Pentoxifylline decreases body weight loss and muscle protein wasting characteristics of sepsis

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Pentoxifylline decreases body weight loss and muscle protein wasting characteristics of sepsis. Am. J. Physiol. 265 (Endocrinol. Metab. 28): E660–E666, 1993.—Sepsis induces metabolic disorders that include loss of body weight, muscle wasting, and acute-phase protein synthesis in liver. Cytokines are generally recognized as active mediators of these disorders, and the implication of tumor necrosis factor (TNF) has been frequently discussed in the recent past. However, the identity of the active agent in alterations of protein metabolism is still controversial. To improve our understanding of the role of cytokines in mediating muscle wasting observed in sepsis, we investigated muscle and liver protein metabolism in the following three groups of rats: infected control rats (INF-C); infected rats pretreated with pentoxifylline (PTX-INF), which is a potent inhibitor of TNF secretion; and pair-fed rats for the PTX-INF group pretreated with pentoxifylline. Pentoxifylline nearly completely suppressed TNF secretion but did not influence the transient fall in rectal temperature, the decreased hematocrit, and the increased liver protein mass and synthesis observed in INF-C rats. Pentoxifylline decreased the anorexia, the loss of body weight and muscle protein observed in INF-C animals, and partially prevented the decrease in muscle protein synthesis induced by infection. The overall data indicate that pentoxifylline is an effective agent in mitigating the characteristic muscle protein wasting induced by sepsis and confirm the limited role of TNF in the mediation of the acute-phase protein synthesis. Our results suggest a probable implication of TNF in the regulation of protein balance in muscle but do not allow discarding possible implication of other mediators that would be inhibited by pentoxifylline.

Infection; tumor necrosis factor; acute-phase protein; liver; protein synthesis

METABOLIC ALTERATIONS in patients with sepsis, trauma, or cancer are characterized by a marked loss of weight and body protein, generalized muscle wasting, and acute-phase protein synthesis (1, 3, 13, 16, 24, 30). It is generally considered that both the decreased protein synthesis rate and increased protein breakdown participate in muscle protein wasting (1, 12, 13, 28, 30). The mediation of these alterations has been recently well documented but is still unclear in muscle. To investigate this mediation, two different methods can be used. The first one consists of administering the putative mediator to healthy animals with the aim of reproducing the pathophysiological alterations observed in sepsis. Various researchers failed to decrease muscle protein synthesis when they incubated rat muscles in vitro with recombinant interleukin (IL)-1 or tumor necrosis factor (TNF; see Ref. 20) or after infusion of TNF in vivo in rats (8). However, other works indicated that injections of TNF (5) or IL-1 (2) in rats induced a transient but acute drop in muscle protein synthesis rates measured in vivo. Likewise, continuous infusion of TNF over several days in rats was found to induce either accelerated muscle protein wasting when compared with respective pair-fed animals (9) or a similar loss of muscle mass (15, 18). All of these studies taken together do not, however, make it possible to decide whether cytokines are primary mediators of muscle wasting or not. The second way of studying this mediation consists of inhibiting the endogenous secretion of the putative mediators in septic animals. We chose the second solution for our study.

Recent studies demonstrated that pentoxifylline (PTX; 3,7-dimethyl-1-(6-oxoheptyl) xanthine), which is used in the treatment of peripheral arterial and arteriovenous circulatory disorders, was able to inhibit the appearance of TNF in serum of lipopolysaccharide-treated mice (27), rats (23), and humans (31). PTX was also found to possess high protecting potential in endotoxic shock (4, 23, 27) and to inhibit the inflammatory action of TNF on neutrophil function (4, 23). It was suggested that the beneficial effects of PTX in septic or endotoxic shock were due to inhibition of TNF synthesis and bioactivity (23, 27), but nothing is known about modifications of protein metabolism by PTX in septic situations. We have therefore investigated the effect of pretreatment of rats with PTX on the alterations in skeletal muscle protein metabolism induced by sepsis.

