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COMPARISON OF THE RESPONSE TO 'STREPTOCOCCUS PNEUMONIAE', 'SALM--ETC(U)  
AUG 78 W L THOMPSON, R W WANNEMACHER

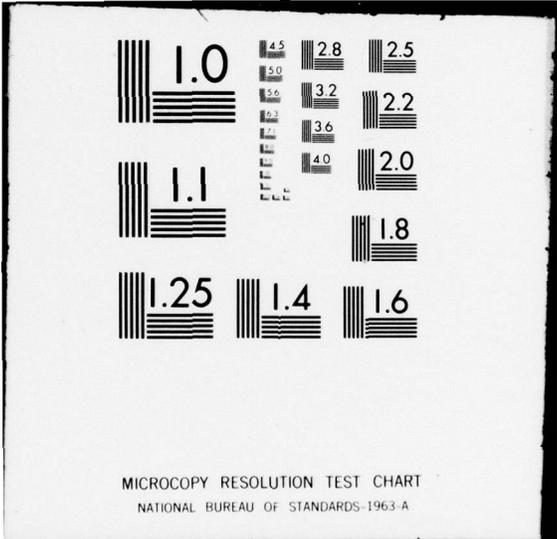
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expense of the free ribosomes during the peak hours of the RNA response. However, in studies using Salmonella typhimurium and its endotoxin, more involvement of the free ribosome fraction during the early stages of the infection is apparent. These data suggest that the hepatic RNA response takes place in two stages, an early "endotoxin" response, resulting in redistribution of cytoplasmic RNA into free ribosomes, and a later "infection" response, involving the mobilization of the bound ribosomes.

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Running title: HEPATIC RNA RESPONSE TO INFECTION

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The views of the authors do not purport to reflect the positions of the Department of the Army or the Department of Defense.

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## ABSTRACT

Recently a technique has been devised for quantitative subfractionation of rat liver. This procedure has been used to study the effects of several infectious organisms on hepatic RNA production and distribution, in order to better understand the alterations in hepatic protein production in response to infection. Results from these and previous studies demonstrate an increase in the rate of RNA production in response to Streptococcus pneumoniae infection which reaches significant levels by 10 h and continues to rise through 16 h after infection. Redistribution of RNA into the bound ribosomal fraction takes place at the expense of the free ribosomes during the peak hours of the RNA response. However, in studies using Salmonella typhimurium and its endotoxin, more involvement of the free ribosome fraction during the early stages of the infection is apparent. These data suggest that the hepatic RNA response takes place in two stages, an early "endotoxin" response, resulting in redistribution of cytoplasmic RNA into free ribosomes, and a later "infection" response, involving the mobilization of the bound ribosomes.

It has been shown recently that in response to infection and inflammatory stimuli, there is an increase in concentration of certain serum proteins, known as acute-phase proteins (3, 14) along with an increased flux of amino acids into hepatic cells (4, 7, 12), a depression in serum zinc and an increase in serum copper (1).

Studies on the effects of Salmonella typhimurium endotoxin (P. Z. Sobocinski, W. J. Canterbury, and C. A. Mapes. Abstr., Fed. Proc. 36:1100, 1977) and infection (8) on hepatic cells have shown it to cause a rapid increase in the uptake of zinc and production of intracellular metallothioneins. Others have shown that the endotoxin acts on hepatic cells by direct penetration of the cells causing numerous metabolic alterations (15).

A previous report on the effects of Streptococcus pneumoniae infection on hepatic RNA distribution and activity described an increase in the rate of transcription of RNA which is directed predominantly toward the bound ribosome fraction (9). However, the commonly used technique for the isolation of ribosomes used in that study (2) was not a good quantitative method for the study of RNA distribution and activity. It has been shown that an initial centrifugation step results in the loss of more than 60% of the ribosome RNA, most of which is associated with the bound ribosomes (10).

The purpose of this study was to use a current technique for the quantitative recovery of undegraded hepatic RNA subfractions in order to compare the effects of S. pneumoniae and S. typhimurium infection and the latter's endotoxin on hepatic RNA distribution and activity. This would then provide information on the involvement of hepatic RNA in the regulation of some of the responses listed above. Comparisons were made

between the responses seen with the old and new techniques, the two types of infections studied and the early endotoxin response compared to the later infection response.

