INFECTION AND IMMUNITY, July 1970, p. 89-95

Vol. 2, No. 1
Printed in U.S.A.

Uptake of ^{3}H-Dihydrostreptomycin by Macrophages in Culture

PETER F. BONVENTRE[1] AND JOHN G. IMHOFF

Department of Microbiology, University of Cincinnati, College of Medicine, Cincinnati, Ohio 45219, and Lister Institute of Preventive Medicine, London, England

Mouse peritoneal macrophages, in culture, concentrate significant amounts of ^{3}H-dihydrostreptomycin, provided that the incubation period is sufficiently extended. Macrophages cultured in vitro from both stimulated and unstimulated animals concentrate the antibiotic from growth or maintenance media. The increase in cell-associated radioactivity is linear for almost a week before a plateau is reached. Calculations based on intracellular volumes of the cells indicate that the intracellular concentration of dihydrostreptomycin may attain levels greater than five times that of the external milieu. No uptake is measurable at 4 C, suggesting an active mechanism of transport into the cell. Phagocytosis of killed bacteria during incubation did not increase uptake of the antibiotic nor did the addition of poly-L-ornithine to the medium augment uptake. A nonphagocytic cell line (BHK-21) concentrated ^{3}H-dihydrostreptomycin to a lesser extent than the macrophages. These observations suggest that a wide variety of mammalian cells may be permeable to the antibiotic, and thus potential bactericidal action on intracellular bacteria cannot be ignored.

Several years ago we reported (2) that mouse peritoneal macrophages were relatively impermeable to dihydrostreptomycin. That conclusion was based on observations made in short-term experiments where the cells were incubated with the antibiotic for a period of several hours. This conclusion must be modified now, in view of more recent experiments where macrophages in culture were incubated with dihydrostreptomycin for periods up to 1 week. Analysis of the kinetics of antibiotic uptake by macrophages reveals that dihydrostreptomycin is accumulated intracellularly over extended periods of time; furthermore, the process does not occur by simple diffusion, but it is probably mediated by an active mechanism of transport. This brief communication describes our more recent experiments.

MATERIALS AND METHODS

Animals. Swiss albino mice reared in the animal facilities of the Lister Institute of Preventive Medicine (London) and guinea pigs from Glaxo Laboratories (London) were used as the source of peritoneal cells for the initial experiments. Albino mice and Hartley strain guinea pigs for the later experiments were supplied by Laboratory Supply, Indianapolis, Ind., and Paul Hamm Rabbitry, Greenwood, Ind., respectively.

Collection of peritoneal macrophages. Both stimulated and unstimulated peritoneal cells were used in the experiments. In the former case, 3 ml of sterile glycogen (0.1%) was injected intraperitoneally (ip) 3 to 4 days prior to harvesting. Peritoneal cells were collected from the abdominal cavity by aspiration with Eagle's minimum essential medium (MEM) containing heparin. The cells were pooled and kept in an ice bath during the collection procedure; they were then washed once in cold medium and resuspended in MEM to a concentration of 10^6 cells per ml.

Cell culture procedure. The uptake experiments were done primarily with macrophage cultures grown in Leighton tubes with flying cover slips. Cell suspension (1 ml) was added to each culture tube, the cells were allowed to settle for 1 to 2 hr, and the medium was replaced by MEM plus 40% horse serum. This medium sustained the macrophage cultures for periods of up to 10 days without fluid change, provided an atmosphere of air and 5% CO_2 was maintained. In specific experiments, the peritoneal cells were grown in 60-mm petri dishes (Falcon 3002) rather than Leighton tubes.

Several experiments were done with BHK-21 cells. This established cell line was chosen so that the uptake of dihydrostreptomycin by a nonphagocytic cell line could be measured. The hamster kidney cell culture was obtained from the virology unit of the Lister Institute of Preventive Medicine. The cells were acid phosphatase negative and did not engulf killed *Staphylococcus aureus* after an incubation period of 1 hr at 37 C.

BHK-21 cells were cultured in a medium consisting of MEM, tryptose phosphate broth (0.3%), and 10% fetal calf serum. The cells were seeded in Leighton tubes at a concentration of 5×10^5 ml and incubated in an atmosphere of 5% CO_2 and air.

[1] Most of the investigation was carried out at the Lister Institute of Preventive Medicine, London, England, where P.F.B. was a visiting scientist.

