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Ribonucleic Acid and Protein Synthesis in Guinea Pig Liver During Q Fever

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Protein and ribonucleic acid (RNA) syntheses increase in the liver during Q fever, coincident with progressive increments in cortisol levels. Cell-free protein-synthesizing systems indicate that the pH 5 enzyme fraction was chiefly responsible for enhanced activity. Although the polysome profiles from normal and infected livers were similar, the number of liver ribosomes doubled during infection. There was a concomitant increase in orotate incorporation in the 28*s*, 18*s*, and 4*s* RNA species of infected liver.

During experimental infection of the guinea pig with *Coxiella burneti*, there is a depletion of liver glycogen and a fatty infiltration of the liver within 24 h postinfection (30, 31, 34). The changes are sharpest by 84 to 96 h postinfection. Despite the pronounced changes in the liver, most liver cells themselves are not invaded by the rickettsiae (13). The disease is overtly an emaciating one, yet protein and ribonucleic acid (RNA) synthesis is actually enhanced in the livers of guinea pigs and other host animals (25, 34). The present report seeks to establish a basis for augmented synthesis of protein and RNA in liver during Q fever.

MATERIALS AND METHODS

Reagents. Acrylamide and *N,N'*-methylenebisacrylamide (Eastman) were recrystallized before use. *N,N,N',N'*-tetramethylene-diamine and ammonium persulfate were from Biorad Laboratories. Sucrose (ribonuclease [RNase] free, density gradient grade) was from Schwarz/Mann, and cortisol sodium succinate (Solu-cortef) was from Upjohn. Pancreatic RNase and RNase-free deoxyribonuclease (DNase) were from Worthington Biochemical Corp.

Radiochemicals were: ^{14}C-reconstituted protein hydrolysate, Schwarz mixture, 13 amino acids, sp act 156 to 468 mCi/mmol (Schwarz/Mann); ^{14}C-L-leucine, sp act 11.3 mCi/mmol (International Chemical and Nuclear Corp.); and ^{3}H-5-orotic acid, sp act 1.67 mCi/mmol (New England Nuclear Corp.).

Organisms and animals. *C. burneti*, Nine Mile strain, phase I, first egg passage, was used to infect Hartley male guinea pigs (31). The animals were obtained from Hilltop Laboratory Animals, Inc., Scottsdale, Pa., and weighed 250 to 300 g at the onset of experiments.

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High-speed supernatant fluid. Guinea pig liver homogenates (33% wt/vol) were prepared in 0.25 M sucrose-TKM [50 mM tris(hydroxymethyl)aminomethane (Tris)-hydrochloride (pH 7.5), 25 mM KCl, 5 M $MgCl_2$] with 10 strokes of a Potter-Elvehjem homogenizer at 1,700 rpm. The homogenate was filtered through gauze, centrifuged at 17,000 × *g* for 10 min at 4 C, and high-speed supernatant fluids containing RNase inhibitor were prepared (2).

Polysomes. Post-mitochondrial supernatant fluids were adjusted to 1.3% deoxycholate (DOC) and centrifuged through 0.5 to 2.0 M sucrose-TKM discontinuous gradients (36); the sucrose solution in the discontinuous gradients contained 10% (vol/vol) high-speed supernatant fluid (RNase inhibitor). The ribosome pellets were suspended in ice-cold distilled water and centrifuged at 770 × *g* for 10 min to remove unsuspended aggregates. Polysome suspensions were layered over 28 ml of 0.3 to 1.0 M linear sucrose-TKM gradients and centrifuged in a Spinco SW25.1 rotor at 63,000 × *g* for 2.25 h at 2 C and allowed to decelerate without braking. Polysome profiles were obtained by monitoring the gradient at 254 nm in an ISCO ultraviolet monitor (model UA-2) connected to an ISCO fraction collector (model D). Polysomal configurations (n, 2n, etc.) were confirmed by electron microscopy.

Total, free, and bound ribosomes. The methods of Blobel and Potter (2, 3) were used to isolate nuclei and prepare postnuclear supernatant fluids, except that nuclei were pelleted through 1.7 M sucrose. Portions (1 ml) of the postnuclear supernatant fluids were used to prepare total cytoplasmic ribosomes and free ribosomes (3); centrifugation was carried out for 14 h to sediment free ribosomes. "Membrane-bound" ribosomes were obtained from the supernatant fluids after sedimentation of free ribosomes. These supernatant fluids, including the membrane fraction, were manually homogenized with a Teflon pestle in a centrifuge tube. To 1.8 ml of 10% DOC (wt/vol) was added 9.0 ml of supernatant fluid; the material was rehomogenized and centrifuged at 105,000 × *g* for 4 h

at 2 C. The pellet was termed "bound ribosomes." All ribosome pellets were stored at −70 C until analyzed.