### MATERIALS AND METHODS

Animals. Male Fisher-Dunning rats weighing 150 to 200 g were supplied by Microbiological Associates (Walkersville, Md.). They were maintained on a 12-h light-dark schedule (6 A.M. to 6 P.M.), at a temperature of 25-26°C and routinely fasted 16 to 20 h prior to killing.

Infecting microorganisms and endotoxin. Virulent S. pneumoniae, Type I strain A5, was used to infect rats by subcutaneous (s.c.) injection of  $10^7$  cells contained in 0.1 ml. S. typhimurium (MIT) was inoculated intraperitoneally (i.p.) at a dose of  $10^8$  microorganisms contained in 0.1 ml. Heat-killed S. typhimurium (56°C, 20 min) was given similarly. S. typhimurium endotoxin, Type B (Difco Laboratories, Detroit, Mich.), was suspended in physiological saline at a concentration of 5 mg/ml and an i.p. injection of 0.1 ml was given to each rat in that group. All control rats were given saline injections of the same volume and route of injection as the agent to which they were being compared. Details concerning the preparation of the inoculated microorganisms and the clinical manifestations of the infections have been published elsewhere (12, 13).

Subfractionation of hepatic cells. All rats were given 5  $\mu$ Ci/100 g body weight 6- $^{14}$ C]orotic acid hydrate (40-60 mCi/mmol, New England Nuclear, Boston, Mass.) 4 h before killing. At the end of the experimental time period the rat livers were perfused in situ with cold saline and subfractionated into nuclear, free and bound ribosomal and soluble fractions using a modification of the procedure of Ramsey and Steele (6). The livers were homogenized in 4 volumes (w/v) of 0.25 M Sucrose in buffer A (50 mM Hepes, pH 7.6, 75 mM KCl, 5 mM MgCl<sub>2</sub>, 3 mM

glutathione) using a Potter-Elvehjem homogenizer. Aliquots of the homogenate were taken for determination of total liver RNA and radioactivity. Portions (16 ml) of the remainder were centrifuged in an SW-27.1 rotor (Beckman Instruments, Silver Spring, Md.) at  $135,000 \times g$  for 12 min at speed. Four milliliters of the supernatant containing the free ribosomes was layered over a two-step discontinuous gradient containing 3 ml each of 2.0 M and 1.38 M sucrose in buffer A. The gradients were spun for 20 h at  $105,000 \times g$  in a 50Ti rotor (Beckman Instruments). The resulting free ribosome pellets and supernatant representing the soluble RNA fraction were collected and stored at  $-20^{\circ}\text{C}$ .

A cell-sap fraction was prepared from the livers of normal rats by homogenizing in 2 volumes (w/v) of 0.25 M sucrose in buffer B (50 mM Hepes, pH 7.6, 250 mM KCl, 5 mM  $\text{MgCl}_2$ , 3 mM glutathione), centrifugation at  $105,000 \times g$  for 3 h, and collection of the supernatant. Each pellet from the initial centrifugation ( $135,000 \times g$ ) was resuspended in 8 ml of the cell sap, treated with 1/10 volume of 10% Triton x-100 and centrifuged at  $1,470 \times g$  for 5 min. The resulting pellets (nuclear fraction) were collected and stored at  $-20^{\circ}\text{C}$ . A 1/10 volume of 13% sodium deoxycholate was added to the supernatants and 4-ml portions of this material layered over a two-step discontinuous gradient containing 3 ml each of 2.0 M and 1.38 M sucrose in buffer B. These were then spun at  $105,000 \times g$  for 20 h. The resulting pellets representing the bound ribosomes were stored at  $-20^{\circ}\text{C}$ .

Determination of RNA, DPM and DNA content. The RNA content and radioactivity of the total homogenate and all subfractions were determined by previously described techniques (11). Aliquots of

each fraction were precipitated and washed twice with cold 0.2 N perchloric acid (PCA). They were then hydrolyzed in 2 ml of 0.3 N KOH for 1 h in a 37°C water bath. The samples were reprecipitated with 0.1 ml of 60% PCA, centrifuged, and the pellets washed twice with 0.2 N PCA, collecting the supernatant containing the hydrolyzed RNA from each spin. All of the samples were brought to a constant volume of 5 ml and aliquots were taken to measure radioactivity and optical density at 260 nm. RNA content was determined from these values using an RNA standard curve of known concentration against absorbance at 260 nm.

