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Chemical and Biochemical Changes in Subcellular Fractions of Guinea Pig Liver During Infection with *Coxiella burneti*

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Livers of uninfected guinea pigs and of guinea pigs infected with *Coxiella burneti* were fractionated into smooth endoplasmic reticulum, rough endoplasmic reticulum (RER), pellet, and cell sap fractions. The ribonucleic acid (RNA) and protein of each fraction were determined, and the phosphorylase, glucose-6-phosphatase, and glucosyl transferase (glycogen synthetase) activities of each fraction were measured. Decreased RNA, protein, and enzyme activities were found in the RER and pellet fractions of infected livers, with the greatest differences in the RER. The evidence indicates a solubilization of the phosphorylase and synthetase, with the enzymes moving from the RER and glycogen-containing pellet fraction to the cell sap. The data suggest the RER as a target during Q fever.

When Q fever is experimentally produced in the guinea pig by infection with the rickettsial agent, *Coxiella burneti*, the infected liver characteristically becomes enlarged and fatty, liver glycogen disappears, and a biochemical lesion occurs apparently at the levels of glycogen synthetase and phosphorylase (26). Recent information on the role of the hepatic endoplasmic reticulum in the control of glycogen synthesis and breakdown (3, 5, 15, 30, 34) led us to reexamine and extend earlier efforts (36) to search for possible correlations between ultrastructural and biochemical changes in the guinea pig liver during Q fever. The enzymes chosen for study in the endoplasmic reticulum and subcellular fractions were phosphorylase, glucose-6-phosphatase, and glycogen synthetase, since up to this time only glycogen synthetase and phosphorylase, among the enzymes involved in glycogen synthesis, have been shown to be affected during Q fever (26; D. Paretsky and J. Stueckemann, Bacteriol. Proc., p. 116, 1966).

MATERIALS AND METHODS

Organism. *Coxiella burneti*, Nine Mile strain, phase I, was propagated in embryonated eggs. The yolk sacs of the first egg passage were harvested, and an inoculum for animal infection was prepared by diluting a 20% yolk sac suspension with an equal volume of 0.1% skim milk. The resultant suspension had an egg LD_{50} of $10^{-5.6}$ per ml.

Animals. Homozygous male guinea pigs, Hartley strain (obtained from Lightner Enterprises, Thompsontown, Pa.), weighing 400 to 470 g at the beginning of the experiment, were divided into groups of two animals each, designated as groups Q and N. Group Q animals were injected intraperitoneally with 1 ml of the *C. burneti* inoculum. The other group was inoculated with a sterile yolk sac suspension. Group N was permitted to eat ad lib. After 84 hr, group N weights increased 9% and Q decreased 24%, consistent with previous reports (26, 36). In one set of experiments, four animals per group were used, and a fasted control set of animals was added (group F). This group had a food intake restricted to the same quantity ingested by group Q, and the animals suffered an 8% weight loss. Animals were sacrificed by decapitation 84 hr postinoculation and exsanguinated.

Fractionation. Livers were rapidly removed from the sacrificed animals, and rinsed twice in 0.15 M KCl-0.01 M tris(hydroxymethyl)aminomethane (Tris), *p*H 7.2. A 33% liver homogenate was prepared in 0.88 M SKM, employing a Teflon-glass Potter homogenizer. Solution SKM consisted of 0.06 M KCl, 0.005 M $MgCl_2$, 0.01 M Tris buffer (*p*H 7.5), 0.001 M ethylenediaminetetraacetate (EDTA), 0.1 M NaF, and sucrose to the indicated molarity.

The homogenate was centrifuged at 25,000 × *g* for 10 min at 4 C, and the postnuclear and mitochondrial supernatant fraction was decanted and saved. The pellet was extracted with 1 volume of 0.88 M SKM and again centrifuged; the supernatant fraction was combined with the previous one (fraction S_2). The S_2, in 0.88 M SKM, was diluted with an equal volume of 1.76 M SKM, so that the resulting suspension was 1.32 M with respect to sucrose.

The S_2 was fractionated as shown in Fig. 1, employing a modified discontinuous gradient technique (22, 27) with gradients of 0.15, 1.23, and 1.32 M SKM.

