Increased albumin plasma efflux contributes to hypoalbuminemia only during early phase of sepsis in rats

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Ruot, Benoît, Isabelle Papet, Fabienne Béchereau, Philippe Denis, Caroline Buffière, Johan Gimonet, Françoise Glomot, Mimoun Elyousfi, Denis Breuille, and Christiane Obled. Increased albumin plasma efflux contributes to hypoalbuminemia only during early phase of sepsis in rats. Am J Physiol Regul Integr Comp Physiol 284: R707–R713, 2003; 10.1152/ajpregu.00483.2002.—The mechanisms leading to hypoalbuminemia in sepsis were explored by measuring plasma volume, albumin distribution, plasma albumin transcapillary escape rate (TER), and efflux (TER × albumin intravascular pool). These parameters were quantified in infected rats, injected intravenously with live Escherichia coli, and pair-fed and well-fed rats using an injection of 35S-albumin and measuring plasma and whole body albumin concentrations. Animals were studied on days 1, 6, and 10 after infection. In pair-fed rats, neither albumin distribution nor exchange rate between the intra- and extravascular compartments was modified. The increase of plasma volume after infection partly explained hypoalbuminemia. Infection resulted in a reduction of the total albumin pool of the body all along the experimental period, indicating a net loss of the protein. Albumin TER (%/day) was significantly increased 1 and 6 days after infection, but the absolute efflux was increased only on day 1. Normal values were observed on day 10. Therefore, an accelerated plasma efflux contributes to hypoalbuminemia only during the early period of sepsis. During this phase, the protein was retained in the extravascular space where it was probably catabolized. Later on, other factors are probably involved.

plasma albumin escape; albumin distribution; infection

One of the most common characteristics of inflammatory diseases is a severe reduction in plasma albumin concentration, which occurs very soon after the onset of the acute-phase response and is maintained during a long period of time. This hypoalbuminemia is associated with physiological disorders such as modification of the intravascular oncotic pressure or alterations in the transport of exogenous and endogenous substrates. However, the mechanisms associated with the prominent decrease of plasma albumin after injury are not completely understood yet. The plasma albumin level is influenced by several mechanisms, including changes in circulating fluid volume, albumin synthesis and catabolism, distribution of the protein in the extravascular space, lymphatic return, and urinary or intestinal losses.

During the initial period after injury, there is increasing evidence in both humans and animals that albumin synthesis is not reduced and even increased (4, 12, 23, 27, 32). This suggests that either extravascular albumin relocation or increased catabolism and losses would be the predominant mechanisms accounting for the rapid fall of albuminemia after the stress of major surgery or infection. There is a continual circulation of albumin between the intra- and extravascular spaces because it leaks from the intravascular space across the capillary membrane and returns to it via the lymphatic system. This flux is 8–10 times the rate of albumin synthesis or degradation, and it has been shown that the transcapillary escape rate (TER) is increased after injury (3, 12, 13). Therefore, it has been suggested that the increased TER has a far more rapid effect on plasma albumin concentration than any alteration in its synthesis or catabolism. However, the net loss of albumin from the intravascular space depends also on its return by the lymph, which is probably also increased severalfold in inflammatory diseases, but this point received little attention. The increased movement of albumin across the capillary wall can lead to a redistribution of albumin between the intravascular and the extravascular pools. It has been shown that the ratio of extravascular to intravascular albumin increased after injury (2, 8). However, the variation of the total albumin pool of the body is unclear.

Hypoalbuminemia is maintained far after the acute phase, and the mechanisms involved in maintaining low plasma albumin levels may be different. Albumin kinetics have been poorly documented in the postacute phase. In a recent study, we have shown that a default

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The aim of the present study was to determine albumin pools and plasma albumin efflux using intrinsically labeled albumin in response to infection. A kinetic study was performed using a long-lasting rat model of sepsis characterized by a persistent hypoalbuminemia (7). To determine specifically the effect of infection and because food deprivation is well known to affect albumin metabolism (26), infected rats were compared with both control rats and with pair-fed noninfected rats.

