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Selective Release of Lysosomal Hydrolases from Phagocytic Cells by Cytochalasin B

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1. Cytochalasin B (10 μg/ml) enhances the release of rabbit polymorphonuclear leucocyte lysosomal acid hydrolases induced by retinol (vitamin A alcohol). 2. This effect is seen at doses of the vitamin that cause selective release of acid hydrolases and those causing more general enzyme release indicated by the loss of lactate dehydrogenase. 3. Cytochalasin B (2–50 μg/ml) has no effect on the release of sedimentable acid hydrolases of intact granules obtained from disrupted polymorphonuclear leucocytes. 4. Cytochalasin B (2–10 μg/ml) causes a time- and dose-dependent release of mouse peritoneal macrophage acid hydrolases. 5. This effect is selective at all doses of cytochalasin B used, since no release of lactate dehydrogenase, malate dehydrogenase and leucine 2-naphthylamidase was detected. 6. Treatment with cytochalasin B at doses of up to 10 μg/ml for as long as 72h did not significantly change the total activities of any of the enzymes measured. 7. The lack of toxicity of cytochalasin B was shown by dye-exclusion tests and its failure to release radioactive colloidal gold stored in secondary lysosomes.

The capacity of various cells to release selectively their lysosomal enzymes, almost certainly by exocytosis, has become apparent in recent years. This phenomenon has been observed in both physiological and pathological situations. The former is exemplified by the remodelling of bone induced by parathyroid hormone (Vaes, 1968) and the latter by the release of large amounts of acid protease from cultures of limb-bone rudiments treated with complement-sufficient antiserum (Dingle *et al.*, 1967).

More recently selective exocytosis of acid hydrolases has been shown to be a concomitant of phagocytosis by polymorphonuclear leucocytes and macrophages (Hawkins & Peeters, 1971; Henson, 1971; Weissmann *et al.*, 1971; Davies *et al.*, 1973). Woodin & Wieneke (1963) had shown that exocytosis of polymorphonuclear-leucocyte acid hydrolases could be induced by low doses of retinol (vitamin A alcohol).

The biological properties of cytochalasin B were first described by Carter (1967), who showed that this compound inhibits cell movement and brings about the formation of multinucleate cells or (in high doses) complete enucleation. These observations have been confirmed by others (Ridler & Smith, 1968; Krishnan & Raychaudhuri, 1969; Allison *et al.*, 1971; Estensen, 1971; Gail & Boone, 1971; Krishnan, 1971; Prescott *et al.*, 1971; Sanger *et al.*, 1971; Spooner *et al.*, 1971; Crawford *et al.*, 1972).

We now report that the disruption of cellular organization caused by cytochalasin B also results in the selective release of acid hydrolases. These experiments have been carried out with two kinds of phagocytic cells, namely rabbit peritoneal-exudate polymorphonuclear leucocytes and the adherent population of cells, predominantly macrophages, obtained by lavage of the mouse peritoneal cavity. A preliminary account of some of this work has already been published (Davies *et al.*, 1972).

Materials and Methods

Materials

Cytochalasin B was obtained from Imperial Chemical Industries Ltd., Pharmaceuticals Division, Alderley Park, Macclesfield, Cheshire, U.K. The compound was dissolved at a concentration of 1 mg/ml in dimethyl sulphoxide for experiments with polymorphonuclear leucocytes and suspended at a similar concentration in calf serum for experiments with mouse peritoneal cells.

Medium 199 was obtained from Grand Island Biological Co., Grand Island, N.Y., U.S.A., in powder form and reconstituted with deionized water. Calf serum was obtained from Tissue Culture Supplies Ltd., Slough, Bucks., U.K. All batches were heated at 56°C for 30min before use.

Crystalline synthetic all-*trans*-retinol, phenolphthalein glucuronic acid (10mM; pH7.0), *o*-nitrophenyl β-D-galactopyranoside, leucine 2-naphthylamide and glycogen (from oyster) were obtained from Sigma (London) Chemical Co. Ltd., London S.W.6, U.K. Heparin (5000 units/ml) was from Boots Pure Drug Co., Nottingham, U.K. Haemoglobin (acid protease substrate powder) was from Worthington

Chemical Corp., Freehold, N.J., U.S.A. Radioactive colloidal gold (specific radioactivity 4–12mCi/mg of Au; particle size up to 20nm) was obtained from The Radiochemical Centre, Amersham, Bucks., U.K.

Preparation of rabbit peritoneal-exudate polymorphonuclear leucocytes

A 0.1% (w/w) glycogen solution in 0.9% NaCl was incubated at 37°C for 24h before intraperitoneal injection of 250ml/rabbit. Harvesting of polymorphonuclear leucocytes was carried out as previously described (Davies *et al.*, 1971). The cells were washed three times in cold Hanks solution after collection and suspended at a final cell concentration of 25×10^6/ml.