Incubation of macrophage cultures with ^{3}H-dihydrostreptomycin. Mouse peritoneal cells were incubated in MEM plus 40% horse serum for several days in Leighton tubes before the experiments were started. This allowed the development of a relatively pure culture of macrophages since neutrophils and lymphocytes are suppressed under these cultural conditions (1). The medium was then changed to one containing tritiated dihydrostreptomycin (uniformly labeled; Nuclear Research Chemicals, Orlando, Fla., and Amersham Radiochemical Centre, Amersham, England) at concentrations of from 2 μc/ml to 10 μc/ml as described in the text. The radioactive purity of the tritiated dihydrostreptomycin was determined by chromatographic procedures (2). The macrophage cultures were incubated at 37 C, and cover slips were removed from the Leighton tubes at desired time intervals. The cover slips were rinsed thoroughly in cold MEM to remove unbound radioactivity and allowed to air dry. Measurements of uptake by the cells were done in quadruplicate at each interval. Radioactivity was expressed as counts per min per 50 μg of protein or counts per min per 10^6 cells. Protein was determined by the method of Lowry et al. (12) as modified by Oyama and Eagle (15) for tissue cultures.

Liquid scintillation counting. The rinsed and dried cover slip cultures were immersed in an upright position in small vials (12 by 45 mm) containing approximately 5 ml of a scintillation mixture of toluene, 2,5-diphenyloxazole, and 1,4-bis-2-(5-phenyloxazolyl)-benzene and sealed with plastic stoppers. The sealed vials containing the cover slips and scintillation fluid were then placed inside regulation-size, screw-capped, glass vials. This arrangement provided a constant geometry and circumvented the problems attendant with sample solubilization. Samples prepared in this manner were counted on a liquid scintillation counter (model CPM200; Beckman Instruments, Fullerton, Calif.).

Calculation of ^{3}H-dihydrostreptomycin uptake by cell cultures. It was assumed that cell-associated radioactivity after incubation at 4 C was antibiotic adsorbed to cell surfaces and did not represent intracellular dihydrostreptomycin. Thus, each experiment was done so that the radioactivity measured at 4 C was subtracted from that obtained at 37 C and the difference represented intracellular dihydrostreptomycin, [i.e., counts/min at 37 C − counts/min at 4 C = counts/min (intracellular)]. The intracellular concentration of dihydrostreptomycin was expressed in terms of a distribution ratio. This is defined as the ratio of the intracellular to the extracellular concentration and was calculated after making several other assumptions.

(i) The volume of 10^6 mouse macrophages was assumed to be 0.005 cm^3; $V = 4/3\ \pi r^3 = [1.33 \times 3.14 \times (1 \times 10^{-3})^3]\ 10^6 = 0.005$ cm^3/10^6 cells. This calculation assumes also that the cells are spherical with a mean radius of 10^{-3} cm (based on microscopic measurements).

(ii) That all of the intracellular volume was available for uptake was assumed; this is not entirely accurate since in most biological systems the intracellular fluids account for approximately 80% of the cell volume (Cirillo, *personal communications*); the assumption was made, however, since it eliminated possible bias in the direction of larger intracellular concentrations of the antibiotic. The distribution ratio was calculated from the relationship: distribution ratio = (counts per minute per 10^6 cells × 200)/(counts per minute per milliliter of medium) = [counts per minute (internal)/counts per minute (external)]. The factor of 200 in the numerator was obtained by dividing 1 ml by 0.005 ml (volume of 10^6 cells) so that the values could be expressed in terms of counts per minute per milliliter of fluid in both the numerator and the denominator. Since this allows cancellation of the units (milliliters), the distribution ratio is a unitless value. A value less than 1 shows that the intracellular concentration is less than that in the menstruum. Conversely, values greater than 1 indicate that the concentration inside the cells is greater than outside. Furthermore, distribution ratios greater than unity suggest an uptake by the cells against a concentration gradient and thus cannot represent simple diffusion.

Autoradiography. The Leighton tube cover slips were rinsed exhaustively in MEM containing no serum to remove all unbound ^{3}H-dihydrostreptomycin. In most instances, no fixative was employed since all of those used caused considerable leaching of the label from the cells. Occasionally, saturated picric acid was used as the fixative (2). The unfixed cells did not stain as well as the fixed cells, but localization of radioactivity was achieved. Cover slips were mounted on glass slides with Permount with cells facing upwards.

