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Proteins of Typhus and Spotted Fever Group Rickettsiae

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Purified radioactive rickettsiae were obtained from irradiated and cycloheximide-inhibited L cells, and their proteins were analyzed by polyacrylamide gel electrophoresis. Rickettsial species could be distinguished by comparing the relative mobilities of constituent proteins after migration of two differentially labeled preparations in a single gel. Distinct differences were observed in gel patterns of rickettsiae from the typhus and spotted fever groups, as well as with different species within a group. Rickettsial organisms causing murine and epidemic typhus were clearly distinguished, as were the causative agents of boutonneuse fever and rickettsialpox. The use of both internal and external molecular weight standards allowed molecular weight estimates for 19 proteins from both *Rickettsia prowazekii* and *Rickettsia conorii*. A flexible system for designating rickettsial proteins is proposed that lends itself to modification as more detailed analysis progresses.

Polyacrylamide gel electrophoresis has been widely used in the analysis of viral and bacterial proteins and has recently been applied to the study of rickettsiae (10). When rickettsial proteins are dissociated with 2-mercaptoethanol and sodium dodecyl sulfate (SDS) and then examined by polyacrylamide gel electrophoresis incorporating SDS, individual proteins may be identified and their molecular weights approximated by reference to the relative mobilities of appropriate standards. This separation and identification of proteins from intact organisms provides a sensitive method for comparison of various rickettsial species (10) and also provides a framework of data to compare and contrast the complexity of immunogenic or antigenic subcellular fractions derived from these microorganisms (15).

Previous studies employing polyacrylamide gel electrophoresis to analyze rickettsial proteins have required relatively large quantities of rickettsiae grown in embryonated eggs and necessarily harvested at poorly defined stages of growth. In our studies, rickettsiae were propagated in L-929 cells and harvested during active growth. These host cells provide a relatively high yield of rickettsiae (1) and are significantly more amenable to separation and purification of rickettsiae than are yolk sac preparations. The use of cycloheximide enabled the radiolabeling of rickettsiae under conditions where protein synthesis of the host cell was almost completely inhibited. The combined use of radioisotopes and cell culture provided for: (i) highly purified rickettsial suspensions; (ii) direct comparison of two differentially labeled preparations in a single gel; (iii) use of a differentially labeled internal marker to assist in molecular weight determinations; and (iv) substantial reduction in amount of rickettsiae needed for characterization of intact organisms.

MATERIALS AND METHODS

Reagents. Radioisotopes (^{14}C-labeled L-amino acid mixture; ^{3}H-labeled L-amino acid mixture) and liquid scintillation material (Formula 949 liquid scintillator; Protosol tissue and gel solubilizer) were purchased from New England Nuclear Corp., Boston, Mass. Acrylamide, *N*-*N'*-methylenebisacrylamide, and SDS were purchased from Bio-Rad Labs, Richmond, Calif. *N,N,N',N'*-tetramethylethylenediamine and Column Coat were obtained from Canalco, Rockville, Md. Fetal bovine serum (FBS), trypsin-ethylenediaminetetraacetic acid, Eagle minimal essential medium with Earle salts and 25 mM *N*-2-hydroxyethylpiperazine-*N'*-2-ethanesulfonic acid (HEPES) buffer (EMEM), Medium 199, and L-glutamine were all purchased from Grand Island Biological Co., Grand Island, N.Y. Cycloheximide was obtained from Calbiochem, Los Angeles, Calif. Density gradient-grade (ribonuclease free) sucrose and bovine albumin were obtained from Schwarz/Mann Co., Div. of Becton, Dickinson & Co., Orangeburg, N.Y. Ovalbumin, chymotrypsinogen, and ribonuclease were purchased from Pharmacia Fine Chemicals, Inc., Piscataway, N.J. Purified Sindbis virus labeled with ^{14}C-labeled amino acid was a generous gift of Joel Dalrymple, Walter Reed Army Institute of Research, Washington, D.C.