Preparation of isolated polymorphonuclear-leucocyte lysosomes

Polymorphonuclear leucocytes were washed and suspended in ice-cold 0.34M-sucrose containing 50 units of heparin/ml. The cells were then disrupted by repeated passage through a Millipore-filter assembly (Davies *et al.*, 1971). Nuclear debris was sedimented by centrifugation at 400*g* and the milky supernatant was used for measurement of lysosomal ability to sediment.

Preparation of mouse peritoneal cells

These cells were collected and cultured by following procedures initially described by Cohn & Benson (1965*a*). Outbred Swiss mice (TO strain) were killed by ether anaesthesia. The abdomen of each animal was thoroughly swabbed with 70% (v/v) ethanol and the skin deflected so as to expose fully the peritoneal wall. The peritoneum was lavaged by injection of 4ml of cold medium 199 containing 50% (v/v) of calf serum and gentle massage, followed immediately by withdrawal of all peritoneal fluid. The cell suspension thus obtained was counted and its cell concentration adjusted to 1×10^6 cells/ml. Portions (5ml) of these suspensions were distributed into plastic Petri dishes (Nunc, Jobling Laboratories Division, Stone, Staffs., U.K.) and cells allowed to adhere in an atmosphere containing 5% CO_2 in air at 37°C. After 3–4h the medium and non-adherent cells were aspirated and the attached cell sheet was thoroughly rinsed four times with phosphate-buffered saline, pH7 (Dulbecco's PBS 'A'; Dulbecco & Vogt, 1954). Fresh culture medium was placed on the cells and within 24h a monolayer of extended cells with only occasional rounded cells was obtained.

Effect of cytochalasin B on retinol-induced release of leucocyte enzymes

Solutions of retinol were prepared in ethanol as near as possible to the time of an experiment and then stored in the dark. Solutions were prepared so that a 100-fold dilution would give the required concentration of the vitamin and 1% (v/v) ethanol. This concentration of ethanol did not influence the release of enzymes from polymorphonuclear leucocytes under the conditions of our experiments. The ethanolic solutions of retinol were added to the cells while they were agitated on a Vortex mixer to ensure rapid and complete mixing. Cytochalasin B was also added in volumes calculated to give a final dimethyl sulphoxide concentration of 1% (v/v). Appropriate controls containing 1% (v/v) of dimethyl sulphoxide and 1% (v/v) of ethanol alone or together were also included. Cells were incubated with cytochalasin B for 30min before the addition of retinol. The cells were incubated for a further 30min at 37°C, after which time the samples were removed and centrifuged at 1000rev./min for 10min at 4°C in an MSE Mistral 6L centrifuge (no. 59548 swing-out head). The supernatants were removed and retained for measurement of enzyme release. Total releasable enzyme activity was determined by incubation of representative samples with 0.1% (v/v) Triton X-100.

Effect of cytochalasin B on isolated polymorphonuclear-leucocyte lysosomes

Portions of the 400*g* post-nuclear supernatant were incubated with increasing concentrations of cytochalasin B from 2 to 50μg/ml for 30min at 37°C. All samples contained 1% (v/v) of dimethyl sulphoxide, and controls containing 1% (v/v) of dimethyl sulphoxide only and no dimethyl sulphoxide were also included. After 30min all samples were centrifuged for 20min at 15000rev./min in a Sorvall S-3 centrifuge (SS-34 head). The supernatants were retained for enzyme assays. Total enzyme release was measured after the addition of 0.1% (v/v) of Triton X-100 to portions of post-granule supernatant.

Effect of cytochalasin B on enzyme activities of mouse peritoneal adherent cells

When the peritoneal cells had formed a layer of extended cells, with only an occasional rounded cell, the medium was removed and replaced with medium 199 containing 50% (v/v) of calf serum. Cytochalasin B was then added to appropriate culture plates as a fine suspension in calf serum. Additions were generally made as 50μl of suspension. Cytochalasin B dissolved in the culture medium at concentrations up to 10μg/ml. Samples were removed for assay of enzyme activities at various times in the following way.

The culture medium was removed and centrifuged at 1000rev./min for 10min at 4°C in an MSE Mistral 6L centrifuge (no. 59548 swing-out head). The supernatant was removed and 3ml was retained for enzyme assay. The pellet was resuspended in 2.0ml of 0.9%

NaCl containing 0.1% (v/v) of Triton X-100. Meanwhile the cell sheet was washed once with 4.0ml of 0.9% NaCl and then removed with 2.5ml of 0.9% NaCl containing 0.1% (v/v) of Triton X-100. Complete cell removal was achieved by vigorous scraping with sterile silicone bungs. The medium and wash samples were made 0.1% (v/v) with respect to Triton X-100 by addition of the required amount of 1% (v/v) solution in 0.9% NaCl. All samples were vigorously stirred at 4°C for 30min.

Enzyme assays

Lactate dehydrogenase. This was assayed by determining the rate of oxidation of 9mM-NADH by measuring the changes in E_{340} in the presence of 0.3mM-pyruvate in 50mM-phosphate buffer, pH7.5, by following exactly the instructions issued with Biochemica Test Combination LDH-UV [Boehringer Corp. (London) Ltd., Ealing, London W.5, U.K.]. Samples were kept on ice and assayed on the same day, since the enzyme was found to be unstable if stored overnight.

