THE UPTAKE AND DIGESTION OF IODINATED HUMAN SERUM ALBUMIN BY MACROPHAGES IN VITRO*

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(Received for publication 23 June 1967)

In his original descriptions of pinocytosis, Lewis suggested that pinocytosed macromolecules were ultimately digested by intracellular hydrolases (1). Since then, his suggestion has been confirmed by studies in a variety of cell types. Evidence for a lysosomal localization of pinocytosed material has accumulated (2, 3) and the digestive function of lysosomes has been established (4). However, most of what is known about intralysosomal digestion has come from studies of the fate of colloidal and particulate (5–7) material ingested by phagocytic cells. Bensch et al. observed that phagocytosed DNA coacervates underwent rapid dissolution within fibroblast lysosomes (5), and found evidence for cellular utilization of the products of digestion (8). In biochemical studies, Cohn showed that mammalian phagocytes digested labeled bacteria to low molecular weight material which they excreted into the medium (6). At present very little is known about the uptake and extent of intracellular digestion of soluble macromolecules, or the fate of the products of digestion.

Methods of harvesting and culturing homogeneous populations of macrophages have recently made possible extensive in vitro studies of pinocytosis. The biochemical and morphological correlates of pinocytosis by cultivated mouse peritoneal macrophages have been described in detail (9–14). When cultivated in the presence of calf serum, macrophages pinocytose continually, accumulating serum proteins and other constituents of the medium. Large numbers of clear, fluid-filled vacuoles are transferred into the perinuclear zone. There they acquire hydrolytic enzymes, become phase dense, and are transformed into secondary lysosomes. Both pinocytosed molecules and hydrolytic enzymes are present within the secondary lysosome, which is presumably the site of intracellular digestion. This article is concerned with the uptake and digestion of iodinated human serum albumin, pinocytosed by macrophages in vitro.

* This investigation was supported in part by Research Grant AI 07012-02, United States Public Health Service, National Institute of Allergy and Infectious Diseases, National Institutes of Health, Bethesda, Md.

Materials and Methods

Harvesting Macrophages.—Methods of harvesting and cultivating mouse peritoneal macrophages were based on those described by Cohn and Benson (9). The mice employed for these studies were males and females of the NCS strain, weighing about 25 g. Mice were killed rapidly with chloroform and the abdominal skin was reflected. 1–2 ml of heparinized phosphate buffer was injected intraperitoneally. The peritoneum was then perforated and the fluid withdrawn. The exudate cells were sedimented by spinning at 800 rpm for 10 min. They were then resuspended in 20% newborn calf serum (nbcs) medium at a concentration of 2-2.5 $\times$ 10^6 cells/ml. The culture medium consisted of medium 199 and nbcs, both from Microbiological Associates, Inc., Bethesda, Md., and penicillin at a concentration of 3000 units/ml.

Cultivating Macrophages.—For quantitative studies, cells were cultured in 15 or 30 cm^2 T-flasks. 4 ml of fresh cell suspension was added to small T-flasks; 10 ml, to the large T-flasks. The flasks were gassed with a 5% CO_2-air mixture and closed with rubber stoppers. The flasks were then kept at 37°C for 1 hr to allow the macrophages to adhere to the glass surface. Then the medium was withdrawn and the attached cells were washed vigorously with medium 199. After removal of the second wash, fresh complete medium was added. The flasks were gassed and incubated at 37°C.

Iodinated Compounds.—^{131}I-labeled human serum albumin (^{131}I-HSA) was obtained as a sterile solution from Abbott Laboratories, North Chicago, Ill., at a specific activity of 0.01 mc/mg. Immediately prior to use it was filtered through Dowex 1X-4 (J. T. Baker Chemical Co., North Phillipsburg, N. J.). Monoiodotyrosine-^{131}I and diiodotyrosine-^{131}I with specific activities of approximately 0.25 mc/ml, were also obtained from Abbott Laboratories. No attempt was made to remove inorganic iodide from the iodinated tyrosine preparations.

^{125}I-HSA was prepared by the method of Helmkamp et al. (15), using 5 mc of ^{125}I for 10 mg of HSA. Crystalline HSA was obtained from Pentex, Inc., Kankakee, Ill. The resulting ^{125}I-HSA had a specific activity of about 0.3 mc/mg. Less than 1.5% of the radioactivity of the ^{125}I-HSA preparations was soluble in cold 10% trichloroacetic acid (TCA). Solutions of ^{125}I-HSA were sterilized by filtration through an 0.45 μ Millipore filter (Millipore Filter Corp., Bedford, Mass.) and used within a week of preparation.

Bovine gamma globulin (Bovine fraction II, Pentex, Inc.) was iodinated by the same method, using 2.5 mc of ^{125}I for 10 mg of protein.

Preparation of Macrophage Cultures for Radioactivity Measurements.—For quantitative studies of iodinated HSA (I*-HSA) uptake, macrophages were cultivated in 15 cm^2 T-flasks in 4 ml of medium containing 10^6-10^7 cpm/ml I*-HSA. At the end of the incubation period, the radioactive medium was removed by aspiration and the cell sheet was washed with six 5 ml aliquots of saline. After the last wash was removed, 1.5 ml of distilled water was added to the flask. The flask was then frozen and thawed five times to detach the cells from the glass surface. 1 ml of the cell suspension was counted in a Nuclear-Chicago automatic well counter (Nuclear-Chicago Corp., Des Plaines, Ill.). The Folin test for protein (16) was performed on the same sample after counting.

The efficacy of the washing procedure was tested by measuring the radioactivity of cells which had been washed various numbers of times. Only four saline washes were required to remove all isotope from cells which had been exposed to radioactive medium (5×10^6 cpm/ml) for 5 min or less. The radioactivity of cells which had been exposed to isotope for 24 hr reached a constant value between three and six washes. Washes in excess of six produced no further decrease in cell-associated isotope or cell protein. These tests established that six saline washes were sufficient to remove adsorbed isotope without apparent loss of intracellular material or cells.

For studies of the fate of I*-HSA, macrophages were cultivated in 30 cm^2 T-flasks, since more cell suspension and medium were required for analysis. Each flask contained 8 or 10 ml

of medium and 10^7 cells. The cell sheets were washed with six 10 ml aliquots of saline and frozen in 3 ml of distilled water.

