Interstitial Albumin-Bound Dye Concentrations in Mice with a Protein-Rich Effusion (43082)

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Abstract. **Levels of intravenously injected Evans blue dye in eluates of the lung and kidney, an index of interstitial fluid albumin concentration, together with water content of these tissues and levels of serum albumin were measured in Ha-icr mice with a tumor cell-induced protein-rich peritoneal effusion. By the fourth day after the intraperitoneal injection of tumor cells, when mean serum albumin levels had fallen to 76% of control values, mean albumin bound dye concentrations in lung and kidney had decreased to 63 and 58%, respectively, of control values. By the tenth day when serum albumin levels had decreased further to 67% of control values, albumin-bound dye concentrations in the lung and kidney had decreased to 58 and 43%, respectively, of control values. During this 10-day period the water content of the lung remained unchanged whereas that of the kidney had decreased by 7%. These observations suggest that the reduction in serum albumin which results from an abnormal distribution of this protein into a nonvascular compartment is accompanied, as in other models of hypoalbuminemia, by a more than proportionate reduction in interstitial albumin concentration in the lung and kidney.** [P.S.E.B.M. 1990, Vol 194]

The colloid osmotic pressure of plasma as well as that of interstitial fluid is regulated principally by the levels of albumin in these fluids (1). Hypoalbuminemia induced experimentally by either a protein poor diet (2), plasmaphoresis (3, 4), excess administration of noncolloidal solutions (5, 6), or by a protein losing nephropathy (7, 8) lowers the colloid osmotic pressure of plasma and is accompanied by a disproportionate reduction in interstitial albumin levels. Information available from computer simulations as well as direct experimental observations is consistent with the view that in each of these models a decrease in the transcapillary escape of albumin along with an increase in net fluid filtration and lymph flow combine to dilute albumin in the interstitial compartment (4, 9, 10). By maintaining a transcapillary oncotic gradient at or near normal levels these compensatory adjustments prevent formation of edema until serum levels of albumin decline to approximately 50% of normal values.

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Hypoalbuminemia develops in patients with a protein-rich malignant effusion, but in contrast to the experimental models cited above, the decrease in circulating levels of albumin is the direct result of an abnormal distribution of albumin between the vascular and an extravascular compartment (11). A close experimental counterpart of this derangement exists in mice injected intraperitoneally with Ehrlich ascites tumor cells. Within 3 days of this injection, these animals develop a progressively severe leak of protein from peritoneal capillaries, which leads to the formation of a protein-rich peritoneal effusion (12). This abnormal leak of protein into peritoneal tissues is the factor which distinguishes these animals from earlier models of hypoalbuminemia. An attempt to determine whether the reduction in circulating albumin which results from this particular derangement is accompanied by a reduction in interstitial fluid content of albumin-bound dye in tissues remote from the peritoneal effusion formed the basis for this study.

Materials and Methods

All studies were performed in adult male Ha-icr mice (34–45 g body weight) maintained on a diet of Purina Lab Chow containing 0.4% sodium and 23.4% protein. A protein-rich peritoneal effusion was induced

in experimental animals by the intraperitoneal injection into conscious mice of 0.3 ml of a saline suspension of Ehrlich ascites tumor cells (1×10^5 cells/ml). Alterations in lung and kidney interstitial albumin levels were estimated by a method developed by Landis (13) and modified by Nachman *et al.* (14) and by Ackerman (15) which is based on the knowledge that intravenously injected Evans blue dye rapidly combines with circulating albumin. Concentrations of the albumin-bound dye were measured in eluates of sections of lung and kidney removed 30 min after the injection of 0.2 ml of a 0.5% solution. Mice were anesthetized with ether and sections of lung and kidney were excised, weighed, and 100-mg samples were eluted for 72 hr in 1.5 ml of Formamide. The color intensity of the eluate was read at 620 μm in a Beckman DU spectrophotometer (model 2400). Albumin-bound dye content was expressed as optical density units/100 mg of tissue. Five control mice were compared with two experimental groups of seven mice each receiving the dye on the 4th or 10th day after the injection of tumor cells.

Albumin levels in samples of plasma obtained from 10 additional mice with tumor cell-induced effusions were determined by the bromcreosol green method (16) at 4 and 10 days after the injection of tumor cells and compared with values in four additional control animals. The albumin content of ascitic fluid was measured by the same method in 8 of these animals 10 days after the injection of tumor cells.

As an index of alterations in tissue water content, differences between wet and dry weight of lung and kidney obtained from eight experimental animals sacrificed on the 10th day after tumor cell injection were compared with those in eight controls. In each case the left lung and kidney were removed under ether anesthesia, blotted dry, and placed in individual aluminum containers that had been treated with HNO_3 to remove grease and moisture. The basket with its specimen was weighed in a Sartorious balance, placed in a dessicator dish, and then dried in an oven at 85°C for 24 hr. Tissues were then reweighed and the results expressed as percentage changed per unit of wet weight (wet − dry/wet).

Statistical Analysis. Standard statistical methods were used. All values are expressed as mean ± SE. Comparison between controls and experimental groups were made with unpaired Student's t test and analysis of variance.