After washing out the hydrolyzed RNA, the DNA content of the total homogenate and nuclear fraction was determined by treating the pellets with 1.5 ml of 0.5 N PCA for 45 min at 95°C. The pellets were centrifuged and washed twice with 0.5 N PCA; the supernatant fractions were brought to 5 ml volume. The absorbance was then read at 265 and 284 nm; the difference was used to determine the DNA content according to a standard curve.

For each time period of each study, six control rats were compared to six infected or endotoxin-treated rats. Each time period of each study represents a separately run experiment although all data are presented in a single table. Group mean values were compared by Students t test and the difference between two means was considered significant at  $P < 0.05$  under the null hypothesis.

### RESULTS

The distribution of RNA and the percent of total RNA in the four subfractions of normal rat liver are shown in Table 1. Between 75 and 80% of the total hepatic RNA is associated with the ribosomes, three-quarters of which is found in the bound ribosome fraction. The sum of the four fractions is within the range of values for the total homogenate indicating quantitative recovery of RNA in the four subfractions.

The raw data for each of the subfractions at each time period studied for the various treatments is presented in Tables 2 to 5 in order to demonstrate the real differences and levels of significance seen in the course of these studies. Variations in the level of activity between experiments may have been due to differences in the level of specific activity of the radioisotope or the metabolic state of the rats. However, these between-experiment variations have no bearing on the within-experiment comparison of control versus treated rats for each of the subfractions at each time period. In each of the tables, the appearance of newly formed RNA in the total homogenate and four liver cell fractions is shown in the upper portion by expressing the results in terms of DPM [ $^{14}\text{C}$ ]orotic acid incorporated into RNA per microgram of total DNA. The distribution of RNA in each of these fractions during the course of the infection or endotoxin treatment is shown in the lower portion of the table by expressing the results in terms of units of RNA present per unit of DNA. For the purpose of graphically demonstrating the differences between the results seen in response to the two infectious models and endotoxin treatment,

the data are also presented as a percent of control values (Figures 1-3) for the three fractions of greatest interest, the total homogenate, and free and bound ribosome fractions. These time course profiles provide better visual comprehension of the interrelationship in responses seen in the various fractions during the course of infection or endotoxin treatment.

Table 2 shows the results of a comparison of S. pneumoniae-infected rats to control rats at 8, 12 and 16 h. Significant increases in radioactivity in the bound and soluble fractions of the infected rats as compared to controls appear at 8 h and remain elevated at 16 h. The total homogenate also demonstrates a significant increase in the appearance of newly formed RNA over control values at 16 h (See also Fig. 1). Previous studies (9) have shown similar results with significant increases in radioactivity in all but the nuclear fraction by 20 h postinfection. However, there appears to be little change in RNA distribution at these three time periods, except for a decrease associated with the free ribosomes starting at 12 h, followed by an increase in RNA in the bound ribosomal fraction (right side of Figs. 2 and 3).

Table 3 shows the results from S. typhimurium infection at 8, 14, 24 and 48 h. A significant increase in specific activity is seen in all fractions by 14 h. Three notable differences exist between S. pneumoniae and S. typhimurium infections. First, a peak response in radioactivity over control values is seen in all subfractions of the S. typhimurium infection at an earlier time period (14 h). This can be seen graphically in Figs. 1-3 by directly comparing the time-course profiles of the total homogenate and two ribosome fractions

in response to the two infections. The greatest response of the three in appearance of newly formed RNA shown on the left portion of the figures is in the bound ribosome fraction. Second, the nuclear fraction demonstrates a build-up in newly formed RNA during the peak hours of S. typhimurium infection and in total RNA during the later stages. Third, although the RNA associated with the S. typhimurium bound ribosomes again increases during the course of the infection, no significant change in RNA associated with the free ribosomes is observed (Figs. 2 and 3). Studies on the effects of S. typhimurium endotoxin were carried out both indirectly using heat-killed S. typhimurium (Table 4) and by direct use of S. typhimurium endotoxin (Table 5). Although similar responses to those seen with the live organisms were apparent at 15 h (Table 4), several new observations were made at the early time periods. There were no significant changes in radioactivity in any of the fractions in response to heat-killed organisms and endotoxin (upper portion of Tables 4 and 5). However, both of them caused an early increase in RNA associated with the free ribosomes (See Fig. 3).