It has been suggested that muscle proteolysis would supply different substrates for acute-phase protein synthesis (12, 24, 26). The shift of amino acids from muscle to liver would be a beneficial regulation; some authors suggested that the limitation of muscle proteolysis may disturb this regulation and result in deleterious effects (12, 26). On the other hand, the acute-phase protein synthesis is mainly initiated by cytokines released by stimulated macrophages, essentially IL-1 (21), IL-6 (14, 21), and TNF (17, 25). The inhibition of cytokine secretion would therefore modify liver protein metabolism either by direct mediation or by suppression of the shift of amino acids from muscle to liver. In this context, we simultaneously studied the effect of PTX on liver and muscle protein metabolism in septic rats.

MATERIALS AND METHODS

Rats. Male Sprague-Dawley rats, 160–170 g body wt (Iffa Credo, St. Germain sur l’Arbresle, France), were individually housed in wire-bottom cages in a temperature-controlled room (22–23°C) with a 12:12-h light-dark cycle. For a 6-day period of acclimatization, all rats had free access to water and to a semisynthetic diet containing 12% protein distributed by an automatic device.

Experiment 1. At 200–210 g body wt, rats were divided into three groups. The infected control group of rats (INF-C, n = 5) was injected intraperitoneally with saline 30 min before the injection of 5.4 ± 0.4 × 10^7 live Escherichia coli into a lateral tail vein. The effect of PTX was studied by comparing INF-C rats...
with the PTX infected group of rats (PTX INF, n = 7). In this group, PTX was injected intraperitoneally (100 mg/kg) 30 min before E. coli administration. Because PTX increased voluntary feed intake, a healthy pair-fed group pretreated with PTX (PTX-PF, n = 7) was given the same restricted food intake as the PTX-INF group. These rats received the injection of PTX and 30 min later an intravenous saline injection.

To estimate food intake, individual tared syringes were filled with the diet and inserted into a timed motor-driven device. Six times a day, at 0300, 0700, 1100, 1500, 1900, and 2300 h, a controlled quantity of diet was thereby delivered into a tared ramenik for each animal. Food intake of ad libitum-fed rats was determined in a previous study, and the same daily quantity of food was offered to INF-C and PTX INF rats. This automatic device was used to avoid temporal differences in intake, especially in PTX-PF rats who were severely restricted. Because determination of food intake of INF-C rats was time consuming, the PTX-PF group was staggered such that the rats in this group were studied 2 days later than the other groups.

In each rat, blood samples were taken 90 min after E. coli administration for measuring plasma TNF concentration. In a preliminary study, we carried out a kinetic study of TNF production from 30 min to 5 h postinfection. All rats (n = 5) produced TNF as soon as 30 min after infection, peaked at 90 min, and returned to baseline between 2 and 4 h. Rectal temperature was measured at different times before and after infection. Animals were weighed 24 and 48 h after infection, and food intake was followed for 2 days after infection. Rats were killed 53 h after infection for measurement of protein synthesis rates and protein mass in the gastrocnemius muscle and liver. On the day of death, weight of rats was 193 ± 11, 209 ± 7, and 209 ± 4 g, respectively, for INF-C, PTX-INF, and PTX-PF groups.

Experiment 2. To examine the long-term effect of PTX on a severe infection, the experiment was repeated for INF-C and PTX-INF groups with an injected dose of live E. coli 10 times higher than in experiment 1. Food intake and growth were monitored for 6 days after infection.

The protocol of experiments 1 and 2 was reviewed and approved by the local Institutional Animal Care Committee.

Skeletal muscle and liver protein synthesis. The rate of protein synthesis was measured in vivo 53 h after infection by a large dose of L-[1-13C]-valine. Each rat received between 1400 and 1600 h a bolus injection of 150 μmol L-valine/100 g body wt (6) within 15–20 s into the lateral tail vein. Unlabeled valine (Sigma Chemical, St. Louis, MO) was combined with L-[1-13C]-valine (99 atom percent; Tracer Technologies, Somerville, MA) to give a level of isotope enrichment of 25.0 atom percent excess. The volume injected was 0.4 ml/100 g body wt. In each group, one rat was killed by decapitation after anesthesia at the following times: 5, 10, 15, 20, 30, 40, and 50 min after injection. Long times (30, 40, and 90 min) were not taken into account for determination of protein synthesis rate in liver because the incorporation of the tracer was not linear after 20 min due to the secretion of exported protein. The shortest times of incorporation into muscle protein (5 and 10 min) were too small to allow confident measurement of the enrichment. In this case, the plot of incorporation of valine vs. time was not linear in the short term, and these values were deleted. These methodological considerations explain the variable number of rats given in different tables.