Cycloheximide inhibition of irradiated L-929 cells. A suspension of L-929 cells was irradiated with 3,000 R (17) in a Gammacell 220 (Atomic Energy of Canada, Limited, Commercial Products, Ottawa,

Canada), diluted in Medium 199 containing 10% FBS, seeded at 2.5×10^6 cells/petri dish (60 mm; Falcon Plastics, Oxnard, Calif.), and incubated at 36 C in a humidified atmosphere of air containing 5% CO_2. After 24 h, the growth medium was removed from one-half of the plates and replaced with fresh medium containing either 1 or 2 μg of cycloheximide per ml. Control plates were replenished with fresh growth medium containing no cycloheximide. The remaining plates were similarly treated after 48 h. Tritiated amino acids were added to each plate at a final concentration of 3 μCi/ml 72 h after seeding.

The relative incorporation of ^{3}H-labeled amino acids into cycloheximide-treated cells as compared to uninhibited controls was determined by precipitation with trichloroacetic acid. At 24 and 72 h after the addition of isotope, cells were scraped into the growth medium and pelleted by centrifugation at $240 \times g$ for 10 min at 26 C. The plastic plates were washed three times with 1 ml of normal saline to recover any cells remaining in the plates. The washes were added to the pelleted cells, mixed with 3 ml of 20% (wt/vol) trichloroacetic acid, and incubated overnight at 4 C. One milliliter of the trichloroacetic acid-precipitated material, representing 4.0×10^5 cells, was dried onto glass fiber filters (Whatman GF/C, Maidstone, England) and counted with 949 liquid scintillator in a Packard Tri-Carb scintillation spectrometer (Packard Instrument Co., Inc., Downers Grove, Ill.).

Growth and radiolabeling of rickettsiae. Seed suspensions of *Rickettsia rickettsii* (Sheila Smith strain), *Rickettsia akari* (Hartford strain), *Rickettsia conorii* (Casablanca strain), *Rickettsia typhi* (Wilmington strain), and *Rickettsia prowazekii* (Breinl strain) had been prepared by standard methods (14) from the yolk sacs of embryonated chicken eggs (Spafas, Inc., Norwich, Conn.), shell-frozen in dry ice-95% ethanol, and stored at −70 C.

Rickettsiae were grown in spinner cultures of gamma-irradiated L-929 cells. Spinner cultures were prepared from heavy monolayer cultures which were dispersed with 0.05% trypsin-0.02% ethylenediaminetetraacetic acid, adjusted to 10% with FBS, and irradiated with 3,000 R. Viable cells were enumerated by trypan blue exclusion. The cells were pelleted by centrifugation at $240 \times g$ for 10 min at 26 C, resuspended in 1.5 ml of cold brain heart infusion (Difco Laboratories, Detroit, Mich.), and infected with rickettsial seed suspensions at a multiplicity of infection of approximately 0.2 plaque-forming unit per cell. Rickettsiae were allowed to adsorb for 1 h at 26 C with occasional mixing. Infected cells were washed twice with EMEM and 10% FBS, centrifuged at $240 \times g$ for 10 min at 26 C, and then resuspended to a final concentration of 3×10^6 cells/ml in EMEM containing 10% FBS, 2 mM L-glutamine, and 1 μg of cycloheximide per ml. Cultures were maintained at 34 C in water-jacketed spinner flasks (Wheaton, Millville, N.J.) attached to Lauda constant-temperature water circulators (Brinkmann Instruments, Inc., Westbury, N.Y.).

Radioisotopes were added 18 h postinfection at 3 μCi/ml for tritium labeling and 1 μCi/ml for carbon labeling. Cultures were monitored daily and harvested when approximately 75% of the cells were infected and each cell contained an average of 100 rickettsiae. Cultures were usually harvested at 2 to 3 days postinfection, but *R. rickettsii* infections were sometimes maintained up to 5 days to obtain an acceptable yield. Since the rickettsiae had not yet maximally filled the cells, they were considered to be still actively growing, although not in the first cycle of a one-step growth curve as described for *R. prowazekii* (18).