#### DISCUSSION

An effective procedure for the quantitative isolation of rat liver subfractions has been recently developed (6) and used to study and compare the hepatic RNA response to S. pneumoniae and S. typhimurium infections and S. typhimurium endotoxin. Results from this study and previous observations (9) provide evidence that the S. pneumoniae infection stimulates a gradual increase in the transcription of RNA reaching a peak response around 16 to 20 h after infection. Since there is no significant change in RNA activity associated with

the nuclear fraction, the newly formed RNA appears to be processed as quickly as it is being made. The only change in actual amounts of RNA associated with the subfractions appears to be a redistribution of cytoplasmic RNA from the free to the bound ribosomal fraction at the peak of the RNA response. This fits well with the proposed function of the bound ribosomes in the production of extracellular proteins, since an increase in certain plasma acute-phase proteins takes place during this stage of the infectious process (5).

A similar response in hepatic RNA production was observed with the S. typhimurium infection reaching a peak response around 14 h after infection. A notable exception was the response seen in the nuclear fraction. A buildup of newly formed RNA during the peak response along with an overall buildup of RNA during the entire course of the infection may be due to differences in the infectious model. The early and rapid enhancement of transcriptional rates in response to S. typhimurium might result in the production of RNA at a rate greater than the transport of RNA from the nucleus to the cytoplasm can take place. Also, some impairment in transport of RNA from the nucleus to the cytoplasm may take place due to the presence of endotoxin, since it has been shown to enter parenchymal cells and become associated with both cytoplasmic and nuclear fractions (15). Although no significant depression in RNA associated with the free ribosomes was noted with Salmonella infection, the RNA content of the bound ribosomal fraction increased during the later stages of the infection along with an earlier increase in radioactivity. This indicates once again a preferential movement of newly formed RNA to the bound ribosomes during the peak and later stages of the infectious response. The

different pattern during the early stages of S. typhimurium infection appears to be due to endotoxin effects. A similar response is seen with both heat-killed S. typhimurium and the purified S. typhimurium endotoxin. This endotoxin response is associated with an earlier increase in RNA in the free ribosome fraction along with a decrease in the RNA content of the bound ribosome fraction. This is an opposite effect to that seen during the later "infectious" stage. These data along with information available in the literature on the mechanism of endotoxemia (15) provides strong support for a two-stage response in hepatic RNA regulation to an infection by endotoxin-containing bacteria.

The first stage, due to the early entry of endotoxin into the hepatic cells, causes the mobilization of the intracellular machinery for its defense. Many possibilities exist for regulatory mechanism(s) involved in the redistribution or mobilization of cytoplasmic RNA. It may be due to a direct effect of the endotoxin, stimulation of soluble cytoplasmic regulatory factors, the presence of rapidly transcribed mRNA produced in response to the presence of endotoxin, or any of the above combinations. Future studies on translational rates of free and bound ribosomes and soluble cytoplasmic fractions from control and endotoxin treated hepatic cells are required to elucidate the mechanism of action of endotoxin on hepatic RNA metabolism.

The second stage, which is similar in both infectious models studied, involves increased rates of RNA transcription and a subsequently greater involvement of the bound ribosomal fraction presumably for the increased production of specific acute-phase serum proteins. This latter stage appears to be a nonspecific response to inflammatory stimuli.

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TABLE 1. Distribution of RNA in subfractions from normal rat liver

Fraction	mg RNA/g liver (mean $\pm$ SE)	% of total RNA
Total homogenate	6.63 $\pm$ 0.07	
Nuclear	0.49 $\pm$ 0.03	6.96
Soluble	1.08 $\pm$ 0.01	15.34
Free ribosomal	1.25 $\pm$ 0.02	17.76
Bound ribosomal	4.22 $\pm$ 0.02	59.94

TABLE 2. The effect of *S. pneumoniae* infection on the production (top) and distribution (bottom) of RNA in total homogenate and four subfractions of liver (n = 6)