Microsomes, pH 5 enzyme, and S_4. Modified procedures of Keller and Zamecnik (16) were employed. Guinea pig livers were minced in 2.5 vol of 0.35 M sucrose-5 mM $MgCl_2$-5 mM β-mercaptoethanol (BME)-25 mM KCl-35 mM Tris-hydrochloride (pH 7.6), and homogenized in a Potter-Elvehjem homogenizer as described above. The homogenates were centrifuged at 15,000 × *g* for 10 min at 4 C. The supernatant fluid, S_2, was carefully decanted so that the fat layer, characteristic of infected livers, remained on the walls of the tube. The S_2 was diluted with 3 vol of 0.9 M sucrose-5 mM $MgCl_2$-5 mM BME-25 mM KCl (medium D), mixed well, and centrifuged in a Spinco 30 rotor at 78,000 × *g* for 1.5 h. The supernatant fluid (S_3) was decanted from the microsome sediment, diluted with an equal volume of medium D, and the pH was adjusted to 5.2 by the dropwise addition of 1 N acetic acid, with stirring. The suspension was allowed to stand on ice for 30 min and then was gently stirred and centrifuged at 15,000 × *g* for 20 min at 4 C. The supernatant fluid, S_4, was decanted and saved. The pelleted pH 5 enzyme was resuspended in 0.25 M sucrose-25 mM KCl-5 mM $MgCl_2$-5 mM BME-10 mM acetate buffer, pH 5.2, by using 10 ml for every 40 ml of S_3. The enzyme fraction was recentrifuged and suspended in medium S (0.25 M sucrose-5 mM $MgCl_2$-25 mM KCl-2 mM dithiothreitol-35 mM Tris, pH 7.6), by using 1 ml for every 2 g of liver. The microsomal pellets were also suspended in medium S and gently homogenized with a Dounce homogenizer (loose-fitting pestle). The pH of the S_4 fraction was adjusted to 7.6 to 7.8 with dry $KHCO_3$.

Amino acid incorporation. Reactions were conducted by employing freshly prepared fractions, in 0.5-ml vol at 37 C, in a shaking water bath for 20 min. In addition, the reaction mixtures contained ^{14}C-L-leucine, 0.08 mM, sp act 25 μCi/μmol; Na_2-adenosine triphosphate, 2 mM; Na-guanosine triphosphate, 1 mM; Na_3-*P*-enolpyruvate, 7.8 mM; pyruvate kinase, 7.5 enzyme units; $MgCl_2$, 6 mM; KCl, 15 mM; BME, 2 mM; and Tris, 60 mM (pH 7.6). The amino acids were 0.1 mM (25). Reactions were initiated by the addition of pH 5 enzyme. Portions of the reaction mixtures were assayed after incubation by a filter disk method (27) employing liquid scintillation spectrometry.

