The response of liver albumin synthesis to infection in rats varies with the phase of the inflammatory process

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ABSTRACT

To discriminate between the effects of infection and of anorexia associated with infection, liver albumin synthesis was measured in well-fed rats, in rats injected with live Escherichia coli and in pair-fed rats at different stages of the inflammatory response (1, 6 and 10 days after infection) using a large dose of L-[1-14C]valine. Albuminaemia and albumin mRNA levels were unchanged following food restriction. However, absolute albumin synthesis was decreased in pair-fed rats compared with control animals after 1 day of food restriction, and had returned to normal values by day 10 when food intake was restored. Infection was characterized by a decrease in the plasma albumin concentration (35%, 45% and 28% as compared with pair-fed rats at 1, 6 and 10 days after infection respectively). Albumin mRNA levels and relative albumin synthesis were reduced in infected rats as compared with both control and pair-fed animals at all stages of infection. However, during the early acute response, the albumin absolute synthesis rate was similar in infected rats and pair-fed rats, indicating no specific effect of infection at this stage. Later in the course of infection, the amount of albumin synthesized by the liver was lower in infected than in pair-fed rats, and hypoalbuminaemia was probably maintained due to a lack of stimulation of synthesis despite increased food intake.

INTRODUCTION

Among proteins, albumin is one of the major products of the liver. While the plasma albumin concentration is normally relatively constant, one of the most consistent changes in response to infection, associated with anorexia, is a decline in the concentration of plasma albumin. However, the plasma albumin level is the net result of three active physiological processes occurring simultaneously: synthesis, distribution and degradation.

For a long time, in both humans and animals, hypoalbuminaemia has been considered to be a consequence of a decreased rate of synthesis of the protein [1]. In particular, tumour necrosis factor α, turpentine and lipopolysaccharide injections in animals are always associated with a rapid decline in albumin mRNA levels in the liver, and based on these results authors have concluded that there is decreased albumin synthesis in acute stress [2–4]. Nevertheless, albumin synthesis measured in animals, in vivo or ex vivo, has revealed...
conflicting results. In response to severe inflammation, albumin synthesis was found to be unchanged [5], decreased [6,7] or increased [8]. However, in most studies where albumin synthesis was reported to decrease in response to injury, treated animals were compared with well-fed animals [6,7,9]. However, it is well known that the nutritional state is important in regulating albumin synthesis [10,11], and in these previous studies the effect of injury cannot be separated from the effect of reduced food intake.

Recent investigations carried out in patients support the idea that hypoalbuminaemia is not the result of a failure of synthesis [12,13]. However, measurements of albumin synthesis were only made at one time point following injury, and it is possible that albumin synthesis responds differently depending on the stage of the inflammatory process. In particular, a biphasic response (no change, then an increase) of albumin synthesis has been demonstrated in a rat model of sepsis [8].

Using a long-lasting model of sepsis in rats, characterized by three distinct phases [14], we have studied the kinetics of liver albumin synthesis rates during the early acute phase (1 day post-infection), the chronic phase (6 days post-infection) and the late phase (10 days post-infection). The first period, during which the body weight loss of 300 g rats reached 40–50 g and food intake was greatly depressed, occurred between 0 and 3 days post-infection. During the second phase, rat body weights remained stable until day 6–8, despite a progressive increase in food intake. The third and final phase corresponds to the beginning of body weight recovery. These three phases are characterized by constant hypoalbuminaemia. Molecular mechanisms of liver albumin synthesis were also investigated by determining albumin mRNA levels. To discriminate the effects of infection from those of anorexia induced by infection, infected rats were compared with both control rats and pair-fed non-infected rats.

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**MATERIALS AND METHODS**

**Animals**

A total of 42 male Sprague–Dawley rats (IFFA-Credo, l’Arbresle, France), weighing approx. 250 g, were maintained in individual cages in a temperature-controlled room (22 °C) on a 12 h light/12 h dark cycle (lights on at 07.00 hours) with free access to water. They received a semi-synthetic diet containing 12% (w/w) protein in six meals per day via an automatic feeder [15]. Body weight and food consumption were recorded daily.