β-Glucuronidase. This was assayed by the method of Talalay *et al.* (1946).

β-Galactosidase. This was assayed by the method of Conchie *et al.* (1959).

N-Acetyl-β-D-glucosaminidase. This was assayed by the method of Woolen *et al.* (1961), with *o*-nitrophenyl *N*-acetyl-β-D-glucosaminide as substrate.

Acid protease. This was assayed by the method of Anson (1937), the tyrosine content of the trichloroacetic acid supernatant being determined with Folin–Ciocalteu reagent.

Leucine 2-naphthylamidase. This activity was determined by the method of Goldbarg & Rutenberg (1958) as described by Davies *et al.* (1970).

Malate dehydrogenase. This activity was determined by measurement of the changes in E_{340} when 12mM-NADH is oxidized to NAD^+ during the conversion of oxaloacetate into malate. The assay was carried out with materials contained in Biochemica Boehringer MDH Test Pack by following exactly the manufacturer's instructions.

Dye-exclusion tests

Culture medium was removed and replaced with a 0.1% solution of Trypan Blue. The cultures were then incubated at 37°C for 1h, at which time the Trypan Blue was removed and replaced by 0.9% NaCl. The number of cells taking up dye was measured in a field containing 400 cells.

Release of radioactive colloidal gold from macrophage cultures

Cultures were incubated with 0.5μCi of radioactive colloidal gold per plate for 4h at 37°C. At this time the medium was removed and the cell sheet thoroughly washed four times with phosphate-buffered saline. Fresh medium was then added to all plates; cytochalasin B (10μg/ml) was added to some plates, others being retained as controls. At 24h intervals medium was removed from cell cultures and cell sheets were detached by the addition of 2.5ml of 0.1% (v/v) Triton X-100 in 0.9% NaCl. Both were examined for their content of radioactive colloidal gold and β-glucuronidase activity. The radioactivity of each sample was determined in a Walac γ-ray counter.

Results

Effect of cytochalasin B on retinol-induced release of polymorphonuclear-leucocyte enzymes

Fig. 1 shows that increasing concentrations of retinol induce a progressive release of β-galactosidase. In the presence of cytochalasin B (10μg/ml) enzyme release is significantly ($P<0.01$) increased at all concentrations of retinol used. Similar findings were obtained when the release of another lysosomal hydrolase, namely β-glucuronidase, was measured (Table 1). Fig. 2 shows that cytochalasin B fails to increase the release of lactate dehydrogenase induced by retinol at doses of 50μg/ml and above. This implies that cytochalasin B disrupts cellular organization in such a way that extrusion of lysosomal hydrolases is enhanced during the 30min period, at the end of which enzyme release was measured.

Effect of cytochalasin B on the ability to sediment of the lysosomes obtained from disrupted polymorphonuclear leucocytes

Cytochalasin B, in concentrations from 2 to 50μg/ml, fails to influence the ability to sediment of isolated polymorphonuclear-leucocyte lysosomes. Table 2 shows that there are no significant changes in the release of β-glucuronidase and *N*-acetyl-β-D-glucosaminidase in samples incubated for 30min at 37°C with cytochalasin B compared with controls incubated with solvent only.

In marked contrast samples incubated with 0.1% Triton X-100 showed an immediate loss of turbidity, normally associated with intact lysosomes. This was accompanied by the release of a large amount of acid hydrolase into the 20000*g* supernatant. Therefore it seems clear that the increased loss of acid hydrolases from polymorphonuclear leucocytes induced by cytochalasin B is not due to a direct labilization of lysosomal membranes.

Release of mouse peritoneal-macrophage acid hydrolases by cytochalasin B

Temple *et al.* (1973) reported that guinea-pig peritoneal-exudate cells incubated with chicken

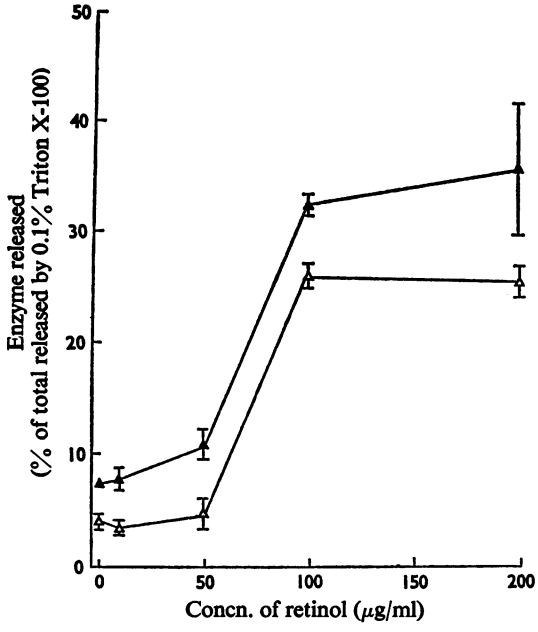

Fig. 1. *Effect of cytochalasin B (10 μg/ml) on the release of β-galactosidase from polymorphonuclear leucocytes induced by increasing concentration of retinol*

