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MITOCHONDRIAL RESPIRATION AND THE THROMBIN-INDUCED RELEASE REACTION OF PLATELETS

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Abstract—Respiration has been monitored polarographically in suspensions of washed platelets isolated from pig blood. Induction of the release reaction by addition of thrombin to such suspensions resulted in a stimulation of respiration. A respiratory stimulation of similar magnitude was achieved by adding 2 deoxy-D-glucose or uncouplers of oxidative phosphorylation. Oligomycin caused a pronounced inhibition of respiration which could be reversed by adding uncouplers but not thrombin or deoxyglucose. Antimycin A caused a total inhibition of oxygen uptake and prevented the respiratory stimulation otherwise observed on addition of thrombin. It is concluded that the stimulation of respiration caused by thrombin reflects a response of platelet mitochondria to a change in the intracellular ratio of ATP/ADP, consequential upon ATP breakdown during the release reaction. The nature of the energy-dissipating reactions stimulated by thrombin is discussed.

THE EXTRACELLULAR release of metabolically inert adenine nucleotides and other platelet constituents in the release reaction[1] is associated with breakdown of metabolically active ATP inside the cell.[2–4] This breakdown is considered directly to reflect the energy requirement of a step occurring during the release reaction.[4,5]

Platelets have a pronounced capacity for glycolysis[6] and utilisation of ATP during the release reaction is associated with a marked increase in lactate production.[5,7] However, oxidative phosphorylation can contribute significantly to total ATP synthesis in platelets, at least where this contribution has been determined under resting conditions.[8,9] Moreover, studies with metabolic inhibitors have shown that ATP derived solely from glycolysis or solely from oxidative phosphorylation can support energy-dependent platelet functions, including the release reaction.[10] Changes have also been reported that are indicative of an increased activity of the citric acid cycle resultant upon ATP utilisation in the release reaction. Thus, several workers have observed increased production of $^{14}CO_2$ following induction of the release reaction in suspensions of platelets that had been preincubated with ^{14}C labelled glucose.[11–13] However, these investigations have involved measurement of $^{14}CO_2$ production over time periods which were so long as to be of dubious relevance to the release reaction *per se*, or else have been carried out under conditions where changes in platelet function additional to the release reaction were induced, precluding the unambiguous association of the increased CO_2 production with the latter process.

The polarographic technique, because of its fast response time and high sensitivity, provides a convenient means of following changes in mitochondrial respiration during the release reaction. A "burst" in oxygen consumption has in fact been measured

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polarographically following addition of thrombin to suspensions of human platelets.[10,14] However, this "burst" of oxygen utilisation was insensitive to the addition of antimycin A and apparently represented a non-mitochondrial process. There appears to be no report of an unambiguous increase in mitochondrial respiration in platelets during the release reaction.

In the present studies, respiration was monitored polarographically in suspensions of washed porcine platelets. We report here evidence of an increase in mitochondrial respiration associated with the thrombin-induced release reaction. We also report the effects on platelet respiration, in the presence and absence of glucose, of inhibitors specific for various steps in mitochondrial energy transfer. We discuss the effects of these inhibitors in terms of the nature of the thrombin-induced increase in respiration of platelet mitochondria.

MATERIALS AND METHODS

Thrombin was obtained from Koch–Light Laboratories Ltd., Colnbrook, Bucks. All other reagents were of analytical grade and supplied by Sigma Chemical Co. (London). Samples of carbonyl-cyanide *p*-trifluoromethoxy-phenylhydrazone (FCCP) and 4,5,6,7-tetrachloro-2-trifluoromethyl-benzimidazole (TTFB) were kindly donated by Professor R. B. Beechey, Shell Research, Sittingbourne, Kent.