Fractions were obtained and designated as SER (smooth endoplasmic reticulum), S_3S (SER supernatant fraction), S_3R (SER subnatant fraction) and a pellet. The pellet was resuspended in 0.25 M SKM, to which was added 0.01 M $Na_4P_2O_7$, 0.01 M $NaKHPO_4$, and 0.08 M sodium citrate, and layered over 2.1 M SKM. After centrifuging, the pellet was separated into the RER (rough endoplasmic reticulum), S_4R (RER supernatant fraction), S_4P (RER subnatant fraction), and pellet fractions (glycogen + free ribosomes + ribosomes removed from RER). The SER, RER, and pellet fractions were each washed once in 0.25 M SKM-pyrophosphate-citrate and centrifuged at 105,000 × *g* at 0 C for 1 hr. The sediments, containing the appropriate microsomal fraction, were finally suspended in 0.25 M SKM.

Protein was measured by the methods of Lowry et al. (14).

Glycogen was measured by reacting 0.5 ml of sample, containing 50 to 500 μg of glycogen, with 0.5 ml of α-amylase solution [4 μg/ml of hog pancreas amylase, type 1A (Sigma Chemical Co., St. Louis, Mo.)] and 1.0 ml of 0.02 M phosphate buffer (*p*H 6.9) in 0.006 M NaCl. The mixture was incubated for 10 min at 30 C. Reducing sugars formed were measured colorimetrically (23). Recrystallized glycogen was used as a standard.

Glycogen synthetase (uridine diphosphate glucose: α-1,4-glucan α-4-glucosyltransferase, EC 2.4.1.11) activity was measured in the presence and absence of exogenous 0.01 M glucose-6-phosphate (G-6-P), for the inactive (D) and active (I) forms, respectively (9, 19). Assays were performed in triplicate (35), as described in Table 3. The reaction was stopped by the addition of 0.3 ml of 60% KOH, and 5 mg of glycogen (rabbit liver, type III, recrystallized, Sigma Chemical Co.) was added as carrier. After KOH digestion, glycogen was precipitated by the addition of 3 volumes of absolute ethanol at 0 C. The glycogen was redissolved and reprecipitated 10 times, finally resuspended in 2.0 ml of water, and 0.4 ml was removed and counted in a Packard scintillation spectrometer.

Phosphorylase (α-1,4-glucan:orthophosphate glucosyltransferase, EC 2.4.1.1) activity was measured in the direction of glycogen synthesis (33).

Inorganic phosphate was measured according to Fiske and SubbaRow (6).

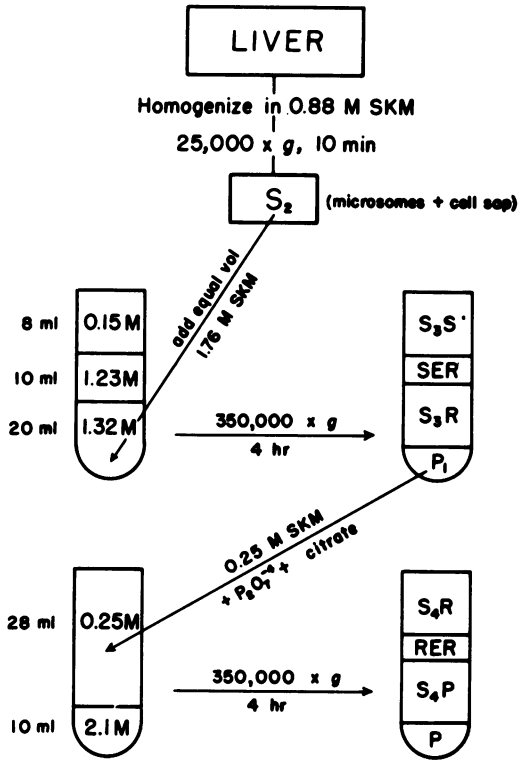
Nucleic acids were estimated spectrophotometrically (21).

Glucose-6-phosphatase (D-glucose-6-phosphate phosphohydrolase, EC 3.1.3.9) activity was determined by measuring inorganic phosphate liberated enzymatically from G-6-P (8).

RESULTS

The SER, RER, pellet, and other liver subcellular fractions were prepared, and the protein and ribonucleic acid (RNA) content of the fractions are presented in Table 1. The RNA-protein ratios show an approximate 10-fold difference between the normal SER and RER, and a value of 0.25 for the pellet, which is consistent with other reports (4, 7). A different picture is seen for the RNA and protein of comparable fractions obtained from the infected liver. There was a diminution of protein and RNA in the RER, whereas the SER and pellet fractions suffered little change.

