JOURNAL OF BACTERIOLOGY
Vol. 88, No. 1, p. 137-142 July, 1964

SOME BIOCHEMICAL CHANGES IN THE GUINEA PIG DURING INFECTION WITH *COXIELLA BURNETII*

D. PARETSKY, C. M. DOWNS, AND C. W. SALMON

Department of Microbiology, The University of Kansas, Lawrence, Kansas

Received for publication 28 February 1964

ABSTRACT

PARETSKY, D. (University of Kansas, Lawrence), C. M. DOWNS, AND C. W. SALMON. Some biochemical changes in the guinea pig during infection with *Coxiella burnetii*. J. Bacteriol. **88:** 137-142. 1964.—Guinea pigs infected with *Coxiella burnetii*, the rickettsial agent of Q fever, were studied for 11 days postinfection. Maximal changes in liver lipids, liver phosphorylase, and uridine diphosphate glucose (UDPG)-glycogen glucosyltransferase activities occurred 3 to 4 days postinfection. In this period, total liver lipids increased from 1.26 to 5.46 mg/mg of N, with the largest increment in the glyceride fraction. Liver glycogen virtually disappeared by the second day, with no chemically detectable restoration until the eleventh day. A pattern of altered phosphorylase and UDPG-glycogen transglucosylase activities was observed, with maximal phosphorylase and minimal glucosyltransferase activities at the third and fourth days. Histochemical observations confirmed chemical analyses for lipids and glycogen.

The clinical picture, histopathology, and epidemiology of Q fever in many animals, and the cultivation of the rickettsial causative agent, *Coxiella burnetii*, in a wide variety of hosts, has been well described and reviewed (Burnet and Freeman, 1937; Cox and Bell, 1939; Lillie, 1942; Perrin and Bengston, 1942; Huebner, Jellison, and Beck, 1949; Weiss and Pietryk, 1956; Kordova and Rehacek, 1959; Roberts and Downs, 1959; Zdrodovskii and Golinevich, 1960). Although the biochemistry of *C. burnetii* has also received critical attention (Paretsky et al., 1958; Myers and Paretsky, 1961; Paretsky, Consigli, and Downs, 1962; Consigli and Paretsky, 1962; Mallavia and Paretsky, 1963), there is as yet little information on the biochemical changes in the host as a consequence of the rickettsial infection. Myers, Downs, and Paretsky (1963) described changes in the vitamin B series of infected chick embryos, and Mattheis, Silverman, and Paretsky (1963) reported patterns of the folate group in developing chick embryos infected with *C. burnetii*. The present report extends the observations on altered host biochemistry during the course of Q fever, and describes some biochemical changes in guinea pigs infected with *C. burnetii*.

MATERIALS AND METHODS

Organism. C. burnetii Nine Mile strain, phase I, second egg passage, was propagated in chick embryos; the infected yolk sacs were aseptically harvested and stored at −40 C. Prior to use, a 20% yolk sac homogenate was prepared which had an egg LD_{50} of $10^{-5.6}$. The inoculum for guinea pigs was prepared by diluting the homogenate with an equal volume of sterile 0.1% skim milk.

Guinea pigs. Male guinea pigs, Hartley strain, with a high degree of homozygosity, were obtained 3 weeks before inoculation from the Tumblebrook Farm, Brant Lake, N.Y. The animals, weighing 400 to 500 g each, were fed Rockland Guinea pig diet, "C" fortified (A. E. Staley Manufacturing Co., Decatur, Ill.) Animals were individually numbered, and daily weight and temperature records were kept for each animal. At the time of infection, the animals weighed 500 to 600 g.

Infection and sacrifice. Each of 20 animals was inoculated intraperitoneally with 2.0 ml of the inoculum, and the animals were divided into five equal groups. Control guinea pigs (12) were inoculated with uninfected yolk sacs which had been processed in a manner identical to the infected sacs. These control animals were divided into three equal groups. At intervals of 2, 3, 4, 7, and 11 days postinfection, a group of animals was sacrificed by means of CO_2 anesthesia. The animals were rapidly exsanguinated, and the livers and spleens were quickly excised. Impression smears made of the excised organs were stained for rickettsiae. Liver sections were cut, prepared by the paraffin method, stained for glycogen with Best's carmine and for morphology and lipids

with hematoxylin and eosin; the organs were then rapidly frozen and stored at −15 C.