Blood samples were centrifuged, and the plasma was frozen in liquid nitrogen. Liver and gastrocnemius muscles were quickly excised, weighed, and immediately frozen in liquid nitrogen. All samples were stored at −20°C until analysis.

Four healthy rats were killed without injection for determination of basal enrichment of valine in muscle and liver. For each rat the fractional rate of protein synthesis (Ks) was calculated as previously described by Garlick et al. (10) from the formula

\[ K_s = \frac{(P_t - P_b) \times 100}{AUC} \]

where Pb and Pt are, respectively, basal enrichment and enrichment of valine in tissue protein at the end of the incorporation period (atom percent excess), and AUC is the area under the curve of free amino acid enrichment between time 0 and real time (atom percent excess X time in days). Muscle basal enrichment measured in rats accustomed to the same diet over 6 days was 1.1012 ± 0.00060 atom percent.

The absolute synthesis rate of protein (ASR) was calculated as the product of the fractional rate and protein mass.

In each group the average Ks and ASR was calculated, and statistical significance of differences between means of 4 or 5 values depending on the tissue was tested.

Analytical methods. Liver and muscle samples were first pulverized in liquid nitrogen with a ball mill (Dangoumeau; Pro- labo, Paris, France), then homogenized in 8 vol of 10% trichloroacetic acid with a Polytron (Kinematica). The acid-soluble fraction was separated by centrifugation, then desalted by cation-exchange chromatography (Amberlite AG 50 × 8, 100–200 mesh, H+ form; Bio-Rad, Richmond, CA) in minidisposable columns. Aminu acids were eluted with 4 M NH4OH. After evaporation, the eluate was resuspended in 0.1 N HCl and used for measurement of the enrichment of free valine by gas chromatography-mass spectrometry on a Nermag R10–10C quadruple spectrometer coupled to a Delsi gas chromatograph (Delsi-Nermag, Argenteuil, France). Valine was measured as the tertiary-isobutyldimethylsilyl derivative under electron-impact ionization conditions with selective-ion monitoring. The trichloroacetic-insoluble material was resuspended two times in trichloroacetic acid and centrifuged. The pellet was neutralized with cold 13.6% sodium acetate-methanol (wt/vol) then delipidated with methanol-chloroform (1:2 vol/vol) and dried. An aliquot of the lipid-free pellet was hydrolyzed in 6 N HCl for 48 h at 110°C. The HCl was removed by evaporation under vacuum, and amino acids were resuspended in 0.2 M lithium citrate buffer, pH 2.2. Amino acids were separated by ion-exchange chromatography (40 × 1 cm column, resin Aminex AG 50; Bio-Rad). The valine-containing fractions were collected, and citrate was removed on a small column of AG-50 cation-exchange resin (Bio-Rad). Valine was eluted with 4 M NH4OH, evaporated under vacuum, and resuspended in 0.1 N HCl to a final valine concentration of ~10 μmol/ml for mass spectrometric analysis of protein-bound enrichment. Sample (200 μl) and degassed lithium citrate buffer (800 μl; 0.1 M, pH 2.2) were introduced into 20 ml Vacutainer tubes and degassed for 30 min at 80°C. The tubes were placed on ice, and 20 mg ninhydrin were added and evacuated at a pressure of 10 Pa. 13CO2 was generated from the COOH-group of valine by heating at 100°C for 1 h. The tubes were allowed to stand at room temperature and centrifuged for 2 min at 1,000 g to remove condensation. The 13C enrichment of the CO2 was measured with a gas isotope ratio mass spectrometer VG 903 coupled with a Roboprep G gas chromatograph (Europa Scientific). Values were calculated as atom percent excess by reference to a standard (PeeDee Belemnite).