Time period (h)	Group	Total				Fraction			
		homogenate	Nuclear	Free ribosome	Bound ribosome	Soluble			
8 h	Control	147.8 ± 7.8	48.3 ± 4.1	24.8 ± 3.1	41.9 ± 4.6	18.8 ± 2.6			
	Infected	154.3 ± 11.3	51.4 ± 3.0	23.7 ± 1.9	54.1 ± 6.1	21.5 ± 3.7			
12 h	Control	179.3 ± 8.9	55.7 ± 4.4	25.9 ± 1.3	57.2 ± 1.6	20.7 ± 1.7			
	Infected	197.3 ± 12.8	56.5 ± 7.6	27.3 ± 2.0	90.2 ± 8.5 <sup>b</sup>	28.0 ± 2.7 <sup>a</sup>			
16 h	Control	163.1 ± 9.1	58.3 ± 4.9	24.6 ± 2.4	39.6 ± 8.7	15.5 ± 1.5			
	Infected	180.5 ± 15.1 <sup>a</sup>	46.2 ± 4.5	25.0 ± 1.9	65.1 ± 8.2 <sup>b</sup>	20.6 ± 5.9 <sup>a</sup>			
8 h	Control	3.08 ± 0.08	0.19 ± 0.02	0.53 ± 0.02	1.48 ± 0.09	0.54 ± 0.02			
	Infected	3.10 ± 0.10	0.20 ± 0.01	0.49 ± 0.06	1.55 ± 0.10	0.58 ± 0.04			
12 h	Control	3.24 ± 0.18	0.20 ± 0.02	0.66 ± 0.04	1.95 ± 0.08	0.60 ± 0.06			
	Infected	2.98 ± 0.14	0.22 ± 0.02	0.51 ± 0.03 <sup>a</sup>	1.92 ± 0.11	0.54 ± 0.04			
16 h	Control	3.09 ± 0.21	0.18 ± 0.02	0.54 ± 0.01	1.25 ± 0.05	0.37 ± 0.02			
	Infected	3.39 ± 0.13	0.24 ± 0.03	0.51 ± 0.01 <sup>a</sup>	1.74 ± 0.07 <sup>b</sup>	0.44 ± 0.03			

<sup>a</sup> P < 0.05.

<sup>b</sup> P < 0.01.

TABLE 3. The effect of *S. typhimurium* infection on the production (top) and distribution (bottom) of RNA in total homogenate and four subfractions of rat liver ( $n = 6$ )

Time period (h)	Group	Total homogenate				Fraction			
		Nuclear	Free ribosome	Bound ribosome	Soluble				
8 h	Control	114.9 ± 2.8	42.9 ± 1.8	14.4 ± 0.5	28.3 ± 1.8	13.1 ± 0.4			
	Infected	129.6 ± 8.9	37.5 ± 2.6	19.8 ± 1.4 <sup>b</sup>	42.7 ± 3.6 <sup>b</sup>	19.0 ± 1.7 <sup>b</sup>			
14 h	Control	109.7 ± 7.6	56.0 ± 3.9	14.6 ± 1.2	29.4 ± 2.2	11.3 ± 1.2			
	Infected	197.4 ± 11.4 <sup>b</sup>	71.8 ± 4.6 <sup>a</sup>	27.3 ± 1.9 <sup>b</sup>	79.0 ± 5.7 <sup>b</sup>	30.2 ± 4.6 <sup>b</sup>			
24 h	Control	192.8 ± 7.6	67.6 ± 4.3	21.7 ± 2.4	48.8 ± 2.6	17.7 ± 1.3			
	Infected	226.6 ± 27.3	91.2 ± 5.2 <sup>b</sup>	28.9 ± 2.9	89.8 ± 13.3 <sup>a</sup>	31.7 ± 4.0 <sup>b</sup>			
48 h	Control	196.7 ± 9.3	99.5 ± 10.3	36.0 ± 4.2	75.7 ± 6.0	27.3 ± 3.1			
	Infected	200.1 ± 9.0	90.6 ± 6.1	35.4 ± 3.0	100.1 ± 7.0 <sup>a</sup>	33.0 ± 3.0			
RNA/DNA ratio (mean ± SE)									
8 h	Control	2.89 ± 0.17	0.20 ± 0.02	0.51 ± 0.03	1.33 ± 0.09	0.51 ± 0.02			
	Infected	2.90 ± 0.05	0.22 ± 0.01	0.51 ± 0.03	1.40 ± 0.04	0.52 ± 0.03			
14 h	Control	3.22 ± 0.08	0.30 ± 0.01	0.64 ± 0.03	1.41 ± 0.05	0.53 ± 0.03			
	Infected	3.53 ± 0.11 <sup>a</sup>	0.40 ± 0.02 <sup>b</sup>	0.59 ± 0.03	1.48 ± 0.06	0.61 ± 0.03			
24 h	Control	3.06 ± 0.05	0.21 ± 0.01	0.55 ± 0.02	1.65 ± 0.08	0.48 ± 0.03			
	Infected	3.58 ± 0.10 <sup>b</sup>	0.33 ± 0.02 <sup>b</sup>	0.56 ± 0.02	2.22 ± 0.13 <sup>b</sup>	0.58 ± 0.03 <sup>a</sup>			
48 h	Control	3.11 ± 0.06	0.22 ± 0.01	0.63 ± 0.03	1.97 ± 0.08	0.59 ± 0.02			
	Infected	3.42 ± 0.07	0.31 ± 0.02 <sup>b</sup>	0.60 ± 0.03	2.22 ± 0.03 <sup>a</sup>	0.63 ± 0.03			

<sup>a</sup>  $P < 0.05$  when comparing infected and control values.

