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Protein Synthesis in HeLa or Henle 407 Cells Infected with *Shigella dysenteriae* 1, *Shigella flexneri* 2a, or *Salmonella typhimurium* W118

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The incorporation of [^{14}C]leucine into protein was studied in two mammalian cell lines which had been infected with strains of *Shigella dysenteriae* 1, *Shigella flexneri* 2a, or *Salmonella typhimurium* W118. These cell lines differed in susceptibility to the effects of exogenously applied Shiga cytotoxin. All invasive shigella strains (which synthesize this toxin to a greater or lesser degree) were found to inhibit protein synthesis in both cell lines with equal efficiency. Leucine accumulation continued in these cells, but the labeled amino acid was preferentially incorporated into bacterial protein. *S. typhimurium* W118, which has not been shown to elaborate a Shiga-like toxin, had little effect on protein synthesis in infected host cells.

It has been recognized for many years that culture filtrates of *Shigella dysenteriae* 1 contain a biologically active material which causes paralysis and death when injected parenterally into rabbits or mice. Early workers found that the "neurotoxin" was an antigenic protein which was distinct from lipopolysaccharide endotoxin (25). Later it was suggested that the neurotoxicity of this material was a secondary manifestation of more generalized cytotoxic activity (3). This was supported by the subsequent isolation of a partially purified fraction of Shiga toxin which was neurotoxic for mice and cytotoxic for mammalian cells cultured in vitro (17). Interestingly, this material also exhibited enterotoxic properties when injected into ligated rabbit ileal loops (17).

Cytotoxic products have potential etiological significance in the pathogenesis of shigellosis because severe colitis is a hallmark of this infection. Anticytotoxin antibody has been detected in convalescent-phase sera of patients infected with *S. dysenteriae* (20), indicating that Shiga toxin is produced in natural infections. Since colitis is elicited only by shigella strains which invade and multiply within colonic epithelial cells (21, 22), cytotoxin produced during the intracellular growth of these organisms may contribute to destruction of the colonic mucosa.

The nature of the physiological derangement induced by Shiga cytotoxin has recently been characterized by a number of workers. Exogenous toxin inhibits protein synthesis in intact HeLa cells (4, 26; M. S. Osato, T. A. Brawner, and D. J. Hentges, Am. J. Clin. Nutr. 32:268, 1979), in human lymphocytes (G. T. Keusch, P. R. Papenhausen, and M. Jacewicz, Clin. Res. 24: 287A, 1976), and in cell-free preparations of rat liver (30), rabbit reticulocytes (5), or *Escherichia coli* (24). Thus, inhibition of protein synthesis in infected colonic epithelial cells may be the initial insult which underlies tissue destruction associated with shigellosis. In the present study, mammalian cell lines which vary in sensitivity to exogenous cytotoxin were employed as in vitro models of the colonic epithelium. Protein synthesis was measured in monolayers exposed to a variety of shigella strains which differ in invasive potential and in vitro toxin production. In addition, protein synthesis was measured in cells which were infected with *Salmonella typhimurium* W118. This strain has not been shown to synthesize a cytotoxin analogous to Shiga toxin. The data indicate that intracellular shigellae inhibit host cell protein synthesis, and cytotoxin elaborated by intracellular organisms was implicated in this inhibition.

MATERIALS AND METHODS

Microorganisms. Experiments were performed with *S. dysenteriae* 1 strains 3818 T, 3818 O, and 725. The parent strain 3818 T was isolated during the 1969 epidemic in Central America. This strain readily invades the intestinal mucosa and is overtly toxigenic; i.e., it elaborates readily detectable Shiga toxin in unconcentrated cultural filtrates (8). Strain 3818 O, an avirulent colonial variant of strain 3818 T, is also fully toxigenic but noninvasive (8). Strain 725 is a virulent chlorate-resistant mutant of 3818 T which is invasive (8) but hypotoxigenic; i.e., it produces at least 1,000-fold less Shiga toxin than the parent strain (20). Studies were also carried out with virulent *Shigella flexneri* 2a strain M4243 and the avirulent colonial variant of

this strain, which is designated 2457 O (21). Like strain 725, strain M4243 is invasive and hypotoxigenic (23). *S. typhimurium* W118 was also employed in this study. This strain has been used in previous work (12, 29).

