Changes in the subcellular distribution of the cytochrome b_{-245} on stimulation of human neutrophils

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Cytochrome b_{-245} of neutrophils has a bimodal distribution in sucrose density gradients. The lighter component ($d = 1.14$) is shown to be associated with the plasma membrane by the similarity between its density and that of markers of this organelle, as well as a parallel increase in the density of the cytochrome and plasma membrane after treatment with digitonin or dimethyl suberimidate. The cytochrome b_{-245} of monocytes and cytoplasts, the latter produced by the removal of nuclei and granules from neutrophils, was located only in the plasma membrane. The denser peak of cytochrome ($d = 1.19$), which contained approximately half of the cytochrome b of neutrophils, had a similar density-distribution profile to the specific granules. After hypo-osmotic disruption of this denser material, the cytochrome distributed with the density of membranes, suggesting an original location within the membrane of the intracellular structure. Redistribution of the cytochrome from the granules to the membranes was observed after stimulation of respiratory activity with soluble agents or opsonized particles. This translocation is not responsible for activation of the oxidase system. There was poor agreement between the kinetics of the transfer of cytochromes from the dense component to the membranes, and degranulation of specific-granule contents, suggesting that the cytochrome may be located in another intracellular structure or that its localization becomes further modified after granule fusion.

The 'professional' phagocytic cells, neutrophils, monocytes, macrophages and eosinophils, undergo a burst of non-mitochondrial respiration when they interact with microbes (Sbarra & Karnovsky, 1959; Selvaraj & Sbarra, 1966; Badwey & Karnovsky, 1980). This respiration, which has an important microbicidal function, is accomplished by an electron-transport chain containing a very-low-potential cytochrome b_{-245} (Segal & Jones, 1979a; Cross *et al.*, 1981, 1982). In the neutrophil this molecule has a dual localization on isopycnic centrifugation on sucrose gradients. The lighter component distributes with markers for the plasma membrane, whereas the denser component is found with the specific granules (Segal & Jones, 1979b).

The present study was conducted to characterize further the subcellular distribution of this cytochrome and to investigate the changes in this distribution accompanying phagocytosis, activation of the respiratory burst and degranulation.

Methods

Purification of cells

Neutrophils were isolated from buffy-coat residues from normal donors as described previously (Segal *et al.*, 1980). Mononuclear cells were obtained from a patient with polycythaemia (Wetherley-Mein & Pearson, 1982) by centrifugation on Ficoll/sodium metrizoate (Boyum, 1968) and subsequently enriched in monocytes by centrifugation on a discontinuous gradient of Percoll (Ulmer & Flad, 1979). The final mixture contained 69% non-specific-esterase-positive cells (Yam *et al.*, 1971) and less than 1% neutrophils.

The cells were suspended in RPMI 1640 medium (Flow Laboratories, Irvine, Ayrshire, Scotland, U.K.) containing heparin (5 units/ml) unless stated otherwise.

Subcellular fractionation

All the procedures were carried out at 0–4°C. Washed cell pellets were homogenized in 0.26 M-

sucrose containing the proteinase inhibitors phenylmethanesulphonyl fluoride (1 mM) and Trasylol (200 Kallikrein Inactivator units/ml; Bayer U.K. Ltd., Haywards Heath, W. Sussex, U.K.), as described previously (Segal *et al.*, 1983). Post-nuclear supernatants of neutrophils and monocytes (8–10 ml) were fractionated by centrifugation through continuous gradients of sucrose (25–27 ml, density range 1.10–1.27 g/ml) on a 3 ml cushion of sucrose (1.29 g/ml). All sucrose solutions contained 1 mM-EDTA (pH 7.4) and heparin (5 units/ml). The gradients were spun at 26000 rev/min in an AH 627 swing-out rotor, in an OTD 50B Sorvall centrifuge (integrated angular velocity = $2.71 \times 10^{11} \text{ rad}^2 \cdot \text{s}^{-1}$; $g_{\text{max.}}$ 125000, $g_{\text{min.}}$ 54500, $g_{\text{av.}}$ 89900). The gradients were unloaded by upward displacement with dense (60%, w/w) sucrose; 20–30 fractions (1.0–1.5 ml) were collected.