Purification. Infected cultures were homogenized for three 1-min cycles in a Sorvall Omnimixer (Ivan Sorvall Inc., Norwalk, Conn.) and centrifuged at 240 $\times g$ for 10 min at 4 C to pellet aggregates of L-929 cell material. The rickettsiae in the supernatant were pelleted by centrifugation at $5,000 \times g$ for 1 h at 4 C. The organisms were resuspended in a small volume of cold brain heart infusion and layered over 26-ml linear sucrose gradients prepared as 5 to 30% (wt/wt) sucrose in TEN buffer [0.05 M tris(hydroxymethyl)aminomethane (Tris), pH 7.5, 0.001 M ethylenediaminetetraacetic acid, and 0.1 M NaCl]. Gradients were centrifuged at $2,600 \times g$ for 35 min at 4 C in a Beckman L2-65B ultracentrifuge using an SW25.1 rotor (Beckman Instruments, Inc., Fullerton, Calif.). Fractions (0.8 ml) were collected from the bottom of the tube, and 100-μl aliquots were counted after drying on glass fiber filters (Whatman GF/A). Labeled organisms were concentrated by diluting peak radioactive fractions with 0.05 M Tris, pH 7.5, and centrifuging at $57,700 \times g$ for 1 h at 4 C. Pellets were resuspended in a small volume of 0.05 M Tris (pH 7.5) containing 20% sucrose (wt/wt), shell-frozen in dry ice-95% ethanol, and stored at −70 C.

Analytical and serological procedures. Protein concentration of purified rickettsial suspensions and antigens was determined by the Folin-biuret method (8), using bovine albumin as a standard. The complement fixation test qas performed by the CF-52 method (5) modified for the microtiter technique (13). Rickettsial antisera were obtained from infected guinea pigs. L-929 cell antiserum was obtained from rabbits immunized by intradermal inoculation at multiple sites with a total of 5×10^7 cells in complete Freund adjuvant. The rabbits were boosted with a similar inoculum 35 days after initial immunization.

Polyacrylamide gel electrophoresis. A modification of the method of Maizel (9) was used to prepare 8% SDS-polyacrylamide gels. The following stock solutions were mixed: 30% acrylamide-1% *N-N'*-methylenebisacrylamide, 5.34 ml; 1 M sodium phosphate buffer, pH 6.9, 2.00 ml; 10% SDS, 0.20 ml; *N,N,N',N'*-tetramethylethylenediamine, 0.01 ml; distilled water, 12.20 ml; and 10% ammonium persulfate, 0.20 ml. The mixture was stirred and poured into glass tubes (5-mm inside diameter by 75- or 125-mm length) which had been previously treated with Column Coat.

Rickettsiae were prepared for electrophoresis by boiling for 10 min in the presence of 2.5% mercaptoethanol and 1% SDS. Prior to electrophoresis, 10 μl of glycerin and 5 μl of a saturated aqueous solution

of bromophenol blue were added to each sample. Samples of 100 μl were loaded on either 7- or 12-cm gels and were subjected to electrophoresis in a Pharmacia gel electrophoresis apparatus (Pharmacia Fine Chemicals, Inc.) equipped with a Buchler regulated power supply (Buchler Instruments Div., Nuclear-Chicago Corp., Fort Lee, N.J.). Gels 7 cm long were subjected to electrophoresis under a constant current of 10 to 12 mA/gel for 3.5 h; 12-cm gels were subjected to electrophoresis at 4 mA/gel for 16.5 h or until the tracking dye had reached the bottom of the gel. Migration is shown in all the graphs from left to right (cathode to anode).

After electrophoresis, gels were extruded from the tubes and either stained with Coomassie brilliant blue R-250, 0.25% (wt/vol) in absolute methanol-distilled water-glacial acetic acid (5:5:1), or quick-frozen on dry ice and cut into 1-mm segments. Gels were stained for 1 h and then destained with absolute methanol-distilled water-glacial acetic acid (2:10:1). Gel slices were swollen overnight at 37 C in 10 ml of 949 liquid scintillator containing 3.35% Protosol, chilled to 4 C, and counted.

RESULTS

The effect of cycloheximide on incorporation of radioactive amino acids by irradiated L-929 cells. Previous studies of Weiss et al. (17) have shown that typhus and spotted fever group rickettsiae, *R. typhi* and *R. akari*, grow well in irradiated L-929 cells and that their growth is uninhibited by low levels of cycloheximide in the growth medium. Table 1 indicates the effect of cycloheximide on irradiated L-929 cell uptake of radioactive amino acids. Concentrations of 1 or 2 μg/ml were sufficient to inhibit L-929 cell protein synthesis by 95%. Little difference in incorporation was observed regardless of whether the inhibitor was added at 48 or 24 h prior to the labeling of the cells. Although isotope uptake was slightly more inhibited in the presence of 2 μg of cycloheximide per ml, this concentration was not used as it caused a gradual destruction of the cells. The standard concentration of 1 μg/ml sufficiently depressed L-929 cell incorporation of radioactive amino acids so that polyacrylamide gel electrophoresis analysis yielded no detectable radiolabeled proteins.