Tissue cultures. Henle 407 cells (embryonic intestine, human), ATCC strain CCL-6, were maintained in Eagle basal medium (Flow Laboratories, McLean, Va.) supplemented with 15% fetal calf serum (Flow), 5 mM glutamine (GIBCO, Grand Island, N.Y.), 100 IU of penicillin per ml, and 100 μg of streptomycin per ml (GIBCO). Monolayers of HeLa cells (Flow, no. 03-117) were maintained in Eagle minimal essential medium (Flow) with 10% fetal calf serum and supplemented as described above. Preliminary experiments employing a quantitative microassay of cytotoxicity (9) showed that Henle 407 cells were at least 10,000-fold more resistant than HeLa cells to the effects of exogenous Shiga toxin. Since isozyme studies have indicated that Henle 407 cells are HeLa clones (7), considerable divergence has apparently occurred in vitro.

Infection procedures. Nonconfluent monolayers consisting of 1.0×10^5 to 2.0×10^5 cells (100 to 200 μg of protein) were grown for 18 h in 35-mm plastic dishes (Falcon Plastics, Oxnard, Calif.). Approximately 3 h before exposure to bacteria, the culture medium was replaced with antibiotic-free medium. Flasks of Luria broth (10) were inoculated with bacteria from stock cultures and incubated for 3 h at 37°C with aeration. The logarithmic-phase cultures were then washed in physiological saline and suspended in Eagle minimal essential medium at a concentration of 2.0×10^8 colony-forming units per ml.

Monolayers were washed in 1.0 ml of Earle balanced salts solution (HEM Research Inc., Rockville, Md.) and overlaid with 2.0 ml of bacterial suspension. These monolayers were centrifuged in a Sorvall GLC-2B centrifuge (Ivan Sorvall, Norwalk, Conn.) at 4,000 rpm for 5 min and then incubated for 30 min at 37°C in 5% CO_2. During this incubation period the extracellular bacterial population doubled. Four washes of Earle balanced salts solution then served to remove approximately 99.9% of extracellular bacteria. Representative plates were fixed in methanol and stained with Giemsa solution. The percentage of host cells infected was determined by microscopic analysis of stained monolayers as previously described (10). Quantitative plate counts on disrupted cells which had been exposed to Eagle minimal essential medium with 15 μg of kanamycin (kanamycin sulfate, Kantrex; Bristol Laboratories, Syracuse, N.Y.) per ml for 30 min at 37°C showed that noninvasive strains of shigella were reduced to less than 0.01 colony-forming unit per host cell. Invasive strains of shigella and salmonella maintained a population of at least 10 colony-forming units per host cell in the presence of extracellular kanamycin.

Sonication of bacteria. Cultures of *S. flexneri* 2a M4243 were grown for 18 h at 37°C in medium containing, per liter: K_2HPO_4, 10.5 g; KH_2PO_4, 4.5 g; $MgCl_2$, 0.05; $(NH_4)_2SO_4$, 1.0 g; and glucose, 1.0 g. This medium was supplemented with, per ml, 5 μg of nicotinic acid, 20 μg of aspartic acid, and 5 μCi of L-^{3}H-amino acid mixture (NET-250, New England Nuclear Corp., Boston, Mass.). The labeled bacteria were washed three times in Earle balanced salt solution and then suspended at a concentration of 10^7 colony-forming units per ml in distilled water with 0.02% ethylenediaminetetraacetic acid, and a sample was digested for 30 min at 50°C in 0.9 ml of NCS solubilizer (Amersham, Arlington Heights, Ill.). The remaining bacterial suspension was sonicated for 2 min in an Ultrasonic Cleaner (Mettler Electronics Corp., Anaheim, Calif.) and centrifuged for 5 min in an Eppendorf centrifuge (model 5412, Brinkmann Instruments Co., Westbury, N.Y.). After centrifugation, 0.1 ml of the supernatant fraction was digested for 30 min at 50°C in NCS. The digested samples were added to 9.0 ml of Aquasol (New England Nuclear Corp.); the Aquasol was neutralized by the addition of acetic acid, and the samples were counted for 60 s in a Packard Tri-Carb scintillation counter.