Macrophage cultures were prepared for autoradiography in a photographic dark room equipped with Ilford "S" safelight filters. When Ilford K5 emulsion was used, autoradiograms were prepared by the procedures outlined by Rogers (17). The emulsion-coated slides were exposed for several weeks at 4 C in plastic, light-tight boxes before developing in Ilford ID-19 developer. Care was taken to allow the slides to equilibrate at room temperature and for the developing solutions to be at approximately 20 C during the developing procedures, to avoid contraction and lifting of the emulsion from the glass surface. The cells were stained with double-strength Giemsa stain through the emulsion after developing of the autoradiograms. Photomicrographs were obtained with a Zeiss research microscope. Kodak NTB-3 emulsion was used to prepare autoradiograms for the later experiments done in Cincinnati. These are indicated in the text.

Chemical identity and biological activity of intracellular tritiated dihydrostreptomycin. Attempts were made to ascertain the chemical identity of the radioactive material taken up by the cells in culture. In view of the extended incubation periods used in the experiments, it was considered possible that the radioactivity found inside cells might be due to materials other than dihydrostreptomycin, i.e., degradation products or products of cell metabolism. Macrophages cultured in 60-mm petri dishes incubated with 10 μc of ^{3}H-dihydrostreptomycin per ml for a period of 1 week were washed exhaustively in cold MEM to re-

move unbound radioactive materials. The cells were disrupted by high-frequency sonic treatment, and the extracts of eight dishes were pooled and concentrated by evaporation. Fifty-microliter samples of the concentrated cell extracts were then spotted and chromatographed on paper together with reference samples of reagent-grade dihydrostreptomycin (Nutritional Biochemicals, Inc., Cleveland, Ohio). The radioactive peak was found to coincide with the peak of biological activity as assayed with a streptomycin-sensitive strain of *Bacillus subtilis* (2). Thus, it was concluded that the material concentrated within the cells represented intact dyhydrostreptomycin.

Attempts were also made to determine whether the cell extracts exhibited biological activity against the strain of *B. subtilis*. However, it was found that 0.2 ml (ca. 80 μg of protein) of a 10× concentrated cell extract was required before a demonstrable zone of inhibition was obtained. Because comparable quantities of extracts obtained from cells not incubated with the radioactive antibiotic also demonstrated a zone of inhibition, it was not possible to draw any conclusions as to the biological activity of the intracellular antibiotic (Fig. 1). Apparently, the absolute amounts of antibiotic were too small to be detected by the biological assay employed.

RESULTS

Stimulated mouse macrophages. Mice were injected (ip) with 3.0 ml of sterile glycogen (0.1%) 3 or 4 days before the peritoneal exudates were harvested. The mouse peritoneal cells were cultured in Leighton tubes at a concentration of approximately 10^6/ml in MEM plus 40% horse serum. After 24 to 48 hr, the fluid was changed and the cultures were incubated in the same medium as before, with the addition of 2.0 μc of ^{3}H-dihydrostreptomycin per ml.

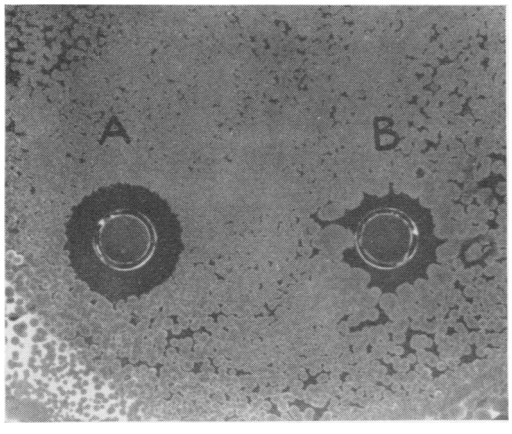

FIG. 1. *Inhibition of growth of B. subtilis by extracts of mouse peritoneal macrophages. A, Concentrated cell extract (0.2 ml; 80 μg of protein) from cultures incubated with 10 μc of ^{3}H-dihydrostreptomycin per ml for 1 week. B, Concentrated cell (0.2 ml) extract from cells incubated without tritiated dihydrostreptomycin.*