MATERIALS AND METHODS

Animals. All procedures were performed in accordance with the current legislation on animal experimentation in France and the “Guiding Principles for Research Involving Animals and Human Beings” of the American Physiological Society (1). Fifty-six male Sprague-Dawley rats (IFFA-Credo, l’Aberre, France) weighing ~250 g were maintained in individual cages at a temperature-controlled room (22°C) on a 12:12-h light-dark cycle (light at 0700) with free access to water. They received a semisynthetic diet containing 12% protein given in six meals distributed every 4 h via an automatic feeder (6). Body weight and food intake were recorded daily.

After an acclimatization period of 6 days, the rats weighed ~280 g. They were divided into seven groups (8 rats/group) of equal mean body weight. One group was the control well-nourished group studied on day 0. Rats from three other groups (infected rats) were injected with live Escherichia coli (serotype 0153 K^-H^-; 7 × 10^8 colony-forming units) into a lateral tail vein as described previously (7). Rats developing diarrhea and having lost >80 g in 6 days were euthanized by injection of a lethal dose of pentobarbital sodium (Sanofi Santé Animale, Libourne, France). There was no mortality in the infected groups. Animals were studied during an acute septic phase (1 day postinjection), a chronic septic phase (6 days postinjection), and a late septic phase (10 days postinjection) and compared with control rats (day 0). Rats from the last three groups were injected likewise with saline. Because infection induced a strong anorexia, the amount of food given in meals to saline-injected rats (pair-fed rats) was equal to the food consumed by infected animals. Pair-fed rats were also studied 1, 6, or 10 days after saline injection.

Preparation of [35S]-albumin. Injections of 2 mCi of L-[35S]-cysteine, 20 mCi/mmol (Amersham Pharmacia Biotech, Saclay, France), were made into a lateral tail vein of five rats weighing 250 g. After 75 min, animals were anesthetized with pentobarbital sodium (6 mg/100 g body wt ip) and exsanguinated. Blood was taken from the abdominal aorta, and plasma was separated by centrifugation (500 g, 15 min). About 5 ml of plasma were obtained from each rat. Albumin was purified by affinity chromatography on blue Sepharose CL6B (Amersham Pharmacia Biotech) as described previously (28). The albumin-containing fractions were dialyzed in the cold against pure water. The pH was then brought to 5.85, and pure ethanol was added to the solution to get a final concentration of 19% (vol/vol). After centrifugation to eliminate insoluble material, the albumin solution was freeze-dried. The final preparation was >97% pure as judged by PAGE. Radioactivity in albumin was determined by liquid scintillation counting with QuickSafe A as scintillation medium (Zinsser Analytic, Frankfurt, Germany) in a counter (TRI-CARB 2100TR, Packard Instruments SA, Rungis, France). The typical final yield from 10 ml of plasma was 62 mg albumin, and the specific activity was ~0.126 μCi/mg albumin.

Experimental design. Albumin TER, plasma volume, and albumin pools were determined in all groups. The day of the study, under general anesthesia (Imalgène, Rhône-Mérieux, France), catheters were inserted into the carotid artery (Vygon, Ecouen, France). One hundred microliters of blood were taken for albumin concentration measurement. Injection of 0.30 μCi and 2.5 mg of [35S]-albumin per 100 g body wt in 500 μl of water was made into a lateral tail vein. Injected radioactivity was determined from the difference of the dosyringe weight before and after injection. The time of injection was immediately noted. Blood samples were taken from the carotid artery every 5 min from 5 up to 25 min after the injection of radioactive albumin, the exact time of sampling being noted. The sampling procedure was as follows. At each sampling point, 200 μl of blood were withdrawn from the cannula and retained. The sample (200 μl) was taken later, and the initial 200 μl were immediately replaced. During the course of the experiment, <5% of the total blood volume was removed. After separation by centrifugation, plasma was removed and kept at ~20°C until analysis.