Radioautography.—Cells were grown on coverslips in Leighton tubes in medium containing about 10^8 cpm/ml ^{125}I-HSA. After 24 hr of incubation, the cells were washed twice with 20% nbcs and then rinsed in a large volume of warm saline. The cells were fixed at 4°C for 10 min in 1.25% glutaraldehyde, pH 7.5. The dried coverslips were dipped in Ilford L-4 emulsion as described by Cohn and Benson (10) and exposed for 7–10 days. After development, the coverslips were mounted in distilled water and examined under oil emersion, phase contrast illumination with a Zeiss Ultraphot Model II (Carl Zeiss, Inc., N.Y.).

Analysis of Radioactive Material Released by Cells.—To prepare quantities of material excreted by pulsed cells, the cells in six large T-flasks were pulsed for 24 hr in 50% nbcs containing ^{125}I-HSA, washed gently, and reincubated in isotope-free 1% nbcs. After 24–48 hr of "cold" incubation, the media were removed and pooled. 10 ml of cold 30% TCA was added to each 20 ml of medium. After sitting for 1 hr or more on ice, the suspensions were centrifuged. The TCA supernatants were extracted 10 times with equal volumes of ether to remove TCA. No radioactivity was lost during the ether extraction. The pooled supernatants were then evaporated to dryness and redissolved in about 3 ml of distilled water for chromatography.

Paper chromatography was performed on Whatman No. 1 paper with butanol:acetic acid:water (4:1:1) as a solvent (7, 17). Samples were applied to two separate 1.5 inch segments of a line drawn parallel to the solvent front. The reference compound, monoiodotyrosine-^{131}I, was applied in 10 μl on one 1.5 inch segment. Both 1.5 inch segments were then overlaid with 75–100 μl of the culture medium sample. This procedure insured that the sample and the reference compound were exposed to the same concentrations of salts and other acid-soluble components of medium 199 during development of the chromatogram. After development by descending chromatography for 16 hr, the paper was dried and cut into 1 cm strips parallel to the solvent front. The strips were cut into halves corresponding to the sample and to the reference compound and counted in the well counter.

Chromatography was also performed on a 45 × 1 cm column of Biogel P-2 (BioRad Laboratories, Richmond, Calif.) in 0.05 M phosphate buffer, pH 6.8. The void volume was determined with blue dextran-2000, molecular weight two million (Pharmacia, Uppsala, Sweden). 1 ml of sample was applied to the column and 2 ml fractions were collected for radioactivity measurements.

For analysis of the whole media, the 1% nbcs washout media from six flasks were pooled and concentrated 10 times by flash evaporation. 1 ml of concentrate was applied to a 45 × 1 cm column of Sephadex G-200 (Pharmacia) and eluted with 0.05 M tris(hydroxymethyl)-aminomethane (Tris) 0.1 M NaCl, pH 8.53.

Other Chemicals.—Dextran sulfate of molecular weight two million (Sodium Dextran Sulfate 2000) was obtained from Pharmacia. D,L-parafluorophenylalanine was from Nutritional Biochemical Corp., Cleveland, Ohio. 2,4-dinitrophenol was obtained from Eastman Organic Chemicals, Rochester, N.Y.

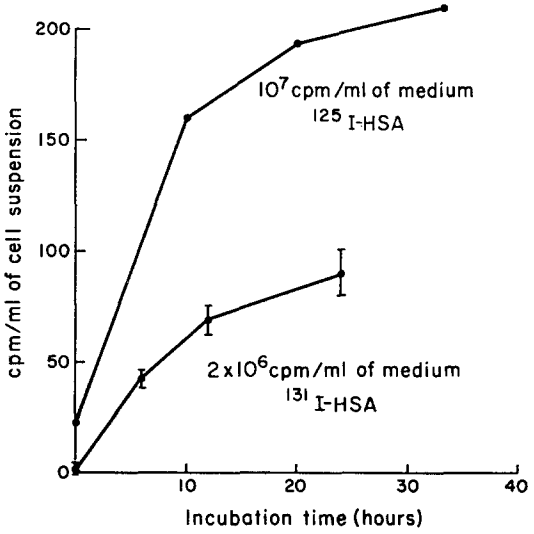
Stock solutions and dilutions were prepared immediately prior to the experiment. The pH of all stock solutions was adjusted to 7.3 with either 1 N NaOH or HCl.

RESULTS

Uptake of I-HSA.*—Initial experiments were performed to find conditions under which macrophages ingested adequate amounts of I*-HSA and to establish that the mechanism of uptake was pinocytosis.

For uptake experiments, freshly harvested macrophages were exposed to

I*-HSA in 50% nbcs from the beginning of their in vitro cultivation. The resulting amount of cell-associated radioactivity depended on the length of cultivation and the activity of the medium, as shown in Text-fig. 1. Using medium containing 2×10^6 cpm/ml of ^{131}I-HSA, the amount of isotope accumulated by cells in 24 hours corresponded to 3×10^{-2} μg of albumin per ml of cell suspension, or about 10^{-2} μg per million cells. On the basis of these experiments, a "pulse" extending 24 hr from the start of cell cultivation was chosen for the ingestion period in further studies. To obtain adequately labeled cells, 5×10^7 cpm/ml ^{125}I-HSA was used in the medium. Then, after 24 hr, the activity of the cell suspension was in the order of 1000 cpm/ml.



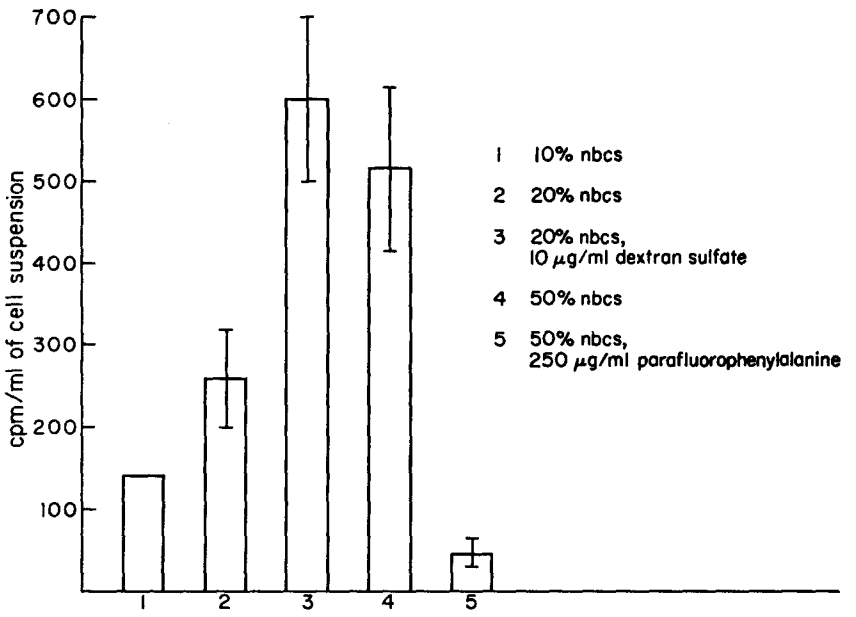
TEXT-FIG. 1. The uptake of I*-HSA by macrophages cultivated in 50% nbcs.