The nitrogen contents of muscle and liver were determined by a Kjeldahl method, and protein was calculated as N × 6.25.

Plasma glucose was assayed by a glucose oxidase method (GOD-PAP assay kit; Boehringer, Mannheim, Germany). Plasma insulin was determined by radioimmunoassay with a commercial kit (SB-INSI-1; CEA). Plasma TNF-α concentration was assayed with an enzyme-linked immunosorbent assay (ELISA) kit purchased from Genzyme (Boston, MA). According to the manufacturer the sensitivity is 50 pg/ml.
Data are expressed as means ± SD. The statistical significance of differences between means was assessed by one-way analysis of variance and by Student’s *t* test where appropriate.

RESULTS

Administration of PTX greatly attenuated the rise in plasma TNF after injection of live bacteria. PTX reduced plasma TNF levels >17-fold compared with nontreated animals (Table 1).

Pretreatment of infected rats with PTX did not modify the hematocrit since PTX-INF and INF-C rats showed a similar hematocrit (Table 1). Also, the hematocrit was not normalized by PTX since PTX-INF values were 11% below PTX-PF animals. Plasma glucose concentrations were not significantly different between the three groups. Insulin concentrations were 2.5 times higher in INF-C rats compared with PTX-PF animals, although they had a smaller food intake. Pretreatment with PTX significantly diminished plasma insulin concentrations but did not normalize it at the PTX-PF control level (Table 1).

As can be seen in Table 2, infection resulted in a decreased rectal temperature 1.5 and 4 h after infection compared with initial temperature. The PTX-INF rats showed the same hypothermic reaction at 1.5 h, but this effect was abolished as early as 4 h later. At this point in time, PTX-INF and PTX-PF rats had similar temperatures.

Infection markedly decreased food intake. In experiment 1, the intake of INF-C rats on postinfection days 1 and 2 was, respectively, 30 and 63% of their intake before the infection. This anorexia was significantly limited by pretreatment with PTX since intakes were 13.5 ± 3.0 and 21.9 ± 0.7 g dry matter/day in the PTX-INF group on days 1 and 2, respectively, vs. 6.5 ± 5.2 and 13.8 ± 5.8 in the INF-C group. Figure 1 shows that rats ate even less under a more severe septic shock and that food intake increased in both infected groups from day 1 to day 3 but steadied at the same level on days 4–6 (expt 2).

Growth rates of animals for the 3 days before infection were similar in the three groups of rats. During the 1st day after infection, the PTX-INF group lost significantly less body weight than the INF-C group (2.4 ± 2.7 and 12.6 ± 6.2 g, respectively). This difference increased during the 2nd day since the cumulative weight change of days 1 and 2 was 10.5 ± 3.0 g in the PTX-INF group and −4.1 ± 8.0 g in the INF-C group (expt 1, Fig. 2). By contrast, both PTX-INF and PTX-PF groups showed similar changes in body weight. Experiment 2 revealed that the difference between a PTX-INF and an INF-C group increased from day 1 to day 3 and reached 22.8 g on day 6 (Fig. 3).

Pretreatment of infected rats with PTX did not alter the muscle mass-to-body weight ratio observed in the INF-C rats (Table 3). However, PTX INF rats exhibited a 14% increase in gastrocnemius muscle mass and protein mass as compared with INF-C animals (Table 3). This represents a limitation of the septic muscle atrophy by PTX, which is confirmed by the fact that the muscle protein-to-body weight ratio of PTX-INF rats was intermediate between the ratio of INF-C animals (the smallest) and PTX-PF rats (the highest), even if the PTX-INF values were not significantly different from those of either of the other groups. This limitation of muscle atrophy was only partial since PTX-INF rats still exhibited gastrocnemius weight and protein mass significantly lower than in the PTX-PF group (respectively, 10 and 12%).

Protein synthesis rate in the gastrocnemius muscle, i.e. Kₚ and ASR, were, respectively, 17.5 and 32.7% higher in the PTX-INF group than in the INF-C group (P < 0.06 and < 0.05, respectively). On the other hand, Kₛ was not significantly different in PTX-INF and PTX-PF rats, but ASR was still 18.8% lower in PTX-INF rats than in PTX-PF animals (Table 4).