Quantitation of protein synthesis. Monolayers which had been exposed to bacteria and washed free of most extracellular organisms were overlaid with 1.0 ml of incorporation medium which consisted of Eagle minimal essential medium without leucine (GIBCO) containing, per ml, 1.0 mg of bovine serum albumin, 15 μg of kanamycin, and 1.0 μCi of [*U*-^{14}C]leucine (New England Nuclear Corp.). At designated times, the incorporation medium was removed, the monolayers were washed three times in cold Earle balanced salt solution, and 1.0 ml of distilled water with 0.02% ethylenediaminetetraacetic acid was added to osmotically lyse the cells. Ten minutes later, the lysates (with a few remaining intact cells) were removed from culture dishes and sonicated for 2 min in an Ultrasonic Cleaner. Portions of the cell lysates were processed as follows: 0.1 ml was added to 0.9 ml of NCS, incubated at 50°C for 30 min, and added to 9.0 ml of Aquasol; 0.2 ml was used for protein determination by the Bradford method (1); and 0.3 ml was centrifuged for 5 min in an Eppendorf centrifuge (model 5412). After centrifugation of the latter, 0.3-ml samples, 0.1 ml of each supernatant fraction was added to 0.1 ml of 0.5 M NaOH containing 1 mg of bovine serum albumin. After 30 min, 0.8 ml of cold 20% trichloroacetic acid was added, and the samples were held overnight at 4°C. The precipitate in each sample was then pelleted by low-speed centrifugation, and the supernatants were added to scintillation vials containing 9.0 ml of Aquasol. The pellets of trichloroacetic acid-precipitable material were solubilized by incubating for 30 min at 50°C in 0.9 ml of NCS. The digest of each sample was then added to 9.0 ml of Aquasol. Vials containing NCS tissue solubilizer and Aquasol were neutralized with acetic acid.

All samples were counted for 60 s in a Packard Tri-Carb liquid scintillation counter. Total [^{14}C]leucine uptake was determined by counting a fraction of the whole-cell lysate. Leucine incorporated into the soluble proteins of the host-cell cytosol was measured by counting trichloroacetic acid-precipitable material present in the supernatant fraction of the cell lysate. Leucine incorporated into the particulate fraction of the lysate (i.e., bacterial cells, mitochondria, and large membranous vesicles) was determined by subtracting the counts present in the supernatant fraction from total counts in the whole-cell lysate. Because there were variations in the number of host cells present in individual monolayers, the values were standardized

to counts per minute per milligram of cellular protein. Statistical analysis of paired control and experimental means was accomplished by Student's t test with a value of $P \leq 0.05$ regarded as significant.

RESULTS

Infection of host cells. Centrifugation of bacterial suspensions onto host cell monolayers facilitated the uptake of invasive shigellae. Table 1 shows that more than 90% of the cells in HeLa or Henle 407 monolayers were infected 30 min after exposure to *S. dysenteriae* 1 strains 3818 T and 725 or *S. flexneri* 2a strain M4243. A 2-h period of incubation was required to achieve comparable levels of infection with *S. typhimurium* W118. Centrifugation of suspensions of avirulent shigellae (i.e., *S. dysenteriae* 1 strain 3818 O or *S. flexneri* 2a strain 2457 O) onto HeLa or Henle 407 cells did not enhance uptake of these bacteria.

Host cell survival. The proportion of cellular protein associated with culture dishes after exposure to various shigella strains is illustrated in Fig. 1. In HeLa or Henle 407 monolayers exposed to invasive strains 3818 T, 725, or M4243, there was a progressive loss of protein reflecting the detachment of infected cells. In contrast, neither HeLa nor Henle 407 monolayers infected with *S. typhimurium* W118 lost protein over a 4-h period (data not shown). With the exception of toxin-sensitive HeLa cells exposed to overtly toxigenic strain 3818 O, there was no loss of protein from monolayers exposed to noninvasive strains. Approximately 15% of cellular protein was lost from HeLa monolayers exposed to the above strain (Fig. 1).

