Biochemical Pharmacology, Vol. 36, No. 22, pp. 3841–3846, 1987.
Printed in Great Britain.

0006-2952/87 $3.00 + 0.00

MITOGENIC RESPONSE OF RAT LUNG TO ENDOTOXIN EXPOSURE

MICHAEL ALLAN HASS* and DONALD MASSARO

Oak Asthma Research and Treatment Facility, Pulmonary Research Laboratories, University of Miami School of Medicine, Miami, FL 33136; and the V.A. Medical Center, Miami, FL 33125, U.S.A.

(*Received* 13 *September* 1986; *accepted* 16 *April* 1987)

Abstract—Adult rats exposed to hyperoxia are protected from lung injury by treatment with bacterial endotoxin. Experiments were undertaken to determine whether endotoxin treatment produces a mitogenic effect on the lung. Endotoxin treatment caused a significant ($P < .05$) loss of body weight (8%) in rats exposed to either air or >95% O_2 for 24 hr. Therefore, experiments were also undertaken in which both saline- and endotoxin-treated rats were starved for the duration of the experiments to make equal any nutritional imbalance. The rate of DNA synthesis in lung slices from fed rats treated with endotoxin did not differ from that of saline-treated controls. In contrast, lung DNA synthesis in starved rats treated with endotoxin increased 50%. The effect of endotoxin treatment was similar in rats breathing air or >95% O_2, and lung protein synthesis generally paralleled lung DNA synthesis. These results indicate that endotoxin does exert a mitogenic effect on the lung and that this effect can be masked by the nutritional imbalance resulting from endotoxin administration.

Seventy to eighty percent of adult rats exposed to normobaric hyperoxia die within 72 hr of exposure, with severe pulmonary edema caused by disruption of the cellular components of the air–blood barrier in the lung [1]. The presence of an increased amount of lung water has been detected in untreated rats at 48 hr but not at 24 hr of exposure to hyperoxia [2]. Administration of a single low dose of endotoxin (500 μg/kg) within 24 hr of the onset of exposure prevents the development of pulmonary edema, increases lung antioxidant enzyme activity, and produces nearly 100% survival during 72 hr of hyperoxia [3, 4]. However, further delay in treatment reduces the protective effect of endotoxin [2].

This raised the possiblity that part of the protective action of endotoxin is produced by a stimulation of cell replication that maintains the cellular integrity of the air–blood barrier, thereby preventing pulmonary edema during the second 24 hr of exposure to hyperoxia before the increase in lung antioxidant enzyme activity takes place [2]. Such a possibility was also raised by previous work from our laboratory in which lungs from O_2-breathing rats given two doses of endotoxin had an increased rate of [^{3}H]thymidine incorporation 48 hr after the first dose and the onset of exposure [5]. Following 48 hr of hyperoxia, however, significant abnormalities are already present in the lungs, including altered cell permeability and increased cellularity due to the influx of neutrophils and other inflammatory cells [6–8]. Thus, the effects of endotoxin on endogenous lung metabolism during the critical initial 24-hr O_2 exposure remain undefined.

The present work was undertaken to determine if treatment with endotoxin increased the synthesis of DNA within 24 hr of a single injection of endotoxin followed immediately by the exposure of rats to >95% O_2. In addition, the possible effect of endotoxin on lung DNA synthesis by the alternate thymidine synthetic pathway was examined, and alterations in lung DNA synthesis due to the effect of endotoxin on the nutrition of the rats were determined.

MATERIALS AND METHODS

Animals. Male Sprague–Dawley rats, weighing 200–300 g, were obtained from the Charles River Breeding Laboratories and maintained in the Animal Care Facility, University of Miami.

Materials. Aquasol, [2,6-^{3}H]phenylalanine (40–60 Ci/mmol), [*methyl*-^{3}H]thymidine (6.7 Ci/mmol), and [^{14}C]sodium formate (4 mCi/mmol) were obtained from New England Nuclear, Boston, MA. Endotoxin (*Salmonella typhimurium* lipopolysaccharide, phenol-water extraction) and all other biochemicals were obtained from the Sigma Chemical Co., St. Louis, MO. Reglan was a gift from the A. H. Robbins Co., Richmond, VA.