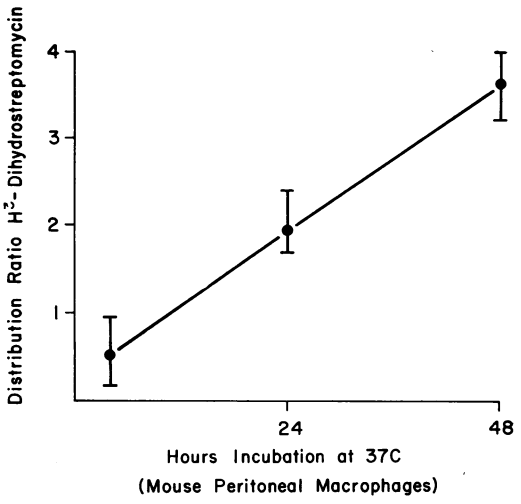

FIG. 2. *Distribution ratio of ^{3}H-dihydrostreptomycin in stimulated mouse peritoneal macrophages in culture. Macrophage cultures incubated with ^{3}H-dihydrostreptomycin at a concentration of 2.0 μc/ml. Composite data of three experiments with quadruplicate samples at each point.*

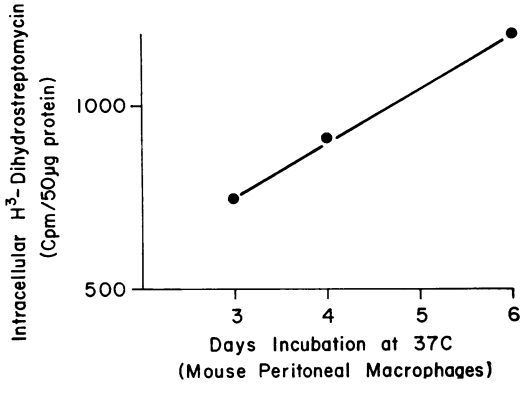

FIG. 3. *Kinetics of ^{3}H-dihydrostreptomycin uptake by stimulated mouse peritoneal macrophages in culture. Conditions same as given in legend to Fig. 2.*

After an initial period of 2 to 4 hr, when no measurable intracellular radioactivity could be detected, the antibiotic was concentrated by the macrophages in a linear fashion during the first 2 days of incubation at 37 C (Fig. 2). In view of the slow rate of uptake and the absence of a plateau which might indicate saturation, stimulated macrophages were incubated for longer periods of time. Figure 3 shows that such cultures continued to increase in cell-associated radioactivity for 6 days. Although significant variation

in uptake occurred in different samples, the distribution ratio attained a mean value of greater than 3 in 48 hr and continued to increase over the next 4 days.

Poly-L-ornithine, a basic polyamino acid, has been shown to act on plasma membranes. It enhances uptake of human serum albumin (18) and alters the membranes of diphtheria toxin-resistant L cells in such manner as to render them sensitive to the action of the toxin (13). The mechanism which has been invoked to account for these observations is stimulation of pinocytosis. When polyornithine was incubated with mouse macrophages at a concentration of 3 μg/ml, no enhancement of dihydrostreptomycin uptake was observed. Concentrations of 5 μg/ml and greater were toxic for the macrophages and destroyed the cell integrity within several hours. This would indicate that phagocytic cells are more sensitive to the action of polyamino acids than are nonphagocytic, established tissue cultures such as Sarcoma-180 and L cells. Differences in the serum concentration between 5 and 40% also had no significant effect on dihydrostreptomycin uptake by mouse macrophages.

Unstimulated mouse macrophages. In view of the possibility that stimulated macrophages might be different in membrane-associated activities than their normal counterparts, cells from the unstimulated mouse peritoneum were also tested for their ability to concentrate dihydrostreptomycin. The macrophage cultures were established as with the stimulated cells and allowed to grow for 3 to 4 days before addition of the tritiated dihydrostreptomycin. The kinetics of ^{3}H-dihydrostreptomycin uptake by unstimulated mouse macrophages over an 8-day period is shown in Fig. 4. The intracellular concentration of antibiotic increased during the initial 6-day period to the same extent as was observed with the stimulated macrophages. Incubation beyond 6 days did not result in further intracellular accumulation, indicating that a saturation had been achieved. The intracellular concentration of dihydrostreptomycin after 6 days in this set of experiments was five times greater than in the external medium.

In contrast, iodinated human serum albumin (HSA-^{125}I; Radiochemical Centre, Amersham, England) under the same experimental conditions was virtually excluded during a 3-day incubation. Small but significant amounts of serum albumin are taken into mammalian cells by a process of pinocytosis (18), but the turnover rate of foreign macromolecules within cells is quite rapid (7). Therefore, it is possible that the constant low level of activity associated with the macrophages incubated with HSA-^{125}I represents an equilibrium established between uptake of the protein and digestion by lysosomal enzymes.

