Biochimica et Biophysica Acta, 640 (1981) 223—230

BBA 79061

ROLE OF Ca^{2+} AND Mg^{2+} IN NEUTROPHIL HEXOSE TRANSPORT

J.T. O'FLAHERTY, S. COUSART, C.L. SWENDSEN, L.R. DeCHATELET, D.A. BASS, S.H. LOVE and C.E. McCALL

Division of Infectious Diseases, Wake Forest University, Bowman Gray School of Medicine, Department of Medicine, 300 South Hawthorne Rd., Winston-Salem, NC 27103 (U.S.A.)

(Received June 2nd, 1980)

Key words: Hexose transport; Ca^{2+}; Mg^{2+}; Ionophore; Chemotactic factor, Arachidonic acid; (Neutrophil)

Summary

The influence of extracellular Ca^{2+} and Mg^{2+} on the transport of 2-deoxy-[^{3}H]glucose into human polymorphonuclear neutrophils was studied. Omission of these cations from the cell suspensions had little effect on resting hexose uptake. Furthermore, the addition of the bivalent cation chelator, EDTA, depressed uptake only slightly. Similarly, neither cation was essential for the enhanced 2-deoxy-D-[^{3}H]glucose uptake stimulated by two chemotactic factors (C5a and *N*-formylmethionylleucylphenylalanine) and arachidonic acid: enhanced uptake was only partially depressed by the omission of Ca^{2+} and Mg^{2+} from the suspensions and was still prominent in the presence of EDTA. Two other neutrophil stimulants, the ionophores, A23187 and ionomycin, also enhanced hexose uptake but their actions were heavily dependent upon extracellular bivalent cations and were totally abrogated by EDTA. In all instances, extracellular Ca^{2+}, but not Mg^{2+}, supported optimal enhanced hexose transport induced by stimuli.

Activation of 2-deoxy-D-[^{3}H]glucose uptake by each of the five stimuli was totally blocked by cytochalasin B (a blocker of carrier-mediated hexose transport) and D-glucose but not by L-glucose. The data indicate, therefore, that a variety of neutrophil stimulants activate carrier-mediated hexose transport. Although this transport can be triggered by the movement of extracellular Ca^{2+} into the cell (as exemplified by the action of the two ionophores), such Ca^{2+} movement is not required for the actions of chemotactic factors or arachidonic acid. Other mechanisms, such as a rearrangement of intracellular Ca^{2+}, may be involved in mediating the activation of hexose transport induced by the latter stimuli.

Introduction

Ca^{2+} commonly appears to act as a modulator of cellular response [1]. In the polymorphonuclear neutrophil, for instance, many responses induced by chemotactic factors and arachidonic acid require extracellular Ca^{2+} [2—12]. Indeed, the same stimuli cause neutrophils to take up extracellular Ca^{2+} [6,7, 9,12—15] and agents which inhibit this uptake similarly inhibit diverse cellular responses [4,6,7,9,11,16]. Furthermore, an ionophore, A23187, which selectively binds Ca^{2+} [17,18] and transports it into the neutrophil [13,15], possesses bioactivities similar to those of chemotactins and arachidonate [2,4,6,10,12,13,19,20]. The concept has evolved, therefore, that Ca^{2+} movement into the neutrophil alters the level of this cation in the cytosol or at some subcellular site; this alteration, in turn, mediates the action of diverse stimuli by triggering events which culminate in functional responses.

In addition to extracellular Ca^{2+}, various neutrophil responses require energy derived from glucose metabolism [21—23]. We have found that neutrophils take up extracellular hexoses and that this uptake is enhanced by chemotactic factors and arachidonic acid but not by insulin [24,25]. Here, we report on studies of the role of Ca^{2+} in this hexose uptake.