Sonication of bacteria. Measurement of host cell protein synthesis was predicated upon the removal of bacterial protein from a lysate of infected cells. To assess the effect of osmotic shock and sonication upon bacterial cells, a culture of *S. flexneri* 2a strain M4243 was intrinsically labeled by growth in a L-^{3}H-amino acid mixture, incubated in distilled water with 0.02% ethylenediaminetetraacetic acid for 10 min, and sonicated for 2 min. Centrifugation of this bacterial suspension for 5 min resulted in the removal of 97.5% of radiolabel from the supernatant fraction. Thus the procedure used for lysis of mammalian cells did not induce the release of substantial amounts of soluble bacterial protein.

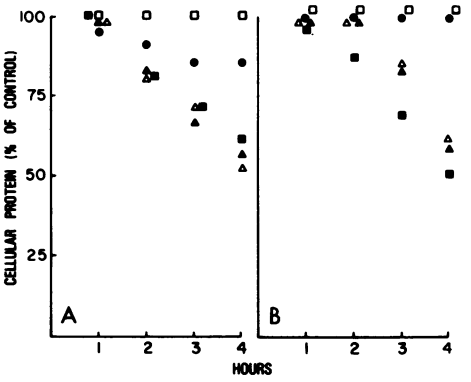

FIG. 1. *Proportion of cellular protein remaining in monolayers exposed to various shigella strains for 30 min and incubated in Eagle minimal essential medium with 15 μg of kanamycin per ml for up to 4 h. (A) HeLa cells; (B) Henle 407 cells. Strains:* (□) *2457 O;* (●) *3818 O;* (△) *725;* (▲) *3818 T;* (■) *M4243.*

TABLE 1. *Infection of mammalian cell lines by strains of Shigella or Salmonella*

Strain	% of host cells[a] infected within 30 min	
	HeLa	Henle 407
Shigella flexneri 2a M4243[b]	95.0 ± 0.7	94.0 ± 0.5
S. flexneri 2a 24570	None	None
Shigella dysenteriae 1 3818 T[b]	96.8 ± 0.4	90.8 ± 1.2
S. dysenteriae 1 725[b]	98.5 ± 0.1	97.0 ± 0.1
S. dysenteriae 1 3818 O	None	None
Salmonella typhimurium W118[b]	79.3 ± 20.0[c]	85.7 ± 1.7

[a] Mean ± standard error.
[b] Virulent strain.
[c] Percentage of host cells infected within 2 h (mean ± standard error).

Protein synthesis in cells exposed to shigellae. Incorporation of [^{14}C]leucine into host cell macromolecules was monitored in monolayers which had been exposed to various shigella strains. Results shown in Fig. 2 confirm that cells exposed to a suspension of noninvasive strain 2457 O synthesize protein at a rate not significantly different from control monolayers. Toxin-sensitive HeLa cells exposed to noninvasive, overtly toxigenic strain 3818 O ceased to incorporate radioactive label into macromolecular constituents after 1 h (Fig. 2A). Protein synthesis also ceased in HeLa cells which were infected with strains 3818 T, 725, or M4243. Within 3 h, this inhibition was statistically significant. The kinetics of protein synthesis in toxin-resistant Henle 407 cells exposed to the above shigella strains is shown in Fig. 2B. Invasive strains rapidly inhibited incorporation of [^{14}C]leucine into host cell macromolecules, whereas strain 3818 O exerted no detectable effect. Inhibition of protein synthesis in infected Henle 407 cells was statistically significant within 4 h.