<sup>b</sup>  $F < 0.01$  when comparing infected and control values.

TABLE 4. The effect of heat-killed *S. typhimurium* on the production (top) and distribution (bottom) of RNA in total homogenate and four subfractions of rat liver ( $n = 6$ )

Time period (h)	Group	Total homogenate		Fraction			
		Nuclear	Free ribosome	Bound ribosome	Soluble		
4 h	Control	158.7 ± 9.7	69.3 ± 5.9	23.2 ± 1.6	43.6 ± 3.9	20.9 ± 1.6	
	Heat-killed	167.5 ± 11.0	62.2 ± 5.4	27.3 ± 1.8	43.4 ± 3.4	25.2 ± 1.6	
8 h	Control	155.6 ± 4.4	65.0 ± 9.7	19.6 ± 1.2	36.6 ± 2.0	18.6 ± 2.2	
	Heat-killed	166.4 ± 19.7	62.3 ± 5.6	27.7 ± 3.6	41.4 ± 5.3	23.2 ± 2.6	
15 h	Control	165.4 ± 4.9	51.6 ± 3.8	19.3 ± 1.4	48.2 ± 3.2	17.1 ± 1.0	
	Heat-killed	218.5 ± 8.9 <sup>b</sup>	65.3 ± 1.5 <sup>a</sup>	30.8 ± 1.3 <sup>b</sup>	75.3 ± 3.2 <sup>b</sup>	30.5 ± 1.1 <sup>b</sup>	
4 h	Control	3.57 ± 0.05	0.37 ± 0.03	0.71 ± 0.01	1.77 ± 0.06	0.59 ± 0.02	
	Heat-killed	3.72 ± 0.02 <sup>a</sup>	0.41 ± 0.02	0.79 ± 0.01 <sup>b</sup>	1.55 ± 0.03 <sup>b</sup>	0.66 ± 0.03	
8 h	Control	2.58 ± 0.06	0.32 ± 0.03	0.48 ± 0.02	1.16 ± 0.05	0.43 ± 0.01	
	Heat-killed	2.72 ± 0.02 <sup>a</sup>	0.21 ± 0.01 <sup>a</sup>	0.53 ± 0.01	0.97 ± 0.09	0.51 ± 0.02 <sup>b</sup>	
15 h	Control	3.04 ± 0.14	0.17 ± 0.01	0.48 ± 0.03	1.62 ± 0.12	0.72 ± 0.04	
	Heat-killed	3.50 ± 0.04 <sup>a</sup>	0.26 ± 0.01 <sup>b</sup>	0.58 ± 0.01 <sup>a</sup>	1.78 ± 0.10	0.86 ± 0.01 <sup>b</sup>	

<sup>a</sup>  $P < 0.05$  when comparing treated and control values.

<sup>b</sup>  $P < 0.01$  when comparing treated and control values.

TABLE 5. The effect of *S. typhimurium* endotoxin in the production (top) and distribution (bottom) of RNA in total homogenate and four subfractions of rat liver (n = 6)