Cells were preincubated with cytochalasin B (▲) or 1% (v/v) dimethyl sulphoxide (△) for 30min at 37°C and then exposed to 1% (v/v) ethanol or increasing concentrations of retinol for a further 30min at 37°C in the continuing presence of cytochalasin B. At this time the samples were centrifuged at 1000rev./min for 10min to sediment the cells and the supernatants were assayed for enzyme activity. 100% enzyme activity is that released by 0.1% Triton X-100; 100% = 23.50±0.92nmol of *o*-nitrophenol/h per 10^6 cells. Each point represents the mean±S.D. of three experiments.

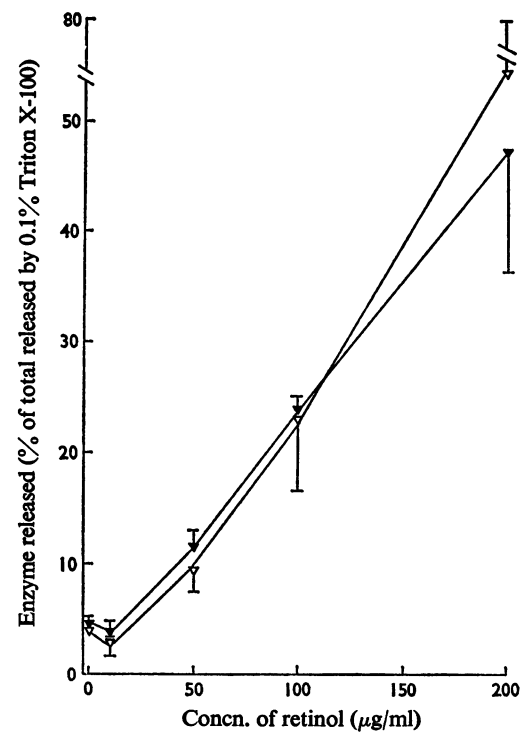

Fig. 2. *Effect of cytochalasin B (10 μg/ml) on the release of lactate dehydrogenase from polymorphonuclear leucocytes induced by increasing doses of retinol*

Experimental conditions were as indicated in Fig. 1. ▼ indicates the presence of cytochalasin B (10 μg/ml) and ▽ the presence of 1% (v/v) dimethyl sulphoxide only. Each point represents enzyme release expressed as a percentage of that released by 0.1% Triton X-100 (100% = 295.6±12.4munits/10^6 cells). Each point is the mean±S.D. of three experiments.

Table 1. *Effect of cytochalasin B (10 μg/ml) on the release of β-glucuronidase from polymorphonuclear leucocytes induced by increasing doses of retinol*

Experimental conditions were as indicated in Fig. 1. Each value represents enzyme release expressed as a percentage of that released by 0.1% Triton X-100 (100% = 11.90±0.52nmol of phenolphthalein/h per 10^6 cells). Each value is the mean±S.D. of three experiments.

	% of enzyme released	
Concn. of retinol (μg/ml)	Dimethyl sulphoxide (1%, v/v)	Cytochalasin B (10 μg/ml)
0	4.4± 0.2	7.0±0.5*
10	3.2± 0.2	6.8±0.6*
50	7.6± 0.4	14.3±1.0*
100	24.7±12.2	40.8±4.0
200	31.4± 7.1	49.0±2.9

* Significantly different from dimethyl sulphoxide-treated samples ($P<0.01$).

Table 2. *Effect of increasing concentrations of cytochalasin B on the ability to sediment of lysosomes from post-nuclear supernatants of polymorphonuclear leucocytes*

Enzyme release is expressed as a percentage of that released by 0.1% Triton X-100. For β-glucuronidase 100% = 17.25nmol of phenolphthalein/h per mg of protein; for *N*-acetyl-β-D-glucosaminidase 100% = 160.1nmol of *o*-nitrophenol/h per mg of protein. Each value represents the mean±S.D. of four separate experiments.

	% of enzyme released	
Concn. of cytochalasin B (μg/ml)	β-Glucuronidase	*N*-Acetyl-β-D-glucosaminidase
2	10.6±0.7	27.9±1.0
5	9.9±0.7	28.2±0.7
10	11.0±1.1	28.2±0.7
20	10.6±0.7	27.9±0.3
50	11.0±0.7	28.2±0.7
Dimethyl sulphoxide (1%, v/v)	10.6±0.7	27.6±0.7
0	9.7±1.5	27.6±0.7