Accumulation of [^{14}C]leucine in cells ex-

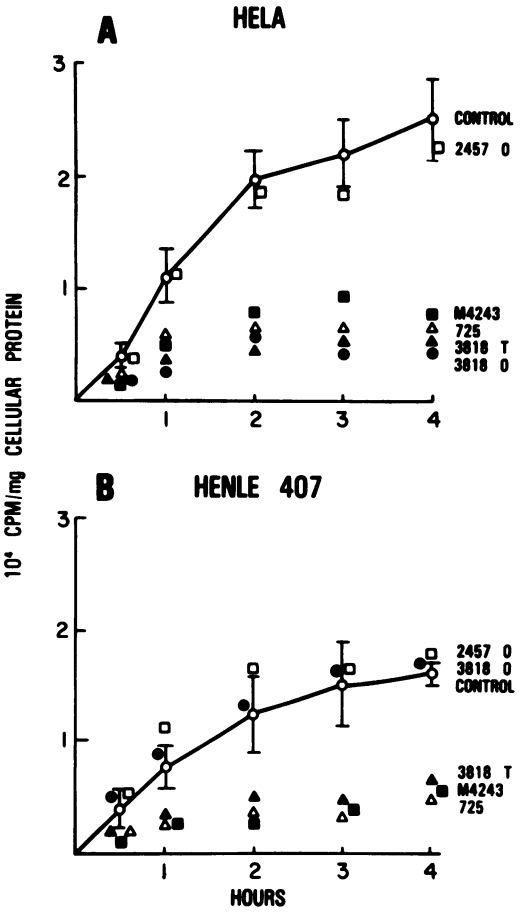

FIG. 2. *(A) Incorporation of [^{14}C]leucine into trichloroacetic acid-precipitable material in the cytosol of HeLa cells. (B) Incorporation of labeled leucine into trichloroacetic acid-precipitable material in the cytosol of Henle 407 cells. Monolayers were exposed to various shigella strains for 30 min, washed free of extracellular bacteria, and incubated for up to 4 h in Eagle minimal essential medium with, per ml, 1.0 μCi of [^{14}C]leucine, 15 μg of kanamycin, and 1.0 mg of bovine serum albumin. The cells were lysed, intracellular bacteria were removed by centrifugation, and acid-precipitable label in the supernatant was counted. Results are presented as the mean of four experiments, and the standard error of control experiments is included.*

posed to shigellae. Uptake of amino acids by infected cells was reflected as intracellular accumulation of [^{14}C]leucine. Accumulation was not significantly depressed in toxin-sensitive HeLa cells infected with invasive shigella strains, whereas cells exposed to noninvasive, overtly toxigenic strain 3818 O reached an equilibrium level within 1 h and subsequently failed to accumulate additional quantities of this amino acid (Fig. 3A). In contrast to HeLa cells, toxin-resistant Henle 407 cells which had been exposed to strain 3818 O continued to accumulate [^{14}C]leucine in quantities comparable to those found in control monolayers (Fig. 3B). Henle 407 monolayers which were infected with invasive shigel-