In contrast to muscle, the liver protein mass was the same in the two septic groups, and PTX-INF animals had a higher liver protein mass and liver protein per gram body weight than PTX-PF rats (10 and 17%, respectively, Table 3).

In liver, Kₛ and ASR values were higher in the two infected groups than in PTX-PF animals. Whether PTX limits the well-documented activation of Kₛ and ASR in liver by infection is not completely clear since Kₛ decreased by 10.2% (PTX-INF vs. INF-C, P < 0.06), but ASR was still 18.8% lower in PTX-INF rats than in PTX-PF animals (Table 4).

DISCUSSION

In our study, PTX was observed to have the following two main effects: 1) limitation of anorexia and 2) limitation of body weight loss and attenuation of muscle protein wasting induced by sepsis.

Our results confirmed a strong inhibition of TNF-α production by pretreatment with PTX 30 min before administration of live *E. coli* in rats. Indeed the TNF-α plasma level was >17 times lower in PTX-INF rats than in INF-C rats (the exact TNF-α plasma level of the latter group is not known since samples were all over the ELISA limit of detection at the plasma dilution we used).

These data fit in well with recent in vivo studies performed in mice (27), rats (23), and humans (31) and in

### Table 1. Effect of pentoxifylline on hematocrit, plasma glucose, insulin, and TNF-α concentration of infected rats (expt 1)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>TNF-α, ng/ml</th>
<th>Glucose, g/l</th>
<th>Insulin, μU/ml</th>
<th>Hematocrit</th>
</tr>
</thead>
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<tr>
<td>INF-C</td>
<td>4</td>
<td>&gt;10</td>
<td>182±0.25</td>
<td>34.7±9.5</td>
<td>37.4±0.9</td>
</tr>
<tr>
<td>PTX-INF</td>
<td>7</td>
<td>0.56±0.06*</td>
<td>189±0.17</td>
<td>19.8±6.29b</td>
<td>36.9±1.9b</td>
</tr>
<tr>
<td>PTX-PF</td>
<td>7</td>
<td></td>
<td>169±0.23</td>
<td>13.9±4.1</td>
<td>41.6±1.3</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. TNF-α, tumor necrosis factor α; INF C, infected control rats; PTX INF, infected rats pretreated with pentoxifylline; PTX-PF, pair-fed rats of PTX-INF group pretreated with pentoxifylline. Measurements were made 0130 h after infection for TNF-α concentration and 48 h after infection for plasma glucose and insulin concentrations. a P < 0.05 vs. INF-C rats. b P < 0.05 vs. PTX-PF rats.
Table 2. Effect of pentoxifylline on rectal temperature of infected rats (expt 1)

<table>
<thead>
<tr>
<th></th>
<th>n</th>
<th>0</th>
<th>1.5</th>
<th>4</th>
<th>24</th>
</tr>
</thead>
<tbody>
<tr>
<td>INF-C</td>
<td>4</td>
<td>37.1±0.7a</td>
<td>35.7±0.9b</td>
<td>36.2±0.9b</td>
<td>37.2±0.9a</td>
</tr>
<tr>
<td>PTX-INF</td>
<td>7</td>
<td>36.7±0.5a</td>
<td>35.4±1.2ab</td>
<td>37.7±0.4c</td>
<td>37.4±0.3bc</td>
</tr>
<tr>
<td>PTX-PF</td>
<td>7</td>
<td>36.5±0.4a</td>
<td>36.5±0.7a</td>
<td>37.4±0.3b</td>
<td>36.7±0.3a</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. a, b, c Different letters within line mean that temperature was different at different time of kinetic. a, b, c Different letters within line mean that temperature was different at different time of kinetic in same group.

Fig. 1. Long-term effect of pentoxifylline on anorexia in severe septic shock (expt 2). Squares, infected control rats (INF-C); triangles, infected rats pretreated with pentoxifylline (PTX-INF). *P < 0.05 and **P < 0.001 vs. INF-C. Daily food intake was measured individually, as explained in MATERIALS AND METHODS.