Time period (h)	Group	Total					Fraction			
		homogenate	Nuclear	Free ribosome	Bound ribosome	Soluble				
4 h	Control	147.1 ± 12.4	56.7 ± 5.8	19.5 ± 1.8	41.4 ± 5.3	18.6 ± 1.8				
	Endotoxin	142.9 ± 8.2	39.6 ± 5.2	13.7 ± 1.6	35.8 ± 2.7	20.0 ± 1.7				
6 h	Control	158.0 ± 13.5	62.0 ± 5.9	22.3 ± 2.3	49.4 ± 4.1	16.9 ± 2.0				
	Endotoxin	158.4 ± 23.2	52.4 ± 10.3	24.4 ± 4.2	44.2 ± 8.8	18.7 ± 3.1				
RNA/DNA ratios (mean ± SE)										
4 h	Control	3.24 ± 0.06	0.26 ± 0.01	0.58 ± 0.02	1.62 ± 0.06	0.52 ± 0.02				
	Endotoxin	3.18 ± 0.05	0.22 ± 0.02	0.66 ± 0.01 <sup>b</sup>	1.39 ± 0.09	0.64 ± 0.02				
6 h	Control	3.30 ± 0.22	0.34 ± 0.02	0.66 ± 0.05	1.92 ± 0.10	0.50 ± 0.03				
	Endotoxin	2.98 ± 0.16	0.39 ± 0.03	0.69 ± 0.05	1.35 ± 0.07 <sup>b</sup>	0.49 ± 0.02				

<sup>a</sup>  $\bar{p} < 0.05$  when comparing endotoxin treated and control values.

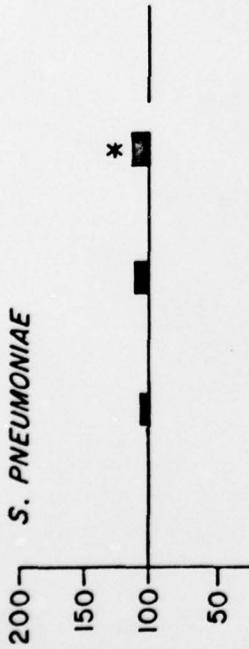
<sup>b</sup>  $\bar{p} < 0.01$  when comparing endotoxin treated and control values.

LEGENDS TO FIGURES

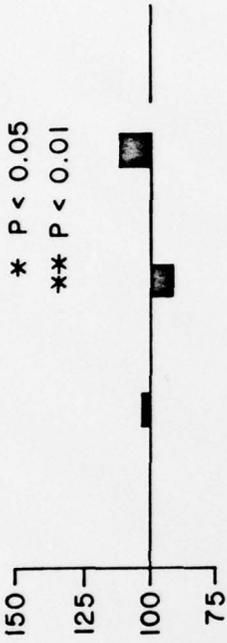
- FIG. 1. Percent of control time course profiles in total homogenate fraction in response to the four treatments studied.  
Incorporation of labeled orotic acid into RNA per  $\mu\text{g}$  total cellular DNA shown on left. Quantity of RNA per total cellular DNA on right. Each bar represents a separate experiment containing 6 control and 6 treated rats.
- FIG. 2. Percent of control time course profiles in free ribosome fraction in response to the four treatments studied.  
Incorporation of labeled orotic acid into RNA per  $\mu\text{g}$  total cellular DNA shown on left. Quantity of RNA per total cellular DNA on right. Each bar represents a separate experiment containing 6 control and 6 treated rats.
- FIG. 3. Percent of control time course profiles in bound ribosome fraction in response to the four treatments studied.  
Incorporation of labeled orotic acid into RNA per  $\mu\text{g}$  total cellular DNA shown on left. Quantity of RNA per total cellular DNA on right. Each bar represents a separate experiment containing 6 control and 6 treated rats.