In view of the possibility that adsorption and pathological permeability changes might account for some or all of the observed I*-HSA uptake (18), experiments were undertaken to determine whether the mechanism of uptake was pinocytosis. As shown in Text-fig. 2, conditions for I*-HSA uptake correlate well with conditions favorable for pinocytic vesicle formation. Optimal uptake occurred in 50% nbcs, in which macrophages are known to pinocytose vigorously and increase in protein content and hydrolase activity (11). At lower serum concentrations, in which macrophages are less active pinocytically and metabolically (11), less I*-HSA uptake occurred.

Morphological studies have shown that parafluorophenylalanine, an inhibitor of protein synthesis, inhibits pinosome formation and macrophage maturation in 50% nbcs (10, 19). At a pinocytosis-inhibiting and sublethal concentration,

parafluorophenylalanine practically abolished the uptake of I*-HSA, as shown in Text-fig. 2. On the other hand, dextran sulfate, a polyanionic stimulator of pinocytosis (20), enhanced the uptake of I*-HSA above that observed in 20% nbcs alone.

The Intracellular Localization of ^{125}I-HSA.—The localization of cell-associated ^{125}I-HSA was studied by autoradiography. Figs. 1 and 2 show cells which had been incubated for 24 hr in 50% nbcs containing ^{125}I-HSA. The silver grains,



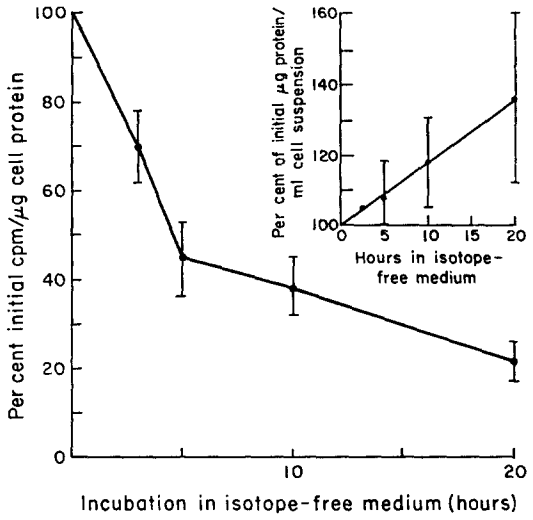
TEXT-FIG. 2. The uptake of I*-HSA in various media after 24 hr of incubation. All media contained 5×10^6 cpm/ml ^{131}I-HSA or ^{125}I-HSA.

which are dark in these photomicrographs, are localized over the perinuclear region of the cells, where granules of pinocytic origin congregate. The number of grains over the nucleus and peripheral cytoplasm did not exceed background. The absence of peripheral label indicates that ^{125}I-HSA was dilute in inward-migrating pinosomes, becoming more concentrated in the resulting central granules. The concentration of grains in the perinuclear granules would result from the continual input of fresh label, as incoming pinosomes fuse with pre-existing granules (11). Further concentration could result from the loss of low molecular weight contents which presumably accompanies the shrinking and increasing phase density of perinuclear granules (11). In contrast to the usual distribution of grains, occasional dead cells displayed heavy and uniform intra-

cellular label, suggesting a pathological increase in their permeability to macromolecules. The perinuclear distribution of grains in the vast majority of cells provides direct evidence that pinocytosis was the normal mechanism of ^{125}I-HSA uptake.

Fate of Pinocytosed ^{125}I-HSA.—The fate of intracellular ^{125}I-HSA was next investigated.

Freshly harvested macrophages were pulsed in 50% nbcs for 24 hr. At the end of the pulse, the radioactive medium was removed and the cell sheets were washed gently in 10 ml of me-

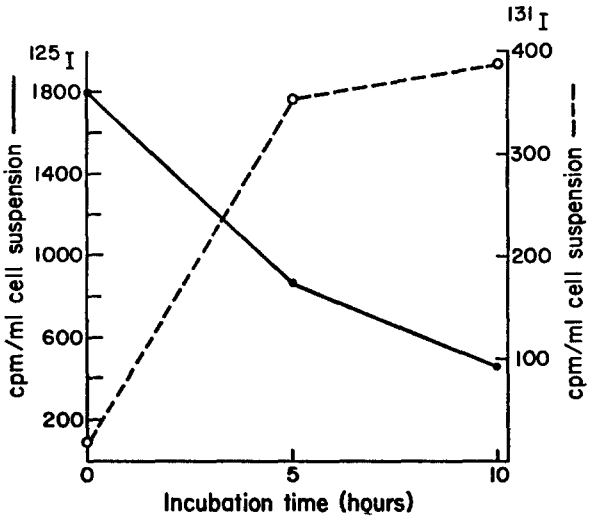


TEXT-FIG. 3. The loss of isotope from pulsed cells during a washout period in 50% nbcs. The insert shows the relative increase in cell protein during the same period.

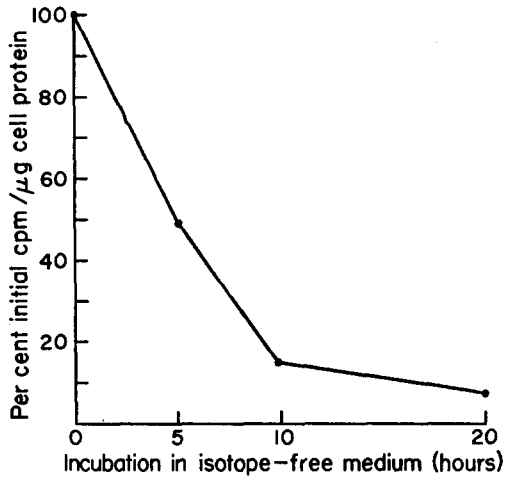
dium 199 and then in 10 ml of 20% nbcs. A final wash consisted of a 15 min incubation in 20% nbcs at 37°C, followed by the addition of fresh medium. This washing procedure, chosen to minimize disturbance of the cells, did not quantitatively remove adsorbed isotope from the flasks. However, the residual radioactivity in the final medium, about 500 cpm/ml, was much too low to result in measurable pinocytic uptake, and was easily removed by the final saline washes.