Fig. 2. Reduction of body weight loss by pentoxifylline in septic rats (expt 1). Filled bars, infected control rats (INF-C); stippled bars, infected rats pretreated with pentoxifylline (PTX-INF); open bars, pair-fed of PTX-INF (PTX-PF). *P < 0.05 vs. INF-C.

IL-1 and TNF-α regulate food intake in mice (19), rats (22), and humans (18); it is therefore possible that the inhibition of TNF-α secretion that we observed may be responsible for the limitation of anorexia. However, PTX-INF rats were still anorexic after infection. Because IL-1 seems to be an important regulator of food intake (22), the nonsuppressive effect of PTX on anorexia could be due to the secretion of IL-1 after the infection. We cannot check this hypothesis in our experiment, since we did not measure IL-1 secretion and since no data have been reported on the effects of PTX on IL-1 production.

It is well known that a reduction in food intake decreases the rate of muscle protein synthesis and diminishes muscle growth in young animals. Therefore, it is not surprising that PTX induced a limitation in loss of muscle mass and muscle protein content via reduced anorexia. However, it is generally considered that septic animals lose more muscle protein than pair-fed controls (30). In this context the finding that the muscle protein-to-body weight ratio was not significantly different in PTX-INF rats compared with PTX-PF rats is more striking (the PTX-INF ratio was intermediate between the INF-C and PTX-PF ratios). This clearly shows that PTX limited the specific loss of muscle protein observed in sepsis (1, 16, 28, 30). Various authors have described the characteristic redistribution of body proteins from

vitro experiments with monocytes and macrophages in response to endotoxins (7), which all showed the inhibitory effect of PTX on TNF-α production.

The anorexia induced by infection was very acute in experiments 1 and 2 since INF-C rats ate on day 1 post-infection, respectively, 30 and 11% of their food intake and, respectively, 63 and 30% on day 2. PTX pretreatment strongly limited this anorexia since PTX INF rats ate two times more than INF-C rats. It is well known that
Peripheral to visceral organs after long-term administration of TNF or IL-1 in rats; Hoshino et al. (15) observed the same muscle protein wasting in TNF-perfused rats and in their pair-fed controls. They suggested that loss of muscle mass induced by TNF should only be ascribed to reduction of food intake. On the other hand, Fong et al. (9), using cytokine doses four times higher than Hoshino et al. (15), reported that IL-1 and/or TNF reproduced the preferential muscle protein wasting in chronic diseases. If PTX effects are due to inhibition of cytokine secretion, our experiment suggests a possible primary importance of TNF in the mediation of dietary restriction and in the specific effect of this cytokine on the muscle wasting observed in sepsis. However, we cannot discard the hypothesis that these modifications may be due to inhibition of other cytokines or other mediators secondary to the effect of PTX.

Muscle protein atrophy results from a negative balance between the rate of protein synthesis and breakdown. Sepsis induces a reduction in muscle protein synthesis (1, 16, 30). Pretreatment with PTX mitigated this effect since $K_s$ and ASR were significantly higher in PTX-INF rats than in INF-C animals. Part of this increase in protein synthesis induced by PTX was probably due to the limitation of anorexia, but the fact that $K_s$ was only 8.7% lower in PTX-INF than in PTX-PF rats also indicates that PTX partially prevented the specific decrease of protein synthesis induced by PTX in septic rats.

Liver weight, protein content, and ASR were similar in both infected groups (INF-C and PTX-INF rats), but $K_s$ was 10.2% lower in PTX-INF than INF-C rats ($P < 0.06$). By contrast, PTX INF rats exhibited an increased protein content, protein-to-body weight ratio, $K_s$, and ASR compared with PTX-PF animals. This represented the well-known specific anabolic response of liver to infection (1, 16, 17, 24, 26), which is part of the acute-phase response and which is assumed to be beneficial to the injured organism (14). Therefore, except for $K_s$, we did not observe any significant difference in the liver re-