**Experimental design**

At the end of a 6-day acclimation period, the rats weighed approx. 300 g. They were then divided into seven groups (six rats per group) of equal mean body weight. Group 1 was the control, well-nourished group studied on day 0. Rats from groups 2–4 were injected with live *Escherichia coli* (serotype 0153:K:H7; 7 x 10^8 colony-forming units) into a lateral tail vein as described previously [14]. Rats from groups 5–7 were injected likewise with saline. Since infection induced considerable anorexia, saline-injected rats received the same amount of food as infected animals (pair-fed rats). Injected animals were studied during an acute septic phase (1 day post-injection), a chronic septic phase (6 days post-injection) and a late septic phase (10 days post-injection), and compared with control rats (day 0). The protocol was approved by the Ethics Committee of the INRA Research Centre of Clermont-Ferrand, and was conducted in accordance with the guiding principles in the care and use of laboratory animals.

**Measurement of protein synthesis in vivo**

Protein synthesis rates were measured *in vivo* using the flooding dose method, which is the method of choice for proteins with a high rate of turnover and exported proteins, since it allows measurements over short periods [16]. Valine was chosen as tracer, as detailed previously [15,17]. Each rat received a bolus injection of L-[1-14C]valine [150 μmol (0.5 ml)/100 g body weight; 0.05 μCi/μmol; Amersham Life Science, Courtaboeuf, France]. One animal from each group was killed after anaesthesia with sodium pentobarbital (6 mg/100 g body weight) at 8, 11, 14, 17, 20 and 23 min after the tracer injection. Blood was collected from the abdominal aorta, and plasma was separated by centrifugation at 3000 g for 5 min and kept at –20 °C until analysis. The liver was rapidly removed, rinsed in cold 0.9% NaCl to remove blood, blotted, weighed and frozen in liquid nitrogen. The times were chosen in order to take into account the synthesis of liver exported proteins. Moreover, we verified in a preliminary experiment that no significant amount of labelled albumin had been released into the systemic circulation at these times.

**Total liver protein synthesis**

The specific radioactivities of free and protein-bound valine were measured as described by Breuillé et al. [15]. Briefly, liver was finely powdered in liquid nitrogen in a ball mill (Dangoumeau*; Prolabo, Paris, France). An aliquot of this frozen powder (2 g) was homogenized in 8 vol. of ice-cold 0.6 mol/l trichloroacetic acid. The acid-soluble fraction, containing free amino acids, was separated from the protein precipitate by centrifugation (9000 g, 15 min, 4 °C). Trichloroacetic acid was removed.
Liver albumin synthesis

Liver albumin biosynthesis was determined as a fraction of total liver protein synthesis in the same rats as those used to measure liver protein synthesis, by immunoprecipitation as described previously [18]. Liver tissue (0.5 g) was homogenized in 3 vol. of 0.35 mol/l sucrose and 50 mmol/l Tris/acetate buffer, pH 7.4. The liver homogenate protein content was determined according to the method of Smith et al. [19] using a colorimetric reaction with bichinominic acid (Pierce, Rockford, IL, U.S.A.), and albumin was measured in the initial homogenate by single radial immunodiffusion [20] using anti-(rat albumin) antibody (ICN, Cappel, Turnhout, Belgium).

To determine the radioactivity in total liver proteins, 100 µl of homogenate was treated with 5 ml of cold 0.6 mol/l trichloroacetic acid and centrifuged (10000 g, 20 min, 4 °C). The pellet was dissolved in 0.5 ml of water and 0.5 ml of 0.6 mol/l NaOH and incubated at 37 °C. After 1 h, 5 ml of cold 0.6 mol/l trichloroacetic acid was added and the mixture was centrifuged (9000 g, 15 min, 4 °C). The pellet was washed with 5 ml of cold 0.3 mol/l trichloroacetic acid, dissolved into 2 ml of 0.3 mol/l NaOH and kept overnight at room temperature. Two 500 µl aliquots of this solution were counted for radioactivity in Quicksafe A scintillation fluid (Zinsser Analytic, Maidenhead, Berks, U.K.) using a liquid scintillant spectrometer (Packard 460 CD; Packard Instrument, Downers Grove, IL, U.S.A.).