Text-fig. 3 shows the change in intracellular radioactivity during a washout period in 50% nbcs. The results are expressed in terms of relative specific activities to facilitate the comparison of experiments employing different media. During the first 5–10 hr of the washout period, intracellular isotope decreased in much the same manner whether expressed in terms of absolute or specific activities. Thus, neither changes in cell number nor total cell protein could account for the decrease in intracellular isotope. In fact, during a washout period in 50% nbcs, total cell protein tended to increase (Text-fig. 4), as expected for maturing

macrophages (9). Further evidence of cell viability during a 50% nbcs washout period was provided by the demonstration of ongoing pinocytosis. If cells which had been pulsed with ^{125}I-HSA were washed and reincubated in medium containing ^{131}I-HSA, normal uptake of the second isotope occurred, as shown in



TEXT-FIG. 4. The release of ^{125}I-activity from pulsed cells and the simultaneous uptake of ^{131}I-HSA (5×10^6 cpm/ml of medium), added to the washout medium.

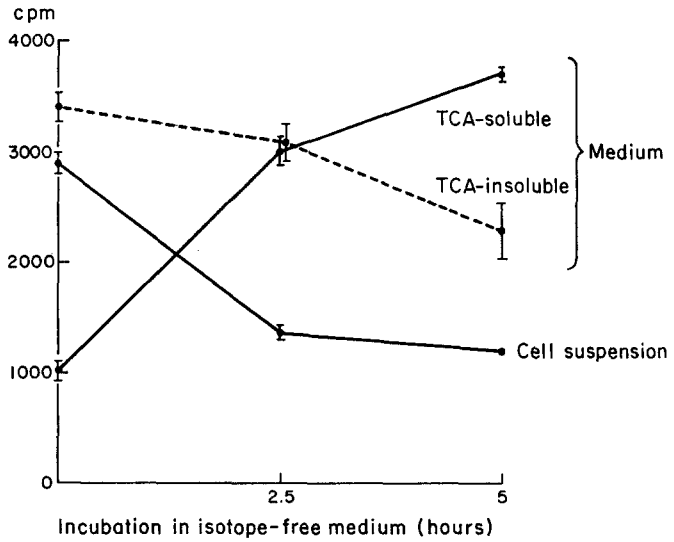


TEXT-FIG. 5. The loss of isotope from cells pulsed with ^{125}I-bovine gamma globulin.

Text-fig. 4. Thus the decrease in cell-associated isotope could not be attributed to impairment of normal cellular activity.

A similar loss of cell-associated radioactivity occurred after a short pulse of ^{125}I-bovine gamma globulin, as shown in Text-fig. 5.

During the washout period the TCA solubility of the radioactivity in the medium increased, as shown in Text-fig. 6. The increase in TCA-soluble counts in the medium was about equal in magnitude to the decrease in cell-associated radioactivity. The amount of TCA-insoluble isotope in the medium showed no



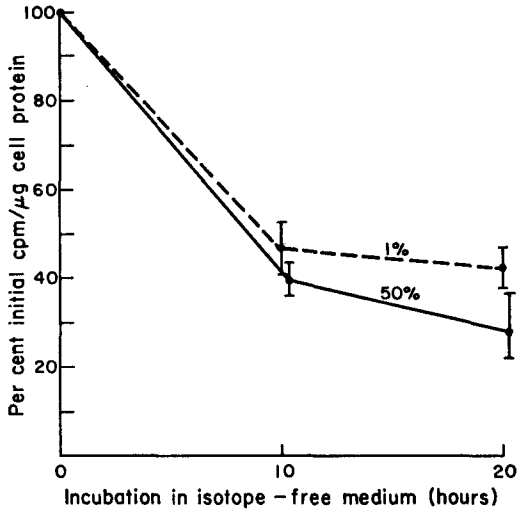
TEXT-FIG. 6. The loss of isotope from pulsed cells and the increase in TCA-soluble isotope in the medium.

consistent change and its presence was attributable to intact ^{125}I-HSA remaining from the pulse.

Control experiments established that the observed increase in TCA-soluble counts was not due to extracellular digestion of residual ^{125}I-HSA in the medium. Cells were pulsed with ^{125}I-HSA, washed in medium containing ^{131}I-HSA, and finally incubated in 50% nbcs. Thus both residual ^{125}I-HSA and ^{131}I-HSA were present in the washout medium. The TCA-solubility of the ^{125}I activity in the medium, but not of the ^{131}I activity, increased during a 20 hr washout period. Thus it seemed clear that the TCA-soluble isotope appearing in the medium resulted from the intracellular digestion of pinocytosed ^{125}I-HSA.

Effect of Metabolic Inhibitors on the Loss of Intracellular Isotope.—It was of interest to determine whether ongoing pinocytosis, protein synthesis, and associated metabolic activities are required for the processing of ingested albumin.

Macrophages cultivated for 24 hr in 50% nbcs are engaged in active pinocytosis and protein synthesis (11). These cellular activities may be inhibited by a decrease in the serum concentration of the medium or by the addition of certain metabolic inhibitors to 50% nbcs (12, 19). To test the effect of reduced serum concentration on the loss of isotope from pulsed cells, cells were pulsed as usual in 50% nbcs, washed, and reincubated in 1% nbcs. During the washout period in 1% nbcs, the cells decreased in total protein, as expected (12). But as shown in Text-fig. 7, reduced serum concentration had no significant effect on the loss of isotope, indicating that the rate of processing of ingested ^{125}I-HSA did not depend on the rate of pinocytosis.

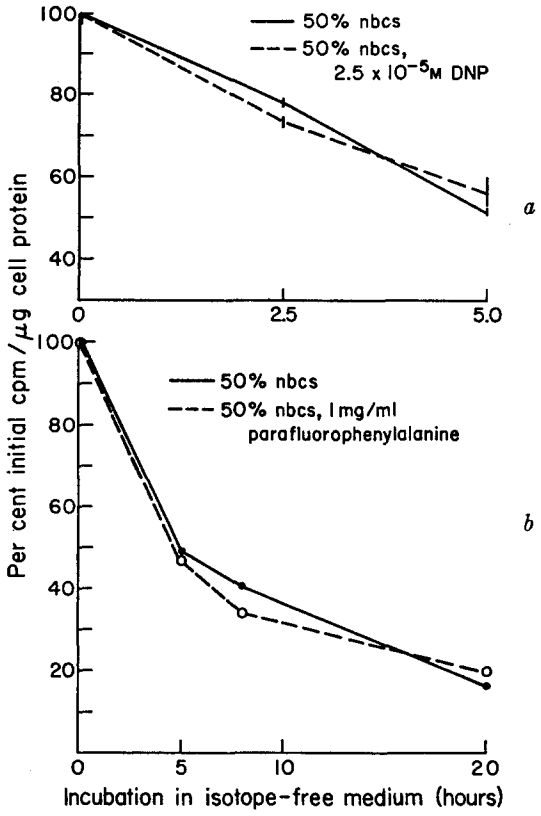


TEXT-FIG. 7. The effect of serum concentration on the loss of isotope from pulsed cells.