The distribution of phosphorylase activity was examined; the data in Table 2 present the specific activity and total activity of phosphorylase in each fraction. Virtually all of the phosphorylase was accounted for. Although the phosphorylase activities were similar in the SER of the uninfected and normal animals, there were striking changes in the phosphorylase content of the RER and pellet fractions. In the infected animal, phosphorylase disappeared from the denser RER and pellet fractions, but more phosphorylase appeared in the S_3R, indicating a possible solubilization of phosphorylase. It should be noted



Fractionation of Liver Microsomes

FIG. 1. *Fractionation of liver microsomes. Reagents as described in text. SER, smooth endoplasmic reticulum; P_1, first pellet, containing glycogen, ribosomes, and rough endoplasmic reticulum (RER); P, pellet, containing free and bound ribosomes and glycogen; S_3S, S_4R, and S_4P, supernatant fractions of SER, RER, and P, respectively; S_3R, cell sap.*

TABLE 1. *Composition of subcellular fractions of liver*

Fraction	Milligrams of RNA per gram of liver		Milligrams of protein per gram of liver		Ratio of RNA to protein	
	N[a]	Q[b]	N	Q	N	Q
S_2	4.80	4.83	87.7	84.8	0.055	0.057
S_3S	0.064	0.108	0.36	0.57	0.181	0.189
SER	0.056	0.085	4.66	4.03	0.012	0.021
S_3R	2.95	2.96	69.9	67.7	0.044	0.044
S_4R	0.27	0.23	2.3	1.8	0.118	0.127
RER	0.39	0.026	2.6	0.81	0.151	0.032
S_4P	0.40	0.77	0.93	0.92	0.433	0.839
P	0.17	0.11	0.69	0.61	0.248	0.181
Per cent recovery	89.6	88.4	92.9	90.1		

[a] Uninfected animals.
[b] Infected animals.

TABLE 2. *Distribution of phosphorylase*[a]

Fraction	Specific activity (micromoles of P_i per milligram of protein)		Total activity (micromoles of P_i per liver)		Per cent of S_2	
	N	Q	N[b]	Q[c]	N	Q
S_2	2.68	2.35	4,435	3,820		
S_3S	2.08	0	14	0	0.31	0
SER	0.24	0.27	21	21	0.47	0.54
S_3R	2.50	2.84	3,185	3,660	71.8	95.5
S_4R	1.13	1.74	50	60	1.1	1.6
RER	6.39	0.37	312	6	7.0	0.14
S_4P	12.9	0.1	225	2	5.1	0.05
P	35.0	0	455	0	10.3	0

[a] Reaction mixture (in micromoles): Tris maleate buffer (*p*H 6.5), 50; G-1-P, K_2, 36; 3′,5′-adenosine monophosphate, 1.04; NaF, 110; pyridoxal 5′-phosphate, 40 μg; glycogen, 3.6 mg; enzyme, 0.2 ml; final volume, 1.1 ml. The mixture was incubated at 37 C for 20 min. The reaction was halted by the addition 1 ml of 10% trichloroacetic acid, and inorganic phosphate (P_i) was measured in the protein-free supernatant fraction.
[b] Recovery, 96%.
[c] Recovery, 98%.

that the pellet fraction of uninfected animals was rich in glycogen, but the infected liver had no chemically detectable glycogen.

Glycogen synthetase distribution was studied in normal, partially fasted, and infected animals (Table 3). Two types of glycogen synthetase activities were examined, in the absence and the presence of 0.01 M (G-6-P). The former measured the active, or independent (I), form, and the latter the total of the I and inactive, or dependent (D), form (9, 19). Enzyme preparations from infected liver subfractions had no detectable endogenous G-6-P, and no G-6-P was found in the pellet and SER fractions of uninfected liver. The uninfected liver RER contained only trace amounts of G-6-P.

In both normal and fasted animals, the I form of the synthetase was distributed chiefly between the RER and the pellet (Table 3A), whereas in the infected animal livers the I form of the enzyme was fairly uniformly distributed in the SER, RER, and pellet fractions. Although there was a diminution of the active (I) form in the fractions of the fasted animals, it was still present in greater abundance than in comparable fractions of the infected animals. The microsomal distribution pattern of the total glycogen synthetase (D + I forms) was similar in the normal, fasted, and infected animal livers, with greatest activities in the pellet fractions. There was no stimulation by exogenous G-6-P of synthetase activity in the SER, indicating the absence of

TABLE 3. *Glycogen synthetase distribution*[a]