The initial homogenate was centrifuged (13000 g, 10 min, 4 °C); the supernatant was retained and the pellet was washed with 1 ml of PBS. The supernatants were pooled. A 100 µl portion of anti-(rat albumin) antibody (produced from goat in our laboratory) was incubated with 50 µl of Protein G–Sepharose® (Sigma) for 1 h at room temperature and then for 3 h at 4 °C. The monoclonality of the antibody was confirmed by testing the antiserum against the antigen by the Ouchterlony technique and immunoelectrophoresis. After incubation, 300 µl (for infected rats) or 200 µl (for pair-fed rats) of the pooled supernatants was added. The amount of antibody added was determined in preliminary experiments as the amount that gave maximal precipitation of radioactivity. The mixture was then incubated for 1 h at room temperature under slow agitation and centrifuged (5500 g, 5 min, 4 °C). The pellet was washed twice with 400 µl of buffer A (140 mmol/l NaCl, 5 mmol/l EDTA, 1% Triton X-100 and 50 mmol/l Tris, pH 7.5) and with 400 µl of buffer B (140 mmol/l NaCl, 5 mmol/l EDTA and 50 mmol/l Tris, pH 7.5). The final pellet was taken up in 1 ml of 0.3 mol/l NaOH overnight at room temperature, and the radioactivity of this solution was determined by a Packard 460 CD liquid scintillant spectrometer.

Albumin mRNA isolation and hybridization

Total RNA was extracted from 0.2 g of liver by the method of Chomczynski and Sacchi [21]. A 20 µg sample of RNA was separated by electrophoresis in formaldehyde/agarose (1%) gels, and transferred electrophoretically to nylon membranes (Gene Screen; NEN Research Products, Boston, MA, U.S.A.). RNA was covalently bound to the membrane by UV cross-linking. Membranes were hybridized with a cDNA albumin probe. Hybridizations were conducted overnight at 65 °C with [32P]cDNA fragments labelled by random priming. After washing at the same temperature, filters were autoradiographed at −80 °C with intensifying screens on Hyperfilm MP (Amersham). After stripping of the probe, the filters were re-probed with a mouse 18 S ribosomal probe (no. 63 178; A.T. C.C., Rockville, MD, U.S.A.). Autoradiographic signals were quantified by digital image processing and analysis (NIH Image 1.54) and normalized using the corresponding 18 S rRNA signals to correct for uneven loading.

Plasma concentrations of acute-phase proteins

Plasma proteins were measured by single radial diffusion using antibodies against rat fibrinogen and rat albumin (ICN), and rabbit antibodies produced in our laboratory against rat α1-acid glycoprotein (α1 GPA) and α2-macroglobulin (α2 M). Calculations

The fractional synthesis rate (FSR) of liver protein, which is the percentage of liver protein synthesized per day, was
calculated according to the method described by Garlick et al. [16] from the formula:

$$\text{FSR} = 100 \times \frac{S_a(t)}{S_b(t)} \times \frac{S_a(t)}{S_a(t)} \times dt$$

where $S_b$ is the specific radioactivity of protein-bound valine, and $t$ is the time elapsed between the middle of the bolus injection and the killing of the animal (in days). $S_a$ is the specific radioactivity of tissue free valine, which is considered as the precursor pool for protein synthesis. The $S_a$ time course was assessed by calculating the area under the curve between time 0 and real time $[S_a(t)]$, as suggested by Breuillé et al. [15]. Briefly, by using each individual specific radioactivity of tissue free valine $[S_a(t)]$ measured in a given experimental group, killing the animals at different time points after the tracer injection allowed us to draw the linear regression of $S_a$ against time for that group. Then the estimated individual areas under the curve were calculated by multiplying the value of $S_a$ obtained at half-time (from the linear regression of $S_a$ against time for the whole group) by $S_a(t)/S_a(t)$, where $S_a(t)$ is the individual value of $S_a$ at time $t$ and $S_a(t)$ is the $S_a$ value of the linear regression at time $t$.

The absolute synthesis rate (ASR) was calculated by multiplying FSR by tissue content, and was expressed in mg/day. It represents the quantity of protein synthesized per unit time.