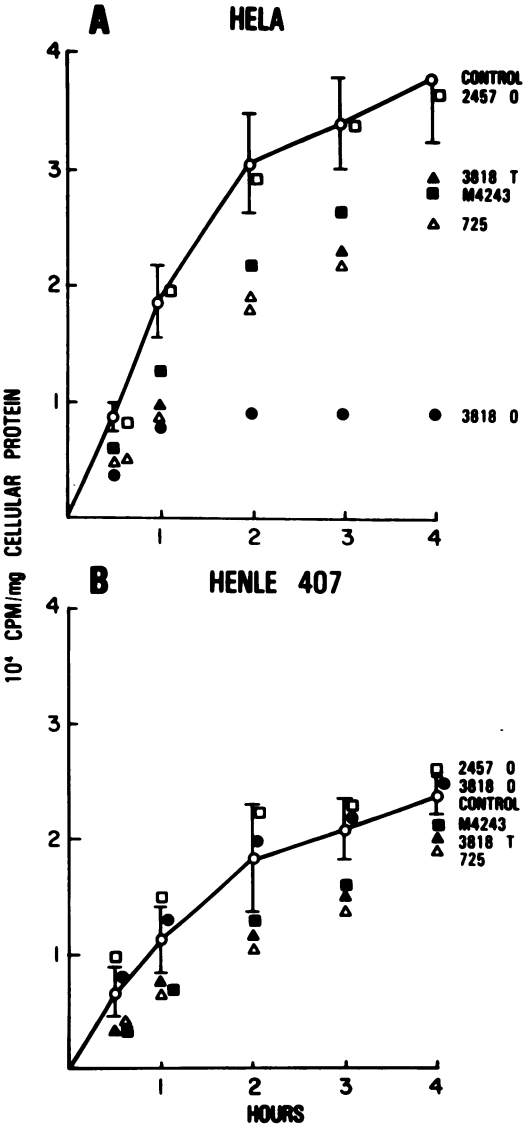

FIG. 3. *(A) Total accumulation of labeled leucine in HeLa cells exposed to various strains of shigella. (B) Total accumulation of [^{14}C]leucine in Henle 407 cells exposed to various strains of shigella. Infection and labeling were carried out as described for Fig. 2, and total radioactivity in the unfractionated cell lysates was counted. Results are presented as the mean of four experiments, and the standard error of control experiments is included.*

lae accumulated only slightly decreased levels of intracellular [^{14}C]leucine during a 4-h period.

Incorporation of [^{14}C]leucine into particulate material. Figure 4 shows the levels of [^{14}C]leucine incorporated into the particulate fraction of lysed mammalian cells. In control cells and in cells exposed to noninvasive strains 2457 O and 3818 O, this fraction represents newly synthesized peptides which have been incorporated into mammalian membranes. HeLa cells exposed to overtly toxigenic strain 3818 O ceased to synthesize protein (see Fig. 3A), and they also ceased to incorporate radioactive label into the particulate fraction (Fig. 4A). In infected cells, the particulate fraction consisted mainly of bacterial cells, and HeLa cells exposed to invasive strains (i.e., 3818 T, 725, and M4243) incorporated constantly increasing amounts of [^{14}C]leucine into this fraction. These data reflect intracellular bacterial growth with incorporation of free amino acids into bacterial protein. Henle 407 monolayers which were infected with shigellae also incorporated [^{14}C]leucine preferentially into the particulate fraction (Fig. 4B). Monolayers which had been exposed to strain 3818 O incorporated normal amounts of radioactive label into membranous organelles. This reflects the normal rate of protein synthesis in Henle 407 cells exposed to this strain (see Fig. 3B).

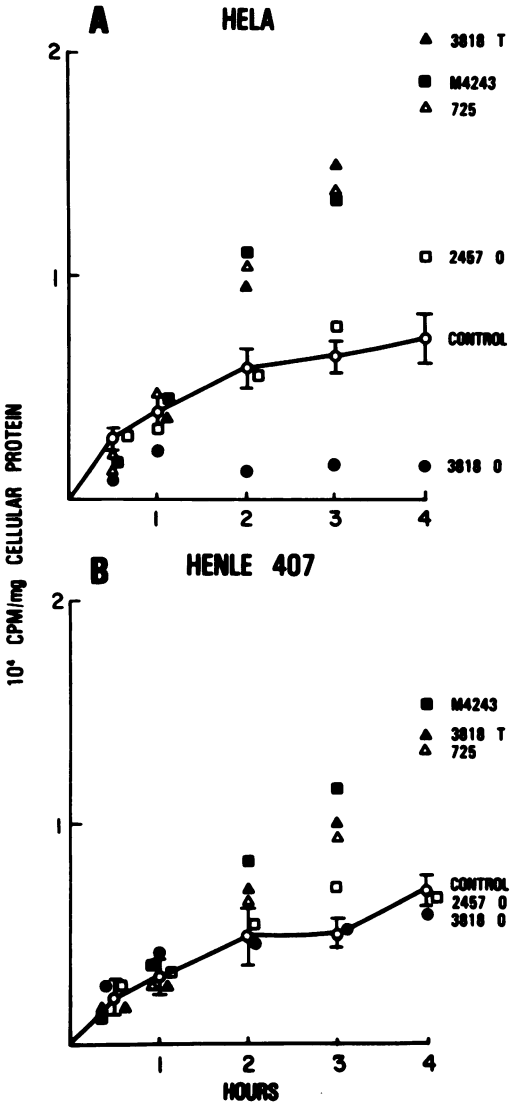

FIG. 4. *(A) Incorporation of [^{14}C]leucine into the particulate fraction of HeLa cells exposed to various strains of shigella. (B) Incorporation of labeled leucine into the particulate fraction of Henle 407 cells. Infection and labeling was carried out as described for Fig. 2, and incorporation of [^{14}C]leucine into the particulate fraction was deduced by subtracting counts in the supernatant fraction of the cell lysate from counts in the total lysate. Results are presented as the mean of four experiments, and the standard error of control experiments is included.*