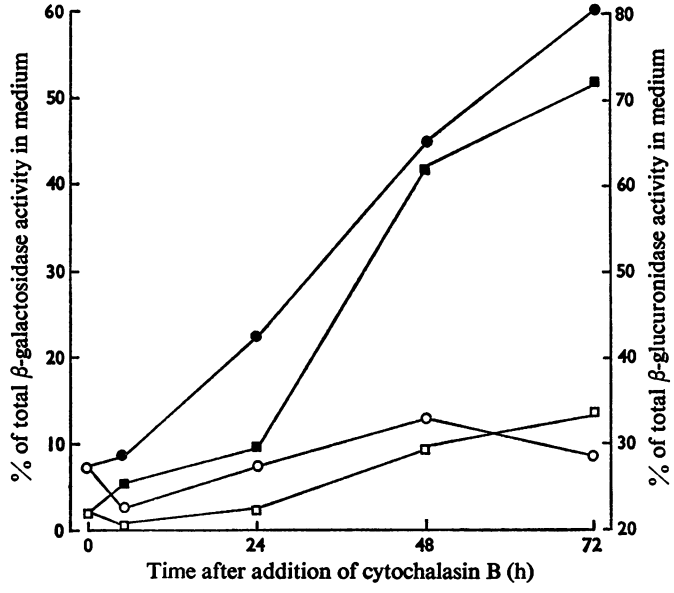

Fig. 3. *Time-dependent release of two acid hydrolases (β-glucuronidase and β-galactosidase) from mouse peritoneal macrophages cultured in vitro in the presence and in the absence of cytochalasin B* (10μg/ml)

Enzyme release into the medium is expressed as a percentage of the total activity found in both cells and medium β-galactosidase release in the absence (○) and in the presence (●) of cytochalasin B; β-glucuronidase release in the absence (□) and in the presence (■) of cytochalasin B.

erythrocytes coated with specific antibody release a large proportion of their acid hydrolases in the presence of cytochalasin B during a period of 5h. This release is selective, since lactate dehydrogenase loss is not increased. In the present experiments the cells were not exposed to a phagocytic stimulus, and it is clear from Fig. 3 that the release of β-glucuronidase is delayed for at least 24h after the addition of cytochalasin B. Between 24 and 72h large amounts of β-glucuronidase and β-galactosidase are released from the cells into the culture medium. The amount of enzyme released is dependent on the dose of cytochalasin B. Table 3 shows that after 72h increasing doses of cytochalasin B induce the release of increasing amounts of β-glucuronidase into the culture medium.

Effect of cytochalasin B on enzyme activities in macrophage cultures

Despite the major redistribution of enzyme activity the total amount of enzyme was not significantly changed (Table 4). Further evidence that cell viability was not compromised is provided in Table 4. This shows that activities of two non-lysosomal enzymes, namely lactate dehydrogenase and leucine 2-naphthylamidase, in macrophage culture exposed to increasing concentrations of cytochalasin B for 72h are not significantly changed. Moreover cytochalasin B (10μg/ml) did not affect the activity of malate dehydrogenase over a 72h incubation period (Table 4).

In marked contrast with our results with acid hydrolases we were unable to detect any lactate dehydrogenase, leucine 2-naphthylamidase and malate dehydrogenase activities in the culture medium in any of the experiments described above. However, we do not consider that this constitutes good evidence of the lack of toxicity of cytochalasin B. Both lactate dehydrogenase and malate dehydrogenase are unstable if released from cells by detergent treatment, so that small quantities released over prolonged time-intervals would not be detectable. The calf serum used in these experiments also contains non-specific NADH-oxidizing activity that would further mask small quantities of enzyme activity. Calf serum also contains leucine 2-naphthylamidase activity, and this prevents detection of low amounts of released intracellular enzyme. We therefore carried out more definitive tests for cytotoxicity, which we describe below.

Dye-exclusion tests

In view of the marked effects of cytochalasin B on both morphological and biochemical aspects of macrophage function, it is important to exclude non-specific toxic effects of the drug. No significant differences in the number of cells taking up Trypan Blue

Table 3. *Release of β-glucuronidase into the medium from macrophages cultured in the presence of increasing concentrations of cytochalasin B for 72h*

Values are expressed as means±S.E.M. of the numbers of observations indicated in parentheses.

Dose of cytochalasin B (μg/ml)	% of total enzyme activity in culture medium
0	28.0±3.2 (17)
2	34.5±7.1 (15)
5	53.7±4.1* (14)
10	70.5±2.3* (15)

* Significantly different from control ($P<0.001$).

Table 4. *Effect of cytochalasin B (2–10μg/ml) on the enzyme content of macrophage cultures*

Cultures were exposed to cytochalasin B for 72h. Enzyme activity is expressed as nmol of tyrosine released/h per plate for acid protease, as nmol of phenolphthalein released/h per plate for β-glucuronidase, as munits of activity/plate for lactate dehydrogenase, as nmol of β-naphthylamine released/h per plate for leucine 2-naphthylamidase and as munits/plate for malate dehydrogenase. Values are expressed as means±S.E.M. of the numbers of observations indicated within parentheses.