The specific (or secondary) granules are found at an equilibrium density of $1.19 \pm 0.01 \text{ g/ml}$ and were identified by the markers lactoferrin and cyanocobalamin-binding protein. The azurophil (or primary) granules have a density of 1.21–1.25 g/ml. They were identified by the marker enzyme myeloperoxidase. Lysozyme is found in both the specific and azurophil granules.

In one experiment, the bands that were visible in the gradient were collected by aspiration from above, and that enriched in the activity of the specific-granule markers was frozen and thawed, diluted to a density of 1.07 g/ml with 1 mM-EDTA (pH 7.4), and centrifuged at 100000 g for 30 min in a Sorvall T 865.1 angle rotor. The pellet was resuspended in 1 mM-EDTA (pH 7.4), homogenized in a Dounce homogenizer (Kontes Glass, Vineland, NJ, U.S.A.) with 30 strokes of a tight-fitting B pestle and kept at 0°C for 15 min. The density was then increased to 1.04 g/ml by adding 0.64 M-sucrose, and the preparation was fractionated as described above.

In another experiment, digitonin (3 μmol) in sucrose (0.26 M; 5 ml) containing EDTA (1 mM) was added to an equal volume of post-nuclear supernatant containing 60 mg of protein, and immediately fractionated on a sucrose gradient. A control experiment was conducted simultaneously on the same post-nuclear supernatant by omitting the digitonin.

Treatments of cells

(a) *Surface labelling.* Intact neutrophils (10^{10}) were incubated in 11.5 ml of RPMI medium (pH 7.0) containing galactose oxidase (4.3 Sigma units/ml) and NaB^3H_4 (130 $\mu\text{Ci/ml}$) for 60 min, at room temperature (Fukuda *et al.*, 1979). Cells were also incubated with NaB^3H_4 in the absence of galactose oxidase to determine non-specific radio-labelling. Incubations were terminated by the addition of NaBH_4 (1 mg), and the cells were then

washed with $3 \times 50 \text{ ml}$ of cold 0.15 M-NaCl, with centrifugation at 250 g for 3 min between washes. The cells were then fractionated as described above.

(b) *Incubation with dimethyl suberimide.* A suspension of neutrophils (1.7×10^9) in RPMI medium (4 ml) was adjusted to pH 8.0 with NaOH and incubated with 1 mg of dimethyl suberimide for 20 min at room temperature, with occasional stirring (Davies & Stark, 1970). Glycine (100 mg) was then added, and after 5 min the cells were washed with cold 0.15 M-NaCl and fractionated as described above.

(c) *Stimulation of degranulation and respiration.* Neutrophils were stimulated with (a) the soluble agents phorbol myristate acetate or *N*-formyl-methionyl-leucylphenylalanine, the latter in the presence of cytochalasin B, and (b) particles [paraffin-oil droplets (Stossel *et al.*, 1972), opsonized latex (Segal & Jones, 1979b), or opsonized heat-killed *Staphylococcus aureus* (Segal & Coade, 1978)].

The rate of oxygen consumption was measured with an oxygen electrode as described by Segal *et al.* (1980).

At the end of the incubations the cells were adjusted to 50 ml with ice-cold 0.15 M-NaCl and centrifuged at 250 g for 5 min. The pellets were washed once more with cold 0.15 M-NaCl and then fractionated as described above.

(d) *Preparation of cytoplasts.* Neutrophil cytoplasts were prepared exactly as described by Roos *et al.* (1983). The cytoplasts were then pelleted at 200 g for 5 min, washed, resuspended in sucrose (0.26 M), homogenized in the same way as for intact neutrophils, and then sonicated (5 s at amplitude 4; MSE ultrasonic disintegrator model 150W) to complete disruption.

Determination of cytochrome b

The concentrations of cytochrome *b* were determined from the peak of absorption at 559 nm in reduced-minus-oxidized difference spectra (Cross *et al.*, 1982).

Markers

Lysozyme (EC 3.2.1.17) was measured by the method of Klass *et al.* (1977), and myeloperoxidase (EC 1.11.1.7) as described by Bretz & Baggiolini (1974). 5'-Nucleotidase (EC 3.1.3.5) was determined by a modification (Segal *et al.*, 1983) of the method of Douglas *et al.* (1972). Cytochrome oxidase (EC 1.9.3.1) was measured by the oxidation of reduced cytochrome *c* (Cooperstein & Lazarow, 1951), and cyanocobalamin-binding protein as described by Kane *et al.* (1974). Lactoferrin was determined by a double-antibody radioassay (Fedail *et al.*, 1978), with lactoferrin purified as described by Bläckberg & Hernell (1980) as standard.