Two metabolic inhibitors of known specificity, parafluorophenylalanine and 2,4-dinitrophenol (DNP), were tested for their effects on the loss of isotope from pulsed cells. Both compounds inhibit pinocytic vesicle formation in 50% nbcs. The time course and dose-response relationships of their effects on pinocytosis have been studied in detail (19). In the present experiments, the inhibitors were used at concentrations 2.5 times greater than the concentrations which produce an 80% reduction in pinosome formation in less than 1 hr (19). As shown in Text-fig. 8 *a*, DNP, an inhibitor of oxidative phosphorylation, had no apparent effect on the loss of isotope from pulsed cells. At concentrations up to 1 mg/ml parafluorophenylalanine was also without effect, as shown in Text-fig. 8 *b*.

The effects of temperature on the release of isotope were monitored with light microscopic observations of cell morphology. At 4°C, macrophages retract their

pseudopods and become rounded, but do not detach from the glass surface. The morphological changes are reversible after up to 6 hr at 4°C. Text-fig. 9 shows the results of 5 hr washout experiments conducted at 4°C. Total cell protein and isotope were retained during the cold incubation. Upon reincubation at 37°C, there was a normal loss of isotope from the cold-treated cells. Thus, the

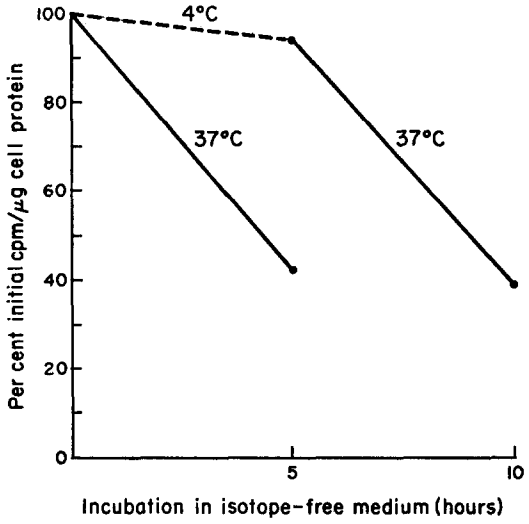


TEXT-FIG. 8. *a*. The effect of DNP on the loss of isotope from pulsed cells. *b*. The effect of parafluorophenylalanine on the loss of isotope from pulsed cells.

processing of ingested albumin was temperature dependent, but did not require protein synthesis, oxidative phosphorylation, or ongoing pinocytosis.

Nature of the Released Material.—Various fractionation procedures were undertaken to determine the size of the radioactive molecules released into the medium by pulsed macrophages. Preliminary attempts to analyze the TCA-soluble fraction on Sephadex G-50 and Sephadex G-25 columns established that the radioactivity was associated with very low molecular weight compounds. Paper chromatography in butanol:acetic acid:water (4:1:1) revealed a fairly

consistent pattern of radioactivity. However, the presence in the sample of large and variable amounts of salts and other acid-soluble components of medium 199 made it difficult to identify the observed peaks by their R_f's. Therefore monoiodotyrosine-^{131}I (MIT-^{131}I), applied to the paper with an overlay of the sample (see Materials and Methods), was used as a reference compound. As shown in Text-fig. 10, the distribution of ^{125}I counts from macrophage culture medium corresponded well with that of the ^{131}I counts from MIT-^{131}I. The slower peak, corresponding to free iodide in this solvent system (16), is a major component of the sample, but only a trace component of the commercially



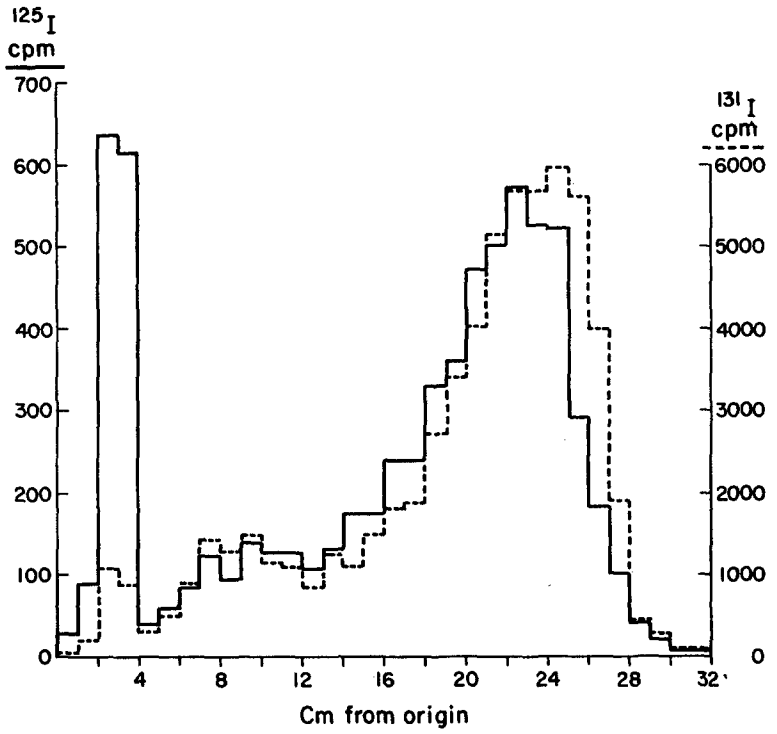
TEXT-FIG. 9. The effect of temperature on the loss of isotope from pulsed cells.

available MIT-^{131}I. The presence of free iodide was not dependent on cellular activity, since it was also found in the TCA-soluble fraction of ^{125}I-HSA incubated in 50% nbcs in the absence of cells. MIT-^{125}I could not be detected in cell-free media, even after 48 hr at 37°C.

The use of Biogel P-2, with an exclusion limit of molecular weight 1600, for the separation of iodopeptides, iodotyrosines, and free iodide has been described recently (21). Text-fig. 11 shows the elution pattern of the TCA-soluble fraction of culture medium on Biogel P-2. The elution pattern of MIT-^{131}I is shown for comparison. The faster moving radioactive material, fractions 30–42 and 42–48, probably corresponds to free iodide, which is eluted in two peaks under these conditions (21).