TABLE 1. *Radioactive amino acid incorporation into trichloroacetic acid-precipitable components of irradiated uninfected cells*

Concn of cycloheximide (μg/ml)	Pretreatment time with cycloheximide (h)	Isotope incorporation 24 h after addition	Isotope incorporation 72 h after addition
0		46,700[a]	84,000
1	24	1,900	4,100
	48	2,700	3,300
2	24	1,200	1,200
	48	1,000	1,100

[a] Counts per minute per 4.0 × 10^5 cells.

Purification of rickettsiae. Irradiated L-929 cells, inhibited by cycloheximide, infected with *R. prowazekii*, and labeled with ^{14}C-labeled amino acids, were combined with uninfected, uninhibited L-929 cells labeled with ^{3}H-labeled amino acids. The mixture was subjected to the described purification scheme with final separation on a 5 to 30% sucrose gradient. Figure 1 indicates that the rickettsiae banded uniformly in the gradient with no detectable contamination by host cell protein. Typhus organisms purified in this manner routinely yielded about 5 μg of rickettsial protein per ml of infected L-929 spinner culture. The specific activity was approximately 4 × 10^5 counts/min per mg of protein for ^{3}H-labeled organisms and 9 × 10^5 counts/min per mg of protein for ^{14}C-labeled rickettsiae. A representative preparation of purified *R. prowazekii* showed a complement fixation titer of 1:256 with typhus antisera prepared in guinea pigs, and no detectable reaction was observed with L-929 cell antisera prepared in rabbits. Table 2 indicates the purification and concentration of rickettsiae achieved at each step of the procedure.

Comparison of polyacrylamide gels sliced for radioisotope counting and stained with Coomassie blue. To establish the authenticity of radiolabeled gel patterns for comparison of proteins from various rickettsial species, it was first necessary to demonstrate meaningful correlates between the radioisotope and classical staining techniques. A radioactive preparation of *R. prowazekii* was used to demonstrate separation of rickettsial proteins on duplicate 7-cm gels. One gel was loaded with sufficient rickett-

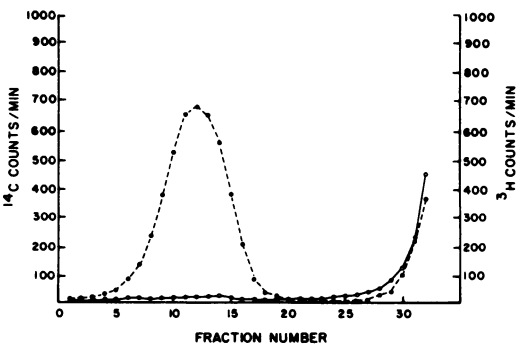

FIG. 1. *Final purification of R. prowazekii by rate zonal centrifugation in a linear 5 to 30% sucrose gradient. Symbols: (●) Rickettsiae labeled with ^{14}C-labeled amino acid; (○) uninfected L-929 cells labeled with ^{3}H-labeled amino acid.*

TABLE 2. *Purification and concentration of epidemic typhus rickettsiae as measured by the complement fixation test at various steps in separation scheme*

Antiserum	Antigen[a]				
	Infected cells after blending	Rickettsiae after differential centrifugation	Rickettsiae after sucrose gradient	*R. prowazekii* complement-fixing antigen[c]	L-929 cell complement-fixing antigen
R. prowazekii antiserum	8[b]	8	256	256	<8
L-929 cell antiserum	32	32	<8	<8	32

[a] All antigen preparations were standardized at 680 μg of protein per ml prior to use in the complement fixation test.

[b] Reciprocal of the serum dilution end point.

[c] Grown in SPAFAS embryonated chicken eggs.