Methods

Reagents and buffers

2-Deoxy-D-[^{3}H]glucose and Aquasol® were purchased from New England Nuclear, Boston, MA. EDTA, arachidonic acid, cytochalasin B, D-glucose and L-glucose were purchased from Sigma Chemical Co., St. Louis, MO. A23187 and ionomycin were generously provided by Dr. Hamill, Eli Lilly Co., Indianapolis, IN, and Dr. W.C. Lui, Squibb Institute for Medical Research, Princeton, NJ, respectively. *N*-fMet-Leu-Phe was purchased from Peninsula Labs, Inc., San Carlos, CA. C5a was purified by sequential column chromatography [26]. The final preparation contained five major bands (one of which co-migrated with authentic C5a on polyacrylamide gel electrophoresis (performed as described in Ref. 27)) and had a protein concentration of 4 mg/ml. The buffer used throughout these studies was a modified Dulbecco's salt solution containing (mM): NaCl (154), KCl (2.7), Na_2HPO_4 (8.1), and KH_2PO_4 (1.5). Where indicated, D-glucose, L-glucose, cytochalasin B, $CaCl_2$ and/or $MgCl_2$ were added to the buffer.

Cellular preparation

Polymorphonuclear neutrophils were obtained by twice sedimenting whole blood over Isolymph (Gallard Schisinger, Cade Place, NY) to obtain leukocyte preparations containing more than 95% neutrophils and less than two platelets per 100 leukocytes. The preparation was twice washed before suspending at $2 \cdot 10^6$ cells/ml in buffer. The erythrocyte-to-neutrophil ratio in this final preparation was 3 : 1. In control studies we found that the erythrocytes did not influence uptake of the radioactive label. Thus, purified erythrocyte populations suspended at $6 \cdot 10^6$ cells/ml took up less than 10% of the amount of 2-deoxy-D-[^{3}H]glucose taken up by $2 \cdot 10^6$ neutrophils and this erythrocyte

uptake was uninfluenced by any of the stimulants or conditions studied here. Furthermore, neutrophil suspensions twice exposed to hypotonic medium to remove virtually all of the erythrocytes gave results qualitatively similar to the results obtained with neutrophil-erythrocyte preparations. However, the 'resting' uptake of radioactive label in preparations exposed to the hypotonic medium was, on occasion, elevated 2—3-fold. Therefore, all results are reported using the mixed cellular preparations.

2-Deoxy-D-[^{3}H]glucose uptake

The 2-deoxy-D-[^{3}H]glucose uptake assay was modified slightly from that previously described [24]. All reagents and buffers were adjusted to pH 7.4 and 37°C before use. A stimulus contained in 0.1 ml of buffer was added to 0.1 ml of a cell suspension and incubated for 15 min before exposure to 0.1 ml of buffer containing 0.5 μCi of label (specific activity 50 mCi/mol). After an additional hour of incubation, the reaction was stopped by the addition of 1 ml of buffer (4°C) and placement on ice. Shortly thereafter, the cell suspensions were rapidly centrifuged (8000 $\times g \cdot 10$ s), washed with 1 ml (4°C) buffer, and recentrifuged. After aspiration of the supernatant fluid, the centrifuge tubes containing the pelleted cells were placed in Aquasol and vigorously shaken. We found that the cells handled in this way were freed of greater than 99.95% of the extracellular fluid in which they were suspended. Ca^{2+}, Mg^{2+}, cytochalasin B, D-glucose, or L-glucose were added to the cell suspensions 20 min before addition of the stimulus, where indicated. Cellular toxicity was measured by assay of the release of lactic acid dehydrogenase [26]. We found that the reagents and stimuli used here did not induce appreciable release of this cytosolic enzyme.

Results

2-Deoxy-D-[^{3}H]glucose uptake in resting cells

Unstimulated neutrophils took up the label progressively over a 60 min

TABLE I

INFLUENCE OF BIVALENT CATIONS AND EDTA ON HEXOSE UPTAKE BY RESTING AND STIMULATED NEUTROPHILS

Neutrophils were incubated with the indicated stimulus and 1.4 mM Ca^{2+} and 0.7 mM Mg^{2+}, as indicated, for 20 min and then exposed to 2-deoxy-D-[^{3}H]glucose for 60 min. Results are expressed as cpm ($\times 10^3$), ± S.E., found in cells 60 min after exposure to the radioactive label.