At 60 min after the injection, animals were killed by collecting total blood from the abdominal aorta. This time was chosen to have enough acid-soluble radioactivity in tissues that can be used as an index of albumin catabolism. The amount of blood taken off was weighed. The liver was rapidly removed, blotted to remove superficial blood, weighed, and frozen in liquid nitrogen. The whole intestine, from duodenum to anus, was isolated, flushed with ice-cold NaCl (9 g/l), blotted, weighed, and frozen in liquid nitrogen. The carcass was weighed and frozen in liquid nitrogen.

Measurement of radioactivity in plasma and tissues. Plasma albumin-bound radioactivity was determined after addition of 800 μl 10% TCA to 100 μl plasma followed by centrifugation (8,000 g, 15 min). The protein pellet was washed two times with 10% TCA and solubilized by addition of 1 ml 0.3 N NaOH. Determination of radioactivity was performed by liquid scintillation as described above.

The whole carcass and tissues were finely powdered in liquid nitrogen in a ball mill (Dangoumeau, Prolabo, France). Weighed portions (2 × 1 g) of frozen tissue powders were homogenized in 8 vol of ice-cold 10% TCA. Homogenates were centrifuged (6,000 g, 20 min, 4°C). The protein pellet was washed twice with 10% TCA. Radioactivity of the combined supernatants containing free amino acids was determined in duplicate by liquid scintillation as described above.

Measurement of albumin concentrations. Plasma albumin was measured by single radial diffusion using anti-rat albumin antibodies (ICN, Cappel, Belgium) as previously described (6). For tissues, weighed portions of frozen powder were homogenized in 6 vol of 0.35 M sucrose 50 mM Tris acetate buffer, pH 7.4. Homogenates were centrifuged 10 min at 10,000 g, and albumin was determined in the supernatant as above.

Calculations. Albumin TER, expressed as percentage per hour, was determined from the measurement of the amount of plasma labeled albumin over the first 25 min after its intravascular injection. Plasma albumin-bound radioactivity was expressed as counts per milliliter of plasma and natural log (ln) counts per milliliter were plotted against time (Fig. 1). The slope of the linear curve yielded the initial rate constant, which represents the TER of albumin. The initial plasma activity was obtained by extrapolation of the semilogarithmic plot to the y-axis (time 0). The plasma volume...
injected dose. Values are means ± SD for n = 8 in each group. LN, natural log.

was calculated by dividing the injected dose by the initial plasma radioactivity. The intravascular albumin mass was expressed as the product of plasma albumin concentration times plasma volume. The absolute plasma albumin efflux was calculated by multiplication of TER with the intravascular albumin pool.

The total albumin pool was calculated from the sum of albumin found in tissues and in blood taken at the end of the experiment. The extravascular pool was obtained by difference between the total pool and the intravascular pool.

Statistical methods. Data are presented as means ± SD. The significance of differences was analyzed by one-way ANOVA and subsequent Scheffe’s F-test when appropriate. Differences were considered significant when P < 0.05.

RESULTS

Animal characteristics. Infection decreased food intake, especially during the acute period because rats ate only 5–15% of the preinfection intake (20–25 g) (data not shown) as observed classically on our model (6, 28). Thereafter, food intake of infected animals progressively increased to reach values similar to those observed before infection. During all the experimental period, infected and pair-fed rats had similar food intake. In this study, the body weight change course was also similar to that observed in previous studies. On day 1 postinfection, the body weight loss was similar in infected and pair-fed rats. Thereafter, infected rats had a lower body weight than pair-fed rats (Table 1).

During the acute phase, the weight of liver and intestine was significantly increased in infected rats compared with pair-fed animals (40 and 11% for liver and intestine, respectively). Thereafter, infection significantly decreased the liver weight. By contrast, the weight of whole intestine was significantly greater in infected rats than in pair-fed rats 10 days after infection (Table 1). Infection significantly decreased the carcass weight during the chronic phase.