Concn. of cytochalasin B (μg/ml)	Acid protease	β-Glucuronidase	Lactate dehydrogenase	Leucine 2-naphthylamidase	Malate dehydrogenase
0	2295±179 (17)	431.9±29.6 (17)	350.1±39.3 (17)	1198±153 (14)	488.0±22.5 (4)
2	2414±234 (16)	417.1±36.0 (15)	384.9±37.4 (16)	1334±228 (15)	
5	2186±198 (14)	391.0±34.4 (14)	376.8±30.0 (15)	1523±209 (13)	
10	2068±220 (14)	401.2±30.9 (15)	336.6±43.2 (14)	1612±198 (12)	470.1±51.3 (4)

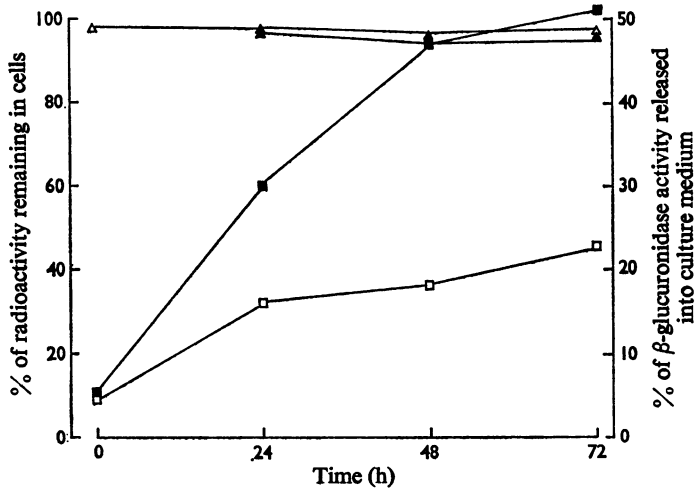

Fig. 4. *Effect of cytochalasin B* (10 μg/ml) *on release of radioactivity from macrophage cultures prelabelled with radioactive colloidal gold*

Each plate was incubated with radioactive colloidal gold (0.5 μCi) for 4 h at 37°C before exposure to cytochalasin B. At 24 h intervals up to 72 h plates were examined for release of radioactive label and β-glucuronidase into the culture medium: percentage of radioactivity remaining in control (△) and cytochalasin B-treated (▲) cells; percentage of β-glucuronidase released into culture medium in control (□) and cytochalasin B-treated (■) cells.

were seen between control and cytochalasin B-treated cultures, indicating that the drug is not toxic. Both in control cultures and at all concentrations of cytochalasin B more than 95% of the cells excluded Trypan Blue.

Retention of radioactive colloidal gold

Cohn (1968) showed that macrophages accumulate colloidal gold in secondary lysosomes in a form not readily released (Cohn & Benson, 1965*b*). We have confirmed this observation with radioactive gold, and Fig. 4 shows that macrophages labelled for 4 h before exposure to cytochalasin B for 72 h lose less than 5% of the label during this time. In marked contrast large quantities of β-glucuronidase are released from treated cultures during this time (Fig. 4). Other experiments (P. Davies, A. C. Allison & A. D. Haswell, unpublished work) have established that macrophages accumulate radioactive gold in a time- and temperature-dependent manner.

Discussion

The capacity of phagocytic cells to release selectively acid hydrolases under certain conditions has attracted attention recently because of the possible importance of this phenomenon in the mediation of various types of tissue damage involving polymorphonuclear leucocytes (Cochrane, 1968) and macrophages (Page *et al.*, 1972). Such release has been found to accompany phagocytosis by macrophages (Weissmann *et al.*, 1971) and polymorphonuclear leucocytes (Weissmann *et al.*, 1971; Henson, 1971; Hawkins & Peeters, 1971). Our group has shown elsewhere that this type of selective release is stimulated by cytochalasin B in both polymorphonuclear leucocytes (Davies *et al.*, 1973) and peritoneal macrophages (Temple *et al.*, 1973) when these cells are presented with a phagocytic stimulus. The experiments described in the present paper show that cytochalasin B also causes the selective release of acid hydrolases from these cells in the absence of a phagocytic stimulus. In polymorphonuclear leucocytes treated with cytochalasin B phagocytosis of bacteria is completely inhibited, and electron microscopy shows that lysosomes accumulate in a position immediately subjacent to the plasma membrane sites at which bacterial attachment occurs (Davies *et al.*, 1973). In macrophage cultures treated with cytochalasin B for 72 h and then allowed to recover for 3 h the cells regain their normal shape, but lysosomes are distributed throughout the peripheral cytoplasm, in contrast with their perinuclear position in untreated cells (P. Davies, A. C. Allison & A. D. Haswell, unpublished work). Therefore it is clear that the biochemical findings reported in this present paper have distinct morphological correlates.

Davies *et al.* (1973) have postulated that cytochalasin B exerts its effect by interfering with the function of a contractile microfilament system that normally plays a part in the maintenance of cellular organization, including the prevention of lysosomal access to the plasma membrane. This function is emphasized by the observation that microfilaments form a continuous meshwork around the periphery of phagocytic cells (Allison *et al.*, 1971).