Preparation of ^{3}H-RNA. Guinea pigs were inoculated intraperitoneally with 500 μCi of [5-^{3}*H*] orotate, sp act 1.67 mCi/μmol, and were sacrificed after 2 h. To 1-ml samples of liver homogenate (33% wt/vol) was added 9.0 ml of 0.1 M NaCl containing 0.5% (wt/vol) sodium dodecyl sulfate and buffer (50 mM Tris-hydrochloride, pH 7.6). Ten milliliters of phenol-cresol mixture (21) was added, and nucleic acids were extracted by vigorous shaking for 10 min at room temperature. After centrifugation, the aqueous phase was removed and reextracted with an equal volume of phenol-cresol for 5 min. The aqueous phase was removed and RNA was precipitated by adding 2.5 vol of absolute ethanol. The precipitate was collected and washed twice with cold 95% ethanol. The pellet was dissolved in 9.0 ml of Tris buffer (50 mM Tris-hydrochloride (pH 7.6)-5 mM $MgCl_2$). DNase (RNase free) was added to a final concentration of 20 μg/ml and incubated at room temperature for 30 min. The RNA was vigorously extracted for 5 min with an equal volume of phenol-cresol. The aqueous phase was removed, made to 1 M with respect to NaCl, and precipitated with 3 vol of cold absolute ethanol. After standing for 1 h at −20 C, the precipitate was collected and dissolved in 0.1 M NaCl-50 mM Tris buffer, pH 7.6. The RNA was then separated into 1 M NaCl-soluble and -insoluble fractions (A. Fried, personal communication, modification of Crestfield et al. [7]). The RNA dissolved in buffer was made to 1 M NaCl by the addition of 4 M NaCl and frozen at −70 C. After slow thawing, the flaky precipitate which remained insoluble in the 1 M NaCl was collected by centrifugation; the supernatant fluid was decanted and saved. The pellet was washed twice in ice-cold 1 M NaCl and dissolved in 0.2 to 0.5 ml of acetate buffer (0.1 M sodium acetate-0.1 M NaCl-1 mM Na_2-ethylenediaminetetraacetic acid, pH 5.1). The salt-soluble RNA was precipitated from the supernatant fraction by the addition of 2.5 vol of cold absolute ethanol, stored at −20 C overnight, collected by centrifugation, and then dissolved in 0.2 to 0.3 ml of acetate buffer. Both fractions were stored at −70 C until used. RNA preparations typically had A_{260}/A_{280} ratios of 1.9 to 2.0. The extraction and preparation of RNA species for marker use have been described (35).

Polyacrylamide gel electrophoresis of RNA. The procedures for gel electrophoresis have been described (35). Gels were cast in cellulose acetate-butyrate tubes to facilitate manipulations. After completion of electrophoresis, the gels were scanned and were then frozen and sliced into 1-mm sections with a battery of razor blades. Slices were placed into counting vials, and 0.5 ml of an aqueous solution of RNase (50 μg/ml) was added. The vials were capped and allowed to stand for 24 h at room temperature with intermittent shaking. Samples were assayed by liquid scintillation spectrometry. Quantitation of the 28*s*, 18*s*, and 4*s* RNA species in polyacrylamide gels was obtained by planimetry of the tracings. Standards were prepared by electrophoresis of known quantities of *Escherichia coli* tRNA.

Chemical analysis. Cortisol was analyzed fluorometrically (6) by employing cortisol sodium succinate as a standard. RNA in liver homogenates and subcellular fractions was analyzed by the methods of Munro and Fleck (29) by employing $E^{1\%}_{260}$ equals 312. DNA was extracted with 0.5 N $HClO_4$ at 90 C and analyzed colorimetrically (5), except that the color reagent consisted of 1.5 g of recrystallized diphenylamine dissolved in 100 ml of glacial acetic acid, 1.5 ml of concentrated sufuric acid, and 0.5 ml of 2.0% aqueous acetaldehyde. Protein was measured by the methods of Lowry et al. (23).

RESULTS

Guinea pigs infected with *C. burneti* suffered the characteristic changes of body weight, temperature, and liver size previously described (30, 34).

Polysome patterns. Protein and RNA synthesis increased in livers of animals during Q

fever (25, 34). An explanation for this phenomenon was sought in an analysis of polysome populations of infected and uninfected animal livers, because polysome sedimentation patterns may be indices of the relative state of translation activity in cells. Polysomes from infected and uninfected guinea pig livers were prepared from DOC-treated post-mitochondrial supernatant fluids. Both preparations had similar RNA-protein ratios (0.72) and velocity sedimentation characteristics. Both preparations had prominent monomer peaks and resolvable polymer classes (n, 2n, 3n . . .). The area of each class was obtained and expressed as a percentage of the total profile. Ribosome polymers 3n and greater represented, for uninfected, 90%, and, for infected, 92% of their respective areas. For polymers 9n and greater, the values were 53% for uninfected and 52% for infected. In another set of experiments, the comparable values obtained were 47 and 50%. We could find no obvious differences between the profiles of uninfected and infected liver polysomes.