Plasma volume, plasma albumin concentration, TER, and absolute efflux. Plasma volumes were similar in well-fed and pair-fed animals. Infection significantly increased plasma volume (Table 2). Plasma albumin concentrations were reduced by ~30% as soon as the first day after infection compared with control rats or with pair-fed rats. Infection is associated with a long-lasting hypoalbuminemia, because plasma albumin concentration was still decreased 6 and 10 days after infection, by about 50 and 40%, respectively, and the intravascular albumin pool was decreased too (Table 2).

Albumin TER was similar in all groups of pair-fed rats and control rats (studied on day 0) (Fig. 2A). One and 6 days after infection, albumin TER was greater in infected rats than in pair-fed rats. It returned to normal values at the end of the experimental period. The absolute albumin efflux, which is an estimate of the amount of albumin loss across the vascular endothelium, was increased 1 day postinfection in infected rats compared with pair-fed rats (53%) and control rats (33%) (Fig. 2B). Similar values were obtained in infected and pair-fed animals 6 and 10 days postinfection.

Albumin pools and distribution. Infection significantly reduced the total albumin pool and the intravascular albumin pool (Table 2). The albumin content in the extravascular pool was similar in infected and pair-fed rats 1 day after infection but lower in infected rats than in pair-fed rats on days 6 and 10. The ratio of the extravascular pool to the intravascular pool was unchanged during the acute phase. It was increased by infection on day 6.

One hour after the injection of labeled albumin, the percentage of the dose remaining in plasma albumin is similar in well-fed and pair-fed animals but lower in infected rats on days 1 and 6 after infection (Table 3).

![Graph showing plasma albumin-bound radioactivity time course after injection of 35S-labeled albumin](image)

**Fig. 1.** Plasma albumin-bound radioactivity time course after injection of 35S-labeled albumin in infected rats (•) and pair-fed rats (○) 1 day after infection. For comparison, values have been normalized for injected dose. Values are means ± SD for n = 8 in each group. LN, natural log.

### Table 1. Effect of infection on cumulative body weight change and tissue weights

<table>
<thead>
<tr>
<th>Day 0 (Control)</th>
<th>PF</th>
<th>Infected</th>
<th>PF</th>
<th>Infected</th>
<th>PF</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Cumulative body wt change, g</td>
<td>-19.1 ± 5.3</td>
<td>-24.9 ± 3.9</td>
<td>-14.2 ± 2.9</td>
<td>-25.4 ± 13.2*</td>
<td>17.6 ± 7.4</td>
<td>9.3 ± 1.6*</td>
</tr>
<tr>
<td>Carcass, g</td>
<td>236 ± 5</td>
<td>221 ± 12†</td>
<td>217 ± 5†</td>
<td>239 ± 4</td>
<td>214 ± 9‡</td>
<td>248 ± 8‡</td>
</tr>
<tr>
<td>Liver, g</td>
<td>10.1 ± 0.5</td>
<td>7.5 ± 0.4†</td>
<td>10.5 ± 0.6*</td>
<td>9.4 ± 0.5</td>
<td>8.3 ± 1.3‡</td>
<td>10.7 ± 0.9</td>
</tr>
<tr>
<td>Whole intestine, g</td>
<td>7.2 ± 0.5</td>
<td>6.2 ± 0.4†</td>
<td>6.9 ± 0.4*</td>
<td>7.0 ± 0.4</td>
<td>7.2 ± 0.5</td>
<td>7.0 ± 0.5</td>
</tr>
</tbody>
</table>

Values are means ± SD for 6–8 rats in each group. Infected, infected rats; PF, pair-fed rats. Initial body weight (day 0) was similar in the different groups and equal to 276 ± 8 g. Statistical significance: *P < 0.05 vs. PF rats; †P < 0.05 vs. control rats (day 0).
remain unclear. These include synthesis (exclusively in ever, the mechanisms involved in hypoalbuminemia bations and poor prognosis in acutely ill patients. How matic syndrome associated with physiological pertur sures and albumin pools at various stages of the acute-phase reponse using a long-lasting septic that produces a persistent and severe hypoalbuminemia (14). Moreover, the time course after injury of the mechanisms responsible for hypoalbuminemia is not documented. Thus the aim of the present study was to examine the effect of infection on albumin distribution and rates of exchange between the intra- and extravascular pools at various stages of the acute-phase reponse using a long-lasting septic that produces a persistent and severe hypoalbuminemia (6, 7).