Mouse peritoneal macrophages were incubated with killed *S. aureus* and ^{3}H-dihydrostreptomycin to determine whether the phenomenon of "piggyback" phagocytosis (19) could be demonstrated. During a 24-hr period, however, actively phagocytizing and nonphagocytizing macrophage cultures concentrated the antibiotic to the same extent. Prolonged experiments were not possible since the phagocytizing cells were ultimately destroyed by overloading with ingested bacteria. This evidence suggests that the movement of dihydrostreptomycin into the cell is a specific phenomenon and does not represent bulk flow of external medium into the cell by the process referred to as liquid endocytosis (9).

Guinea pig macrophages and nonphagocytic cells. Phagocytic cells from another species were tested for their ability to concentrate dihydrostreptomycin. Albino guinea pigs were injected ip with 30 ml of sterile glycogen (0.1%) 3 days before the exudates were obtained. The cells were seeded into Leighton tubes at a concentration of 1.5×10^{6}/ml in serum-free MEM. After 2 hr, the attached cells were rinsed and incubated in MEM containing 20% guinea pig serum and ^{3}H-dihydrostreptomycin at a concentration of 2 μc/ml. The kinetics of uptake by the

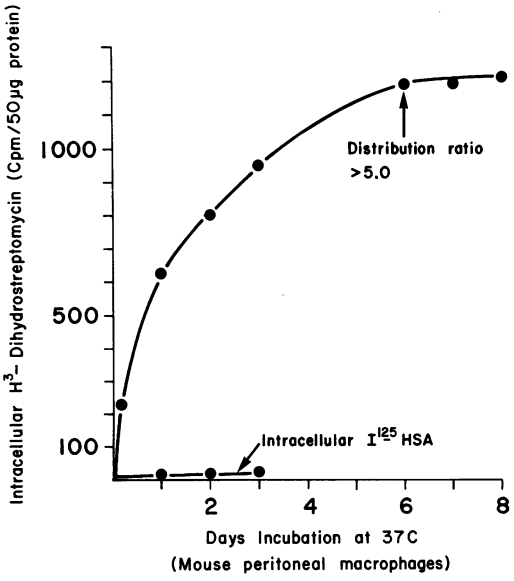

FIG. 4. *Intracellular concentration of ^{3}H-dihydrostreptomycin and human serum albumin (HSA)-^{125}I in unstimulated mouse peritoneal macrophages. Cultures were incubated with ^{3}H-dihydrostreptomycin or HSA-^{125}I at a concentration of 2 μc/ml of tissue culture fluid.*

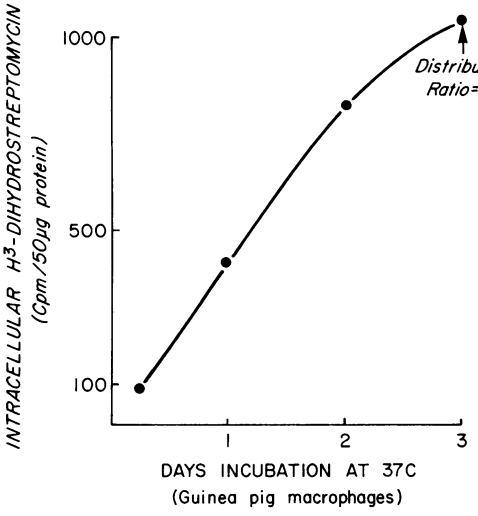

FIG. 5. *Kinetics of dihydrostreptomycin uptake by stimulated guinea pig peritoneal macrophages in culture. Conditions same as given in legend to Fig. 2.*

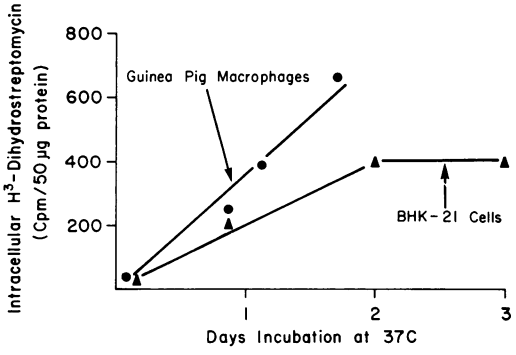

FIG. 6. *Uptake of ^{3}H-dihydrostreptomycin by guinea pig macrophages and BHK-21 cultures. Conditions same as given in legend to Fig. 2.*

guinea pig macrophages are shown in Fig. 5. The rate and extent of streptomycin concentration within guinea pig macrophages were essentially the same as observed in the mouse cell cultures (Fig. 4).