Hepatic albumin synthesis was calculated as a fraction (%) of total liver protein synthesis, i.e. the fraction of the radioactivity in total liver proteins that was precipitated by anti-albumin antibody. The albumin ASR was then calculated by multiplying albumin synthesis as the fraction of total liver protein synthesis by the absolute rate of total liver protein synthesis.

**Statistics**

Data are presented as means ± S.E.M. The significance of differences was analysed by one-way ANOVA and subsequent Scheffe $F$-test and Student’s $t$-test when appropriate. Differences were considered significant at $P < 0.05$.

**RESULTS**

**Characteristics of the animal model**

Food intake and body weight changes are presented in Figure 1. During the acute phase (days 1–3 after infection), infection induced strong anorexia that was associated with a loss of body weight in pair-fed rats, but this loss was less pronounced than that of infected animals. Thereafter, body weights began to recover as food intake increased progressively, to reach values similar to those observed before infection at the end of the late septic phase (day 10). Infected rats lost more weight than pair-fed rats.

![Figure 1. Effects of infection on cumulative body weight loss and food intake of rats](image)

<table>
<thead>
<tr>
<th>Day of Infection</th>
<th>Food Intake (g of dry matter / Day)</th>
</tr>
</thead>
<tbody>
<tr>
<td>1</td>
<td>20</td>
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<tr>
<td>2</td>
<td>10</td>
</tr>
<tr>
<td>3</td>
<td>0</td>
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<tr>
<td>4</td>
<td>-10</td>
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<td>7</td>
<td>-40</td>
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<tr>
<td>8</td>
<td>-50</td>
</tr>
<tr>
<td>9</td>
<td>-60</td>
</tr>
<tr>
<td>10</td>
<td>-70</td>
</tr>
</tbody>
</table>

Plasma concentrations of acute-phase proteins were measured as markers of the acute-phase response (Table 1). Plasma concentrations of $\chi, \text{GPA}, \alpha_2\text{M}$ and fibrinogen were increased significantly in infected rats compared with pair-fed rats.

Anorexia induced no modifications of albuminemia, since the levels in pair-fed animals were always similar to those in control animals. In contrast, plasma albumin levels were reduced in infected rats as early as day 1 after infection, and were still decreased at 6 and 10 days after infection.

**Liver protein metabolism**

After 1 day of food restriction, liver weights of pair-fed rats were reduced by 40% compared with those of control rats (day 0) (Table 2). No differences were found on days 6 and 10. At 1 day after infection, liver weights of infected rats were 1.5 times higher than those of pair-fed animals, but similar to those of control rats. On days 6 and 10 post-infection, liver weights of infected rats were lower than those of both pair-fed and control rats.
The FSR and ASR of liver proteins were similar in control rats and pair-fed rats (Table 3). In contrast, infection always induced an increase in FSR. Liver protein ASR was significantly higher in infected rats than in pair-fed rats on day 1, and also higher than that in control rats on days 1 and 6. On day 10, the ASR of liver proteins in infected rats had returned to basal values.

**Liver albumin synthesis rate and albumin gene expression**

On day 1, liver albumin concentrations (in mg/g) were similar in control and in pair-fed rats (Table 2). Thereafter values were decreased in pair-fed rats compared with controls. Infection progressively decreased the liver albumin concentration in septic rats. The total liver albumin content was lower in both pair-fed and infected rats than in control rats throughout the experimental period. In addition, albumin content was decreased in infected rats compared with pair-fed rats on day 10.

When expressed relative to total liver protein, albumin synthesis was reduced by 30% in pair-fed rats compared with pair-fed rats on day 10.

When expressed relative to total liver protein, albumin synthesis was reduced by 30% in pair-fed rats compared with control rats after just 1 day of food restriction.

Relative albumin synthesis was always significantly lower in infected rats than in control animals (Table 3). Moreover, infection reduced relative albumin synthesis on days 1, 6 and 10 by 2.3-fold, 2.6-fold and 1.5-fold respectively compared with pair-fed rats.

### Table 1 Effects of infection on plasma acute-phase protein concentrations

Plasma levels of acute-phase proteins were determined by single radial diffusion using anti-protein antibodies. Values are means ± S.E.M. for six rats in each group. Statistical significance: *P* < 0.05 compared with pair-fed rats; †P < 0.05 compared with control rats (day 0).