Incubation conditions. Rats were killed by intraperitoneal injection of sodium pentobarbital (50 mg/kg) followed by exsanguination. The lungs were perfused via the pulmonary artery with 0.154 M NaCl, 0.1 M potassium phosphate (pH 7.4). Following surgical removal, the lungs were sliced on a McIlwain tissue slicer at a setting of 1.0 mm.

To measure the rate of lung protein synthesis, 500 mg lung slices were incubated for 2 hr at 37° in 5 ml medium shaking at 120 oscillations/min and gassed with 95% O_2–5% CO_2. The incubation medium consisted of Krebs Ringer bicarbonate buffer, 5.5 mM glucose, rat plasma levels of nineteen amino acids, and 0.7 mM [^{3}H]phenylalanine (4.5 mCi/mmol) [9]. At 0.7 mM, the specific radio-

* Correspondence: Michael A. Hass, Ph.D., Pulmonary Research Laboratories R120, University of Miami School of Medicine, P.O. Box 016960, Miami, FL 33101.

activities of the intracellular, medium and tRNA-bound phenylalanine pools are the same [10, 11]. Therefore, under these conditions, the medium phenylalanine specific radioactivity can be used in calculating absolute rates of lung protein synthesis.

The incubation medium and conditions used to measure DNA synthesis were similar to those used to measure protein synthesis except that instead of [^{3}H]phenylalanine the medium contained 1.5 to 45.0 μM thymidine. The slices were preincubated for 1 hr at 0° in this medium and then transferred to similar medium containing [^{3}H]thymidine (113 mCi/mmol). This incubation continued for an additional 2 hr at 37°.

Following incubation with medium containing either [^{3}H]phenylalanine or [^{3}H]thymidine, the lung slices were rinsed in saline, blotted, and homogenized using a Brinkmann Instruments Polytron at the top setting for 15 sec in 6 ml of 5 mM potassium phosphate (pH 7.8). Portions (1 ml) of the homogenate were added to 1 ml of cold 20% trichloroacetic acid (TCA) to precipitate [^{3}H]protein or [^{3}H]DNA. The resulting pellets were washed three times with cold 5% TCA and either dissolved in 2 N sodium hydroxide at 56° ([^{3}H]protein) or extracted with 5% TCA at 90° ([^{3}H]DNA). Radioactivity in each extract was determined by liquid scintillation counting in Aquasol. Medium [^{3}H]thymidine was determined by the addition of 1 ml of medium to 1 ml of cold 20% TCA, followed by liquid scintillation counting of a portion of the resulting supernatant solution. Lung DNA was determined in each experiment using the diphenylamine assay [12].

Exposure conditions. Adult rats exposed to hyperoxia were housed in a 3.4 ft^3 plastic chamber in which O_2 (> 95%), CO_2 (< 0.5%), temperature (22–25°) and humidity (60–80%) were monitored. Rats were starved in cages modified to prevent coprophagia and in which water was freely available. Endotoxin was dissolved in saline and was injected intraperitoneally (500 μg/kg) just prior to the start of the exposure period. Control rats were injected with an equal volume of saline. All exposures were conducted so that measurements of DNA and protein syntheses were done at the same time of day, between 9:00 and 11:00 a.m.

Statistical analysis. For each exposure regimen, the significance of the difference between the saline- and endotoxin-treated group means was determined by unpaired *t*-test analysis [13]. An analysis of variance was used to test the significance of differences when more than two group means were compared [14, 15].

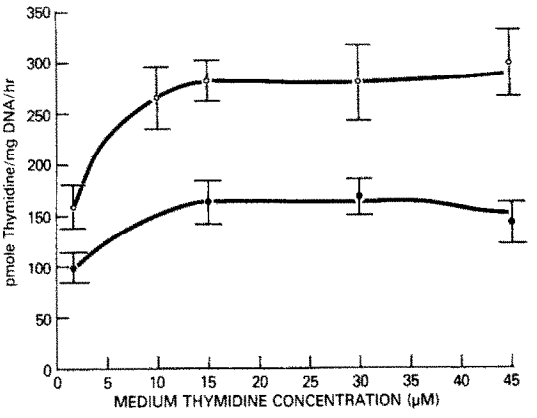

Fig. 1. Incorporation of [^{3}H]thymidine into lung DNA. Points represent the mean ± SE of at least four experiments measuring incorporation of [^{3}H]thymidine into DNA by lung slices incubated in medium containing various concentrations of thymidine. Key: (○—○) fed rats, and (●—●) rats starved for 24 hr.