Because overtly phagocytic cells of the reticuloendothelial system are uniquely active in terms of plasma membrane flow, it was considered important to measure streptomycin uptake by a nonphagocytic cell. The established tissue culture cell line of baby hamster kidney (BHK-21) was used for this experiment. BHK-21 cell cultures concentrated dihydrostreptomycin in measurable quantities, but the extent of uptake was less than that which occurred in the phagocytic cells (Fig. 6). This observation is significant since it suggests that mammalian cells other than those of the reticuloendothelial system are able to concentrate streptomycin. The extent to which this phenomenon occurs should be investigated because it has definite clinical implications.

Autoradiography. Intracellular ^{3}H-dihydrostreptomycin was localized by autoradiography. Mouse macrophages cultured in Leighton tubes were incubated with tritiated antibiotic for 7 to 10 days. In spite of the difficulties attendant with localization of water-soluble compounds, several good preparations were obtained. Figure 7 and 8 show the intracellular localization of streptomycin in the mouse peritoneal macrophages with two different autoradiographic emulsions. Both the fine-grained Ilford and the larger grained Kodak NTB-3 emulsions show a concentration of exposed grains above the cells. Stained preparations revealed that the streptomycin was localized primarily in the cytoplasm since radioactivity above the nucleus was minimal.

DISCUSSION

The polemic as to whether or not mammalian cells are permeable to streptomycin has arisen

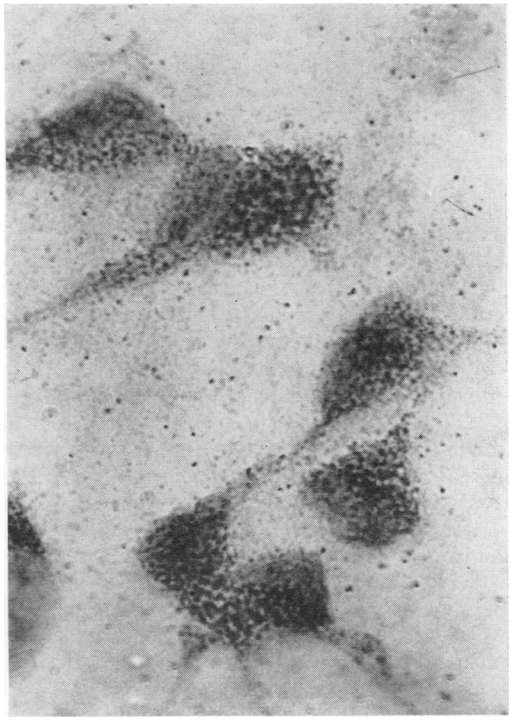

FIG. 7. *Autoradiograph of mouse peritoneal macrophage culture incubated with 4 µc of ^{3}H-dihydrostreptomycin per ml for 10 days. Ilford liquid emulsion K5. Unfixed preparation. Unstained phase-contrast photomicrograph.*

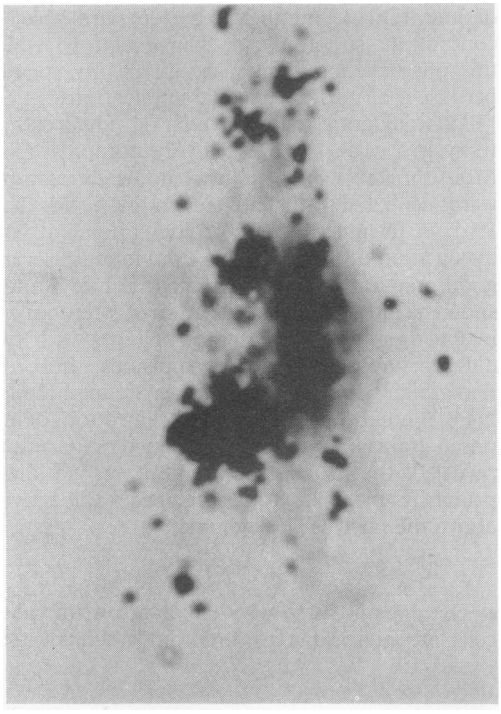

FIG. 8. *Autoradiograph of mouse peritoneal macrophage incubated with 5 μc of ^{3}H-dihydrostreptomycin per ml for 10 days. NTB-3 (Kodak) liquid emulsion. Cells fixed in picric acid prior to coating with emulsion. Giemsa stain.*