<table>
<thead>
<tr>
<th>Protein</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>PGM (mg/l)</td>
<td>11.5 ± 0.7</td>
<td>11.6 ± 1.2</td>
<td>560 ± 45†</td>
<td>39.5 ± 6.6</td>
<td>549 ± 91†</td>
<td>41.9 ± 3.5</td>
<td>404 ± 96†</td>
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<tr>
<td>α1MG (mg/l)</td>
<td>40.0 ± 4.9</td>
<td>42.8 ± 2.9</td>
<td>1097 ± 183†</td>
<td>37.7 ± 4.3</td>
<td>698 ± 80†</td>
<td>40.6 ± 2.9</td>
<td>663 ± 133†</td>
<td></td>
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<tr>
<td>Fibrinogen (g/l)</td>
<td>5.6 ± 0.8</td>
<td>3.3 ± 0.3</td>
<td>7.1 ± 1.3</td>
<td>3.1 ± 0.9</td>
<td>9.3 ± 1.7†</td>
<td>3.8 ± 0.5</td>
<td>12.2 ± 1.0†</td>
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<tr>
<td>Albumin (g/l)</td>
<td>23.6 ± 0.8</td>
<td>21.6 ± 0.7</td>
<td>13.6 ± 0.8 †</td>
<td>21.0 ± 1.7</td>
<td>11.4 ± 0.6 †</td>
<td>19.1 ± 1.3</td>
<td>13.5 ± 0.6 †</td>
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</table>

### Table 2 Effects of infection on liver weight, liver protein content and liver albumin content

Liver total proteins were determined by the bicinchoninic acid procedure. Liver albumin levels were determined by the Mancinni procedure. Values presented are means ± S.E.M. for six rats in each group. Statistical significance: *P* < 0.05 compared with pair-fed rats; †P < 0.05 compared with control rats (day 0).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
</tr>
</thead>
<tbody>
<tr>
<td>Weight (g)</td>
<td>12.64 ± 0.33</td>
<td>8.71 ± 0.15 †</td>
<td>11.65 ± 0.39 *</td>
<td>11.11 ± 0.22</td>
<td>10.08 ± 0.45 †</td>
<td>12.75 ± 0.38</td>
<td>10.48 ± 0.39 †</td>
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<tr>
<td>Proteins (g)</td>
<td>2.41 ± 0.11</td>
<td>1.94 ± 0.03</td>
<td>2.54 ± 0.08 †</td>
<td>1.99 ± 0.15</td>
<td>1.84 ± 0.14 †</td>
<td>2.16 ± 0.07</td>
<td>1.89 ± 0.04</td>
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<tr>
<td>Albumin (mg/g)</td>
<td>3.65 ± 0.33</td>
<td>3.06 ± 0.32</td>
<td>2.32 ± 0.25 †</td>
<td>1.75 ± 0.08 †</td>
<td>1.29 ± 0.09</td>
<td>2.38 ± 0.11 †</td>
<td>1.69 ± 0.14 †</td>
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<tr>
<td>Albumin (mg)</td>
<td>46.1 ± 4.2</td>
<td>26.5 ± 2.7</td>
<td>26.7 ± 2.3</td>
<td>19.4 ± 0.7 †</td>
<td>12.9 ± 0.8 †</td>
<td>30.3 ± 1.3</td>
<td>17.5 ± 1.6 †</td>
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### Table 3 Effects of infection on liver albumin synthesis and total protein synthesis

Rats received an intravenous injection of either live E. coli (infected) or saline (pair-fed). At 1, 6 and 10 days after injection, rats were injected with a flooding dose of L-[1-14C]valine. Results are given as means ± S.E.M. for five or six rats in the two groups. TLPS, total liver protein synthesis. Statistical significance: *P* < 0.05 compared with control rats (day 0).