Protein synthesis in cells infected with salmonellae. Figure 5 shows protein synthesis in HeLa cells infected with *S. typhimurium* W118. In contrast to HeLa monolayers infected with shigellae, these cells incorporated [^{14}C]leucine into macromolecular constituents at rates which were not significantly lower than those observed in control cells. Similar results were obtained in experiments with Henle 407 monolayers which were infected with strain W118 (data not shown).

DISCUSSION

It is well established that exogenous Shiga

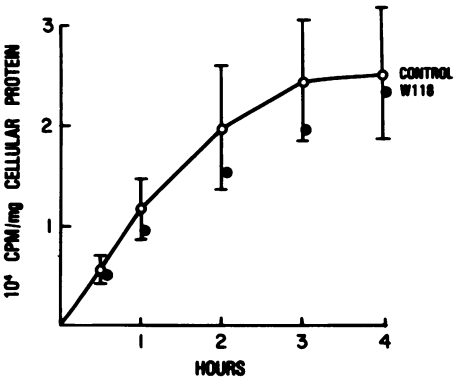

FIG. 5. *Incorporation of [^{14}C]leucine into trichloroacetic acid-precipitable material in the cytosol of HeLa cells infected with S. typhimurium W118* (●). *The protocol was as described for Fig. 2. Results are presented as the mean of four experiments, and the standard error of control experiments* (○) *is included.*

toxin is cytotoxic for certain cultured mammalian cell lines (13, 26, 31), but two observations make the relevance of these experiments unclear. Branham et al. (2) found no gross pathological alterations in the intestinal mucosa when large doses of crude Shiga toxin were added to isolated ileocecal pouches in monkeys. More recently, Donowitz and Binder reported that Shiga toxin injected into rat cecal loops elicited no histological mucosal alterations (6). Thus, Keusch and Jacewicz have proposed that there is a discontinuous distribution of toxin receptors on the intestinal epithelium (18). Receptors on the ileum and jejunum may mediate the enterotoxic (15) and cytotoxic (16) manifestations of Shiga toxin, whereas an absence of receptors on the colonic mucosa may render this organ resistant to the effects of toxin in the lumen of the bowel. Nonetheless, since virulent shigellae invade the colonic mucosa, it is possible that cytotoxin elaborated by intracellular organisms is involved in the destruction of infected epithelial cells.

Until recently it was thought that toxin production was limited to *S. dysenteriae* type 1 (the Shiga bacillus); however, it is now evident that Shiga-like toxins are also synthesized by *S. flexneri* and *Shigella sonnei* (18, 23). The biological activity of extracts from these non-Shiga strains is at least 1,000-fold less than that obtained from wild-type *S. dysenteriae* 1 strain 3818 T. The latter strain also produces at least 1,000-fold more cytotoxin in the cytosol of infected cells than does either hypotoxigenic mutant strain 725 (11) or *S. flexneri* 2a M4243 (unpublished data). It is reasonable to ask whether these hypotoxigenic strains produce sufficient cytotoxin to elicit manifestations in vivo or in vitro.

Inhibition of protein synthesis is the earliest physiological deviation thus far detected in mammalian cells exposed to Shiga toxin (4). Therefore, protein synthesis was used as a measure of cytotoxicity in tissue culture cells infected in vitro. Bacterial suspensions were centrifuged onto the surface of monolayers so that virtually all of the host cells were infected at approximately the same time (see Table 1). Thus, protein synthesis could be assayed in the entire cell population. Since a parasitic relationship existed in these cells, it was necessary to differentiate eucaryotic from procaryotic protein synthesis. This was accomplished by incubating infected cells with [^{14}C]leucine, gently lysing these cells, removing intact bacteria by differential centrifugation, and measuring the amount of radioactive label incorporated into macromolecular constituents of the supernatant fraction. Heavily infected cells have a tendency to detach from culture dishes, so the data were adjusted to compensate for the loss of host cell protein from the monolayers (see Fig. 1).