Amino acid incorporation. Cell-free amino acid incorporation systems were prepared (16) from infected (Q) and uninfected (N) livers and contained microsomes, pH 5 enzymes, and S_4 fractions. When components of the systems were added together and tested for amino acid incorporation (Table 1, experiment 1 and 8), it was found that the Q (pH 5 enzyme-microsome-S_4) combination incorporated more ^{14}C-leucine (486 dpm/mg of reaction protein) than the comparable uninfected system (296 dpm/mg of reaction protein). To determine which component of the infected system was responsible for enhanced incorporation, a series of constituent substitutions were made in the N and Q systems (Table 1). The results (Table 1, experiment 3) show that the Q pH 5 enzyme, incubated with N microsomes + N S_4, had activity comparable to the total Q system (Table 1, experiment 8). The results indicate that the Q pH 5 enzyme fraction was responsible for incorporation stimulation. In other experiments, the Q pH 5 enzyme was consistently the locus of stimulation ($P < 0.01$, t analysis, six separate experiments). The phenomenon of Q pH 5 enzyme stimulation also occurred when ^{14}C-reconstituted protein hydrolysate was used instead of ^{14}C-leucine. Here the total infected system incorporated 840 dpm/mg of reaction protein, compared to the 361 dpm of the total uninfected system.

TABLE 1. *Amino acid incorporation by fractions from infected and uninfected livers*

Expt no.	Components[a]	Protein (dpm/mg)	RNA (dpm/mg)
	N Microsomes +		
1	N Enz + N S_4	296	2,468
2	N Enz + Q S_4	260	2,156
3	Q Enz + N S_4	474	3,900
4	Q Enz + Q S_4	565	4,640
	Q Microsomes +		
5	N Enz + N S_4	241	2,004
6	N Enz + Q S_4	245	2,027
7	Q Enz + N S_4	455	3,736
8	Q Enz + Q S_4	486	3,979

[a] The preparation of components and conditions of incubation are described in the text. Livers from three uninfected and three infected animals were each pooled and processed. The proteins of the components were N (uninfected): enzyme, 2.1 mg; microsomes, 2.6 mg; S_4, 0.15 mg; and Q (infected): enzyme, 2.2 mg; microsomes, 2.1 mg; S_4, 0.14 mg. Enz, pH 5 enzyme. Animals were sacrificed 84 h postinfection.

RNA synthesis. The incorporation of ^{3}H-orotate into liver RNA species was examined by employing gel electrophoresis. Figure 1 shows the electrophoresis patterns of NaCl-insoluble (higher-molecular-weight) RNA. The 45*s*, 28*s*, and 18*s* and 12–13*s* species are identified. The isotope labeling was greater in the 28*s* species, and to a lesser extent in the 18*s* RNA of infected livers (Fig. 1a), indicating a more rapid turnover or synthesis of ribosomal RNA (rRNA).

The salt-soluble RNA (lower-molecular-weight) electrophoresis patterns (Fig. 2) showed a greater labeling of the 4*s* RNA of infected livers (Fig. 2a), presumably the transfer RNA (tRNA) species. The data are quantitatively expressed in Table 2 and show that the infected-derived RNA species were indeed the most actively labeled.

We have consistently observed that at 84 h postinfection, total liver RNA increases (34). By employing animals carefully matched for age and weight at the onset of the experiment, we found that the RNA of infected animals increased from 54 ± 3 mg per liver to 75 ± 3 mg (standard errors of the means for eight animals per group). Because about 85% of the liver RNA is ribosomal (14), it was of interest to examine the temporal changes in rRNA during infection. Animals were infected and sacrificed 0, 12, 36, 60, and 84 h postinfection. After exsanguination, the livers were rapidly excised. The liver protein and RNA progressively increased during infection (Table 3). The DNA levels remained remarkably stable (Table 3), so that data could be expressed with DNA as the base line. Ribosomes were prepared from these livers and the rRNA was measured (Table 4). Total and bound rRNA increased during infection, and there was a significant increase in the rRNA by 36 h postinfection. The increase of rRNA was