Collection of blood and preparation of platelet suspensions. Pig blood was obtained from the slaughterhouse. After slaughter, animals' throats were cut and blood allowed to flow freely into large; wide necked polythene vessels; containing per 2 l. of blood collected, 160 ml of a solution that was 77 mM in EDTA and buffered to pH 7·35 with Tris base. After gentle agitation, the blood was transferred to pre-cooled glass containers which were then stoppered and kept on ice during transit to the laboratory. On arrival, blood was transferred to pre-cooled 1 l. polypropylene containers and centrifuged at 300 *g* for 20 min at 12° to sediment red cells. All subsequent steps for the isolation of platelets and preparation of suspensions of washed platelets were performed at 1–5° according to a method previously described.[15]

For oxygen polarography and measurement of nucleotide release, platelet pellets were resuspended, after washing, in a medium obtained by mixing in the proportion of 90:8:2 by volume respectively, the following solutions: 0·154 M NaCl; 0·154 M Tris-Cl buffer, pH 7·35, and 77 mM EDTA. For turbidimetric measurement of platelet aggregation, platelet pellets were resuspended finally in a medium obtained by mixing in the proportions of 9:1 by volume respectively the following solutions: 0·154 M NaCl and 0·154 M Tris–Cl buffered at pH 7·35.

Oxygen polarography. Oxygen uptake was measured as the decrease in oxygen activity recorded with a Clark electrode, model number 4004, Yellow Springs Instrument Co., Yellow Springs, Ohio, U.S.A., in conjunction with a "Teflon" membrane (0·001 mil cast "Teflon" film). The electrode chamber was of perspex construction and has previously been described.[16] Periodically, the apparatus was calibrated using the method described by Robinson and Cooper[17] to ensure that the relationship between electrode response and oxygen concentration in the Tris–saline–EDTA medium was linear. This was always found to be the case. Samples of platelet suspension were equilibrated to the desired temperature in a water bath and then introduced into the electrode chamber, which contained a volume of Tris–saline–EDTA

medium at the same temperature, such as to give a final volume of 3·0 ml. Approximately 5–8 mg platelet protein/ml of final reaction mixture were used in each test. When measurements of oxygen uptake were taken at low oxygen tensions, a stream of nitrogen was passed over the electrode chamber in order to minimise the rate of back diffusion of oxygen from the atmosphere.

Release of platelet nucleotides. Release of platelet nucleotides was estimated by measurement in a Perkin–Elmer Model 124 Spectrophotometer, of the 257 nM-absorbing material remaining in the platelet-free supernatants after centrifugation of platelet suspensions. The method adopted was exactly as described by Mürer.[10,14]

Turbidimetric measurement of platelet aggregation. Platelet aggregation was measured turbidimetrically as described by Born and Cross[18] in a medium supplemented with potassium and calcium ions.

Protein. Total platelet protein (mg) was estimated by a biuret method[19] after solubilisation in deoxycholate.

RESULTS

Control respiration and the effect of thrombin. When samples of platelet suspensions, stored on ice, were rewarmed to 37° and introduced into the chamber of the oxygen polarograph, their measured rate of oxygen uptake typically increased with time until a limiting respiratory rate was attained (Fig. 1). The relationship between oxygen concentration and electrode current with the measuring apparatus was linear (see Methods), so that the recorded increase in respiratory rate of platelet suspensions during their incubation represented a true increase in respiratory rate with time. This non-linearity of control respiration complicated evaluation of the response to addition of thrombin. Nevertheless, induction of the release reaction by thrombin was associated with an immediate stimulation of control respiration to a rate equivalent to the limiting rate of respiration observed on longer incubation of platelet suspensions in the absence of thrombin (Fig. 1). The stimulation of respiration by thrombin