The effects of cytochalasin B on the lysosomal systems of both polymorphonuclear leucocytes and macrophages occur without loss of cell viability. The augmentation of the effects of low doses of retinol in releasing acid hydrolases from polymorphonuclear leucocytes by cytochalasin B occurs in the absence of the release of lactate dehydrogenase. Moreover, at concentrations of retinol which cause cell lysis, as indicated by lactate dehydrogenase release, cytochalasin B gives acid hydrolase release that is superimposed on that obtained with retinol alone while having no effect on the release of lactate dehydrogenase. In macrophages exposed to cytochalasin B for 72h at doses up to 10μg/ml there was no detectable loss of non-lysosomal enzymes into the culture medium, nor any change in their intracellular activities. In a minority of our experiments the release of acid hydrolases was paralleled by an increase in the total activities of the various lysosomal hydrolases measured. This is reminiscent of the findings obtained by Dingle *et al.* (1967), who showed that the release of acid protease from embryonic limb-bone cultures was accompanied by an increase in total enzyme activities. It is also noteworthy that there is a marked, though not statistically significant, increase in the activity of leucine 2-naphthylamidase in cultures exposed to cytochalasin B (Table 5). The determination of the activity of an enzyme at any given time is only a crude indicator of the behaviour of that enzyme in the cultures, since it does not take into account the turnover of the enzyme within the cell and the decay of activity after release into the extracellular environment. More sensitive methods for detection of enzyme activity and turnover should yield useful information on these points. Others have reported on the lack of toxicity of cytochalasin B and its effects on RNA, DNA, phospholipid (Estensen, 1971) and protein synthesis (Estensen, 1971; Parkhouse & Allison, 1972). Cytochalasin B has, however, been shown to inhibit glucose transport in several cell types at low concentrations (Zigmond & Hirsch, 1972*a*,*b*; Estensen & Plagemann, 1972; Kletzien *et al.*, 1972; Mizel & Wilson, 1972). The significance of this inhibition to the biological effects of cytochalasin B is uncertain, especially since micropinocytosis, a possible entry route for glucose, is unaffected by cytochalasin B in macrophages (Wills *et al.*, 1972).

The failure of cytochalasin B to release radioactive colloidal gold from secondary lysosomes although large quantities of β-glucuronidase are being released deserves comment. This differential release may occur in several ways: (*a*) the gold may be tightly bound to a lysosomal component, such as that described by Dingle & Barrett (1969), which is not released during the loss of acid hydrolases; (*b*) the nature of the experiment described in the present paper does not exclude the possibility that the β-glucuronidase was released from a population of lysosomes formed after labelling with the radioactive gold; (*c*) the β-glucuronidase may have been released from a non-lysosomal site such as the endoplasmic reticulum (Fishman *et al.*, 1969). Although exocytosis seems to be the most likely route of acid hydrolase release in cytochalasin B-treated polymorphonuclear leucocytes and macrophages, we only have morphological evidence for this route of release in the former cell type when presented with a phagocytic stimulus (Davies *et al.*, 1973).

No information is available as to whether cytochalasin B has similar effects on the lysosomal system of non-phagocytic cells. Effects of cytochalasin B on induced release of other packaged secretory products are variable. As reviewed by Allison (1973), cytochalasin B in several systems inhibits such release, whereas in others it increases release, e.g. glucose-induced discharge of insulin from isolated rat pancreatic islet cells (Orci *et al.*, 1972) and antigen-induced release of histamine from sensitized leucocytes (Colten & Gabbay, 1972; Orr *et al.*, 1972). These apparently conflicting results can perhaps be reconciled by supposing that a microfilament network normally keeps granules away from the plasma membrane, and that either relaxation of this network by cytochalasin B, or induced contraction of the microfilament system into bundles leaving spaces between them, may allow the granules to gain access to the plasma membrane and be discharged. Whether cytochalasin B increases or inhibits release will then depend on a delicate balance that varies according to the cell type and experimental conditions used.

It is clear that cytochalasin B promotes the release of acid hydrolases from phagocytic cells under a variety of conditions. This occurs rapidly in the presence of a phagocytic stimulus (Davies *et al.*, 1973; Temple *et al.*, 1973) and under conditions where membranes are labilized by substances such as retinol. In the absence of any stimulus release occurs to a smaller extent in polymorphonuclear leucocytes (Table 1 and Fig. 1) and more slowly in macrophages (Fig. 3). The use of cytochalasin B to induce selective release of acid hydrolases from phagocytic cells may help to elucidate the role of these enzymes in tissue damage in which these cells are involved. Moreover it may facilitate analyses of other cells in which selective release of acid hydrolases or other products plays a role in their function in health and disease.