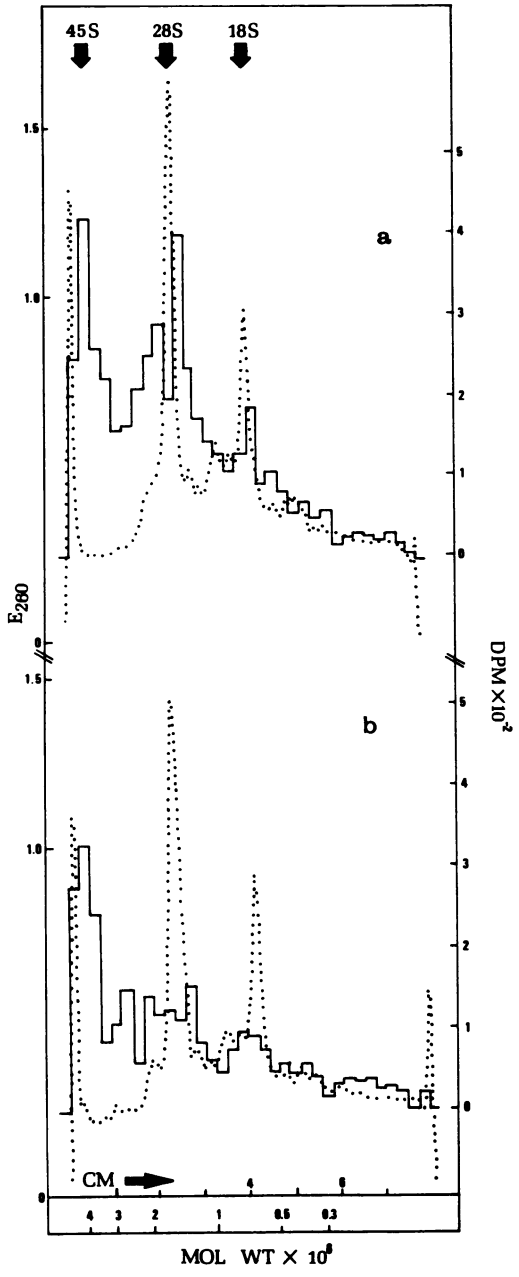

FIG. 1. *Electrophoresis of higher-molecular-weight (NaCl-insoluble) RNA extracted from infected and uninfected guinea pig livers. Animals were injected intraperitoneally with 500 μCi of ^{3}H-orotate (sp act 1,667 μCi/μmol) for 2 h prior to sacrifice. Animals were sacrificed 84 h postinfection. RNA was extracted and purified. About 30 μg of infected liver RNA were layered onto 2.6% gels and subjected to electrophoresis for 180 min at 26 to 28 C, by using 3 mA per gel. Gels were scanned at 260 nm and 2-mm slices were radioassayed. s Values and the molecular-weight scale were determined with E. coli rRNA reference standards. Absorbance, ...; radioactivity, ——. A, infected; B, uninfected. Migration is from left to right.*

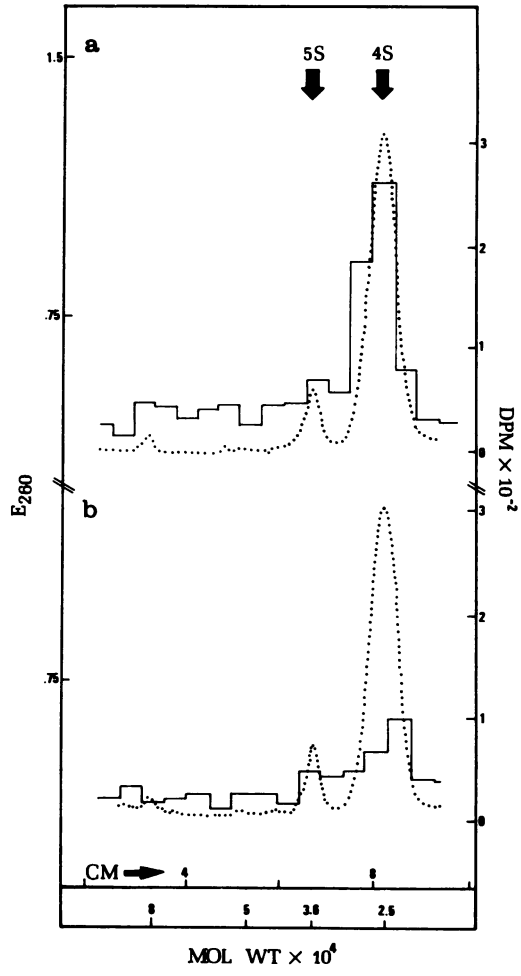

FIG. 2. *Electrophoresis of lower-molecular-weight (NaCl-soluble) RNA extracted and prepared from infected and uninfected guinea pig livers. About 50 μg of RNA was subjected to electrophoresis for 195 min on 8% gels, scanned, and radioassayed. Absorbance, ...; radioactivity, ——. A, infected; B, uninfected. Direction of migration is from left to right.*

parallel to the total liver RNA and protein increases.