Receptors for *N*-formylmethionyl-leucylphenylalanine were determined as described by Bennett *et al.* (1980).

Radiochemicals

N-Formylmethionyl-leucyl[ring-2,6-³H]phenylalanine (50 Ci/mmol) was obtained from New England Nuclear, Boston, MA, U.S.A., and NaB³H₄ (469 Ci/mol), [2-³H]AMP (ammonium salt, 19.3 Ci/mmol), cyano[⁵⁷Co]cobalamin

(220 μ Ci/ μ g) and Na¹²⁵I (100 mCi/ml, for lactoferrin iodination) were from Amersham International, Amersham, Bucks., U.K.

Results

Subcellular distribution of cytochrome b in unstimulated cells

The cytochrome *b* of human neutrophils demon-

Fig. 1. Relationship between the light component of cytochrome *b* (◆) and the plasma membrane. The results from two experiments are shown. In (a) and (b) the cell surface was labelled with galactose oxidase and NaB³H₄ (◇), whereas in (c) and (d) the binding of *N*-formylmethionyl-leucylphenylalanine (□) was used to identify the plasma membrane. The distributions of 5'-nucleotidase (▲) and lysozyme (△), the latter to indicate the localization of the granules, are also shown.

Fig. 2. Effect of dimethyl suberimidate on the density-distribution profile of (a) cytochrome *b*, (b) 5'-nucleotidase, (c) cyanocobalamin-binding protein, (d) lysozyme, (e) myeloperoxidase and (f) lactoferrin
 ----, Non-treated cells; —, treated cells

strated a bimodal distribution after isopycnic centrifugation on sucrose gradients, as described previously (Segal & Jones, 1979b), with $42.5 \pm 5.2\%$ and $42.8 \pm 5.1\%$ (means $\pm$ S.E.M., $n = 13$) of the total cytochrome *b* being associated with the peaks at densities of 1.14 (1.11–1.16) and 1.19 (1.17–1.21) g/ml respectively.

The light peak of cytochrome b

This peak distributed with the membranous components. To locate it specifically in the plasma membrane, various techniques were used to identify this organelle selectively. Heterogeneity of the density-distribution profile of the different markers was observed. The cytochrome was

most closely associated with surface components labelled by galactose oxidase and NaB^3H_4 and with receptors for *N*-formylmethionyl-leucyl-phenylalanine (Fig. 1). However, it did not coincide closely with the activity of 5'-nucleotidase, which showed a light peak at a density of 1.11 g/ml and a denser shoulder at 1.14 g/ml, which sometimes resolved into a second peak which corresponded more closely to the light peak of the cytochrome *b*. The likelihood that 5'-nucleotidase has a dual localization within the cell is heightened by the finding that cytoplasts only had a single peak of activity at 1.14 g/ml, which corresponded exactly with the distribution of the cytochrome of these structures (Fig. 4).

To establish more firmly the association of the light peak of cytochrome with the plasma membrane, we examined the effect of changing the density of this organelle on the distribution of the cytochrome. The first technique used was to add digitonin to a post-nuclear supernatant (Amar-Costesec *et al.*, 1974). This complexes cholesterol and increases the density of the organelles according to their content of this sterol. The densities of 5'-nucleotidase and the light peak of cytochrome *b* were increased from 1.14 to 1.19 g/ml, and that of the markers of the specific granules and the dense peak of cytochrome *b* changed in a bimodal way, from 1.19 to 1.21 and 1.25 g/ml. Smaller increases in the density of the azurophil granules were also observed.

To produce more selective changes in the density of the plasma membrane, we attempted to couple ferritin to the surface of the cells by means of the cross-linking agent dimethyl suberimidate (Peters & Richards, 1977). We found the addition of ferritin unnecessary as the dimethyl suberimidate alone markedly increased the density of the plasma membrane (Fig. 2). The dual distribution of the 5'-nucleotidase, with peaks at 1.11 and 1.145 g/ml, was converted into a major peak with a density of 1.135 g/ml with a shoulder at 1.17 g/ml. Most of the light component of the cytochrome *b* increased in density and moved to form a shoulder at a density of 1.17 g/ml, merging with the lighter components of the denser peak of cytochrome. Treatment with dimethyl suberimidate did not influence the density of the specific or azurophil granules. In addition, it did not appear to damage the 5'-nucleotidase or cytochrome *b*, recoveries of which were 100 and 87% respectively.