RESULTS

Measurement of DNA synthesis. Rates of DNA synthesis are commonly determined by quantitating the incorporation of trace (usually about 1.5 μM) amounts of isotopic thymidine into DNA [16, 17]. Our experiments show that, in lung slices from either fed or starved rats, thymidine incorporation increased with increasing concentration of medium thymidine up to about 15 μM, above which the rate of incorporation remained virtually unchanged (Fig. 1). Plotting the medium thymidine concentration versus the reciprocal of the lung [^{3}H]thymidine incorporation (dpm/mg DNA/hr) of fed or starved rats produced two straight lines ($R = 0.998$), each with an ordinate intercept of −1.4, indicating intracellular isotope dilution equivalent to a pool of 1.4 μM thymidine. Although this value does not precisely define the intracellular thymidine concentration, it does provide a reasonable estimate of the pools that may dilute the incorporation of added [^{3}H]thymidine into DNA. The similarity of location of the ordinate intercepts indicates that fed and fasted rats had similar intracellular thymidine pools. This is consistent

Table 1. Effect of endotoxin treatment on syntheses of DNA and protein in air-breathing rats*

Exposure	Time (hr)	[^{3}H]Thymidine incorporation (pmol/mg DNA/hr)	[^{3}H]Phenylalanine incorporation (nmol/mg DNA/hr)
Saline	12	427 ± 7 (3)	20.1 ± 0.7 (4)
Endotoxin	12	314 ± 36† (4)	22.9 ± 1.7 (4)
Saline	24	304 ± 38 (9)	21.5 ± 0.9 (6)
Endotoxin	24	296 ± 27 (8)	21.6 ± 1.6 (6)

* Lung slices from rats treated with saline or endotoxin for the period indicated were incubated for 2 hr in medium containing 15 μM [^{3}H]thymidine or 0.7 mM [^{3}H]phenylalanine. Values are means ± SE for the number of experiments in parentheses.

† $P < 0.05$ vs 12-hr saline group.

Table 2. Effect of endotoxin treatment on syntheses of DNA and protein in O_2-breathing rats*

Exposure	Time (hr)	[^{3}H]Thymidine incorporation (pmol/mg DNA/hr)	[^{3}H]Phenylalanine incorporation (nmol/mg DNA/hr)
Saline	12	187 ± 23 (4)	19.7 ± 0.6 (4)
Endotoxin	12	162 ± 25 (4)	20.3 ± 2.4 (4)
Saline	24	236 ± 28 (8)	16.7 ± 0.5 (8)
Endotoxin	24	258 ± 14 (8)	18.5 ± 0.6† (7)

* Lung slices from rats treated with saline or endotoxin and exposed to >95% O_2 for the period indicated were incubated for 2 hr in medium containing 15 μM [^{3}H]thymidine or 0.7 mM [^{3}H]phenylalanine. Values are means ± SE for the number of experiments in parentheses.
† $P < 0.05$ vs 24-hr saline group.

with the finding that in both fed and fasted rats the apparent rate of thymidine synthesis became constant at a medium thymidine concentration of 15 μM.

Our data indicate that, at the low medium thymidine concentration commonly used by others (1.5 μM), incorporation of radioactive thymidine into DNA seriously underestimates the actual rate of thymidine incorporation, due to the substantial dilution by intracellular pools. In our subsequent experiments, we used a medium concentration of 15 μM that seems to have expanded the intracellular pool of thymidine to the point where isotope dilution by endogenous thymidine pools is greatly reduced. Incubation of lung slices in medium containing 15 μM thymidine also apparently caused no significant alterations in *de novo* DNA synthesis, as reflected by [^{14}C]formate incorporation into DNA [4.35 ± 0.25 vs 3.78 ± 0.05 nmol incorporated/mg DNA/hr, mean ± SEM, N = 4, for medium with 15 μM or no exogenous thymidine respectively ($P < 0.05$)].