Stimulus	Incubation conditions		
	With cations	Without cations	EDTA
None	6.37 ± 0.98	6.35 ± 0.41	5.08 ± 0.28
C5a (3 μl)	12.61 ± 1.34	13.34 ± 0.68	11.89 ± 0.42
fMet-Leu-Phe (20 nM)	38.73 ± 2.34	38.00 ± 1.38	35.63 ± 1.17
Arachidonic acid (10 μM)	11.17 ± 0.57	10.69 ± 0.40	7.72 ± 0.28
A23187 (100 nM)	18.52 ± 0.78	8.87 ± 0.49	4.65 ± 0.79
Ionomycin (100 nM)	11.44 ± 0.51	8.25 ± 0.47	6.07 ± 0.49
A23187 (1000 nM)	13.75 ± 1.38	14.97 ± 1.43	6.14 ± 0.28
Ionomycin (1000 nM)	3.08 ± 0.74	10.42 ± 0.37	5.23 ± 0.22

TABLE II

INFLUENCE OF L-GLUCOSE, D-GLUCOSE, AND CYTOCHALASIN B ON HEXOSE UPTAKE BY RESTING AND STIMULATED NEUTROPHILS

Neutrophils were incubated with the indicated glucose isomer (20 mM), cytochalasin B (5 μg/ml), or no agent for 20 min, treated with the indicated stimulus for 15 min, and then exposed to 2-deoxy-[^{3}H]glucose for 1 h. Results are expressed as cpm ($\times 10^3$), ± S.E., associated with the cells 60 min after addition of the radioactive label.

Stimulus	Agent cells preincubated with			
	None	L-Glucose	D-Glucose	Cytochalasin B
None	5.94 ± 0.29	5.55 ± 0.32	0.19 ± 0.07	0.81 ± 0.12
C5a (3 μl)	16.41 ± 0.71	15.93 ± 0.20	0.16 ± 0.01	1.46 ± 0.24
fMet-Leu-Phe (20 nM)	24.59 ± 0.39	24.30 ± 0.60	0.18 ± 0.30	1.78 ± 0.50
Arachidonic acid (3 μM)	13.89 ± 0.70	16.63 ± 0.54	0.18 ± 0.12	1.61 ± 0.42
A23187 (100 nM)	20.69 ± 0.55	18.99 ± 0.78	0.28 ± 0.03	0.43 ± 0.14
Ionomycin (100 nM)	12.53 ± 0.62	12.02 ± 0.59	0.12 ± 0.01	1.36 ± 0.08

period and this uptake was rectilinear between 15 s (the earliest time period sampled) and 60 min [24,25]. Resting uptake was minimally influenced by extracellular Ca^{2+} or Mg^{2+}; it was slightly depressed by EDTA (Table I). Since D-glucose and cytochalasin B (a blocker of carrier-mediated hexose transport [28]) inhibited uptake by about 97 and 86%, respectively (Table II), these data indicate that neutrophils take up hexose through a stereospecific transport mechanism which is essentially independent of extracellular bivalent cations.

2-Deoxy-D-[^{3}H]glucose uptake in stimulated cells

As previously reported [24,25], cells exposed to chemotactic factors (i.e., fMet-Leu-Phe and C5a) or arachidonic acid increased their rate of uptake of the radioactive label. As with resting cells, this uptake was rectilinear during 60 min of incubation. Furthermore, at all time points during this period, stimulated cells exhibited a consistently increased amount of radioactive label compared to controls. The amount of uptake found after 60 min of incubation, therefore, is indicative of the stimulator's effect. The solid lines of Fig. 1 give the uptake (in cpm $\times 10^3$) at this time period in cells exposed to increased amounts of each stimulus. The dose-response relationships are evident in this figure. We note that if takes as much, or more, of each stimulator to effect other neutrophil responses such as chemotaxis, degranulation, and aggregation [4–6,10,11].