Glycogen. Liver glycogen was analyzed with anthrone (Kahan, 1953) by use of 5% perchloric acid extraction instead of trichloroacetic acid. Reducing carbohydrate in the extract was measured colorimetrically (Nelson, 1944).

Glycogen synthetase. Uridine diphosphate glucose (UDPG)-glycogen glucosyltransferase (glycogen synthetase) activity was measured by the method of Algranati and Cabib (1962) with the use of 25% liver homogenates. Liver phosphorylase was determined in 15% liver homogenates (Singh, Venkitasubramanian, and Viswanathan, 1961).

Lipids. Total liver lipids were extracted with chloroform-methanol (2:1). One sample of the extract was evaporated under N_2, dried, and weighed; a second sample was titrated with iodine-bromine for absorption number (Association of Official Agricultural Chemists, 1945). Neutral lipids, phospholipids, and sterols were analyzed by the methods of Azarnoff (1962, *personal communication*).

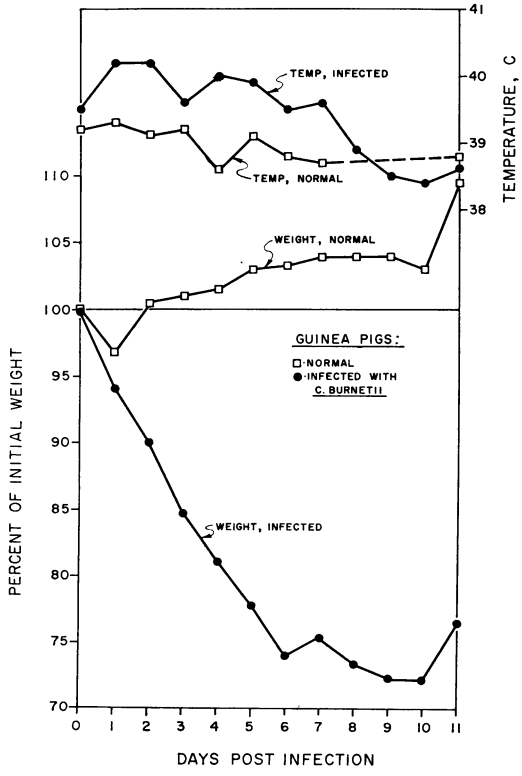

FIG. 1. *Temperature and weight changes in guinea pigs infected with Coxiella burnetii.*

TABLE 1. *Guinea pig liver and spleen changes during infection with Coxiella burnetii*

Days post-infection	Organ	Wet wt	Increase	Amt of N (mg/g of wet wt)	Degree of infection*
		g	%		
0	Liver	22.4	—	29.1	—
	Spleen	0.96	—		—
2	Liver	22.2	−0.9	28.7	±
	Spleen	1.63	69.8		±
3	Liver	25.8	15.2	26.5	+
	Spleen	2.75	186.6		+++
4	Liver	31.1	38.8	24.9	++++
	Spleen	2.32	141.7		++++
7	Liver	20.9	−6.7	27.0	++++
	Spleen	1.92	100.0		++++
11	Liver	22.7	1.3	30.4	—
	Spleen	2.0	108.4		—

* Macchiavello stains of impression smears: ± = one rickettsia per ten fields; + = one rickettsia per one field; +++ = ten rickettsiae per one field; ++++ = too numerous to count.

RESULTS

The daily temperature and weight record (Fig. 1) shows an initial weight decrease of the uninfected animals with subsequent small, steady increases. Although Derrick (1937) did not report weight fluctuation in guinea pigs during Q fever, the infected animals in the present case progressively lost weight until the ninth day postinfection. Uninfected animals maintained a fairly steady temperature range of 38 to 39 C, and infected guinea pigs displayed elevated temperatures for 7 days postinfection. The febrile response shown in Fig. 1 is generally similar to the classic description (Derrick, 1937).