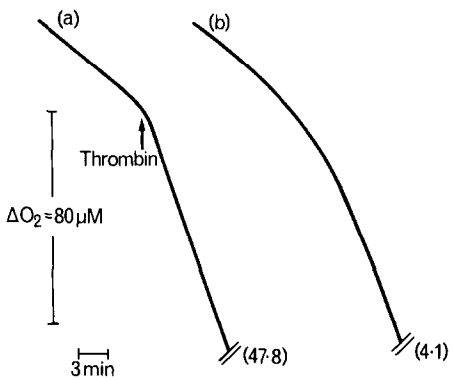

FIG. 1. Effect of thrombin on platelet respiration and release of adenine nucleotides. The medium (see Methods) was at 37° and contained 7·01 mg platelet protein/ml. Thrombin was added to the oxygen electrode chamber as indicated; thrombin 1·0 unit, to (a); no additions (control) to (b). After incubation for the times shown, samples (2·0 ml) of test and control suspensions were taken (//) into pre-cooled polycarbonate tubes and placed immediately on ice. After centrifugation, the amount of adenine nucleotides present in the supernatant from each sample was determined (see Methods). Values obtained are given in parentheses as nmol of adenine nucleotide released/mg platelet protein.

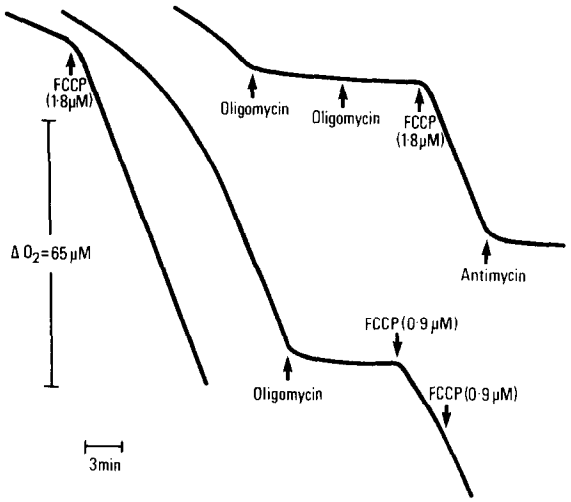

FIG. 2. Effects of oligomycin, FCCP and antimycin A on platelet respiration. In each test the medium contained 5·6 mg platelet protein/ml. Oligomycin (3·0 μM, final concentration), and antimycin A (0·5 μg), were added where indicated. FCCP was added at the final concentrations shown in the figure.

was always observed providing that the respiration rate had not, on incubation in the absence of thrombin, already attained its limiting rate. In the latter case, thrombin addition was without effect on respiratory rate. In spite of the spontaneous increase in respiratory rate in the absence of thrombin, the amounts of adenine nucleotides released from platelets during incubation alone were small in relation to the amounts released into the medium after addition of thrombin (Fig. 1).

Effect of inhibitors of oxidative phosphorylation on platelet respiration and on the response of respiration to thrombin. Addition of oligomycin to a platelet suspension resulted in an inhibition of respiration within a few seconds (Fig. 2). The respiratory rate measured in the presence of oligomycin at concentrations causing a maximum inhibition of respiration (about 0·5 nmole/mg platelet protein) was constant during the course of an experiment with a given preparation of platelets and independent of the steady increase in respiration observed during incubation of platelet suspensions alone.

Suitable concentrations of the uncouplers of oxidative phosphorylation FCCP or TTFB overcame oligomycin-mediated inhibition of respiration (Fig. 2). Concentrations of either uncoupler sufficient to cause an optimal stimulation of respiration (about 0·3 nmole FCCP or TTFB/mg protein) in the presence or absence of oligomycin, produced an immediate stimulation of respiration to a rate equal to the limiting rate of respiration attained by platelet suspensions incubated alone. As with thrombin, the stimulation of respiration by uncouplers was not observed once respiration had, on incubation alone, attained a limiting rate. In contrast to the stimulation of respiration observed in the presence of uncouplers, that produced by thrombin was totally prevented by concentrations of oligomycin causing respiratory inhibition (see next Section and Fig. 4). Moreover, addition of oligomycin to platelet suspensions respiring in the presence of thrombin effected an immediate decrease in respiratory rate. The final rate of respiration observed in the presence of oligomycin plus