as a result of conclusions based on indirect evidence. That is to say, bactericidal activity on intracellular bacteria has, by and large, been the criterion employed. The data presented here are, as far as we can tell, the first which show by direct means that this aminoglycoside antibiotic is concentrated intracellularly by mammalian cells. The kinetics of uptake is unexpected, in that the process appears to be extremely slow as compared to other substances which are transported across cell membranes. There seems to be no doubt, however, that uptake of dihydrostreptomycin by macrophages is an active process which requires metabolic energy since low temperatures ablate uptake. The mechanism by which dihydrostreptomycin is taken into macrophages has not been determined. The kinetics of intracellular accumulation would suggest that the mechanism is pinocytosis rather than a form of permeation, such as active transport or facilitated diffusion. Cohn (*personal communication*) suggests that a quantitative estimate of vesicle formation during uptake and the rate of dihydrostreptomycin egress from the macrophages when they are placed in an antibiotic-free medium would provide information which might make a choice between permeation and pinocytosis possible. Jacques (9), however, feels that such a choice is at times very difficult to make since concentration of an extracellular compound within cell vesicles does not invariably mean that the compound has been taken in by a pinocytic event. He points out that substances such as neutral red reach lysosomes by two successive permeation crossings of the plasma and lysosomal membranes.

The fact that macrophages and nonphagocytic cells can concentrate dihydrostreptomycin, provided that contact between the cell and the antibiotic is sufficiently long, is important from both a clinical and experimental standpoint. In view of the extended time required for intracellular accumulation of streptomycin, it is necessary that the antibiotic not be degraded by cellular enzymes so that bactericidal concentrations within the cells can be reached. Our data on that point are equivocal. Extracts of macrophages incubated with tritiated dihydrostreptomycin contained materials which by chromatographic methods were undegraded antibiotic. Biological activity of these extracts could not be demonstrated with certainty. The intralysosomal accumulation of dyes, drugs (4), dextran, and detergents (substances which apparently do not serve as substrates for lysosomal enzymes; reference 8) is a well-established fact. It should also be made clear that biological activity in the case where the compound is concentrated within vesicles may be minimized by compartmentalization. Unless extensive vesicle fusion occurs, this would constitute a membrane barrier between the antibiotic and the bacteria which also reside within membrane-bound phagosomes.

A search of the literature would strongly suggest that streptomycin is bactericidal for facultative intracellular parasites, provided two conditions are met: (i) extended incubation of cells with the antibiotic, and (ii) relatively high antibiotic concentration in the extracellular menstruum. Recent experiments by Chang (3) appear to prove that is is the case. He circumvented the problem which makes the use of antibiotics necessary, namely, extracellular multiplication of bacteria. This was accomplished by using *Mycobacterium lepraemurium* as a test organism. It is an obligate intracellular parasite, has a generation time of 7 days, and is streptomycin sensitive. Thus, the problem of extracellular division during the 4-week length of the experiment was avoided. *M. lepraemurium* cells residing within the mouse peritoneal macrophages were killed

when the cell cultures were incubated in a streptomycin-containing medium for extended periods. Over the 4-week period, suppression of intracellular bacterial growth was related directly to the length of incubation and the concentration of streptomycin in the external menstruum.

Many others have reported that streptomycin may be bactericidal for intracellular bacteria (5, 6, 10, 11, 14, 16, 20–22), but none of these investigations has demonstrated the fact as unequivocally as that of Chang (3). One can conclude, therefore, that streptomycin and other antibacterial compounds must be used judiciously in studies dealing with intracellular killing of microbes and also in studies of "cell immunity" where the potential bactericidal effects of streptomycin are usually ignored.

ACKNOWLEDGMENTS

The senior author expresses his sincere appreciation to Ashley Miles, the director of the Lister Institute of Preventive Medicine, where this investigation was initiated. The enthusiastic help of Guy and Elinor Meynell of the Lister Institute is also gratefully acknowledged.

The work was supported by the United States Army Medical Research and Development Command under contract DA 49 193 MD 2832, by a Public Health Service Career Development Award (AI-K3 16,999) to P. F. B. from the National Institute of Allergy and Infectious Diseases, and by an interdisciplinary grant from the National Aeronautics and Space Agency to the Institute of Space Sciences, University of Cincinnati.