Employing the above protocol, protein synthesis was measured in toxin-sensitive HeLa and toxin-resistant Henle 407 cells which had been infected with shigellae. Both of these cell lines ceased to synthesize protein when infected with either overtly toxigenic or hypotoxigenic strains (see Fig. 2). Total [^{14}C]leucine accumulation was not significantly less than that found in control monolayers (see Fig. 3). This indicated that transport system L (leucine-preferring) was functional and that inhibition of protein synthesis in these infected cells was not due to defective amino acid uptake. In contrast to monolayers infected with invasive strains of shigella, HeLa cells exposed to strain 3818 O (a noninvasive but fully toxigenic mutant of *S. dysenteriae* 1) not only ceased to synthesize protein but also ceased to accumulate [^{14}C]leucine in the intracellular compartment. Because toxin-resistant Henle 407 cells were not similarly affected, it would appear that inhibition of protein synthesis in HeLa cells exposed to strain 3818 O was mediated by cytotoxin elaborated during the 30-min period of extracellular growth which preceded the addition of medium containing kanamycin. System L does not serve to concentrate amino acids but does mediate exchange of amino acids between endogenous and exogenous compartments (27). Intoxicated HeLa cells (which had ceased de novo protein synthesis) probably established an equilibrium of influx and eflux which precluded accumulation of large amounts of free leucine in the cytosol. Infected HeLa and Henle 407 cells continued to accumulate leucine because this amino acid was incorporated into the particulate fraction, i.e., into bacterial cell protein (see Fig. 4). Apparently intracellular shigellae inhibited host cell protein synthesis while scavenging amino acids from endogenous pools.

An invasive enteric pathogen from a genus which is not known to synthesize a Shiga-like toxin was employed as a nontoxigenic experimental control. *S. typhimurium* W118 infected approximately 80% of host cells after 2 h, and plate counts on disrupted monolayers indicated there were about 30 bacteria per cell (unpublished data). Although these monolayers were heavily infected, there was no loss of cellular protein over a 4-h period (unpublished data) and little inhibition of protein synthesis (see Fig. 5). Thus, infection of mammalian cells with gram-negative bacteria did not nonspecifically cause inhibition of protein synthesis. The implication is that invasive strains of shigellae elaborate a toxic material which specifically inhibits host cell protein synthesis. The toxin produced by these intracellular organisms is probably the

Shiga cytotoxin. Furthermore, this toxin is fully potent when released into the cytosol of Henle cells which are resistant to exogenous cytotoxin. Since toxin resistance in cultured mammalian cells has been equated with the absence (or masking) of a receptor on the plasma membrane (19), it is possible that invasive shigellae circumvent the requirement for a toxin receptor by multiplying intracellularly.

The nature of enteric disease in monkeys infected with *S. typhimurium* W118 or *S. flexneri* reveals the possible significance of the above observations. In the simian model, salmonella gastroenteritis is accompanied by relatively mild ileitis and colitis, whereas shigellosis is characterized by severe colonic involvement with congestion of the mucosa, frequent crypt abscesses, and ulcerative lesions (12). Histological studies showed few salmonellae in intestinal epithelial cells but many in the submucosa and lymphoid patches. Great numbers of shigellae were observed within the colonic epithelium, whereas relatively few organisms were found in submucosal areas (12, 28). It was concluded that salmonellae are transient residents of the intestinal mucosa, whereas shigellae lodge in intestinal epithelial cells, causing mucosal destruction and abscess formation. Ultrastructural analysis has shown that salmonellae are phagocytized by intestinal epithelial cells and transported to the basal membrane in endocytic vacuoles (29). Perhaps this can occur because salmonellae do not produce a Shiga-like toxin which immediately disrupts host cell metabolic functions. If shigellae inhibit protein synthesis in infected colonic epithelial cells with an efficiency analogous to that observed in vitro, they would be trapped within rapidly dying cells. In this manner intracellular shigellae may be confined to lateral spread from focal abscesses rather than being transported through an essentially intact epithelium. Thus the pathogenesis of invasive enteric disease could be influenced by a virulence factor such as the Shiga cytotoxin.

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