Extracellular Ca^{2+} and Mg^{2+} had only a moderate influence on this uptake: in the absence of both bivalent cations, uptake stimulated by fMet-Leu-Phe and arachidonic acid was inhibited by less than 1/3 and uptake stimulated by C5a was virtually uninfluenced (Fig. 1, dashed lines). With reference to the former two stimulators, Ca^{2+} appeared more important than Mg^{2+} in supporting enhanced uptake of the radioactive label. Thus, in the presence of both cations, 1.4 mM Ca^{2+} alone, or 0.7 mM Mg^{2+} alone, 0.2 nM fMet-Leu-Phe induced 8.6 ± 0.5, 8.4 ± 0.5, and 7.7 ± 0.7 cpm $\times 10^3$ (±S.E.), respectively, to be associated with the cells after 60 min of incubation with 2-deoxy-D-[^{3}H]glucose.

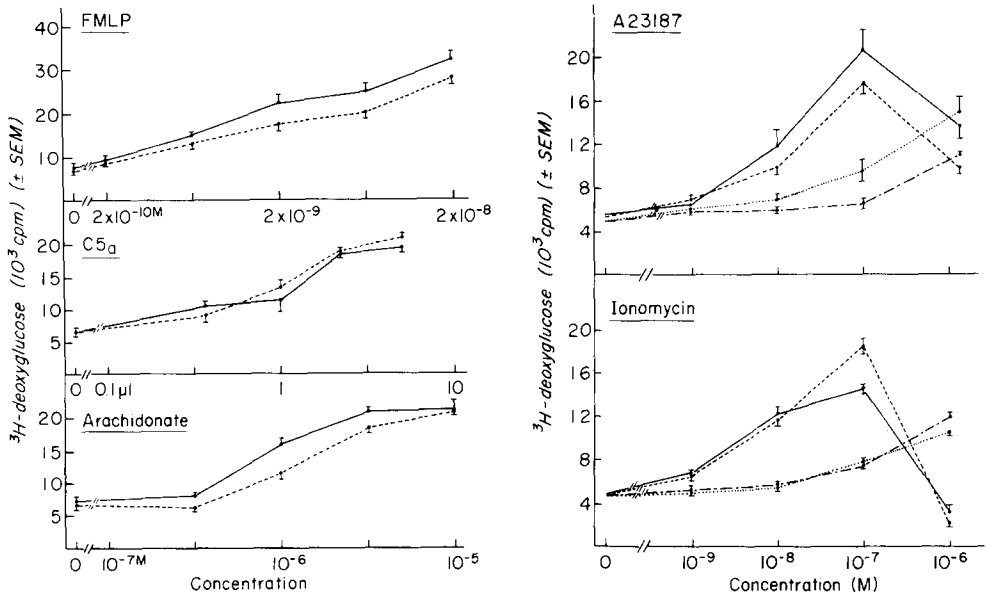

Fig. 1. Uptake of 2-deoxy[^{3}H]glucose in neutrophils exposed to varying concentrations of fMet-Leu-Phe (FMLP), C5a, or arachidonic acid. Cells were incubated with (solid lines) or without (dashed lines) 1.4 mM Ca^{2+} and 0.7 mM Mg^{2+}.

Fig. 2. Uptake of 2-deoxy[^{3}H]glucose in neutrophils exposed to varying concentrations of A23187 or ionomycin. Cells were incubated with 1.4 mM Ca^{2+} and 0.7 mM Mg^{2+} (———), 1.4 mM Ca^{2+} and 0 mM Mg^{2+} (- - - - -), 0 mM Ca^{2+} and 0.7 mM Mg^{2+} (— - —) ,or 0 mM Ca^{2+} and 0 mM Mg^{2+} (· · · · ·).

The same values for cells exposed to 1 μM arachidonic acid were 8.8, 8;7, and 6.6 cpm $\times 10^3$, respectively. The addition of EDTA to the cell suspensions had little influence beyond further depressing the arachidonic acid-induced response (Table I). However, D-glucose and cytochalasin B totally abrogated stimulated uptake (Table II). These data, then, indicate that stimulated uptake, similar to that seen in resting cells, is mediated by a stereospecific transport mechanism which does not require extracellular bivalent cations for its activation. These cations, and in particular Ca^{2+}, may play an enhancing role for the activation induced by certain stimuli.