Determination	Fraction	D + I form (plus exogenous G-6-P)			I form (minus exogenous G-6-P)			D form [(D +I) −I]		
		N	F[b]	Q	N	F	Q	N	F	Q
A. Activity (counts per min per liver, × 10^3)	S_2	10,598	6,964	10,183	2,859	1,869	1,826	7,739	5,095	8,375
	SER	49	58	49	51	60	51	0	0	0
	RER	503	361	50	451	209	38	52	152	12
	P	1,740	338	188	763	140	44	977	198	144
B. Specific activity (counts per min per mg of protein, × 10^3)	S_2	7.16	6.42	6.75	1.93	1.73	1.21	5.23	4.69	5.54
	SER	0.56	1.28	0.89	0.58	1.32	0.93	0	0	0
	RER	26.2	34.7	3.65	23.5	20.1	2.75	2.7	14.6	0.90
	P	187.1	49.0	17.2	82.0	20.4	4.02	105.1	28.6	13.2

[a] Reaction mixture contained in micromoles: Tris maleate buffer (*p*H 7.8), 20; uridine diphosphate glucose-^{14}C, 10^5 counts/min, 1.35; G-6-P·Na_2 (where added), 3; glycogen, 2 mg; enzyme, 0.2 ml; final volume, 0.3 ml. The mixture was incubated at 30 C for 30 min. Abbreviations: D, inactive; I, active; G-6-P, glucose-6-phosphate; S, supernatant fraction; SER, smooth endoplasmic reticulum; RER, rough endoplasmic reticulum; P, pellet.

[b] Fasted animals.

the D, or inactive form. The glycogen synthetase in the RER of all animals was predominantly in the I form [Table 3A; ([D + I form] − [I form] = D form)], but the D form increased in all pellet fractions. The ratios of the I:D forms in the pellets were, for the normal, 0.78; fasted, 0.71; and infected, 0.31. If the total synthetase activities of the normal and infected S_2 fractions are compared (Table 3A), only a small decrease is noted in the infected S_2 fraction. The infected RER and pellet fractions, however, showed about 90% inhibition of total synthetase activity. The same magnitude of inhibition was found in the I forms of the synthetase in the RER and pellet fractions of infected animals.

Table 3B presents the specific activities of glycogen synthetase in the several fractions. The data show that the S_2 fractions from normal, fasted, and infected animals had about the same specific activities, although the total activity units (Table 3A) showed a decrease, especially in the fasted animals. It is further seen that it was the pellet fractions which had the highest specific activities, indicative of association of the enzyme with the high-molecular-weight glycogen. The specific activity of the I form of the infected pellet fraction was higher than the RER, which in turn was greater than the SER, in contrast to the activity values shown in Table 3A. The proportionate distribution of specific activities is indicative of a solubilization of the glycogen synthetase during fasting and infection.

The key position of G-6-P in glycogenesis led to a study of distribution of glucose-6-phosphatase in the microsomal and subcellular fractions of normal and infected animals (Table 4).

TABLE 4. *Distribution of glucose-6-phosphatase*[a]

Fraction	Specific activity (μmoles P_i/mg protein)		Total activity (μmoles P_i/total liver)		Per cent of S_2	
	N	Q	N	Q	N	A
S_2	0.87	0.83	1,435	1,353		
S_3S	0	0	0	0		
SER	3.82	4.53	335	349	23.3	25.8
S_3R	0.51	0.49	651	635	45.4	46.9
S_4R	0	0.88	0	31		
RER	6.32	7.47	308	115	21.5	8.5
S_4P	3.79	1.29	66	23		
P	3.26	2.56	42	30	2.9	2.2
		% recovery	98	87		

[a] Reaction mixture (in μmoles): citrate buffer (*p*H 6.5), 10; G-6-P, K_2, 8; enzyme, 0.1 ml; final volume, 0.2 ml. Incubated 37 C, 15 min. The reaction was halted by the addition of 2 ml of 10% trichloroacetic acid, and P_i was measured in the protein-free supernatant.

The SER of both normal and infected livers had comparable activities, as did the pellet fractions, whereas there was a decreased total activity in the RER of infected animals. The specific activities of the SER, RER, and pellet fractions of uninfected and infected animals within each fraction did not show marked change. The lesser total activity in the RER of the infected animals was correlated with a decreased RER and protein content. There was no detectable G-6-P in any of the microsomal fractions, except that the normal RER had trace amounts.