In monocytes the cytochrome *b* was found to be predominantly located in a single band, corresponding very closely to the plasma-membrane marker 5'-nucleotidase (Figs. 3c and 3d). A minor peak of cytochrome was found in the denser regions of the gradient in association with the mitochondrial marker cytochrome *c* oxidase.

In cytoplasts, i.e. neutrophils depleted of nuclei and granules, the cytochrome *b* was also observed as a single band at a density of 1.14 g/ml, which coincided exactly with the distribution of the 5'-nucleotidase (Fig. 4).

The dense peak of cytochrome b

This peak co-distributed with lactoferrin, cyanocobalamin-binding protein and the light peak of lysozyme, markers of the specific granules. A clear separation of the two peaks of cytochrome *b* from mitochondria was most obvious on a less steep gradient (Fig. 3a). The mitochondrial marker cytochrome *c* oxidase is found between the peaks of cytochrome b_{-245} , and therefore those cytochromes in this 'valley' are most likely to be predominantly mitochondrial in origin. The mitochondrial contribution to the cytochromes in the cell was more obvious in monocytes, which contain much greater numbers of mitochondria than neutrophils (Fig. 3c).

An attempt was then made to clarify the location of the cytochrome *b* within the organelle that co-locates with the specific granules. Fractions enriched in this material were collected from a gradient, disrupted, and fractionated on a second gradient (Fig. 5). Most of the cytochrome was then observed in the lighter regions of the gradient normally occupied by membranes, with only a small residual amount of cytochrome *b* at the original density of the specific granules. The markers of the contents of the specific granules had a different distribution. Most of the lysozyme was solubilized and remained at the light region of the gradient, whereas the other two markers, lactoferrin and cyanocobalamin-binding protein, retained their usual position at 1.19 g/ml.

Translocation of the cytochrome b

Having confirmed the dual localization of the cytochrome *b* in neutrophils, we investigated the changes in the pattern of distribution of this molecule when the cells were stimulated to phagocytose and respire. Of particular interest was the fate of the granule-associated cytochrome. By using particles opsonized with immunoglobulin G, a shift of a proportion of the cytochrome was observed from the dense to the light location in the gradients (Table 1), indicating that the release of granule components was associated with a change in the location of the cytochrome *b*. Not only was this transfer of cytochrome from granules to membranes seen after phagocytosis of light latex particles, but also with *S. aureus*, which is much denser than the specific granules. Although structures associated with the membrane of the phagocytic vacuole might be expected to move into the sucrose gradient together with the dense bacteria,

Fig. 3. Comparison of the density-distribution profile of cytochrome *b* (●) in neutrophils (a and b) and monocytes (c and d) ◇, 5'-Nucleotidase; Δ, cytochrome *c* oxidase; ◆, myeloperoxidase.

the vacuole is probably disrupted by the homogenization procedure. After phagocytosis of bacteria, digestion of bacterial components causes osmotic swelling of the vacuole, making it susceptible to rupture (Segal *et al.*, 1981).

When the neutrophils were given a large number of particles to ingest (Fig. 6), in this case paraffin-oil droplets opsonized with immunoglobulin G, there was a very substantial transfer of cytochrome *b* from the granule to the membrane peak. This effect was dependent on the opsonin. When albumin was used, phagocytosis was decreased by 80%, oxygen consumption by 76%, and transfer of the cytochrome was not detected.

To determine whether the cytochrome was moving specifically to the phagocytic vacuole or

simply fusing with the plasma membrane, we isolated phagocytic vacuoles containing latex particles, by flotation (Segal *et al.*, 1980). These experiments showed the shift of cytochrome from the granules to the vacuoles, with the amount of cytochrome in the membranous fraction remaining constant or increasing slightly.