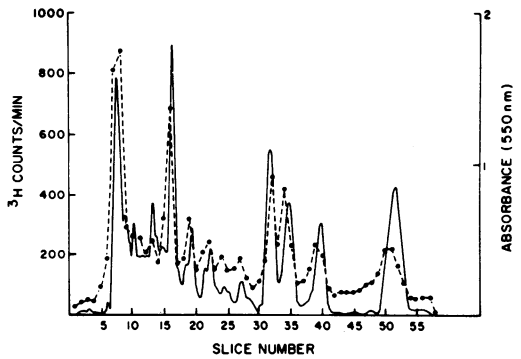

FIG. 2. *Comparison of duplicate R. prowazekii electropherograms (7-cm gels) obtained by Coomassie blue staining and radioisotope counting. Migration is from left to right in all figures (cathode to anode). Symbols: (●) Rickettsiae labeled with ^{3}H-labeled amino acid; (——) densitometer tracing of same labeled preparation in another gel.*

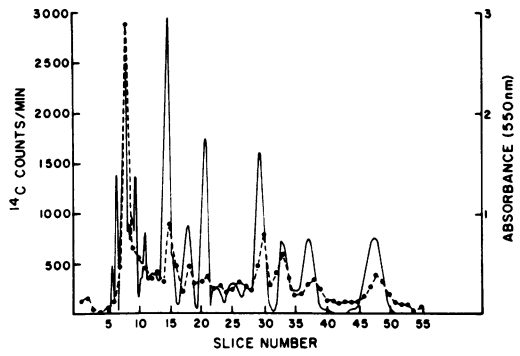

FIG. 3. *Comparison of duplicate R. rickettsii electropherograms (7-cm gels) obtained by Coomassie blue staining and radioisotope counting. Symbols: (●) Rickettsiae labeled with ^{14}C-labeled amino acid; (——) densitometer tracing of same labeled preparation in another gel.*

sial protein to enable final visualization of bands by Coomassie blue staining. The duplicate gel received 20,000 counts of rickettsial protein per min. After electrophoresis, the former gel was stained, decolorized, and scanned with a densitometer. The latter gel was frozen, sliced, digested, and counted. The electropherogram in Fig. 2 illustrates the correlation of radioactive label with stained bands. Excellent correspondence was obtained from six major, or intensely stained, bands and six minor, or less darkly stained, bands. The limitations of our 1-mm slicing apparatus did not allow resolution of closely spaced radioactive bands in the high-molecular-weight region of the gel. Figure 3 shows a similar comparison of a densitometer tracing and a radioactive pattern achieved on duplicate gels of the same radioactive preparation of *R. rickettsii*. There was, again, excellent correlation of radioactive label with stained bands for six major proteins and three minor proteins.

Comparison of radioactive rickettsial proteins. Comparison of multiple electropherograms of each individual rickettsial species revealed no differences in the major proteins regardless of whether the organisms were from the same or from different radioactive preparations. The constancy of these results made appropriate the coelectrophoresis of both different species within a group and species from different groups of rickettsiae. The more sensitive 12-cm gels were used for these comparisons.

Figure 4 shows an electropherogram of *R. prowazekii* labeled with ^{14}C-labeled amino acid and *R. typhi* labeled with ^{3}H-labeled amino acid subjected to coelectrophoresis in the same gel. Differences in the protein patterns were observed in the low-molecular-weight region of the gel between fractions 50 and 65. A similar comparison of two spotted fever group rickettsiae yielded an even more striking variation in peak patterns. Figure 5 illustrates the coelectrophoresis of *R. conorii* labeled with ^{14}C-labeled amino acid and *R. akari* labeled with ^{3}H-labeled amino acid. Major differences in peak

patterns are evident at fractions 37 to 41 and 55 to 60.

Comparisons made between species of different groups of rickettsiae also yielded demonstrable differences. Figure 6 shows an electropherogram of *R. prowazekii* and *R. rickettsii* subjected to coelectrophoresis in the same gel. Differences were observed in the area of fractions 50 to 65, and the low-molecular-weight region again appeared as the major focus of variability.