LITERATURE CITED

1. Bennett, B. 1966. Isolation and cultivation in vitro of macrophages from various sources in the mouse. Amer. J. Pathol. 48:165–181.
2. Bonventre, P. F., R. Hayes, and J. Imhoff. 1967. Autoradiographic evidence for the impermeability of mouse peritoneal macrophages to tritiated streptomycin. J. Bacteriol. 93: 445–450.
3. Chang, Y. T. 1969. Suppressive activity of streptomycin on the growth of *Mycobacterium lepraemurium* in macrophage cultures. Appl. Microbiol. 17:750–754.
4. Dingle, J. T., and A. J. Barrett. 1969. Uptake of biologically active substances by lysosomes. Proc. Roy. Soc. Ser. Biol. Sci. 173:85–93.
5 Ekzemplyarow, O. N. 1966. Effect of tetracycline, chloramphenicol, monomycin and streptomycin on *S. typhimurium* after phagocytosis by macrophages. Fed. Proc. (Translation Suppl.) 25:T309–T312.
6. Freeman, B. A., and L. R. Vana. 1958. Host-parasite relationships in brucellosis. I. Infection of normal guinea pig macrophages in tissue culture. J. Infec. Dis. 102:258–267.
7. Gabathuler, M. P., and H. J. P. Ryser. 1969. The digestive function of lysosomes as studied by the turnover of ingested foreign macromolecules. Proc. Roy. Soc. Ser. Biol. Sci. 173:95–98.
8. Hart, P. D. 1968. *Mycobacterium tuberculosis* in macrophages: effect of certain surfactants and other membrane-active compounds. Nature (London) 162:686–689.
9. Jacques, P. J. 1969. Endocytosis, p. 395–420. *In* J. T. Dingle and H. B. Fell (ed.), Lysosomes in biology and pathology. North-Holland, Amsterdam.
10. Jenkin, C. R., and B. Benecerraf. 1960. In vitro studies on the interaction between mouse peritoneal macrophages and stains of *Salmonella* and *E. coli*. J. Exp. Med. 112:403–417.
11. Kochan, I., and L. Smith. 1965. Antimycobacterial activity of tuberculostatic factor on intracellular bacilli. J. Immunol. 94:220–227.
12. Lowry, O. H., N. J. Rosebrough, A. L. Farr, and R. J. Randall. 1951. Protein measurement with the Folin phenol reagent. J. Biol. Chem. 193:265–275.
13. Moehring, J. M., and T. J. Moehring. 1968. The response of cultured mammalian cells to diphtheria toxin. II. The resistant cell: enchancement of toxin action by poly-L-ornithine. J. Exp. Med. 127: 541–554.
14. Oxman, E., and P. F. Bonventre. 1967. Facilitated uptake of streptomycin by Kupfer cells during phagocytosis. Nature (London) 213:294–295.
15. Oyama, V. I., and H. Eagle. 1956. Measurement of cell growth in tissue culture with a phenol reagent (Folin-Ciocolteau). Proc. Soc. Exp. Biol. Med. 91:305–307.
16. Patterson, R. J., and G. P. Youmans. 1970. Multiplication of *Mycobacterium tuberculosis* within normal and "immune" mouse macrophages cultivated with and without streptomycin. Infec. Immun. 1:30–40.
17. Rogers, A. W. 1967. Liquid-emulsion techniques for grain-density autoradiograms, p. 266–271. *In* Techniques of autoradiography. Elsevier Publishing Co., New York.
18. Ryser, H. J. P. 1968. Uptake of protein by mammalian cells: an underdeveloped area. Science 159:390–396.
19. Sbarra, A. J., W. Shirley, and W. A. Bardawil. 1962. "Piggyback" phagocytosis. Nature (London) 194:255–256.
20. Shepard, C. C. 1957. Use of HeLa cells infected with tubercle bacilli for the study of anti-tuberculous drugs. J. Bacteriol. 73:494–498.
21. Showacre, J. L., H. E. Hopps, H. G. Dubuy, and J. E. Smadel. 1961. Effect of antibiotics on intracellular *Salmonella typhosa*. I. Demonstration by phase microscopy of prompt inhibition of intracellular multiplication. J. Immunol. 87:153–161.
22. Suter, E. 1952. The multiplication of tubercle bacilli within normal phagocytes in tissue culture. J. Exp. Med. 96:137–150.