The soluble stimuli *N*-formylmethionyl-leucyl-phenylalanine (in the presence of cytochalasin B) and phorbol myristate acetate also stimulated the transfer of the granule-associated cytochrome *b* to the membranes (Table 2). The time course of phorbol myristate acetate-induced translocation was studied in particular, and was a prolonged process that proceeds substantially slower than the increase in the rate of oxygen consumption by the

Fig. 4. Density-distribution profile of cytochrome *b* (●) and 5'-nucleotidase (Δ) of cytoplasts

Fig. 5. Density-distribution profiles of components of specific granules after hypo-osmotic disruption
●, Cytochrome *b*; □, lactoferrin; Δ, cyanocobalamin-binding protein; ◆, lysozyme; ■, protein.

Fig. 6. Changes in the subcellular distribution of cytochrome *b* in association with phagocytosis

Neutrophils (2.7×10^9) were incubated in RPMI medium (24 ml) alone (a), with paraffin-oil droplets in the presence of bovine serum albumin (1.7 mg/ml; b), or with immunoglobulin G (1.7 mg/ml; c). After 20 min at 37°C the cells were taken into and washed with cold 0.15 M-NaCl and fractionated. The density-distribution profiles of cytochrome *b* (●) and lysozyme (○) are shown.

cells and continues after the respiration rate reached a maximum.

A discrepancy was observed when the decrease

Table 1. *Effect of phagocytosis on the redistribution of cytochrome b from the specific granule to the membrane compartment*
Granulocytes (2.5×10^7 /ml in Expts. 1 and 3, 7.4×10^7 /ml in Expt. 2) were incubated at 37°C in RPMI medium with or without opsonized particles for the times indicated before they were washed and fractionated. Densities: membranes, 1.11–1.15 g/ml; specific granules, 1.18–1.20 g/ml.

Expt. no.	Phagocytic particle	Ratio particles/neutrophils	Cytochrome b (%)	
			Membranes	Specific granules
1	None	—	42.1	57.9
	<i>S. aureus</i> , 5 min	30	54.0	46.0
	<i>S. aureus</i> , 30 min	30	65.9	34.1
2	None	—	35.0	65.0
	Latex ($1 \mu\text{m}$), 15 min	50	59.5	40.5
3	None	—	35.9	64.1
	Latex ($1 \mu\text{m}$), 5 min	60	49.4	50.6
	Latex ($1 \mu\text{m}$) + cytochalasin ($5 \mu\text{g}/\text{ml}$), 5 min	60	50.2	49.8
	Latex ($5 \mu\text{m}$), 5 min	2	48.6	51.4

Table 2. *Relationship between the translocation of the cytochrome b to the membranes and the release of specific-granule contents*

Granulocytes (1.5×10^9) were incubated for 30 min at 37°C in RPMI medium (50 ml) with the additions indicated, and then washed and fractionated. The different bands after isopycnic centrifugation were collected as a single fraction each by aspiration from above and analysed for cytochrome b and granule markers. The results were normalized against the control according to the content of lysozyme of the intact cells, taking into account the efficiency of cell disruption (range 90–95%). Oxygen-consumption rates were measured during the course of the incubations, and the total oxygen consumption was calculated by integration of the rate-versus-time curves.

	Relative total oxygen consumption	Depletion of cytochrome b from specific-granule compartment (%)	Depletion of specific-granule markers (%)		Depletion of myeloperoxidase from azurophilic granules (%)
			Lysozyme	Cyanocobalamin binding protein	
None	1	—	—	—	—
<i>S. aureus</i> (3:1 ratio)	27.5	18.7	50.0	42.2	11.3
Phorbol myristate acetate ($0.5 \mu\text{g}/\text{ml}$)	18.9	47.2	83.8	81.4	0
Cytochalasin B ($5 \mu\text{g}/\text{ml}$) + <i>N</i> -formylmethionyl-leucylphenylalanine ($0.2 \mu\text{M}$)	13.1	22.6	86.3	85.4	44.2

Table 3. *Lack of inhibition by colchicine of the redistribution of cytochrome b after stimulation with phorbol myristate acetate (PMA)*

Neutrophils (1.8×10^9) were preincubated in RPMI medium (30 ml) with or without colchicine ($50 \mu\text{M}$) at 37°C for 40 min. PMA ($5 \mu\text{g}/\text{ml}$) was then added to half of each mixture, and the incubations were continued for 20 min at 37°C before the cells were washed and fractionated.