Influence of ionophores on 2-deoxy-D-[^{3}H]glucose

A23187 is a bivalent cation ionophore which can transport Ca^{2+} into the neutrophil and, attendant upon this, stimulate diverse cellular responses. In separate studies we found that ionomycin also carries Ca^{2+} into the cells in a fashion similar to A23187 and effects similar responses. Both ionophores stimulated the cells to take up 2-deoxy-D-[^{3}H]glucose. In the presence of Ca^{2+}, dose-response curves were biphasic with maximal effects found at 100 nM (Fig. 2, upper two curves on both panels). Most of this effect was dependent upon extracellular Ca^{2+}: at concentrations below 100 nM, neither agent was active in the absence of Ca^{2+} (Fig. 2, lower two dashed lines of both panels). However, at higher concentrations, each agent stimulated significant uptake in the absence of Ca^{2+}; indeed, at these higher concentrations, Ca^{2+}

inhibited this effect (Fig. 2). EDTA inhibited essentially all of the ionophores activity, regardless of the concentration of ionophore (Table I). And, similar to the results seen with the other stimulants, D-glucose and cytochalasin B blocked uptake of the labeled hexose (Table II). These data imply that the transport of Ca^{2+} into the neutrophil can stimulate hexose transport. This transport also appears to be carrier mediated.

Discussion

Five stimulants cause neutrophils to increase their uptake of 2-deoxy-D-[^{3}H]glucose (Figs. 1 and 2). Stimulated uptake was inhibited by cytochalasin B and D-glucose but not L-glucose (Table I) and exhibited Michaelis-Menten type enzyme kinetics [24,25]. Therefore, it appeared to be carrier facilitated. The agents studied here could act primarily to stimulate neutrophil trapping of the label without directly influencing this carrier-mediated transport. Indeed, essentially all of the label taken up by stimulated or unstimulated cells is rapidly phosphorylated to a derivative which is only minimally metabolized further and which does not equilibrate with the extracellular fluid, 2-deoxy-D-[^{3}H]glucose 6-phosphate [24]. However, hexose kinase, the enzyme responsible for phosphorylation of 2-deoxyglucose, is not elevated in stimulated neutrophils [24]. Enhanced accumulation of the radioactive label, therefore, does not appear to be due to direct stimulation of metabolizing or trapping mechanisms within the cell, at least as these mechanisms may be related to enzymatic pathways. (These studies, however, do not exclude other, unknown mechanisms which might lead to trapping of the label.) Similarly, increased uptake did not require neutrophils to have degranulated or aggregated, nor did it depend upon a burst of oxidative metabolism: these responses are not induced by the stimuli, at the concentrations used here, in the absence of extracellular bivalent cations [2,6,10,11], yet hexose uptake was only moderately inhibited by these conditions. Thus, activation of the hexose-transporting mechanisms appears to occur as a consequnece of cell stimulation but not as a direct result of any specific functional responses. It may be that these agents cause neutrophils to utilize their glucose stores, the depletion of which feeds back to activate membrane transport. Or, alternatively, other events occurring in the stimulated cell may mediate the effect. One possible mechanism for this may be alterations in cellular Ca^{2+}. A23187 and ionomycin transport Ca^{2+} into the neutrophil (Refs. 6, 13, 15; and unpublished observations) and stimulate hexose transport (Fig. 2). Given the relatively well defined mechanism of action of ionophores [17,18], these data clearly imply that Ca^{2+} entry into the cell can directly or indirectly stimulate membrane transport of hexoses. The modest enhancing effect of extracellular Ca^{2+} on stimulated uptake induced by fMet-Leu-Phe and arachidonic acid (Fig. 1) may be related to Ca^{2+} influx induced by these agents [6,11,14,15]. We note that Ca^{2+} entry into the neutrophil triggers a wide variety of functional and mechanistic responses [29]. Thus, Ca^{2+} movements may be only an early step in a series eventuating in hexose transport activation.