Exposure of air-breathing rats to endotoxin. Twelve hours after injection of air-breathing rats with saline, the lung thymidine incorporation rate (Table 1) was higher than in lungs of uninjected rats ($P < 0.05$, analysis of variance, Fig. 1), and returned to untreated rates after 24 hr. Treatment of air-exposed rats with endotoxin for 12 or 24 hr maintained DNA synthesis at the same rate as in uninjected rats, thus preventing the increase observed 12 hr after injection with saline. The rate of lung protein synthesis was the same in saline- and endotoxin-treated rats (Table 1).

Exposure to hyperoxia and endotoxin. Exposure to hyperoxia for 12 hr decreased the rate of DNA synthesis in saline- and in endotoxin-treated rats ($P < 0.05$, analysis of variance, Fig. 1 and Table 2). The rate of DNA synthesis did not differ between saline- and endotoxin-treated rats at 24 hr, neither value at that time being significantly different than uninjected rats (Fig. 1 and Table 2). Following 24 hr of hyperoxic exposure, lung protein synthesis in endotoxin-treated rats was significantly higher than in saline-treated rats, and in this regard differed from the pattern observed in air-breathing endotoxin-treated rats (Table 1). Thus, the decrease in lung protein synthesis that developed between 12 and 24 hr of exposure to hyperoxia ($P < 0.05$, analysis of variance, Tables 1 and 2) was prevented or reversed by endotoxin treatment.

Effect on body weight. One effect of *in vivo* endotoxin administration is pyloric sphinctor constriction, which prevents gastric emptying and apparently leads to a severe loss of appetite [18]. In our experiments, injection with endotoxin led to an average loss of approximately 9% of body weight within 12 hr, with only slight recovery to an average 8% deficit after 24 hr (Table 3). Injection with saline led to only a temporary decrease in body weight that was reversed by 24 hr. Whether the rats were breathing air or O_2 made little difference in the extent of these alterations. Intraperitoneal injection of the pyloric sphinctor relaxant Reglan (metoclopramide hydrochloride) failed at low doses (< 150 mg/kg) to reduce the overnight weight loss resulting from endotoxin treatment and was fatal at higher doses (> 225 mg/kg, data not shown).

Comparison of starvation and endotoxin treatment. So large a loss of body weight in the endotoxin-treated rats suggested to us that the lungs of these rats might experience a transient state of nutritional imbalance, and that relatively well-nourished saline-treated rats might not constitute a proper control group for comparison of lung metabolic activities. Further experiments showed that the body weight loss following endotoxin treatment was comparable to that experienced by saline-treated rats completely deprived of food (Table 3).

Table 3. Effect of >95% O_2 exposure, endotoxin treatment, and starvation on body weight*

	Body weight (% change)			
	Fed		Starved	
Exposure	12 hr	24 hr	12 hr	24 hr
Air–saline	↓2%†	↑2%	↓8%	↓10%
Air–endotoxin	↓7%	↓6%	↓8%	↓11%
O_2–saline	↓2%	↑3%	↓6%	↓ 9%
O_2–endotoxin	↓10%	↓9%	↓6%	↓ 9%

* Rats allowed food or starved for the duration of the experiment were treated with saline or endotoxin and exposed to air or >95% O_2 for the period indicated. Values are expressed as percent change from initial body weight.
† All exposure regimens resulted in final body weights significantly different from initial body weights ($P < 0.05$ determined by Student's *t*-test) except in the 12-hr air–saline group.