The question remained whether any TCA-insoluble albumin fragments were excreted by the cells. It seemed possible that flask-to-flask variations in the amount of ^{125}I-HSA remaining from the pulse could have obscured any systema-

tic increases in TCA-insoluble activity in the washout media. Preliminary fractionations of concentrated whole washout media on Sephadex G-50 had not revealed any TCA-insoluble radioactivity other than that of intact albumin. Text-fig. 12 shows the results of chromatography on Sephadex G-200. The chromatogram of a mixture of ^{131}I-HSA, MIT-^{131}I and diiodotyrosine-^{131}I is shown for comparison. No material of molecular weight intermediate to that of



TEXT-FIG. 10. Paper chromatogram of MIT-^{131}I and the TCA-soluble fraction of the washout medium (^{125}I-activity).

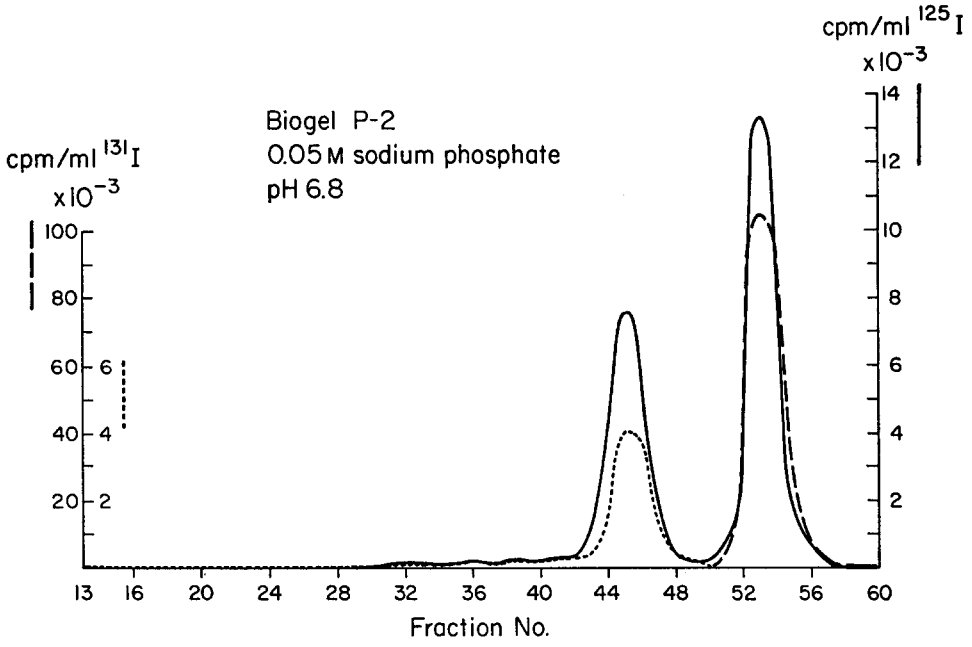
albumin and the iodinated tyrosines could be detected. The radioactive material eluted in the void volume may correspond to polymers of HSA or to iodide bound to serum proteins.

DISCUSSION

The results presented here indicate that pinocytosed albumin is digested by macrophages and that the products of digestion are excreted into the medium. Cohn and Benson (12) have previously reported evidence for turnover of proteins pinocytosed by macrophages. They found that cells which had ingested

fluorescein-labeled serum proteins or lysozyme gradually lost the exogenous protein after transfer to fresh medium. In the present study, the use of a radioactively labeled protein, I*-HSA, permitted quantitative measurement of pinocytic uptake and identification of the labeled products of digestion.

The uptake of I*-HSA by macrophages occurred only under conditions supporting active pinocytosis. It was inhibited in low serum media and in the

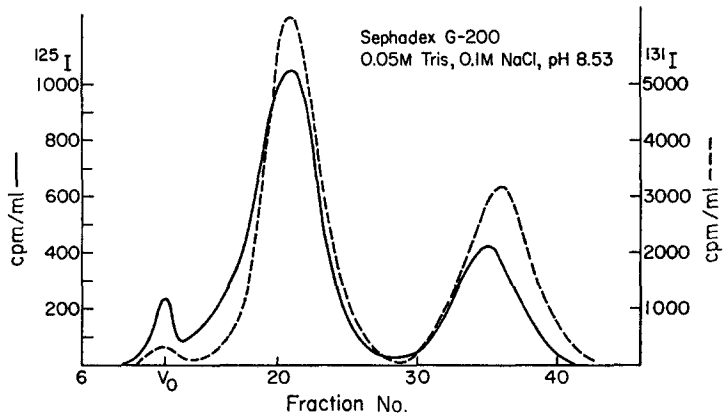


TEXT-FIG. 11. Chromatography of MIT-^{131}I and the TCA-soluble fraction of the washout medium (^{125}I-activity) on Biogel P-2. The void volume was eluted in the first 13 fractions.

presence of parafluorophenylalanine. In contrast, the release of isotope from pulsed cells did not require ongoing pinocytosis, protein synthesis, or respiration. This result is consistent with Cohn and Benson's earlier finding that the loss of lysozyme activity from cells which had pinocytosed the enzyme was not affected by the serum concentration of the medium (12), hence not by the rate of pinocytosis (11) and associated metabolic activity. There are indications that the intracellular degradation of phagocytosed material may also be independent of cellular metabolism in some types of cells. The intracellular digestion of bacteria by macrophages and polymorphonuclear leukocytes does not require glycolysis, the main energy source for phagocytosis itself (22). Silverstein has shown that the intracellular degradation of reovirus coat protein by strain L

cells is not affected by puromycin or actinomycin.[1] Thus the mechanism of catabolism of material engulfed by endocytosis may be quite different from that of endogenous protein, whose breakdown, in some cases, requires ongoing protein synthesis (23, 24).

The only condition which was found to inhibit the release of isotope from pulsed cells was reduced temperature. Inhibition of intracellular digestion of viral coat protein at low temperatures has also been reported (25). Many, if not all of the reactions involved in the processing of pinocytosed material may be temperature dependent. These could include intralysosomal proteolysis and enzyme-facilitated transport of the products of digestion to the exterior of the



TEXT-FIG. 12. Chromatography of whole washout media (^{125}I-activity). The elution pattern of a mixture of ^{131}I-HSA, MIT-^{131}I and diiodotyrosine-^{131}I is shown for comparison. The void volume (V_0) is indicated.

cell. The possibility of separating these reactions by means of specific inhibitors deserves further investigation.