DISCUSSION

The disappearance of liver glycogen observed during Q fever occurs as well in a broad range of other events, including bacterial (2, 12, 13, 26, 31, 38) and protozoan infections (16, 18, 37), starvation (29, 34), chemical poisoning, and tumor transplants (24, 28, 34). Although glycogen depletion may be an end result of diverse pathogenic conditions, little is known of the role of the endoplasmic reticulum in glycogen metabolism during infection (36). The liver endoplasmic reticulum has been implicated in glycogen synthesis (3, 34) and in the synthesis and transport of hepatic enzymes (5, 10, 11, 30). Changes in the endoplasmic reticulum during chemical poisoning have been described (25, 32), but comparable information is not available for animals during infection. The present report extends previous observations (26) on the fate of phosphorylase and glycogen synthetase during Q fever to the hepatic endoplasmic reticulum and subcellular fractions.

Phosphorylase was found in greatest abundance in the glycogen-rich pellet fraction of normal microsomes, but was absent from the pellet during Q fever, coincident with the disappearance of liver glycogen. Glycogen synthetase had a similar distribution; greatest abundance of the enzyme was found in the normal pellet fraction, whereas only 10% of normal activity was present in the infected pellet. Tata (34) had shown that rat liver phosphorylase was normally bound to particulate glycogen, but that when glycogen was depleted the phosphorylase was redistributed in the soluble fraction of the subcellular constituents. Luck (15) reported a similar association of glycogen synthetase with glycogen. When glycogen diminished during fasting, there was an increase in the relative amount of glycogen synthetase in the "soluble" postmicrosomal supernatant fraction. Barber et al. (1) also demonstrated the association of liver phosphorylase and glycogen synthetase with glycogen. Ethionine-poisoned mice displayed a liver glycogen depletion and concomitant decreased glycogen synthetase and increased solubilized phosphorylase (28). The patterns of diminished glycogen correlated with diminished phosphorylase and glycogen synthetase were qualitatively similar to the events during Q fever described in the present report. Phosphorylase in animals infected with *C. burneti* moved from the denser pellet and RER fractions to the "soluble" S_3R cell sap fraction (Table 2). Infected animals had 87% of the phosphorylase activity of normal S_2 fractions, but the infected RER and pellet fractions had virtually no activity. It was therefore in the microsomal fractions that the large decreases of phosphorylase occurred. Glycogen synthetase specific activity was greater in the infected SER than in the normal SER, indicating a degree of solubilization. Some solubilization was also observed in fasted animals, but not to the same degree as during infection.

The distribution of glycogen synthetase during Q fever displayed additional interesting features. The active (I) forms and the total enzyme (I + D) were studied. The I form of glycogen synthetase in the infected S_2 fraction had 64% of the I form in normal S_2, but the *total* (active + inactive forms) synthetase activities were about the same. Only the I form appeared in the SER of normal, fasted, and infected animals. The RER of normal and fasted animals showed some D as well as I form, but there apparently was only the I form of the enzyme in the RER of infected animals. The most notable difference occurred in the pellet fractions, in which a large proportion of the total synthetase was in the inactive (D) form (55% of the normal, 60% of the fasted, and 80% of the infected animals), again showing the loss of I form in infection. Mersmann and Segal (20) showed that in the absence of glycogen, glycogen synthetase was solubilized, with the enzyme in the active form only when it was bound to glycogen. The present report (Table 3) indicates the operation of a similar I to D transition, and it was the I form of the enzyme which showed the major decrease of activity during infection.

Another alteration in the subcellular composition during Q fever was in the RER fraction. Although the S_2 fraction of normal and infected animals had approximately the same RNA, protein, and RNA-protein values, and although the infected SER showed some increase in RNA and decrease in protein, it was the RER which suffered the greatest change during infection. The infected RER showed greater than a 10-fold decrease in RNA and a three-fold decrease of protein. The RNA-protein ratio of the RER was 10 times greater than the SER in the normal animal (Table 1), in good agreement with the

literature (4). In contrast, the infected animal showed less than a twofold greater RNA-protein value between the RER and the SER. When the RER and S_4P values of the RNA are added (Table 1), both normal and infected animals had the same value (0.79 mg of RNA/g of liver). The same addition of the RER and S_4P fractions for protein fails to show an equal additive effect. The data indicate that there is a real decrease in the RNA and protein of the RER during infection, and that, although the RNA may appear in the S_4P fraction, the protein of the infected RER substantially decreased.

The overall picture indicates that the major loci of decreased phosphorylase and glycogen synthetase activities are in the RER and pellet fractions of infected microsomes. The active (I) form is that form of synthetase which had greatest decrease of activity. Glucose-6-phosphatase was chiefly distributed in the SER and RER, and, like phosphorylase, in the cell sap fraction. The RER is the subcellular fraction which seems to be most affected during Q fever. The nature of the RNA of the RER which is lost during infection is under investigation.

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