Most studies on albumin metabolism have used in vitro labeling of albumin with iodine, but it is not clear that the rate of removal of labeled iodine from the protein is the same as the rate of metabolism of the intrinsically labeled protein (9). Moreover, in animal studies, albumin from other species is often used, and albumin of another species will not necessarily show the same behavior. In this study, albumin metabolism was explored after injection of intrinsically labeled rat albumin.

The plasma volume reported in this study, by measuring the dilution of radioactive albumin, is in agreement with the currently reported normal values in rats of similar weights (8, 20, 30) and also with our previous results obtained with the classical blue dye dilution method (27). Schreiber et al. (30) also reported similar values with these two methods. The TER, i.e., the fraction of intravascular albumin that passes to the extravascular space per unit time, ~27%/h, is far greater than the rate of synthesis (~2%/h) as described also by others (20). The high value of albumin TER and absolute efflux from the intravascular space implies that the lymphatic return is also very rapid as shown by the theoretical calculations based on the model presented in Fig. 3. Therefore, the lymphatic return can have an important impact on albumin distribution. The total body pool of albumin reported in the present study for well-fed rats (302 ± 14 mg/100 g body wt) is in agreement with the values reported by Brown et al. (8) and Schreiber et al. (29). In control rats, the intravascular albumin pool represents ~35% of the total pool as described previously (8).
Infection is associated with malnutrition, essentially due to a reduction of food intake, and we used pair-fed animals to determine the effect of infection on albumin kinetics independently of food restriction effects. Transient food restriction appeared to have no effect on plasma volume, albumin pools, TER, and absolute efflux. We have shown previously that 1 day of severe food restriction strongly reduced albumin synthesis (28). Taken together, these results suggest that albumin pools are maintained by a decreased rate of catabolism. However, the amount of free radioactivity was only reduced in the liver. Moreover, protein-energy depletion resulted in no changes in albumin distribution and pool sizes but decreased catabolic rate in the rabbit (31). During the progressive restoration of food intake, we observed little changes in albumin pools, distribution, and rate of exchange. As albumin synthesis progressively returned to normal levels (28), we can hypothesize that catabolism did too, as shown during recovery from protein malnutrition (18, 19).

After injury, hypoalbuminemia could result from a passive enhanced dilution due to plasma volume increase. Plasma volume was increased in infected rats compared with pair-fed rats (Table 2), as shown also 6 days after injury in burned-infected rats (8). In humans, hypovolemia is described during the early shock period after severe injury and patients require a resuscitation procedure. After resuscitation of the patient, a period of overexpansion of the extracellular fluid volume occurs that is partly due to the infusion of intravenous fluids (resuscitation) and nutrients (15, 17, 21, 22). During this period, the plasma volume is increased but to a very variable extent (3, 10, 12, 22). Therefore, the increase of plasma volume after infection and injury partly explained the decrease of albuminemia. However, the decrease of albuminemia, which reaches >50%, was more important than the increase of plasma volume. As a consequence, the intravascular albumin pool decreased markedly after infection or acute diseases both in animals and in humans (3, 8).

A dramatic fall in albuminemia and intravascular albumin pool under stress conditions could result from an increased TER and absolute efflux from the plasma pool. Our data during the first day after infection confirm this hypothesis. However, on day 6, despite a high TER, albumin efflux was normal. This result shows that TER can only partly explain albumin leakage to the extravascular space. Under normal conditions in humans, ~5% of the intravascular albumin passes per hour through the vascular endothelium (13, 25), but this value is two to three times higher (or more) in patients (3, 12, 13). Moreover, Ballmer et al. (3) reported a correlation between low plasma albumin levels and elevated TER in acute infection diseases. The intravascular absolute albumin efflux was only slightly increased 24–48 h after the onset of fever (3). By contrast, absolute albumin efflux was increased in patients, and the proportion of patients showing increased absolute albumin efflux was more important in those studied during the first 5 days after admission in the intensive care unit than in those studied later (12).