<table>
<thead>
<tr>
<th>Parameter</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
<th>Control</th>
<th>Pair-fed</th>
<th>Infected</th>
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<tbody>
<tr>
<td>FSR (%/day)</td>
<td>69.8 ± 2.3</td>
<td>63.0 ± 1.6</td>
<td>115 ± 5 †</td>
<td>86.3 ± 6.9</td>
<td>121 ± 4 †</td>
<td>73.1 ± 3.8</td>
<td>94.0 ± 6.9 †</td>
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<tr>
<td>AIR (g/day)</td>
<td>1.68 ± 0.12</td>
<td>1.22 ± 0.03</td>
<td>2.93 ± 0.17 †</td>
<td>1.67 ± 0.14</td>
<td>2.24 ± 0.21 †</td>
<td>1.57 ± 0.09</td>
<td>1.78 ± 0.13</td>
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<tr>
<td>% of TLPS</td>
<td>13.4 ± 0.5</td>
<td>9.67 ± 0.4 †</td>
<td>4.26 ± 0.37 †</td>
<td>10.4 ± 0.9</td>
<td>4.01 ± 0.25 †</td>
<td>13.0 ± 0.4</td>
<td>8.38 ± 1.1 †</td>
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<td></td>
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</tr>
<tr>
<td>ASR (mg/day)</td>
<td>270 ± 26</td>
<td>123 ± 10 †</td>
<td>116 ± 6 †</td>
<td>174 ± 22 †</td>
<td>88.6 ± 6.8 †</td>
<td>203 ± 9</td>
<td>143 ± 10 †</td>
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</tbody>
</table>
The liver albumin ASR was significantly reduced in the pair-fed groups on days 1 and 6 and in all infected groups compared with the control group. Liver albumin ASR was similar in infected rats and pair-fed rats on day 1 post-infection. Conversely, on days 6 and 10 post-infection, albumin synthesis was not stimulated by food intake as occurred in pair-fed rats, and therefore remained below the values obtained in pair-fed animals.

Liver albumin mRNA levels are presented in Figure 2. Food restriction did not significantly affect albumin mRNA levels, since no difference was observed between control and pair-fed animals, whatever the day of the experiment. In contrast, albumin mRNA levels were always significantly lower in infected rats than in control or pair-fed animals.

**DISCUSSION**

**Effects of anorexia on albumin synthesis**

Hypoalbuminaemia is one of the most common and dramatic events that characterizes the metabolic response to infection in humans and animals. Moreover, injury is associated with malnutrition, essentially due to a reduction in the intake of nutrients and calories [22]. The model used in the present study, consisting of an injection of live bacteria into rats, also induced acute anorexia for several days. One of the most important factors in the regulation of albumin synthesis is the nutritional state [23]. Protein-free diets are associated with depleted plasma albumin levels and reduced albumin synthesis in rats [10]. Fasting for 18 h decreases the rate of albumin synthesis, in both relative and absolute terms [24]. The contribution of albumin synthesis to total liver protein synthesis reported here in well-fed control rats (13.4%) is in agreement with values reported previously, of between 11 and 15% [18,25]. The present study shows that 1 day of severe food restriction reduced the relative and absolute liver albumin synthesis rates. Surprisingly, food restriction appeared to have no effect on albumin mRNA levels at this stage of pair feeding compared with those in well-nourished rats. However, the Northern blot technique allows only the determination of the contribution of albumin mRNA to total RNA. As the total liver RNA content decreases during the early stages of food restriction [26], taken together, these data strongly suggest that the total amount of albumin mRNA also decreased in rats receiving a limited amount of food. This decrease can partly explain the decreased synthesis observed following food restriction. Nevertheless, post-transcriptional mechanisms, such as modifications in the stability or the turnover of albumin mRNA, could also contribute to the fall in synthesis measured in pair-fed rats.

The progressive restoration of food intake was associated with a progressive increase in albumin synthesis, which had returned to normal by day 10. Peters and Peters [24] have suggested that albumin synthesis responds rapidly to a complete nutrient supply, since refeeding restores the ability of the hepatocytes to synthesize albumin within 15–30 min. The albuminaemia of pair-fed rats was similar to that of control rats throughout the experiment, in agreement with previous data showing that albuminaemia was more rapidly affected by protein restriction than by total dietary restriction [27,28]. Therefore the decreased synthesis observed during the initial period of food restriction was probably associated with a decreased rate of catabolism and/or loss of the protein.