The major product of I*-HSA digestion by macrophages was identified as iodotyrosine. Inorganic iodide was also present in the TCA-soluble fraction of the washout medium, but it did not seem to result from the intracellular processing of I*-HSA. Although MIT was the only fragment of I*-HSA detected, the possibility that others were excreted remains open. Non-iodinated peptides and iodinated peptides of low specific activity or low concentration would escape detection. Current studies employing other means of labeling proteins may help determine whether macrophages can, in general, degrade pinocytosed proteins to the level of amino acids.

The available evidence permits only the sketchiest outline of the events involved in the intracellular processing of I*-HSA. Radioautography showed that

[1] Silverstein, S. Personal communication.

^{125}I-HSA, like other pinocytosed proteins, is sequestered within secondary lysosomes in the perinuclear region of the cell. In addition to other hydrolases, these organelles contain cathepsins capable of digesting albumin in vitro[2] and are presumably the site of the intracellular digestion of I*-HSA. Very little is known about the mechanism of excretion of the digestion products, except that bulk excretion, or "regurgitation" of lysosomal contents from mouse macrophages is extremely unlikely. Indigestible pinocytosed material is quantitatively retained by macrophages, indicating that lysosomes do not discharge into the medium by a process of reverse pinocytosis (12). If this did occur, fragments of I*-HSA representing all stages of digestion would be found in the washout medium. In fact, only MIT, the smallest labeled unit of I*-HSA (26) is excreted. Recently, Mego et al. have shown that the digestion of formaldehyde-treated ^{131}I-HSA within isolated mouse liver lysosomes is accompanied by the release of monoiodotyrosine to the medium (27). Their work suggests that in the intact cell MIT may be the only labeled product of digestion to leave the lysosome and enter the cytoplasm. Alternatively, peptides resulting from intralysosomal digestion may enter the cytoplasm and there undergo further digestion by cytoplasmic peptidases, as suggested by Coffey (28). In either case, the question remains how products of digestion are transported across the cytoplasm and thence across the plasma membrane. The present results suggest only that these processes may not require metabolic energy. Clearly further work, including studies of the permeability of lysosomal membranes within intact cells, will be required to fully elucidate the mechanism of disposal of products of lysosomal digestion.

It is perhaps worth considering some implications of the present results for the functions of pinocytosis by macrophages in vivo. In certain types of cells, such as the oocytes of many species, pinocytosis appears to serve as a mechanism for the bulk transport of intact proteins (29). This does not seem to be the case for macrophages, with their rich endowment of hydrolytic enzymes. In fact, it can be inferred from the present results that macrophages effect a continual turnover of the proteins in their medium, ingesting intact molecules and returning some or all of the digestion products to the extracellular space. There seems little reason to doubt that this occurs in vivo, since pinocytosis and lysosome formation occur in much the same manner in the mouse peritoneum as in tissue culture (9, 13). Very likely the general features of pinocytosis and digestion described for cultured peritoneal macrophages obtain throughout the entire reticuloendothelial system. If so, the reticuloendothelial system could account for a quantitatively significant turnover of soluble proteins and might play a major role in the catabolism of plasma proteins.

The present results also suggest a possible function of pinocytosis in the economy of the individual cell. Once they have synthesized the requisite hydro-

[2] Ehrenreich, B. A. Unpublished results.

lases and formed pinocytic vesicles, macrophages can apparently degrade exogenous protein to the level of convenient metabolites without further expenditure of metabolic energy. MIT itself cannot be incorporated into cellular proteins (18), but other amino acids arising from lysosomal proteolysis may possibly be utilized for the synthesis of cellular protein. Metabolites gained by pinocytosis may possibly be required for the rapid growth of monocytes to mature phagocytes at sites of inflammation and tissue necrosis. Some authors have suggested that endocytosis may have a nutritive function in other metazoan cells (30, 31), but its quantitative significance in this role remains questionable (32). The possibility that macrophages may utilize the amino acids arising from the digestion of pinocytosed, internally labeled proteins is currently being investigated.

SUMMARY

Mouse peritoneal macrophages take up I*-HSA from their medium during in vitro cultivation. Conditions which promote I*-HSA uptake are the same as those which stimulate formation of pinocytic vesicles. Autoradiography of cells pulsed with ^{125}I-HSA showed that intracellular isotope is localized in perinuclear granules, or secondary lysosomes.

Following a pulse of ^{125}I-HSA, intracellular radioactivity decreases and the amount of TCA-soluble isotope in the medium increases correspondingly. About 50% of the intracellular isotope is lost in 5 hr. The release of isotope from pulsed cells is not inhibited by parafluorophenylalanine, 2,4-dinitrophenol or by a reduction of the serum concentration of the medium. However, the processing of ingested ^{125}I-HSA is reversibly inhibited by reduced temperature.

The TCA-soluble radioactive material excreted by pulsed macrophages was identified as monoiodotyrosine.

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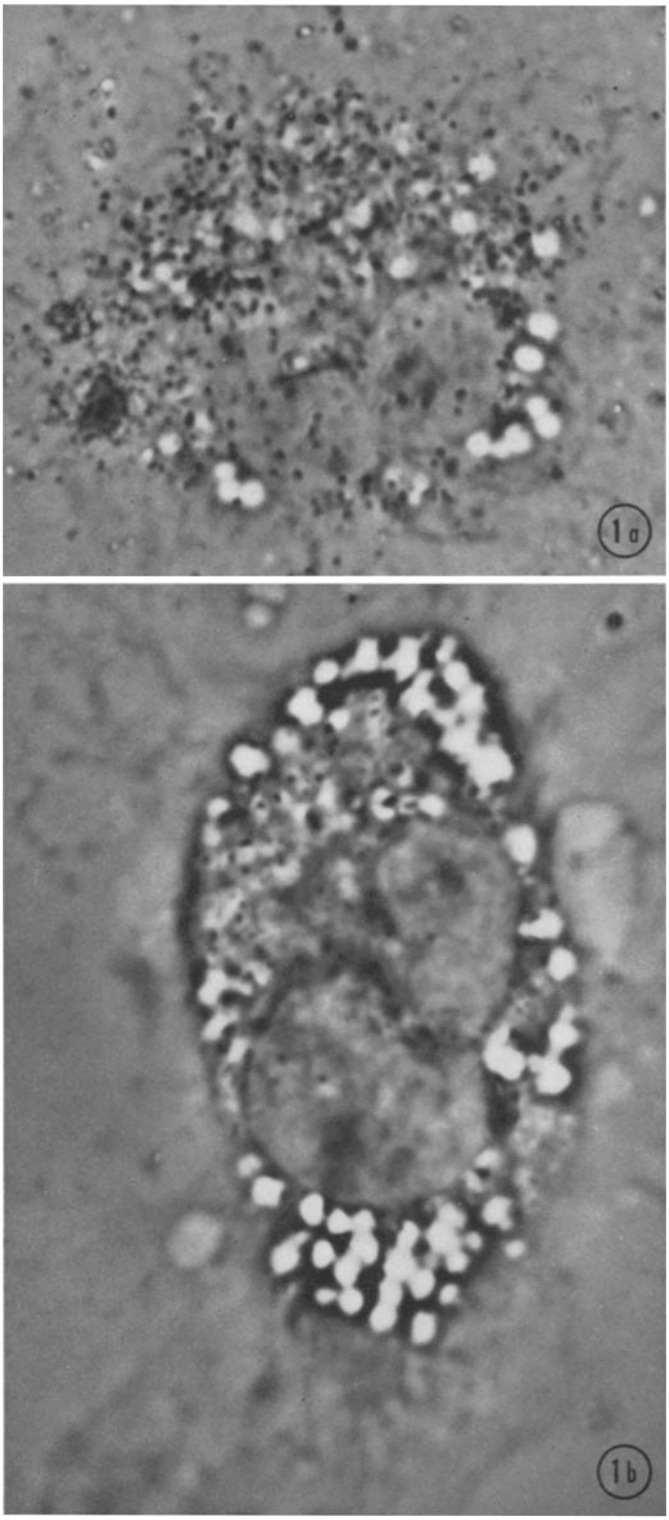
EXPLANATION OF PLATES