During the early phase after injury, the increased albumin escape from the intravascular space could modify the distribution of the protein between the extra- and intravascular compartments. The amount of albumin in the extravascular space was not significantly reduced in infected animals compared with pair-fed animals on day 1 but was significantly reduced on day 6. However, at these stages, albumin was more distributed in the extravascular space as indicated by the ratio of extravascular to intravascular pools and as also suggested by the amount of radioactivity bound to
albumin remaining in the plasma pool. Similar data were obtained in injured patients (2) and in burned or burned-infected rats (8), probably due to albumin sequestration within the lesion sites (16, 24). However, albumin was also retained in tissues after systemic injection of bacteria (24). An impairment of the lymphatic return would also contribute to the sequestration of the protein into the extravascular space, but this factor has never been explored. Nevertheless, the decrease of the vascular pool was never compensated by an increase of the extravascular pool, leading to a net loss of albumin. Indeed, the total albumin pool of the body decreased until day 6 after infection and remained low on day 10.

On day 1 after infection, the greater amount of acid-soluble radioactivity found in the tissues of infected rats than in those of pair-fed animals would suggest an increased catabolism of albumin. The decreased extravascular pool observed despite an increased input from the intravascular pool would lead to the same conclusion. Moreover, we have previously shown that albumin synthesis was not reduced in infected rats compared with pair-fed animals (28). Because the intravascular pool is reduced by infection, these results suggest that the net efflux (total efflux minus lymphatic return) would be greater in infected rats than in pair-fed animals. Therefore, during the early acute phase of sepsis, albuminemia decreased because of an increased net efflux of the protein toward the extravascular space where it is probably more catabolized. In patients, albumin catabolism is correlated with the severity of the injury (11). Nevertheless, the significance of an increased albumin catabolism after injury is unclear. It can be hypothesized that, like muscle proteolysis, this process contributes to provide amino acids required for the anabolic pathways involved in host defenses (6).

On day 6, despite a high TER, albumin efflux was normal. Hypoalbuminemia continued to fall probably because albumin synthesis was very low (28) and/or because of insufficient lymphatic return. The catabolism of the protein probably fell, but the balance between synthesis and catabolism would be negative, leading to a large decrease of the extravascular pool. Finally, on day 10, the amount of albumin in the plasma began to increase probably because synthesis was a little stimulated as shown previously (28). By contrast, the extravascular pool did not change between 6 and 10 days after infection, suggesting no change in catabolism as observed in recovery from protein malnutrition (19).

In conclusion, the present study has allowed us to determine the contribution of albumin plasma efflux to hypoalbuminemia at different stages of the response to infection. In addition to hypoalbuminemia, the total albumin pool of the body was reduced all along the experimental period, as observed until 6 days after turpentine injection (29), indicating a net loss of the protein after infection. The current results and our previous data concerning albumin synthesis (28) demonstrate that hypoalbuminemia is a multifactorial syndrome resulting from changes in plasma volume, plasma efflux, body repartition of the protein, catabolism, and synthesis. During the acute phase of infection, hypoalbuminemia can be mainly explained by a higher flux of albumin toward the extravascular pool where the protein is probably catabolized. During the chronic phase of sepsis, albumin was preferentially located in the extravascular space and was lost from the body probably because of a negative balance between synthesis and catabolism. During the late phase of sepsis, hypoalbuminemia would be maintained by a default of stimulation of synthesis as shown previously (28) and/or a too important catabolism. However, further studies are required to confirm the hypothesis developed on the basis of previous and present data concerning the importance of albumin catabolism. Moreover, other factors, such as the lymphatic return and urine and intestine losses, should be estimated.

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