When no bivalent cations were added, our buffer contained less than 0.2 mM Ca^{2+} and 0.4 mM Mg^{2+}. The stimuli may have caused the cells to take up

a portion of this small amount of Ca^{2+} and this effect could underlie their ability to activate hexose transport in the absence of added Ca^{2+}. However, 2 mM EDTA did not totally inhibit fMet-Leu-Phe-, C5a-, or arachidonate-induced uptake of the label (Table I). Furthermore, neutrophils suspended with only small amounts of $^{45}Ca^{2+}$ do not take up this radioactive label in response to fMet-Leu-Phe unless the suspension contains higher, more physiological, levels of the cation; indeed, under these conditions, they release their cellular Ca^{2+} to the suspending fluid when exposed to the chemotactin [14,37]. This suggests that the small amounts of extracellular bivalent cations found in our buffer may not contribute to, and certainly are not required for, activation of hexose transport by the three previously mentioned stimuli. In contrast, EDTA was effective in totally blocking the stimulating actions of the two ionophores (Table I). The ionophores' actions, then, may be exclusively related to their ability to carry Ca^{2+} into the cell. This possibility is reinforced by noting the influence of Mg^{2+} on ionophore-induced hexose transport (Fig. 2). In the absence of added extracellular Ca^{2+}, Mg^{2+} significantly depressed A23187-induced 2-deoxy-D-[^{3}H]glucose uptake but had no such effect on ionomycin-induced uptake. In separate studies, we have found that Mg^{2+} is at least 10-fold more potent in displacing Ca^{2+} from A23187 than from ionomycin. That is, A23187 has less affinity for Ca^{2+}. Consequently, at high Mg^{2+} : Ca^{2+} ratios, A23187, but not ionomycin, binds much less Ca^{2+}. Hence, the suppressive effects of extracellular Mg^{2+} may indicate that this cation displaces Ca^{2+} from A23187, thereby inhibiting the transport of Ca^{2+} in the presence of trace levels of Ca^{2+}.

Calcium also appears to be involved in membrane hexose transport in other cell types. Extracellular Ca^{2+} enhances resting hexose transport in rat intestinal epithelial cells [30] and is required for insulin-activated hexose transport in isolated fat cells [31,32]. Indeed, A23187 stimulates hexose transport in pigeon erythroyctes [33] and mimics other actions of insulin in fat cells [34]. (Erythrocyte hexose uptake in these studies was stimulated by 50 μM A23187; in our studies on human erythrocytes, up to 1 μM A23187 did not induce this effect.) However, in many cells, Ca^{2+} influx is not required for enhancing hexose uptake [35]. Rather, Ca^{2+} released from sequestered cellular stores appears to be involved in activating membrane hexose transport [35–37]. Similar events may occur within the neutrophil and underlie a portion of the effects studied here. Neutrophils contain sequestered Ca^{2+} which is mobilized by various stimulants and may be involved in mediating cellular response in the absence of Ca^{2+} influxes [36,37]. It may be, therefore, that rearrangements in the cellular Ca^{2+} underlie stimulated hexose uptake occurring in the absence of extracellular Ca^{2+}. Ca^{2+} may thereby move from either an extracellular location or an intracellularly sequestered pool to a site where it activates neutrophil function. Similar events have been proposed for the platelet [38]. Although some of these suggestions are clearly speculative, they do explain a wide range of data. The neutrophil appears to be a valuable cell in which to study these possibilities and the relationships between Ca^{2+} and hexose transport.

Acknowledgements

This work was supported by NIH grants AI 09169, HL 16769, AI 10732, and AI 14929.