Gross changes in liver and spleen during the course of *C. burnetii* infection are presented in Table 1. The liver weight increased to a maximum at the fourth day, with splenomegaly maximal at the third day postinfection. Liver protein (as N) varied inversely with total liver weight. This observation may be explained at least in part by the increased liver lipids, which also were maximal at the fourth day (Table 2, Fig. 2). Histo-

TABLE 2. *Liver lipid fractions of guinea pigs infected with Coxiella burnetii*

Days postinfection	Cholesterol (μg/mg of N)	Glyceride, as glycerol (μg/mg of N)	Phospholipid, as P_i (μg/mg of N)	Total lipid (mg/mg of N)	Unsaturated lipid (μeq of I/mg of N)	Unsaturated lipid / Total lipid
0	50.8	7.2	39.9	1.26	4.30	3.41
2	65.2	106.5	41.8	2.27	0.86	0.38
3	54.3	169.4	36.0	2.86	1.01	0.35
4	64.1	411.4	34.3	5.46	1.42	0.26
7	80.9	329.7	35.9	4.52	1.34	0.30
11	68.0	120.9	30.0	2.43	1.03	0.42

chemical examinations of normal and infected livers at 2, 3, 4, 7, and 11 days postinfection revealed a sharp diminution of glycogen in infected livers at the third day and complete disappearance at the seventh day; glycogen restoration was evident by the eleventh day. Infected livers showed pronounced fatty infiltration at the seventh day, with relief evident at the eleventh day. The histochemical observations are supported by chemical analyses (Fig. 2). The glycogen content of infected livers decreased abruptly from 1.15 mg/mg of N at 0 hr to 0.025 mg/mg of N 2 days postinfection, and was chemically undetectable after 7 days. At 11 days, a restoration of glycogen was apparent with a rise to 0.106 mg/mg of N. Liver reducing sugars followed the same pattern, with a minimal level at 7 days and recovery evident by the eleventh day. An inverse relationship was noted between total liver lipids and glycogen, and total lipids and unsaturated lipids. Further changes in infected liver lipids are presented in Table 2. There was some increase in cholesterol and a large increase of glycerides during infection, with a pattern of recovery by the eleventh day. The phospholipids decreased, while the ratio of unsaturated lipids (as μeq of iodine) to total lipids decreased tenfold 2 days postinfection. The most striking change was the 57-fold increase in glyceride content.

Hepatic glycogen depletion in infected guinea pigs suggested study of some enzymes involved in glycogen synthesis. Glycogen synthetase and phosphorylase activities were measured in infected and uninfected guinea pigs. The data in Table 3 show progressive decrease of UDPG-glycogen glucosyltransferase activity in infected guinea pig livers as the infection progresses, with subsequent recovery apparent by the eleventh day postinfection. At the height of infection, the

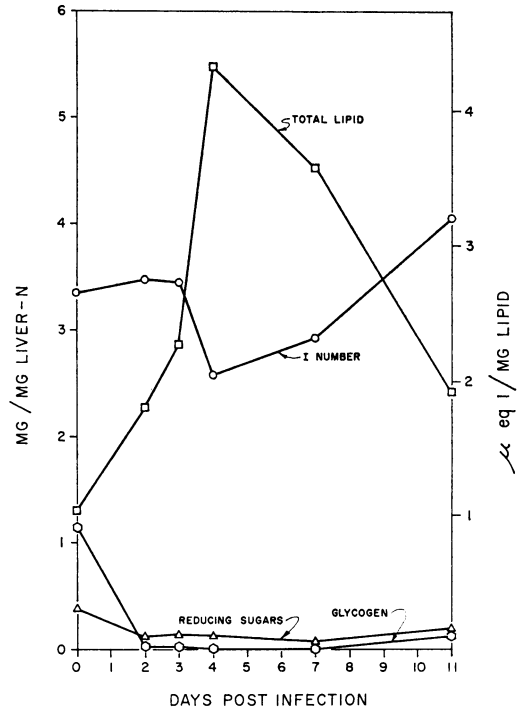

FIG. 2. *Liver carbohydrate and lipid changes in guinea pigs during infection with Coxiella burnetii.*

glycogen synthetase has only 26% of the activity of the uninfected liver enzyme, increasing to about 70% of normal activity at the eleventh day. Table 4 shows the greater phosphorylase activity of infected livers, again with maximal difference at the height of the infection, and approaching uninfected liver phosphorylase activity by the eleventh day.