The implication of glucocorticoid in controlling rRNA, tRNA, and messenger RNA (mRNA) synthesis (1, 18) and the observed increase of RNA during infection suggested a closer examination of temporal cortisol levels during Q fever. A steady increase of cortisol during infection was found (Table 5). The observed increase of ribosomes (Table 4) at 36 h was parallel to the significant increase of cortisol in the plasma ($P < 0.02$) and liver ($P <$

0.01). By 84 h postinfection, the cortisol levels had increased by greater than twofold in the plasma and sixfold in the liver (on the basis of either total liver weight, or per milligram of liver DNA).

DISCUSSION

Liver glycogen disappearance early in Q fever is correlated with diminished glycogen synthetase activity, despite increased activities of adenosine triphosphate-uridine 5'-diphosphate transferase and uridine 5'-diphosphate glucose pyrophosphorylase (34). Protein and RNA synthesis also increase in livers of infected guinea pigs and chicken embryos (25, 34). In many respects the damaged liver in Q fever resembles the regenerating liver after partial hepatectomy (4). In both instances glycogen disappearance and fatty infiltration are found, as well as enhanced synthesis of RNA and protein. Unlike the increased DNA synthesis in regenerating liver, the present report shows that the DNA levels in rickettsial-infected livers remain unchanged (Table 3). The situation which we describe in the infected guinea pig is also comparable to alloxan-diabetic rats and tumor-bearing rats, wherein the total animal lost protein, yet the liver protein increased (28).

TABLE 2. *Specific activity of liver RNA species during infection*[a]

Expt no.	Source[b]	RNA (dpm/mg)[c]		
		28*s*	18*s*	4*s*
1	N (1)[d]	34,800	22,400	19,100
	Q (1)	62,000	72,500	45,600
2	N (2)	35,500	27,000	19,700
	Q (1)	49,800	36,000	32,200
3	N (2)	49,800	37,900	20,200
	Q (2)	69,400	56,400	40,000

[a] Electrophoresis and analysis as described in Fig. 1 and 2. RNA values for each peak were obtained by planimetry. Animals were sacrificed 84 h postinfection.

[b] N, Normal liver; Q, infected liver.

[c] Average values.

[d] Number of animals.

The cell-free protein-synthesizing system prepared from liver (Table 1) contained microsomes and the pH 5 fraction, which includes tRNA and aminoacyl-tRNA synthetase species. The data indicate that it was the pH 5 fraction of infected liver which was chiefly responsible for augmented in vitro protein synthesis. This observation contrasts with a report that liver microsomes of mice infected with *Diplococcus pneumoniae* were responsible for enhanced protein synthesis (24). In findings similar to those reported here, there were no changes in the polysome profiles of mouse livers during infection with *D. pneumoniae*, yet there was a general increase in ^{14}C-orotate incorporation (15). It is of interest to note that *Salmonella typhimurium* endotoxin augmented orotate incorporation into RNA, and leucine into protein, of mouse liver (33). The present report demonstrates that during Q fever infection, although the liver polysome profile was unchanged, there was a quantitative increase in the ribosome number (Table 4). The 28*s*, 18*s*, and 4*s* fractions of infected liver also showed increased orotate incorporation (Fig. 1, 2; Table 3). Augmented protein synthesis (Tables 1 and 3) could be due to several factors. An increase in RNA polymerase, either in specific activity or quantity, would lead to an increase of rRNA, mRNA, or tRNA species, so that by individual or cooperative action enhanced protein synthesis resulted. The data (Table 2) indicate that this may be at least partially the case. As total RNA increased from 39.6 mg to 77.1 mg per liver during 84 h of infection, the protein per liver (Table 3) increased from 1.06 to 1.56 g ($P < 0.05$), confirming the data of Table 2. We were unable to find an expected increase in the higher polymers of ribosomes as a corollary of increased protein synthesis. However, there was a doubling of total ribosomes per cell, which would increase the potential of protein synthesis (Table 4). Welsh and Paretsky (unpublished data) recently found that RNA polymerase from nuclei

TABLE 3. *Temporal changes of RNA, DNA, and protein during infection*[a]

Time post-infection (h)	RNA (mg/liver)	DNA (mg/liver)	Protein (g/liver)	RNA (mg/mg of DNA)	Protein (mg/mg of DNA)
0	39.6 ± 5.9	19.2 ± 4.0	1.06 ± 0.20	2.06	55.1
12	46.5 ± 6.5	17.2 ± 3.5	1.21 ± 0.44	2.70	70.3
36	63.1 ± 5.3	20.4 ± 1.5	1.31 ± 0.29	3.10	63.4
60	67.0 ± 9.0	21.0 ± 6.8	1.47 ± 0.25	3.18	69.9
84	77.1 ± 6.6	21.1 ± 3.8	1.56 ± 0.17	3.65	74.0

[a] The data represent averages of three animals per set, ± standard deviation.