### Table 3. Effect of pentoxifylline on hepatic and muscle protein content of infected rats (expt 1)

<table>
<thead>
<tr>
<th></th>
<th>Liver Weight</th>
<th>Liver Protein</th>
<th>Gastrocnemius Weight</th>
<th>Gastrocnemius Protein</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>g/mg/g body wt</td>
<td>g/mg/g body wt</td>
<td>g/mg/g body wt</td>
<td>mg/mg/g body wt</td>
</tr>
<tr>
<td>INF-C</td>
<td>4</td>
<td>9.52±0.75</td>
<td>49.6±4.3</td>
<td>1.80±0.10</td>
</tr>
<tr>
<td>PTX-INF</td>
<td>7</td>
<td>9.65±0.66</td>
<td>46.1±2.1</td>
<td>1.81±0.09</td>
</tr>
<tr>
<td>PTX-PF</td>
<td>7</td>
<td>9.44±0.72</td>
<td>42.6±3.0</td>
<td>1.61±0.14</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. a P < 0.05 vs. PF. b P < 0.05 vs. INF-C.

### Table 4. Effect of pentoxifylline on protein synthesis in muscle and liver of infected rats

<table>
<thead>
<tr>
<th></th>
<th>Liver</th>
<th>Gastrocnemius</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>n %/day</td>
<td>g/day</td>
</tr>
<tr>
<td>INF-C</td>
<td>4</td>
<td>116.8±9.2</td>
</tr>
<tr>
<td>PTX-INF</td>
<td>4</td>
<td>104.9±3.4b</td>
</tr>
<tr>
<td>PTX-PF</td>
<td>4</td>
<td>88.92±8.61</td>
</tr>
</tbody>
</table>

Values are means ± SD; n, no. of rats. a P < 0.06 vs. INF-C. b P < 0.05 vs. PF. c P < 0.05 vs. INF-C.
response to infection with or without PTX pretreatment. Different studies demonstrated that TNF participates in the regulation of hepatic hyperplasia (1/7) and hepatic acute-phase gene expression (25). However, it is generally assumed that IL-6 is the main mediator of the liver response to infection (14, 21). Zehl et al. (31) showed that PTX induces an inhibition of endogenous TNF secretion but does not affect IL-6 release or IL-6-mediated effects. We may therefore suppose that PTX treatment of our rats did not prevent the IL-6 production and that the hepatic acute-phase protein synthesis would have been induced by this mediator. On the other hand, we observed a decreased K+ value in the liver of PTX-INF rats compared with INF-C animals (10.2%). This small variation may be due to the inhibition of TNF production and would reflect the involvement of TNF in the mediation of the acute-phase protein synthesis. So, our study appears to emphasize the small but real contribution of TNF and the major role of other mediators (probably IL-6) in the induction of the anabolic response of liver to infection.

In summary, the administration of PTX before infection inhibited TNF secretion, partially suppressed anorexia, body weight loss, and muscle protein wasting, and induced a slight reduction of acute-phase protein synthesis in the liver. This suggests that TNF seems to participate in the primary mediation of alterations in muscle protein metabolism but is not a major mediator of the liver response to infection. The experimental model of sepsis probably differs from the progressive and overwhelming syndrome of multiple organ failure in patients. However, our results and other works that demonstrated beneficial effects of P'TX on the outcome of endotoxemia (23, 27, 31) suggest that PTX may prove useful as a prophylactic agent in patients at high risk for overwhelming infection. It would be interesting to test the efficiency of PTX as a therapeutic agent in a sepsis model. In addition, several studies in animals and humans have focused on the shift of amino acids from muscle to liver for increased gluconeogenesis and protein synthesis (12, 24, 26). It has been suggested that this transfer has beneficial effects for survival in sepsis (12, 26) and that inhibition of muscle protein loss may be deleterious for the outcome of patients. Our study demonstrates that this concept is false, since we simultaneously observed in rats a limitation in muscle atrophy, an improvement of physiological state, and maintenance of the acute-phase protein synthesis in PTX-INF rats compared with INF-C animals. This probably reflects a better nutritional supply to the liver in PTX-INF rats compared with INF-C animals secondary to the limitation of anorexia by PTX pretreatment. This emphasizes the importance of nutrition in septic states; if we want to prevent muscle atrophy, it will be necessary to provide suitable nutritional support, particularly for hepatic needs. Any other method may involve the risk of inducing deleterious effects for the patient.

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