DISCUSSION

Anorexia, muscular weakness, and splenomegaly are among the clinical symptoms de-

TABLE 3. *Glycogen synthetase in uninfected and infected guinea pig liver**

Days post-infection	UDPG transglucosylated			
	Amt (mμmoles) per g of wet wt		Amt (mμmoles) per mg of N	
	Uninfected	Infected	Uninfected	Infected
0	252	—	9.0	—
2	—	77.6	—	2.70
3	250	60.4	8.63	2.28
4	—	69.6	—	2.80
7	—	49.5	—	1.84
11	247	17.5	8.02	5.77

* UDPG, 5 mM; tris(hydroxy)aminomethane-maleate buffer (pH 7.5), 0.08 M; shellfish glycogen, 6 mg; phosphoenolpyruvate, 5 mM; phosphoenol-pyruvic kinase, 1.1 units; 0.15 ml of 25% liver homogenate (in 0.154 M KCl, 0.05 M buffer); final volume, 0.25 ml. Incubated for 30 min at 30 C. Pyruvate measured as 2,4-dinitrophenylhydrazone (Algranati and Cabib, 1962).

TABLE 4. *Phosphorylase of uninfected and infected guinea pig liver**

Days post-infection	P_i liberated			
	Amt (μmoles) per g of wet wt		Amt (μmoles) per mg of N	
	Uninfected	Infected	Uninfected	Infected
0	3.79	—	0.136	—
2	—	6.51	—	0.227
3	3.07	7.23	0.107	0.273
4	—	3.97	—	0.240
7	—	2.71	—	0.101
11	3.11	4.52	0.100	0.149

* Glucose-1-phosphate, 25 mM; shellfish glycogen, 2 mg/ml; citrate buffer (pH 5.8), 30 mM; NaF, 54 mM; 15% liver homogenate (in 0.1 M NaF), 0.4 ml; final volume, 2.0 ml. Incubated for 30 min at 30 C.

scribed for several species during Q fever (Lillie, Perrin, and Armstrong, 1941; Lennette, 1959; Zdrodovskii and Golinevich, 1960), but the altered host biochemical responses which give rise to the gross clinical changes are not well understood. In the present report, we describe increased weight of livers of guinea pigs infected with *C. burnetii*, correlated with an increment in total liver lipid. The observed fatty infiltration of the liver is a condition which occurs in a wide diversity of infections and pathological conditions. Selye (1950) described fatty infiltration of the liver as a response to X rays, cold, starvation, and bacterial toxins. Dible (1950) and Boyd (1961) reported fatty infiltration in livers as a consequence of diphtheria toxemia and other poisons. During Q fever in the guinea pig, there is actually a decrease in the proportion of unsaturated to total lipid (Table 2), consistent with Dible's (1950) description of decreased iodine numbers in fatty livers as the fat content increased. The increased liver lipid in guinea pigs infected with *C. burnetii* may be analogous with the demonstration by Gaush and Youngner (1962) of large total lipid increment, especially in the glyceride and sterol fractions, in chick chorioallantoic membranes subsequent to infection with vaccinia virus.

Associated with lipid increase during Q fever is a rapid disappearance of hepatic glycogen. A diminution of muscle and liver glycogen found in mice infected with *Salmonella typhimurium* was attributed to bacterial toxins (Berry and Smythe, 1960). Singh, Venkitasubramanian, and Viswanathan (1963) and Singh et al. (1963) reported a depletion of hepatic glycogen in tuberculous guinea pigs, and attributed this to suppressed glycogenesis resulting from decreased activities of some enzymes of the glycogen cycle. The present paper shows that during the course of Q fever. there are changes in activities of two enzymes involved in glycogenesis: UDPG-glycogen glucosyltransferase (glycogen synthetase) and phosphorylase. If decreased hepatic glycogen is at least partially due to suppressed glycogen synthetase activity, then the diminution of synthetase activity observed during the height of the infection, and restoration of enzyme activity during recovery (Table 3), meets the expectation. Similarly, enhanced phosphorylase activity leading to glycogenolysis could be expected as the infection progressed, and this was actually found. A parallel observation of glycogenolysis accompanied by increased active phosphorylase and decreased glycogen synthetase activities was reported by Belcopitow (1961) in epinephrine-stimulated glycogenolysis. While the present report indicates that decreased glycogen synthetase activity is apparently one site of metabolic impairment in Q fever, the actual mechanism of enzyme inhibition remains to be uncovered. Altered phosphorylase activity could be the result of a series of reactions, and might be hormone-linked, for infection stress in the guinea pig could stimulate epinephrine secretion with resultant phosphorylase activation (Cori and Illingworth

1956; Sutherland and Rall, 1958). Hormonal involvement in glycogen synthesis by insulin-enhanced glycogen synthetase activity was shown by Villar-Palasi and Larner (1961) and Steiner, Rauda, and Williams (1961). Hormonal involvement was also shown in epinephrine-mediated hepatic glycogenolysis in normal mice (Szentivanyi, Fishel, and Talmage, 1963); it was proposed that this effect was obviated in pertussis-sensitized mice by blockage of some adrenergic effector cells.