Table 4. Effect of endotoxin treatment on syntheses of DNA and protein in starved air-breathing rats*

Exposure	Time (hr)	[^{3}H]Thymidine incorporation (pmol/mg DNA/hr)	[^{3}H]Phenylalanine incorporation (nmol/mg DNA/hr)
Saline	12	445 ± 29 (6)	22.8 ± 0.8 (4)
Endotoxin	12	350 ± 29† (7)	25.8 ± 1.0† (4)
Saline	24	232 ± 16‡ (13)	20.6 ± 1.0 (12)
Endotoxin	24	335 ± 28‡ (12)	25.1 ± 1.4† (13)

* Lung slices from rats starved and treated with saline or endotoxin for the period indicated were incubated for 2 hr in medium containing 15 μM [^{3}H]thymidine or 0.7 mM [^{3}H]phenylalanine. Values are means ± SE for the number of experiments in parentheses.
† $P < 0.05$ vs the saline group for the respective exposure period.
‡ $P < 0.05$ vs the 12-hr saline group.

Starvation for 12–24 hr also made virtually equal the weight loss and presumably the nutritional state of the lung of the saline- and endotoxin-treated groups. Therefore, we undertook a second series of experiments in which rats treated with saline or endotoxin, were all starved for the duration of each exposure to air or >95% O_2.

Exposure of starved air-breathing rats to endotoxin. Twenty-four-hour starvation of saline-treated air-breathing rats resulted in a significantly decreased rate of DNA synthesis compared to 12-hr starvation (Table 4). The DNA synthetic rate in endotoxin-treated rats was significantly lower than that of saline-treated controls after 12 hr but did not subsequently decrease, thus resulting after 24 hr in a DNA synthetic rate 50% higher than in saline-treated controls.

The lung protein synthesis rate was not decreased significantly in saline-treated rats starved for 24 hr (Table 4). Endotoxin-treated rats had increased rates of lung protein synthesis after both 12- and 24-hr exposure.

Exposure of starved rats to hyperoxia and endotoxin. The mean value for the rate of DNA synthesis was lower in lungs of starved saline-treated rats exposed to hyperoxia for 24 hr than in those exposed for 12 hr, but this difference fell short of statistical significance ($P > 0.05$) (Table 5). At the 24-hr exposure period, endotoxin treatment led to significantly increased rates of DNA and protein syntheses compared to saline-treated O_2-breathing rats (Table 5), a pattern similar to that observed in starved air-breathing rats (Table 4).

Formate incorporation and thymidine transport studies. To ensure that the increased rate of thymidine incorporation was not merely due to alterations in the thymidine-utilizing "salvage pathway", we measured the rate of [^{14}C]formate incorporation into DNA in lungs from similarly exposed rats. The incorporation of [^{14}C]formate was greater in the lungs of endotoxin-treated rats whether they had been exposed to 24 hr air or to >95% O_2 (Table 6). Nor was there any significant alteration in lung thymidine transport that could contribute to the higher rate of thymidine incorporation in the endotoxin-treated rats (Fig. 2).

In vitro *exposure to endotoxin.* Syntheses of DNA and protein were measured in normal rat lung slices incubated in medium with or without 200 ng/ml endotoxin. This is the serum endotoxin concentration of rats 30 min after intraperitoneal injection of an endotoxin dose (500 μg/kg) sufficient to protect rats from oxygen toxicity.* Lung DNA synthesis was unchanged by endotoxin exposures of up to 4 hr (Table 7); in contrast, protein synthesis was increased significantly after 4-hr pre-exposure to endotoxin.

DISCUSSION

Although not frequently considered, use of the incorporation of radioactive precursors into macro-

* L. Frank, personal communication; cited with permission.

Table 5. Effect of endotoxin treatment on syntheses of DNA and protein in starved O_2-breathing rats*

Exposure	Time (hr)	[^{3}H]Thymidine incroporation (pmol/mg DNA/hr)	[^{3}H]Phenylalanine incorporation (nmol/mg DNA/hr)
Saline	12	210 ± 33 (7)	21.7 ± 2.0 (7)
Endotoxin	12	277 ± 28 (6)	22.0 ± 2.0 (5)
Saline	24	140 ± 17 (6)	22.6 ± 1.1 (7)
Endotoxin	24	233 ± 32† (6)	27.5 ± 1.4† (6)

* Lung slices from rats starved, treated with saline or endotoxin, and exposed to >95% O_2 for the indicated length of time were incubated for 2 hr in medium containing 15 μM [^{3}H]thymidine or 0.7 mM [^{3}H]phenylalanine. Values are means ± SE for the number of experiments in parentheses.
† $P < 0.05$ vs the saline group for the respective exposure period.