Preincubation	Incubation	Cytochrome b (%)	
		Membranes	Specific granules
—	—	38.4	61.6
Colchicine	—	35.4	64.6
—	PMA	68.1	31.9
Colchicine	PMA	63.4	36.6

in the concentration of cytochrome b in the fractions containing specific granules was compared with that of the markers cyanocobalamin-

binding protein and lysozyme (Table 2). Both markers were similarly depleted from the specific granules, whereas the decrease in cytochrome b was appreciably less.

Neither cytochalasin B nor colchicine prevented the transfer of the cytochrome b from the granule to the membrane fraction (Tables 1 and 3), indicating that microfilaments and microtubules are unlikely to play an important role in the translocation process.

Discussion

Initial studies indicated that cytochrome b_{-245} has a dual localization in neutrophils. A light peak was associated with the plasma membrane, and a denser one with the specific granules (Segal & Jones, 1979b). Borregaard *et al.* (1983) have disrupted the cells by nitrogen cavitation and

fractionated them by centrifugation on a gradient of Percoll and obtained different results. They found that almost all the cytochrome was associated with the granules and only a very small proportion with the membrane. They postulated that activation of the oxidase system was produced by translocation of the cytochrome from the granules to the membranes.

The current study clearly shows that in fact about half the cytochrome *b* is associated with the plasma membrane after analytical subcellular fractionation. Evidence that this was not simply released by disruption of the granules during homogenization of the cells is provided by: (1) the absence of the liberation of granule contents, which would distribute with the cytosol; (2) parallel increases in the density of the light peak of cytochrome *b* and the plasma membrane by treatment with digitonin and dimethyl suberimide. The alterations produced by the suberimide were particularly enlightening, because there was a clear increase in the density of the plasma membrane, but not of the intracellular organelles.

Further evidence for the association of the cytochrome *b* with the plasma membrane was the finding that it localized with this organelle in other cells. It closely corresponded to the plasma-membrane marker 5'-nucleotidase in monocytes in this study, and has been previously shown to distribute with the membranes in HL-60 cells, a promyelocytic cell line that lacks specific granules (Roberts *et al.*, 1982). In addition, the removal of granules from neutrophils to form cytoplasts resulted in the loss of the dense cytochrome component, while leaving that associated with the plasma membrane.

The association of the cytochrome with the membranes of all these cells and neutrophil cytoplasts, in the unstimulated state, indicates that there is no need to invoke fusion of the dense component with the membranes as an activation mechanism.

The observation that the dense peak of cytochrome *b* has a similar density distribution to that of the specific granules, together with the lack of this dense material in monocytes and HL-60 cells, which lack these granules, provides evidence for the location of the cytochrome with these granules. However, unlike Borregaard *et al.* (1983), we observed a discrepancy between the rates of transfer of the cytochrome *b* from the region of the specific granules to the membranes and the release of the specific granule contents. If our observations are correct, this indicates that the cytochrome *b* is not in these granules, or that they are heterogeneous, or that there is redistribution of the cytochrome or granule contents through membrane recycling, after fusion. Distinction between these

possibilities and a more detailed examination of the movements of the cytochrome awaits electron-microscopic immunohistochemistry.

A plasma-membrane location for the cytochrome *b* appears logical because, as this membrane invaginates to form the wall of the phagocytic vacuole, its contained cytochrome would be ideally located to reduce oxygen inside the vacuole. The cytochrome is situated in this organelle in monocytes, HL-60 cells and neutrophils. What is less clear is why neutrophils should have a second, denser, pool of the cytochrome. It could act as a reserve pool of membrane containing a functional oxidase system, to replace the plasma membrane consumed in the formation of the wall of the phagocytic vacuole. The studies in which phagocytic vacuoles were isolated clearly showed that the cytochrome *b* that disappeared from the granule compartment could be accounted for in the phagocytic vacuoles. The requirement for a reserve pool of membrane and cytochrome in neutrophils, but not in monocytes and macrophages, could be explained on the basis that, unlike neutrophils (DeChatelet *et al.*, 1973), the mononuclear cells have the capacity for extensive protein synthesis and could more easily replace membrane and its contained electron-transport chain if required. Another possibility is that the neutrophil usually engulfs a larger number of particles and hence needs to replace more plasma membrane than do the other cell types.