The present experiments, which demonstrate enhanced hepatic glycogenolysis and concomitant lipogenesis, indicate a biochemical derangement in the guinea pig as a consequence of *C. burnetii* infection. Some of the gross changes which occur during Q fever are correlated with altered enzyme activities, but the rickettsia-induced mechanisms responsible for changes in host biochemistry remain to be identified.

ACKNOWLEDGMENTS

The authors acknowledge support from Public Health Service training grant 2G-703 from the National Institutes of Health, National Science Foundation grant E-11466R, and the University of Kansas General Research Fund. The technical assistance of J. Steuckemann is gratefully acknowledged. The authors are indebted to D. Azarnoff for his discussions and analysis of lipids.

LITERATURE CITED

ALGRANATI, I. D., AND E. CABIB. 1962. Uridine diphosphate D-glucose-glycogen glucosyltransferase from yeast. J. Biol. Chem. **237**: 1007–1013.

ASSOCIATION OF OFFICIAL AGRICULTURAL CHEMISTS. 1945. Methods of analysis, 6th ed., p. 494–495. Association of Official Agricultural Chemists, Washington, D.C.

AZARNOFF, D. L. 1962. Micromethod for the determination of serum lipids. J. Lab. Clin. Med. **60**:331–338.

BELCOPITOW, E. 1961. The action of epinephrine on glycogen synthetase. Arch. Biochem. Biophys. **93**:457–458.

BERRY, L. J., AND D. S. SMYTHE. 1960. Some metabolic aspects of host-parasite interactions in the mouse typhoid model. Ann. N.Y. Acad. Sci. **88**:1278–1286.

BOYD, W. 1961. Textbook of pathology, 7th ed., p. 18–19. Lea and Febiger, Philadelphia.

BURNET, F. M., AND M. FREEMAN. 1937. Experimental studies on the virus of "Q" fever. Med. J. Australia **2**:299–305.

CONSIGLI, R. A., AND D. PARETSKY. 1962. Oxidation of glucose-6-phosphate and isocitrate by *Coxiella burnetii*. J. Bacteriol. **83**:206–207.

CORI, G. T., AND B. ILLINGWORTH. 1956. The effect of epinephrine and other glycogenolytic agents on the phosphorylase A content of muscle. Biochim. Biophys. Acta **21**:105–110.

COX, H. R., AND E. J. BELL. 1939. The cultivation of *Rickettsia diaporica* in tissue culture and in the tissues of developing chick embryos. Public Health Rept. U.S. **54**:2171–2178.

DERRICK, E. H. 1937. "Q" fever, a new fever entity: clinical features, diagnosis and laboratory investigation. Med. J. Australia **2**:281–299.

DIBLE, J. H. 1950. Pathology, 3rd ed., p. 71–80. J. B. Lippincott Co., Philadelphia.

GAUSH, C. R., AND J. S. YOUNGNER. 1962. Studies on the lipids of virus-infected cells. I. Lipid analysis of a soluble hemagglutinin from chorioallantoic membranes infected with vaccinia virus. Virology **19**:573–579.

HUEBNER, R. J., W. L. JELLISON, AND M. D. BECK. 1949. Q fever—a review of current knowledge. Ann. Internal Med. **30**:495–509.

KAHAN, J. 1953. A rapid photometric method for the determination of glycogen. Arch. Biochem. Biophys. **47**:408–418.

KORDOVA, N., AND J. RAHACEK. 1959. Experimental infection of ticks in vivo and their organs in vitro with filterable particles of *Coxiella burnetii*. Acta Virol. Prague **3**:201–209.

LENNETTE, E. H. 1959. Q fever, p. 880–895. *In* T. M. Rivers and F. L. Horsfall [ed.], Viral and rickettsial infections of man. J. B. Lippincott Co., Philadelphia.

LILLIE, R. D. 1942. Pathologic histology in guinea pigs following intraperitoneal inoculation with the virus of Q fever. Public Health Rept. U.S. **57**:296–306.