INITIAL HOMOGENATE

DPM/ $\mu$ g DNA

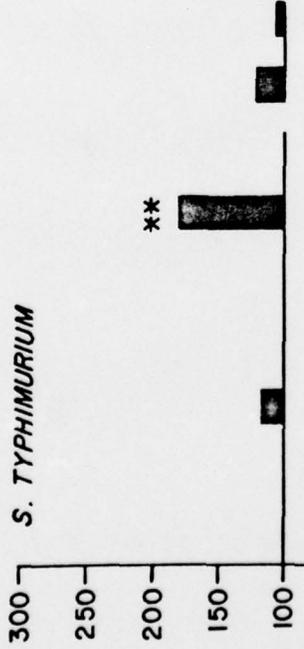


RNA/DNA RATIO

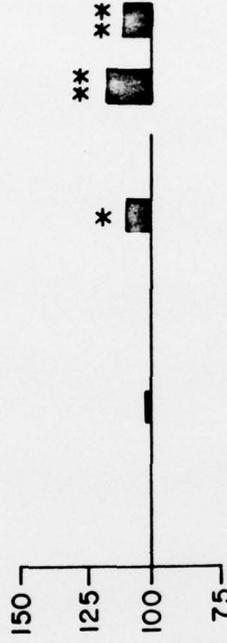


\*  $P < 0.05$   
\*\*  $P < 0.01$

*S. TYPHIMURIUM*



150  
125  
100  
75



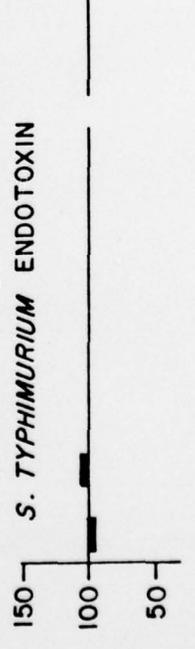
HEAT-KILLED  
*S. TYPHIMURIUM*



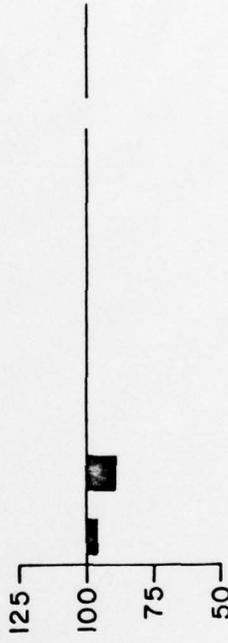
125  
100  
75



*S. TYPHIMURIUM* ENDOTOXIN



125  
100  
75  
50

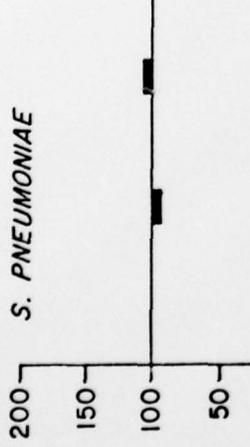


4 6 8 10 12 14 16 24 48

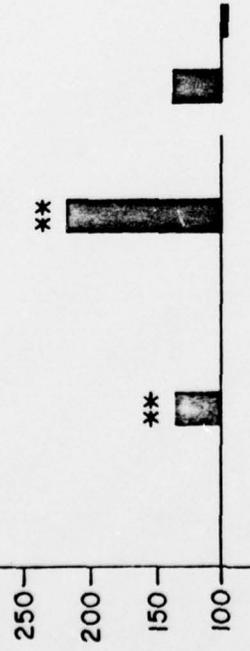
4 6 8 10 12 14 16 24 48

HOURS AFTER EXPOSURE

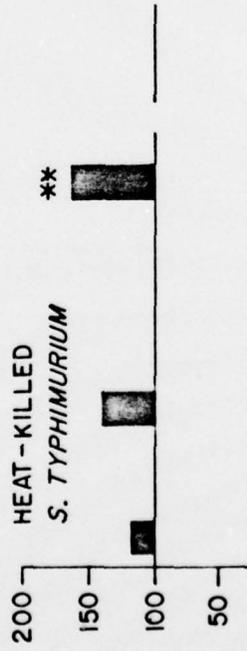
DPM/ $\mu$ g DNA



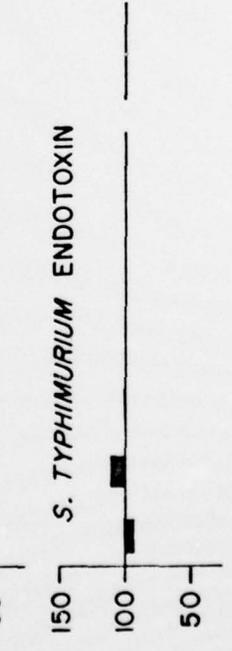
*S. TYPHIMURIUM*



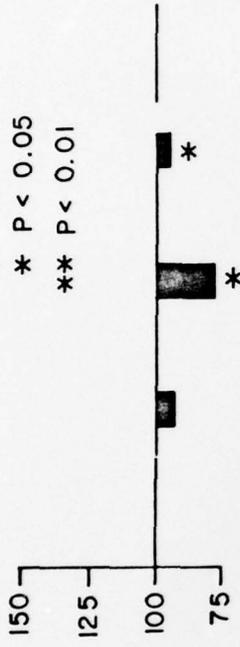
HEAT-KILLED  
*S. TYPHIMURIUM*



*S. TYPHIMURIUM* ENDOTOXIN



RNA/DNA RATIO



\*  $P < 0.05$

\*\*  $P < 0.01$

4 6 8 10 12 14 16 24 48  
HOURS AFTER EXPOSURE

INDUCTION FRACTION

RNA/DNA RATIO

DPM/μg DNA