Estimation of molecular weights of rickettsial proteins. Electrophoresis of the unlabeled molecular weight standards bovine albumin (molecular weight of 67,000), ovalbumin (molecular weight of 45,000), chymotrypsinogen

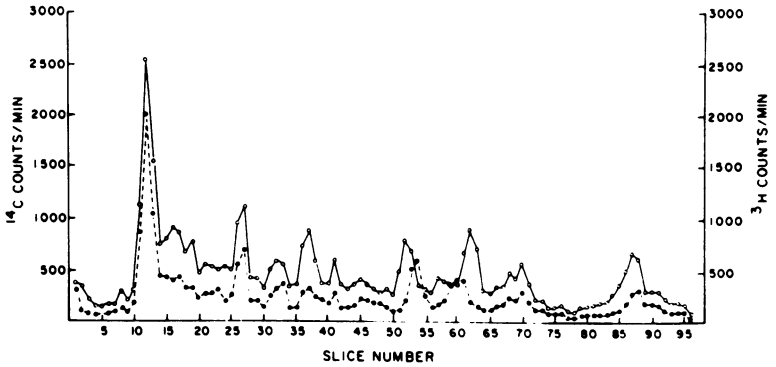

FIG. 4. *Coelectrophoresis of radioactive typhus group rickettsiae (12-cm gel). Symbols: (○) R. typhi labeled with ^{3}H-labeled amino acid; (●) R. prowazekii labeled with ^{14}C-labeled amino acid.*

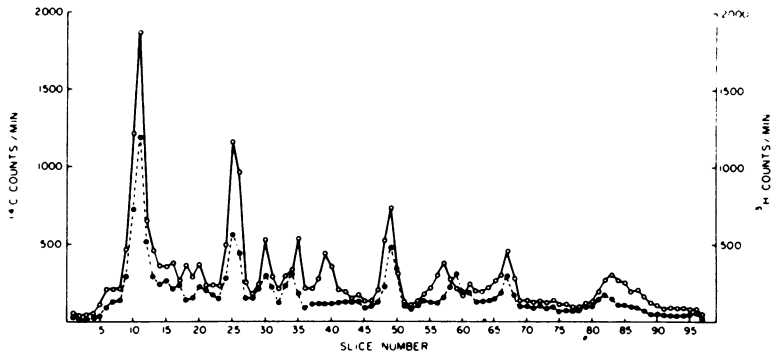

FIG. 5. *Coelectrophoresis of radioactive spotted fever group rickettsiae (12-cm gel). Symbols: (○) R. akari labeled with ^{3}H-labeled amino acid; (●) R. conorii labeled with ^{14}C-labeled amino acid.*

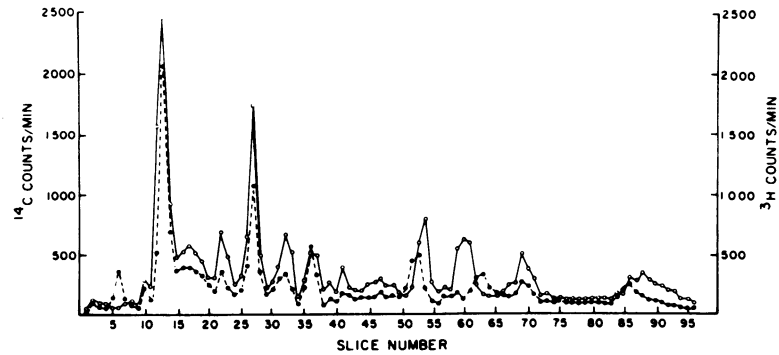

FIG. 6. *Coelectrophoresis of radioactive typhus and spotted fever group rickettsiae (12-cm gel). Symbols: (○) R. prowazekii labeled with ^{3}H-labeled amino acid; (●) R. rickettsii labeled with ^{14}C-labeled amino acid.*