References

Allison, A. C. (1973) *Locomotion of Tissue Cells, Ciba Found. Symp.* in the press

Allison, A. C., Davies, P. & de Petris, S. (1971) *Nature (London) New Biol.* **232**, 153–155

Anson, M. L. (1937) *J. Gen. Physiol.* **20**, 565–574

Carter, S. B. (1967) *Nature (London)* **213**, 261–264

Cochrane, C. G. (1968) *Advan. Immunol.* **9**, 96–162

Cohn, Z. A. (1968) *Advan. Immunol.* **9**, 163–214

Cohn, Z. A. & Benson, B. (1965*a*) *J. Exp. Med.* **121**, 153–170

Cohn, Z. A. & Benson, B. (1965*b*) *J. Exp. Med.* **122**, 455–466

Colten, H. & Gabbay, K. (1972) *J. Clin. Invest.* **51**, 1927–1931

Conchie, J., Findlay, J. & Levvy, G. A. (1959) *Biochem. J.* **71**, 318–325

Crawford, B., Cloney, R. A. & Cahn, R. D. (1972) *Z. Zellforsch.* **130**, 135–151

Davies, P., Krakauer, K. & Weissmann, G. (1970) *Nature (London)* **228**, 761–762

Davies, P., Rita, G. A., Krakauer, K. & Weissmann, G. (1971) *Biochem. J.* **123**, 559–569

Davies, P., Allison, A. C., Fox, R. I., Polyzonis, M. & Haswell, A. D. (1972) *Biochem. J.* **128**, 78P–79P

Davies, P., Fox, R. I., Polyzonis, M., Allison, A. C. & Haswell, A. D. (1973) *Lab. Invest.* **28**, in the press

Dingle, J. T. & Barrett, A. J. (1969) *Proc. Roy. Soc. Ser. B* **173**, 85–93

Dingle, J. T., Fell, H. B. & Coombs, R. B. (1967) *Int. Arch. Allergy Appl. Immunol.* **31**, 283–303

Dulbecco, R. & Vogt, M. (1954) *J. Exp. Med.* **99**, 167–182

Estensen, R. D. (1971) *Proc. Soc. Exp. Biol. Med.* **136**, 1256–1260

Estensen, R. D. & Plagemann, P. G. W. (1972) *Proc. Nat. Acad. Sci. U.S.* **69**, 1430–1434

Fishman, W. H., Ide, H. & Rufo, R. (1969) *Histochemie* **20**, 287–299

Gail, M. H. & Boone, C. W. (1971) *Exp. Cell Res.* **68**, 226–228

Goldbarg, J. A. & Rutenberg, A. M. (1958) *Cancer* **11**, 283–291

Hawkins, D. & Peeters, S. (1971) *Lab. Invest.* **24**, 483–491

Henson, P. M. (1971) *J. Exp. Med.* **134**, 114S–135S

Kletzien, R. F., Perdue, J. F. & Springer, A. (1972) *J. Biol. Chem.* **247**, 2964–2966

Krishnan, A. (1971) *J. Ultrastruct. Res.* **36**, 191–204

Krishnan, A. & Raychaudhuri, R. (1969) *J. Cell Biol.* **43**, 618–621

Mizel, S. B. & Wilson, L. (1972) *J. Biol. Chem.* **247**, 4102–4105

Orci, L., Gabbay, H. & Malaisse, W. J. (1972) *Science* **175**, 1128–1130

Orr, T. S. C., Hall, D. E. & Allison, A. C. (1972) *Nature (London)* **236**, 350–351

Page, R. C., Davies, P. & Allison, A. C. (1972) in *Host Resistance to Commensal Bacteria* (MacPhee, I. T., ed.), pp. 195–201, Churchill-Livingstone, Edinburgh

Parkhouse, R. M. E. & Allison, A. C. (1972) *Nature (London) New Biol.* **235**, 220–222

Prescott, D. M., Kates, J. & Kirpatrick, J. B. (1971) *J. Mol. Biol.* **59**, 505–508

Ridler, M. A. C. & Smith, G. F. (1968) *J. Cell Sci.* **3**, 595–602

Sanger, J. W., Holtzer, S. & Holtzer, H. (1971) *Nature (London) New Biol.* **229**, 121–123

Spooner, B. S., Yamada, K. M. & Wessells, N. K. (1971) *J. Cell Biol.* **49**, 595–613

Talalay, P., Fishman, W. H. & Huggins, C. (1946) *J. Biol. Chem.* **166**, 757–772

Temple, A., Loewi, G., Davies, P. & Howard, A. (1973) *Immunology* in the press

Vaes, G. (1968) *J. Cell Biol.* **39**, 676–697

Weissmann, G., Dukor, P. & Zurier, R. (1971) *Nature (London) New Biol.* **231**, 131–135

Wills, E. D., Davies, P., Allison, A. C. & Haswell, A. D. (1972) *Nature (London) New Biol.* **240**, 58–60

Woodin, A. M. & Wieneke, A. A. (1963) *Biochem. J.* **90**, 498–509

Woolen, J. W., Heyworth, R. & Walker, P. G. (1961) *Biochem. J.* **78**, 111–116

Zigmond, S. H. & Hirsch, J. G. (1972*a*) *Science* **175**, 1432–1434

Zigmond, S. H. & Hirsch, J. G. (1972*b*) *Exp. Cell Res.* **73**, 383–393