Table 6. Effect of >95% O_2 exposure and endotoxin treatment on [^{14}C]formate incorporation into DNA in lungs of starved rats*

Exposure (24 hr)	[^{14}C]Formate incorporation (nmol/mg DNA/hr)
Air–saline	2.71 ± 0.14
Air–endotoxin	4.35 ± 0.57†
O_2–saline	1.01 ± 0.18
O_2-endotoxin	2.09 ± 0.43‡

* Lung slices from starved rats treated with saline or endotoxin and exposed to air or >95% O_2 for 24 hr were incubated for 2 hr with 250 μM [^{14}C]sodium formate. Values are means ± SE for four experiments.
† $P < 0.05$ vs air–saline group.
‡ $P < 0.06$ vs O_2–saline group.

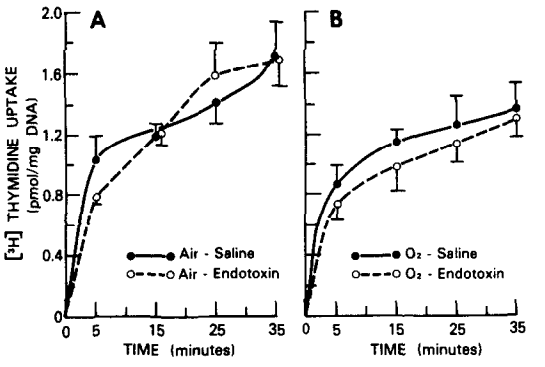

Fig. 2. Thymidine uptake by lung slices. Points represent the mean ± SE of four experiments measuring uptake of [^{3}H]thymidine by lung slices from rats treated with saline or endotoxin and exposed to air (A) or >95% O_2 (B) for 24 hr.

molecules such as DNA or protein as an index of macromolecule synthesis requires that the effects of the treatments on precursor pool sizes be known or substantially diminished [19]. One method to achieve the latter effect is to "flood" the intracellular precursor pool by the addition of a large amount of exogenous precursor [20]. We found, in lung slices from fed and starved rats, that a medium thymidine concentration of 15 μM appeared to flood the intracellular thymidine pool and that further increases in thymidine concentration did not alter the apparent rate of DNA synthesis. Thus, it is likely that the observed intergroup differences in the incorporation of radioactive thymidine into DNA reflect true differences in the rate of DNA synthesis rather than apparent differences due to inequality in the specific radioactivity of thymidine in the precursor pool for DNA synthesis. The results of our experiment on the transport of thymidine into lung cells indicates that differences in thymidine transport did not influence our findings.

Thymidine is an intermediate in the DNA synthesis pathway and, as such, thymidine incorporation into DNA is commonly referred to as the "salvage" pathway for DNA synthesis [21]. The incorporation of formate into DNA is a more direct measure of *de novo* DNA synthesis [22]. Our studies using this agent, as a second precursor molecule in the synthesis of DNA, help establish that the evidence accrued from the experiments using thymidine reflect *de novo* DNA synthesis rather than alterations only in the "salvage" pathway of DNA synthesis.

Although diminished food intake occurs in rats given endotoxin and must therefore be considered a "normal" response of the rat to endotoxin, this change alone could lead to significant alterations in lung metabolism. For example, while starvation does not change the ribosomal profile of the lung, lung RNA content is diminished and significant depression of lung protein synthesis usually occurs within 48–72 hr of the onset of food deprivation [23, 24]. In addition, our studies show that lung DNA synthesis in saline-treated rats starved for 24 hr was lower than in rats starved for only 12 hr. Together, these conditions may also partly resemble the early metabolic state of the endotoxin-exposed lung. Direct comparison of the nutritionally-deprived (endotoxin-treated) lung with a well-nourished (saline-treated) lung runs the risk of considering effects as primary actions of endotoxin, those that are actually responses to the effect of endotoxin on nutrient uptake.