TABLE 4. *Changes in ribosomes during infection*[a]

Time post-infection (h)	Free[b]	Bound[b]	Total[b]
0	0.49 ± 0.10	0.60 ± 0.09	1.28 ± 0.11
12	0.36 ± 0.05	0.81 ± 0.05	1.32 ± 0.07
36	0.65 ± 0.07	1.04 ± 0.01	2.29 ± 0.08
60	0.58 ± 0.08	0.97 ± 0.00	1.64 ± 0.17
84	0.71 ± 0.16	1.31 ± 0.07	2.54 ± 0.11

[a] The values are averages ± standard error of the mean, for three animals per time period.
[b] Milligrams of rRNA per milligrams of DNA.

TABLE 5. *Temporal changes in liver and plasma cortisol levels during infection*[a]

Time post-infection (h)	Cortisol (μg) per			
	Plasma (ml)	Liver (g)	Liver (total)	Liver DNA (mg)
0	0.65 ± 0.6	0.57 ± 0.06	4.50 ± 0.63	0.22 ± 0.036
12	0.83 ± 0.08	0.92 ± 0.13	8.77 ± 1.93	0.44 ± 0.064
36	0.99 ± 0.03[b]	1.18 ± 0.11	12.0 ± 0.42	0.53 ± 0.027
60	0.94 ± 0.06	1.27 ± 0.13	15.8 ± 1.91	0.60 ± 0.106
84	1.54 ± 0.32	2.07 ± 0.43	30.7 ± 7.08	1.36 ± 0.281

[a] Each value represents the average of four animals, ± standard error of the mean.
[b] Average of two animals.

of infected liver has 40 to 60% greater activity than comparable preparations from uninfected liver. These data are consistent with the increased number of ribosomes during infection.

There is abundant literature on the regulation of RNA and protein synthesis in mammalian liver (10, 28, 32) and ample evidence for the key role of glucocorticoids in regulatory mechanisms (1, 8–12, 17, 19, 26, 37). Whether cortisol acts as a gene derepressant to stimulate RNA synthesis (8, 20) or enhances RNA polymerase activity with concomitant increase in transcription and translation (9, 12, 37), the results are increased hepatic RNA and protein synthesis. In the present report, the total liver cortisol (Table 5) doubled in the first 12 h postinfection and increased six to sevenfold during the 84 h postinfection. The same magnitude of increase is seen when the cortisol levels are examined on a per cell basis. Cortisol increase was accompanied by an increase in protein and RNA (Table 3). The RNA increase is reflected by a doubling of ribosomes, with no change in the polysome profile, and increased synthesis of rRNA and what is probably tRNA (Table 2, Fig. 2). The data may be interpreted to indicate that the events after infection are an increase of cortisol, and a stimulation of RNA synthesis and concomitant augmented protein synthesis. The data suggest that the increased protein synthesis may be the result of a quantitative increase in the protein-synthesizing machinery, including ribosomes, mRNA, and tRNA.

Increased activities of uridine 5′-diphosphate glucose and uridine 5′-diphosphate synthetases during Q fever, coincidental with decreased glycogen synthetase activity (34) can be interpreted in light of the increased cortisol levels during infection (Table 5), for cortisol has been reported to induce some enzymes and to repress others (18, 20). Furthermore, these results are consistent with the report (22) of increased activity of crude RNA polymerase preparations after glucocorticoid treatment.

The net result of the infectious process in Q fever seems to be that, although the total animal loses weight, in the liver, there is an increase in ribosomes and other RNA species, with a net increase of protein. In these and other aspects, the infected liver resembles the regenerating liver which results from partial hepatectomy and other forms of liver damage. Increased synthesis of RNA and protein plays a major role in rapid repair of this vital organ.

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