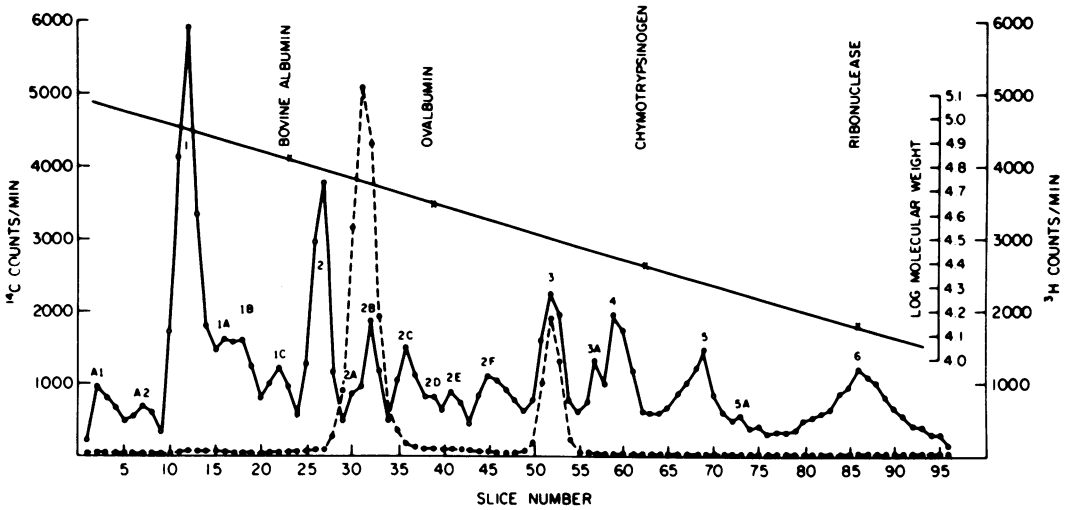

FIG. 7. *Coelectrophoresis of radioactive R. prowazekii and differentially labeled Sindbis virus proteins plotted with a similar gel of Sindbis virus and the indicated protein standards. Sindbis virus served as a common molecular weight reference point in both gels. Symbols: (○) R. prowazekii labeled with ^{3}H-labeled amino acid; (●) Sindbis virus labeled with ^{14}C-labeled amino acid.*

(molecular weight of 25,000), and ribonuclease (molecular weight of 13,700) with radioactive Sindbis virus proteins (molecular weights of 53,000 and 30,000, respectively) established a linear relationship of relative migration to log molecular weight (16) and also allowed subsequent incorporation of Sindbis virus into gels of rickettsial protein to serve as an internal molecular weight marker. Figure 7 indicates the linearity of log molecular weight of these standards plotted against relative migration in the gel and also indicates the migration of radioactive Sindbis virus proteins relative to differentially radioactive rickettsial proteins when subjected to coelectrophoresis in the same 12-cm gel. This experiment allowed estimation of molecular weights for major proteins and for minor proteins observed in 7-cm stained gels but not clearly resolved by the gel slicing required for radioactive preparations. Table 3 indicates the estimated molecular weights of *R. prowazekii* proteins determined from electrophoresis in a 12-cm gel. A similar analysis of *R. conorii* proteins subjected to electrophoresis with radioactive Sindbis virus is shown in Fig. 8. Molecular weight estimates for proteins of this spotted fever group rickettsia are shown in Table 4.

TABLE 3. *Molecular weights of R. prowazekii proteins*

R. prowazekii proteins	Gel (12 cm)	
	Fraction no.	Mol wt
Major proteins		
1	12	87,000
2	27	60,000
3	52	32,000
4	59	27,000
5	69	21,000
6	86	13,000
Minor proteins		
A1	2	112,000
A2	7	100,000
1A	16	79,000
1B	18	76,000
1C	22	68,000
2A	30	56,000
2B	32	54,000
2C	36	48,000
2D	39	45,000
2E	41	43,000
2F	45	38,000
3A	57	28,000
5A	73	19,000

DISCUSSION

This study clearly demonstrated that the preparation of radioactive rickettsiae in cultured mammalian cells, irradiated and treated with a host cell metabolic inhibitor, presents a biological system with substantial potential for the detailed analysis of biochemical and biophysical parameters of rickettsiae. It provides for the growth of organisms under conditions that allow monitoring to ensure harvest during active growth and takes advantage of the tremendous sensitivity afforded by the use of radioisotopes.