**Effects of infection on albumin synthesis**

Actual measurements of the effect of injury on albumin synthesis in humans are more consistent than in animal models. Measurements of albumin synthesis in patients that have suffered head trauma or cancer patients with an acute-phase response clearly indicate that a reduction in plasma albumin levels is not caused by a reduction in albumin synthesis rates [12,13]. Studies in humans have been conducted at only one stage of the inflammatory process, mostly during the acute phase, and it is possible that albumin synthesis varies with time following injury, as shown in a rat model of peritonitis [5,8]. However, albuminaemia was not reported, and the high mortality
observed in this model [29] made it unsuitable for long-term metabolic studies. In the present study, animals were studied for up to 10 days after infection. During this period, infected animals presented a rapid and persistent hypoalbuminaemia, as observed in injured humans [30], as well as sustained levels of positive acute-phase proteins, i.e. fibrinogen, $\alpha_1$GPA and $\alpha_2$M.

On the basis of observed rapid declines in liver albumin mRNA levels in response to various inflammatory stimuli, it has usually been concluded that reduced hepatic albumin synthesis is a major process causing hypoalbuminaemia in response to injury [2,31,32]. In agreement with these previous studies, we report a decrease in albumin mRNA levels in the livers of infected rats compared with control rats as soon as 1 day post-infection. In addition, in response to infection, we found a decrease in both relative and absolute albumin synthesis rates compared with those in well-nourished rats, whatever the phase of the inflammatory process. Estimated from direct $^{14}$C-leucine incorporation into the protein, Schreiber et al. [7] reported a 64% decrease in the albumin synthesis rate 24 h after turpentine injection in rats as compared with control well-fed rats, in agreement with our findings.

To determine specifically the effect of infection, the present experiment also included animals that were pair-fed to the food intake of the infected rats. During the early phase of sepsis there was no specific effect of infection, since liver albumin ASR was not reduced in comparison with pair-fed rats. This result is in agreement with the data of Sax et al. [5], who also described an unchanged albumin synthesis rate measured in the perfused liver in septic rats 16 h after coeliac ligation and puncture compared with sham-operated rats. The liver weight and protein content were increased by infection, probably due to inflammation in the tissue [14]. The total liver RNA content has been reported to be increased by approx. 50% in infected rats compared with pair-fed rats during the acute phase [26]. This increase could compensate for the fall in albumin mRNA levels and explain the unchanged albumin synthesis. Therefore this suggests no alteration in albumin mRNA transcription, but rather increased transcription of other mRNAs, such that the relative amount of albumin mRNA was reduced. In particular, hepatic mRNA levels for $\alpha_1$GPA and fibrinogen were found to increase during the acute response in the same model of sepsis [33]. However, albuminaemia remained lower in infected rats than in pair-fed rats. The plasma albumin concentration is the result of numerous processes, including synthesis, but also catabolism and body distribution [34]. An unchanged albumin ASR suggests that synthesis was not the active mechanism in establishing acute hypoalbuminaemia during the acute phase of sepsis.

During the chronic and late phases of infection, albumin synthesis, both as a fraction of total liver protein synthesis and as an absolute rate, did not increase as it did in pair-fed rats. In animals, and especially in rats, few data of long-term albumin synthesis have been reported in the literature. Krähenbühl et al. [35] observed a similar decrease in liver albumin synthesis 14 days after bile-duct ligation in rats.

**Conclusions**

In summary, the kinetics of albumin synthesis were studied in a septic rat model over several days. Hypoalbuminaemia was observed as soon as day 1 after infection, and was maintained until day 10. By comparison with that in pair-fed rats, the albumin ASR was unchanged 1 day after infection, as observed previously on day 2 [26]. Later in the course of infection, the albumin ASR was reduced compared with that in pair-fed rats. A decrease in plasma albumin levels observed during later phases of infection can be explained mainly by a failure of stimulation of liver albumin synthesis. These results suggest that the response of hepatic albumin synthesis to infection changes with the stage of the inflammatory process, and that other mechanisms are responsible for the hypoalbuminaemia syndrome. In particular, escape of plasma albumin into the extravascular space during the early phase of infection is an attractive hypothesis that needs to be explored.

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