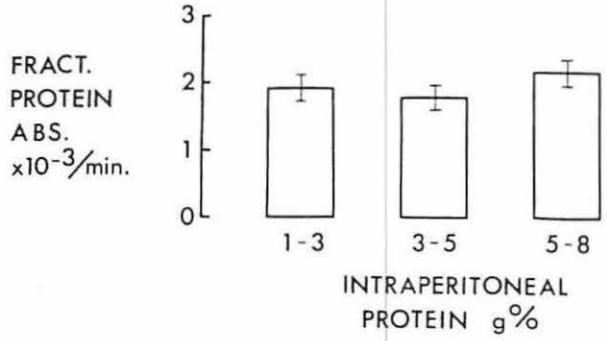

Fig. 3 Fractional absorption rates for protein at different intraperitoneal protein concentrations.

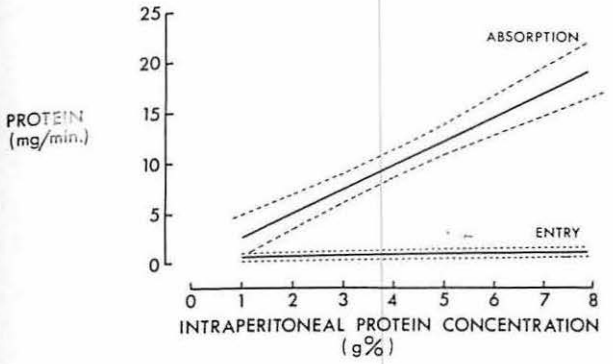

Fig. 4 The lines of best fit and the 95% confidence limits for the correlation of protein absorption (12 experiments) from, or protein entry (6 experiments) into, the intraperitoneal fluid as a function of the concentration of protein in the intraperitoneal fluid at an intraperitoneal pressure of 15 mm Hg.

absorption when the intraperitoneal pressure was increased to abnormal levels. Nevertheless, any conclusions must be considered with the realization that the experiments were conducted with animals that were physiologically normal. The pathological processes which give rise to ascites by the transudation of fluid and protein from the splanchnic and/or hepatic circulation were absent. Thus only the absorption process was investigated. In this context it is important to note that the six experiments which studied protein transfer from the blood to the peritoneal cavity were designed to discount the possibility of recirculation of the radiolabelled protein which was administered intraperitoneally in the other 12 animals. The observed turnover rates are much lower than would be expected if ascites was actively being formed (10). The use of this technique also assumes that purified radiolabelled bovine serum albumin enters and leaves the peritoneal cavity at rates similar to the transfer of unlabelled albumin. The validity of this assumption is supported by the agreement of the protein concentrations as determined by the radioactive measurements and the *Lowry* assay.

The results of this study indicate that protein is absorbed from the peritoneal cavity in an equal proportion to the absorption of fluid. Throughout the experiments the fractional rate of protein absorption was not significantly different from the fractional rate of fluid absorption. Furthermore, both rates were independent of the concentration of protein in the peritoneal cavity which suggests that the removal process involves lymphatic absorption rather than transcapillary absorption. The observation of iso-oncotic removal is thus consistent with the hypothesis of *Courtice* et al (3, 5) that absorption occurs via the lymph vessels of the diaphragm.

The ascites that accumulates in advanced cases of cirrhosis has a low protein content. If cirrhotic patients reabsorb ascites in a manner similar to the absorption studied in the present experimental model, it is clear that the reabsorption process could not be responsible for dilution of the intraperitoneal protein. The source of ascites must be a fluid with a protein content corresponding to that of the intraperitoneal fluid.

Acknowledgements

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