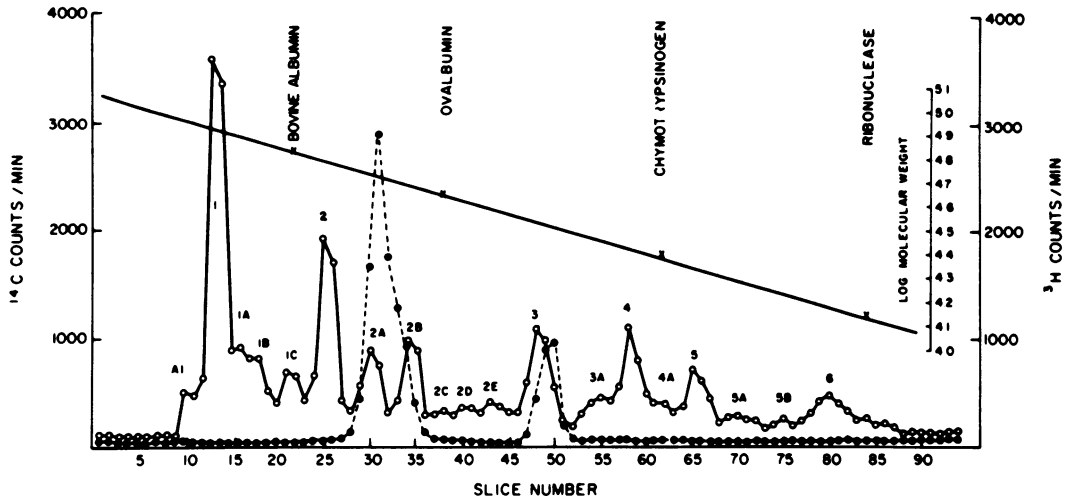

FIG. 8. *Coelectrophoresis of radioactive R. conorii and Sindbis virus plotted with a similar gel of Sindbis virus and protein standards. Symbols: (○) R. conorii labeled with ^{3}H-labeled amino acid; (●) Sindbis virus labeled with ^{14}C-labeled amino acid.*

Our simple purification scheme, employing differential centrifugation followed by rate zonal separation in a linear sucrose gradient, is not grossly different from that used to purify unlabeled spotted fever rickettsiae propagated in L-929 cells (1) and accentuates the ease of purification of rickettsiae derived from cell culture. Organisms of equal purity can be obtained from embryonated eggs, but a complex, multistep technique is required (10).

Polyacrylamide gel electrophoresis was shown to be a sensitive technique for distinguishing between different rickettsiae, especially when the relative mobilities of their constituent proteins could be compared after migration of differentially labeled preparations in a single gel. Those proteins identified were sufficient to demonstrate reproducibly both inter- and intragroup differences among the rickettsiae, but they probably constitute a small proportion of the total rickettsial complement, since the genome size has been estimated at 1.5 $\times$ 10^6 nucleotide pairs (7). Our results are in general agreement with those obtained by others (10) using unlabeled rickettsiae cultivated in embryonated eggs. Although there were some differences in estimation of molecular weights and appearance of high-molecular-weight proteins, they may result from differences in denaturing conditions employed with rickettsiae prior to their incorporation in gels (11) or to differences in growth conditions when organisms were harvested (12). Although more rickettsial proteins might be resolved by using different concentrations of acrylamide or a different supporting menstruum, the present system highlighted one area (25,000 to 35,000 daltons) where typhus and spotted fever group rickettsiae both show distinguishing variability of their proteins. Our immediate interest is to focus on this region of variability and to elucidate the biological significance of these proteins as well as to determine their chemical

TABLE 4. *Molecular weights of R. conorii proteins*

R. conorii proteins	Gel (12 cm)	
	Fraction no.	Mol wt
Major proteins		
1	13	85,000
2	25	63,000
3	48	35,000
4	58	27,000
5	65	22,000
6	80	15,000
Minor proteins		
A1	10	92,000
1A	16	79,000
1B	18	75,000
1C	21	69,000
2A	30	55,000
2B	34	50,000
2C	38	45,000
2D	40	43,000
2E	43	39,000
3A	55	29,000
4A	62	24,000
5A	70	20,000
5B	75	17,000

and physical properties. The numbering system suggested for rickettsial proteins lends itself to modification as detailed study of various macromolecules progresses. Assignment of integers to those proteins presently recognized as "major" or seen in relatively large quantity will allow their use as reference points during more detailed analysis of various regions of the gel. The use of integer-letter combinations allows for description of minor proteins presently recognized but with the possibility of substantial enlargement as other minor proteins are identified and described in relation to one of the reference point proteins.

Electrophoretic analysis of bacterial proteins has proven useful in identification and classification of these organisms (4, 6). Similar analysis of rickettsial proteins can provide supporting evidence for identification of new strains (2, 3) and aid in the classification of presently recognized rickettsiae. Perhaps more importantly, this type of analysis can focus attention on some key proteins which show distinguishing variability among rickettsiae and stimulate a detailed comparative study of their biochemical and immunological properties.

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