PLATE 69

FIG. 1. Radioautographs of macrophages cultivated for 24 hr in the presence of ^{125}I-HSA. Phase contrast × 2500.

a. A cell exhibiting heavy concentration of grains over the granules of the perinuclear region.

b. A less heavily labeled cell which illustrates the strict localization of dark grains to the granules of the centrosphere region. This area is surrounded by clear, refractile lipid droplets which are unlabeled. The peripheral cytoplasm contains no demonstrable isotope.

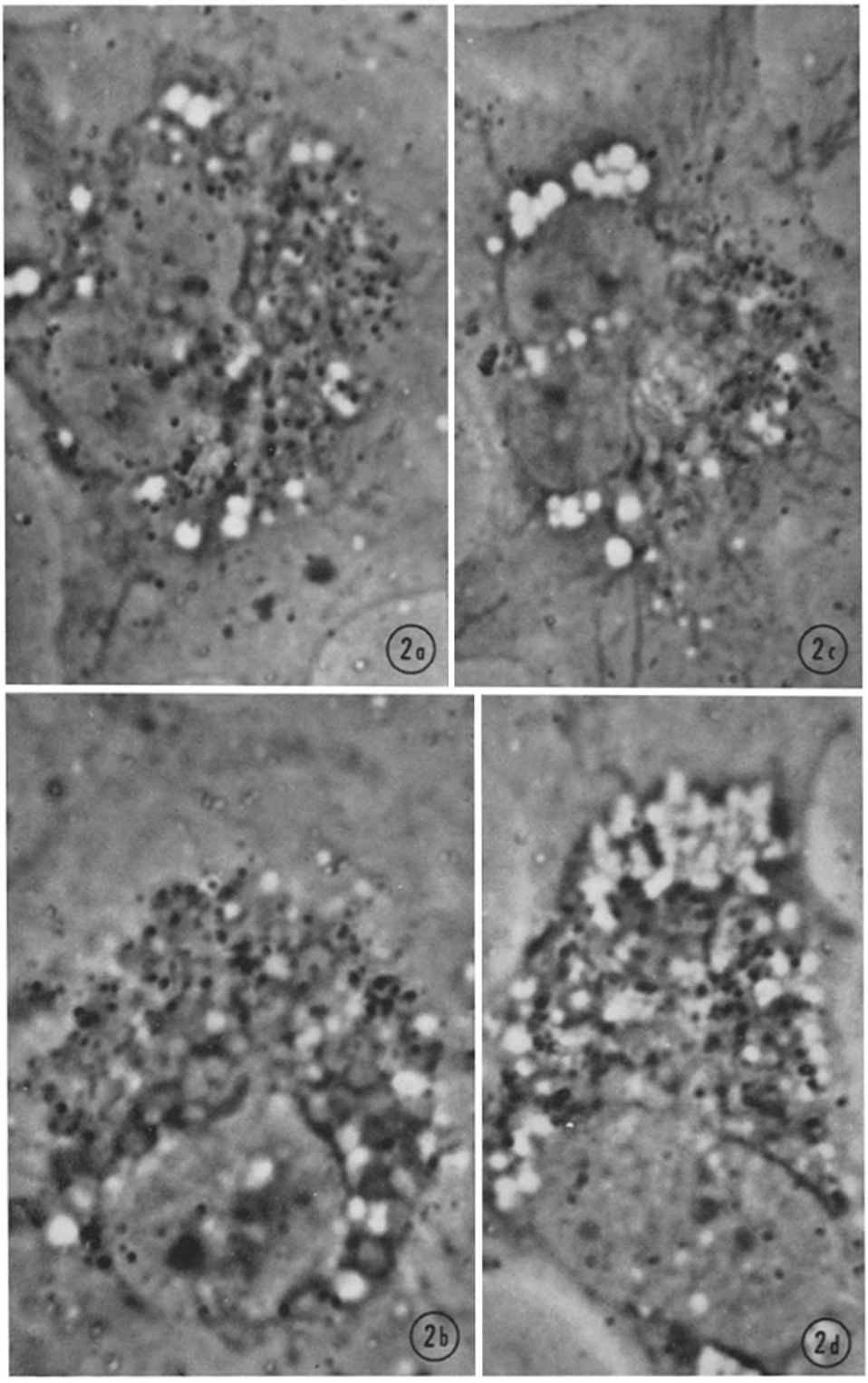


(Ehrenreich and Cohn: Uptake and digestion of albumin by macrophages)

PLATE 70

FIG. 2. The localization of grains in cells exposed to ^{125}I-HSA for 24 hr. Phase contrast × 2500.

a–d. Examples of the intensity and distribution of grains over the granules of the perinuclear region. A portion of the peripheral cytoplasmic radioactivity is related to background. The phase-lucent pinocytic vacuoles of the centrosphere are relatively free of label and the lipid droplets are almost completely unlabeled. The exposure of injured or dead cells to ^{125}I-HSA leads to a uniform and heavy labeling of both cytoplasm and nucleus.



(Ehrenreich and Cohn: Uptake and digestion of albumin by macrophages)