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References

- Amar-Costesec, A., Wibo, M., Thines-Sempoux, D., Beaufay, H. & Berthet, J. (1974) *J. Cell Biol.* **62**, 717–745
- Badwey, J. A. & Karnovsky, M. L. (1980) *Annu. Rev. Biochem.* **49**, 695–726
- Bennett, J. P., Cockcroft, S. & Gomperts, B. D. (1980) *Biochim. Biophys. Acta* **601**, 584–591
- Bläckberg, L. & Hernell, O. (1980) *FEBS Lett.* **109**, 180–184
- Borregaard, N., Heiple, J. M., Simons, E. R. & Clark, R. A. (1983) *J. Cell Biol.* **97**, 52–61
- Boyum, A. (1968) *Scand. J. Clin. Lab. Invest.* **21**, Suppl. **97**, 31–50
- Bretz, U. & Baggiolini, M. (1974) *J. Cell Biol.* **63**, 251–269
- Cooperstein, S. J. & Lazarow, A. (1951) *J. Biol. Chem.* **189**, 665–670
- Cross, A. R., Jones, O. T. G., Harper, A. M. & Segal, A. W. (1981) *Biochem. J.* **194**, 599–606
- Cross, A. R., Higson, F. K., Jones, O. T. G., Harper, A. M. & Segal, A. W. (1982) *Biochem. J.* **204**, 479–485

- Davies, G. E. & Stark, G. R. (1970) *Proc. Natl. Acad. Sci. U.S.A.* **66**, 651-656
- DeChatelet, L. R., McCall, C. E. & McPhail, L. C. (1973) *Infect. Immun.* **8**, 791-795
- Douglas, A. P., Kerley, R. & Isselbacher, K. J. (1972) *Biochem. J.* **128**, 1329-1338
- Fedail, S. S., Salmon, P. R., Harvey, R. F. & Read, A. E. (1978) *Lancet* **i**, 181-182
- Fukuda, M. N., Fukuda, M. & Hakomori, S. (1979) *J. Biol. Chem.* **254**, 5458-5465
- Kane, S. P., Hoffbrand, A. V. & Neale, G. (1974) *Gut* **15**, 953-959
- Klass, H. J., Hopkins, J., Neale, G. & Peters, T. J. (1977) *Biochem. Med.* **18**, 52-57
- Peters, K. & Richards, F. M. (1977) *Annu. Rev. Biochem.* **46**, 523-551
- Roberts, P. J., Cross, A. R., Jones, O. T. G. & Segal, A. W. (1982) *J. Cell Biol.* **95**, 720-726
- Roos, D., Voetman, A. A. & Meerhof, L. J. (1983) *J. Cell Biol.* **97**, 368-377
- Sbarra, A. J. & Karnovsky, M. L. (1959) *J. Biol. Chem.* **234**, 1355-1362
- Segal, A. W. & Coade, S. B. (1978) *Biochem. Biophys. Res. Commun.* **84**, 611-617
- Segal, A. W. & Jones, O. T. G. (1979a) *Biochem. Biophys. Res. Commun.* **88**, 130-134
- Segal, A. W. & Jones, O. T. G. (1979b) *Biochem. J.* **182**, 181-188
- Segal, A. W., Dorling, J. & Coade, S. B. (1980) *J. Cell Biol.* **85**, 42-59
- Segal, A. W., Geisow, M., Garcia, R., Harper, A. & Miller, R. (1981) *Nature (London)* **290**, 406-409
- Segal, A. W., Garcia, R. C., Harper, A. M. & Banga, J. P. (1983) *Biochem. J.* **210**, 215-225
- Selvaraj, R. J. & Sbarra, A. J. (1966) *Nature (London)* **211**, 1272-1276
- Stossel, T. P., Mason, R. J., Hartwig, J. & Vaughan, M. (1972) *J. Clin. Invest.* **51**, 615-624
- Ulmer, A. J. & Flad, H. D. (1979) *J. Immunol. Methods* **30**, 1-10
- Wetherley-Mein, G. & Pearson, T. C. (1982) in *Blood and its Disorders* (Hardisty, R. M. & Weatherall, D. J., eds.), pp. 1269-1316, Blackwell Scientific Publications, Oxford
- Yam, L. T., Li, C. Y. & Crosby, W. H. (1971) *Am. J. Clin. Pathol.* **55**, 283-290