LILLIE, R. D., T. L. PERRIN, AND C. ARMSTRONG. 1941. An institutional outbreak of pneumonitis. III. Histopathology in man and rhesus monkeys of the pneumonitis due to the virus of Q fever. Public Health Rept. U.S. **56**: 149–155.

MALLAVIA, L., AND D. PARETSKY. 1963. Studies on the physiology of rickettsiae. V. Metabolism of carbamyl phosphate by *Coxiella burnetii*. J. Bacteriol. **86**:232–238.

MATTHEIS, M. S., M. SILVERMAN, AND D. PARETSKY. 1963. Studies on the physiology of rickettsiae. IV. Folic acids of *Coxiella burnetii*. J. Bacteriol. **85**:37–41.

MYERS, W. F., C. M. DOWNS, AND D. PARETSKY. 1963. Some biochemical alterations in hosts during *Coxiella burnetii* infection. Iowa State J. Sci. **38**:69–74.

MYERS, W. F., AND D. PARETSKY. 1961. Synthesis of serine by *Coxiella burnetii*. J. Bacteriol. **82**:761–763.

NELSON, N. 1944. A photometric adaptation of the Somogyi method for the determination of glucose. J. Biol. Chem. **153**:375–380.

PARETSKY, D., R. A. CONSIGLI, AND C. M. DOWNS. 1962. Studies on the physiology of rickettsiae. III. Glucose phosphorylation and hexokinase activity in *Coxiella burnetii*. J. Bacteriol. **83**: 538–543.

PARETSKY, D., C. M. DOWNS, R. A. CONSIGLI, AND B. K. JOYCE. 1958. Studies on the physiology of rickettsiae. I. Some enzyme systems of *Coxiella burnetii*. J. Infect. Diseases **103**:6–11.

PERRIN, T. L., AND I. A. BENGSTON. 1942. The histopathology of experimental "Q" fever in mice. Public Health Rept. U.S. **57**:790–798.

ROBERTS, A. N., AND C. M. DOWNS. 1959. Study on the growth of *Coxiella burnetii* in the L strain mouse fibroblast and the chick fibroblast. J. Bacteriol. **77**:194–204.

SELYE, H. 1950. Stress, p. 141–143, 712–715. Acta Inc., Montreal.

SINGH, V. N., U. BHARGAVA, T. A. VENKITASUBRAMANIAN, AND R. VISWANATHAN. 1963. Study of glycogen synthesizing and degrading enzymes of guinea pig liver in experimental tuberculosis. Arch. Biochem. Biophys. **101**: 234–239.

SINGH, V. N., T. A. VENKITASUBRAMANIAN, AND R. VISWANATHAN. 1961. The glycolytic enzymes of guinea pig lung in experimental bagassosis. Biochem. J. **78**:728–732.

SINGH, V. N., T. A. VENKITASUBRAMANIAN, AND R. VISWANATHAN. 1963. Study of glucose tolerance and synthesis of hepatic glycogen from glucose and glycine-1-C^{14} in tuberculous guinea pigs. Arch. Biochem. Biophys. **101**:229–233.

STEINER, D. F., V. RAUDA, AND R. H. WILLIAMS. 1961. Effects of insulin, glucagon and glucocorticoids upon hepatic glycogen synthesis from uridine diphosphate glucose. J. Biol. Chem. **236**:299–304.

SUTHERLAND, E. W., AND T. W. RALL. 1958. Fractionation and characterization of a cyclic adenine ribonucleotide formed by tissue particles. J. Biol. Chem. **232**:1077–1091.

SZENTIVANYI, A., C. W. FISHEL, AND D. W. TALMAGE. 1963. Adrenalin mediation of histamine and serotonin hyperglycemia in normal mice and the absence of adrenaline-induced hyperglycemia in pertussis-sensitized mice. J. Immunol. **113**:86–98.

VILLAR-PALASI, C., AND J. LARNER. 1961. Insulin treatment and increased UDPG-glycogen transglucosylase activity in muscle. Arch. Biochem. Biophys. **94**:436–442.

WEISS, E., AND H. C. PIETRYK. 1956. Growth of *Coxiella burnetii* in monolayer cultures of chick embryo entodermal cells. J. Bacteriol. **72**:219–225.

ZDRODOVSKII, P. F., AND H. M. GOLINEVICH. 1960. The rickettsial diseases. Pergamon Press, New York.