With these considerations in mind, the present work demonstrates that, in rats allowed free access to food (the usual conditions of our experiments on endotoxin-induced tolerance to hyperoxia [31]), treatment with endotoxin did not produce an increased rate of DNA synthesis within 24 hr of treatment and the onset of exposure. However, when the nutritional and mitogenic effects of endotoxin

Table 7. Effect of *in vitro* endotoxin exposure on syntheses of DNA and protein by lung slices*

Exposure	Preincubation period)(hr)	[^{3}H]Thymidine incorporation (pmol/mg DNA/hr)	[^{3}H]Phenylalanine incorporation (nmol/mg DNA/hr)
Control	2	194 ± 26 (3)	15.9 ± 3.5 (8)
Endotoxin	2	160 ± 16 (3)	14.3 ± 3.1 (8)
Control	4	162 ± 5 (3)	17.3 ± 0.5 (5)
Endotoxin	4	151 ± 4 (3)	19.8 ± 1.1† (5)

* Lung slices from untreated rats were preincubated for the indicated length of time in medium with or without 200 ng/ml endotoxin. [^{3}H]Thymidine or [^{3}H]phenylalanine was then added, and the incubation was continued for an additional 2 hr. Values are means ± SE for the number of experiments in parentheses.
† $P < 0.05$ vs 4 hr control group.

were dissociated by starving both the control rats and the treated rats, the rate of DNA synthesis in endotoxin-treated rats exposed to O_2 or air for 24 hr exceeded the rate found in saline-treated rats. The validity of this dissociation of endotoxin effects is supported not only by our finding of similar weight loss after 24 hr in endotoxin-treated rats whether fed or starved but also by our finding that DNA synthesis rates 24 hr after endotoxin were not different in fed and starved rats. These observations extend prior studies in which it was found that fed rats treated with endotoxin (two doses 24 hr apart) maintained a higher rate of DNA synthesis 48 hr after the initial injection and the onset of O_2 exposure than did similarly exposed saline-treated rats [5].

That the mitogenic effect of endotoxin occurs within 24 hr of treatment and the onset of O_2 exposure is consistent with its possible importance in the prevention of the pulmonary edema that typically develops between 24 and 48 hr of O_2 exposure. Whether the relative increase in DNA synthesis simply represents maintenance of a more normal synthesis rate due to stabilization of endothelial membranes by endotoxin is unclear. However, a 70% inhibition of DNA synthesis by dexamethasone was not sufficient to prevent endotoxin's protection from pulmonary edema, suggesting that DNA synthesis in certain key lung cells may have continued and contributed to the observed lung protection.

Under the conditions of our present experiments, a higher rate of DNA synthesis in endotoxin-treated rats compared to saline-treated rats was always accompanied by an elevation in the rate of protein synthesis. In two instances, elevated protein synthesis occurred in the absence of more rapid DNA synthesis in endotoxin-treated than in saline-treated rats. Two interpretations of these data are plausible: (1) endotoxin's stimulation of protein synthesis and the differences in DNA synthesis between saline- and endotoxin-treated rats are independent, and (2) endotoxin leads initially to increased protein synthesis that subsequently triggers DNA synthesis. We attempted to resolve this dilemma (Table 7) but, although endotoxin given *in vitro* increased the rate of protein synthesis, it did not result in a difference from control slices in the rate of DNA synthesis. Hence, the use of an inhibitor such as cycloheximide, which might have enabled us to dissociate the effect of endotoxin on DNA and protein syntheses, was not feasible. Prolongation of the incubation was not attempted since we have found that it is associated with fibroblast overgrowth and alterations in the rate of protein synthesis (unpublished observation). Furthermore, it is unlikely that a sufficient inhibition of protein synthesis could be achieved by the administration of cycloheximide *in vivo* to allow us to answer this question [25].

Acknowledgements—We thank Mrs. Ondina Garcia-Pons for typing the manuscript. These studies were supported by research funds from the American Lung Association, NIH Grant HL20366, Biomedical Research Support Grant 507RR-05363, and V.A. Research Funds. D. Massaro is a Medical Investigator of the Veterans Administration.

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