References

1 Douglas, W.W. (1968) Br. J. Pharmacol. 34, 451—474
2 Goldstein, I.M., Horn, J.K., Kaplan, H.B. and Weissmann, G. (1974) Biochem. Biophys. Res. Commun. 60, 807—812
3 Goldstein, I.M., Hoffstein, S.T. and Weissmann, G. (1975) J. Immunol. 115, 665—670
4 Showell, H.J., Naccache, P.H., Sha'afi, R.I. and Becker, E.L. (1977) J. Immunol. 119, 804—811
5 Lehmeyer, J.E., Snyderman, R. and Johnston, R.B. (1979) Blood 54, 35—45
6 Simchowitz, L. and Spilberg, I. (1979) J. Lab. Clin. Med. 93, 583—593
7 Becker, E.L. and Showell, J. (1972) Z. Immun. -Forsch. Bd. 143S, 466—476
8 Gallin, J.I. and Rosenthal, A.S. (1974) J. Cell Biol. 62, 594—600
9 Boucek, M.M. and Snyderman, R. (1976) Science 193, 905—907
10 O'Flaherty, J.T. (1980) Inflammation 4, 181—194
11 O'Flaherty, J.T., Showell, H.J., Becker, E.L. and Ward, P.A. (1978) Am. J. Pathol. 92, 155—166
12 Gallin, J.I. (1980) J. Clin. Invest. 65, 298—306
13 Smith, R.J. and Ignarro, L.J. (1975) Proc. Natl. Acad. Sci. U.S.A. 72, 108—112
14 Naccache, P.H., Showell, H.J., Becker, E.L. and Sha'afi, R.I. (1977) J. Cell Biol. 73, 428—444
15 Naccache, P.H., Showell, H.J., Becker, E.L. and Sha'afi, R.I. (1977) J. Cell Biol. 75, 635—649
16 Volpi, M., Naccache, P.H. and Sha'afi, R.I. (1980) Biochem. Biophys. Res. Commun. 92, 1231—1237
17 Pressman, B.C. (1973) Fed. Proc. 32, 1698—1703
18 Pfeiffer, D.R. and Lardy, H.A. (1976) Biochemistry 15, 935—943
19 Zabucchi, G. and Romeo, D. (1976) Biochem. J. 156, 209—213
20 Becker, E.L., Sigman, M. and Oliver, J.M. (1979) Am. J. Pathol. 95, 81—97
21 Becker, E.L. and Showell, H.J. (1974) J. Immunol. 112, 2055—2062
22 Carruthers, B.M. (1967) Can. J. Physiol. Pharmacol. 45, 269—283
23 O'Flaherty, J.T., Kreutzer, D.L., Showell, H.J. and Ward, P.A. (1977) J. Immunol. 119, 1751—1756
24 McCall, C.E., Bass, D.A., Cousart, S. and DeChatelet, L.R. (1979) Proc. Natl. Acad. Sci. U.S.A. 76, 5896—5900
25 Bass, D.A., O'Flaherty, J.T., Szejda, P., DeChatelet, L.R. and McCall, C.E. (1980) Prog. Lipid Res., in the press
26 O'Flaherty, J.T., Kreutzer, D.L., Showell, H.J., Vitkauskas, G., Becker, E.L. and Ward, P.A. (1979) J. Cell Biol. 80, 564—572
27 Gerard, C. and Hugli, T.E. (1979) J. Biol. Chem. 254, 6346—6353
28 Zigmond, S.H. and Hirsch, J.G. (1972) Science 176, 1432—1436
29 O'Flaherty, J.T. and Ward, P.A. (1979) Semin. Hematol. 16, 163—177
30 Manning, A.S., McMullan, J.M. and Evered, D.F. (1978) Biochem. Soc. Trans. 6, 914—915
31 Akhtar, R.A. and Perry, M.C. (1979) Biochim. Biophys. Acta 585, 107—116
32 Gould, M.K. and Chaudry, I.H. (1970) Biochim. Biophys. Acta 215, 249—257
33 Carruthers, A. and Simons, T.J.B. (1978) J. Physiol. 284, 49P
34 Vydelingum, N., Kissebah, A.H., Drake, R. and Alquadran, F. (1980) Fed. Proc. 39, 821
35 Czech, M.P. (1977) Annu. Rev. Biochem. 46, 359—384
36 Clausen, T., Elbrink, J. and Dahl-Hansen, A.B. (1975) Biochim. Biophys. Acta 375, 292—308
37 Holloszy, J.O. and Narahara, H.T. (1965) J. Biol. Chem. 240, 3493—3500
38 Massini, P. and Luscher, E.F. (1976) Biochim. Biophys. Acta 436, 652—663