Results

Four days after the injection of tumor cells, the mean concentration of albumin-bound dye (expressed as optical density units/100 mg of tissue) was 4.0 ± 0.3 in lung and 2.6 ± 0.16 in kidney compared with 6.3 ± 0.23 and 4.4 ± 0.18, respectively, in five controls ($P < 0.001$). By the 10th day after tumor cell injection, these values had decreased further to 2.1 ± 0.15 and 1.9 ± 0.14, respectively.

During this 10-day period mean levels of serum albumin in mice with effusions declined from a mean value in four controls of 3.4 g 100/ml ± 0.04 to 2.6 ± 0.1 on Day 4 ($P \leq 0.003$); and to 2.3 ± 0.1 on Day 10.

All 14 animals receiving an injection of tumor cells had evidence of a peritoneal effusion by the 4th day. On the 10th day the mean level of albumin in the effusion was 2.1 ± 0.1 g 100/ml.

The results of the albumin-bound dye studies on the fourth day after tumor cell injection represent a fall in concentration in the lung and kidney of 63% and 48% of control values, respectively, compared with a 76% decline in serum albumin levels. By the 10th day, when the latter had decreased further to 67% of control values albumin bound dye concentrations in the lung and kidney had fallen to 53% and 43%, respectively, of control values.

A comparison of wet and dry weights of lung and kidney removed from mice with a peritoneal effusion 10 days after the injection of tumor cells with those of lung and kidney from control animals disclosed no change in the water content of the lung but a significant decrease in that of the kidney from 55% to 48% ($P = 0.05$).

Discussion

Levels of albumin-bound dye in eluates of the lung and kidney were used in this study as an index of changes in the albumin content of interstitial fluid, an approach that has been applied for similar purposes by others (17, 18). The results demonstrate that hypoalbuminemia induced by an abnormal leak of circulating albumin into the peritoneal cavity lowers the concentration of albumin bound dye in the interstitial fluid of the lung and kidney. The decline was noted from the 4th to the 10th day after intraperitoneal injection of tumor cells when increased peritoneal capillary permeability to protein is known to be well established (12). As Evans blue dye is tightly bound to albumin, it is likely that circulating dye leaked into the peritoneal effusion at the same rate as albumin, thereby reducing in equal proportions the amount of dye available for transcapillary escape into interstitial fluid of the lung and kidney and, in animals not injected with dye, the amount of albumin available for measurement in plasma. But instead of finding a decrease in albumin-bound dye in the interstitial fluid proportional to the decrease in serum albumin, a disproportionate reduction was found. Similar disproportionate reductions in interstitial albumin have been found in other experimental models of hypoalbuminemia (2–8).

A possibility that a significant fraction of the albumin-bound dye measured in the lung and kidney might have been in the vascular rather than the inter-

stitial compartment seems unlikely. Even if filled with blood (which was not the case), the vascular compartment of these tissues would comprise no more than 4% of their weight, the plasma portion approximately half of this amount and the albumin fraction even less.

To consider that this effect of hypoalbuminemia might be due to simple expansion of the interstitial fluid compartment would be to overlook the fact that in the case of the lung the tissue water content remain unchanged whereas that in the kidney actually decreased. It is much more likely, as demonstrated by Wiederhelm and by others (9, 10), that a "protein wash down" effect, that is, a relatively greater transcapillary flux of water than of protein, lowers the protein content of tissue fluid by simple dilution. At the same time, an increase in lymph flow avoids any increase in interstitial fluid volume. This mechanism appears to compensate for hypoalbuminemia even when microvascular pressure gradients (including venous pressure) remain unchanged.

Reduction in tissue fluid content of albumin can be regarded as a compensatory adjustment to hypoalbuminemia which serves to maintain the transcapillary oncotic gradient and thus to limit net transfer of fluid out of the capillary. Functioning effectively only within certain limits, this mechanism fails when serum levels of albumin are lowered to less than 2 g 100/ml, corresponding to serum oncotic pressures lower than 15 mm Hg (10). Below this level (which was not reached in the studies described here) protein concentrations in interstitial fluid can be expected to change only minimally, and as a result the transcapillary oncotic gradient would decline, resulting in the appearance of edema.

Compensatory adjustments to hypoalbuminemia were clearly not possible in the peritoneal capillary bed, which was directly exposed to tumor cells. Levels of albumin in the peritoneal effusion increased to a mean of 2.1 g 100/ml, a value only slightly lower than serum levels, virtually eliminating any transcapillary oncotic gradient.

The excessive transcapillary leak of protein into peritoneal tissues responsible for the peritoneal effusion and the accompanying hypoalbuminemia in this study appear to derive from the release by tumor cells of pharmacologically active substances, kinins and prostaglandins, which increase local capillary permeability to protein (19–21). Based on the results reported here, a possibility that these substances might be absorbed from the peritoneal cavity and circulate to other tissues increasing their permeability seems unlikely.

Whether the findings in this study bear on the problem of malignant effusions in man is uncertain. They do suggest, however, that attempts to correct hypoalbuminemia in such patients by the intravenous administration of colloidal solutions may be unwarranted in those who are free of edema. Moreover, any increase in venous pressure that might accompany such attempts is likely to disrupt existing compensatory adjustments to hypoalbuminemia and thus result in edema.

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