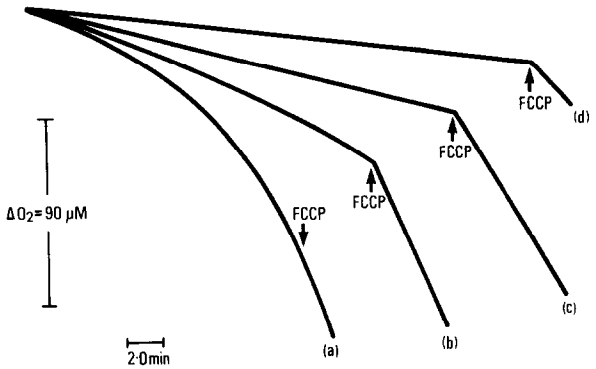

FIG. 3. Effect of glucose on platelet respiration at different temperatures. Superimposed are polarographic recordings of oxygen uptake for platelet suspensions incubated in the absence of glucose at 37°, (a); and in the presence of glucose (final concentration 6 mM) at the following temperatures: 37°, (b); 30° (c); and 25°, (d). FCCP (final concentration 1·8 μM) was added where indicated.

thrombin (whatever their order of addition) was equal to that observed in the presence of oligomycin alone.

Respiration rates measured in the presence of uncouplers were maintained for periods of investigation up to 25 min and were uninfluenced by the addition of several respiratory substrates including pyruvate or succinate, both of which are aerobically metabolised by platelets.[20]

Effect of temperature on platelet respiration in presence and absence of added glucose. When glucose (5–10 mM final concentration) was added to samples of platelet suspension prior to their addition to the electrode chamber, the rate at which respiration increased with time on subsequent incubation was considerably decreased in comparison with the pattern observed in the absence of glucose (Fig. 3 a and b). In some preparations the respiration rate remained constant, and inhibited with respect to control respiration on incubations of 20 min duration.

The response of respiration to incubation in the presence of glucose was investigated at two other temperatures, 25° and 30°. In the presence of glucose at these lower temperatures, respiration was consistently linear in all platelet preparations tested (Fig. 3 c and d). In the presence of glucose, lowering the temperature of incubation also decreased the rate of respiration measured in the presence of uncouplers. Nevertheless, at 30° and in the presence of glucose, the difference between coupled and uncoupled respiration rates was pronounced. Addition of thrombin to platelets respiring in the presence of glucose at 30° caused a marked stimulation of respiration to a rate which was not further increased by addition of uncouplers (Fig. 4). As was observed in the absence of glucose, oligomycin caused an immediate inhibition of respiration in the presence of glucose. For all preparations tested the extent of inhibition fell within the range 75–100 per cent (mean 84 per cent). Again, no increase in respiration was observed on addition of thrombin to platelet suspensions respiring in the presence of glucose plus oligomycin, although inhibition of respiration by oligomycin was totally reversed by addition of uncouplers (Fig. 4).

Effects of 2 deoxy-D-glucose and antimycin A on aggregation and respiration. In order to obtain more information about the metabolic status of the suspensions of washed platelets employed in the present study, the effect on aggregation was investi-

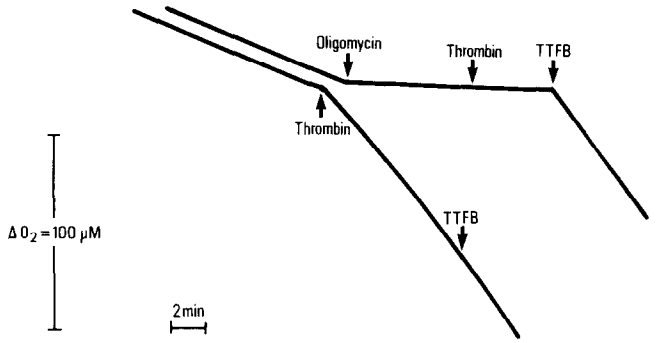

FIG. 4. Effect of oligomycin on the response of respiration to thrombin. Incubations were carried out at 30° in medium containing glucose (6mM final concentration). In each test 6·95 mg platelet protein/ml of incubation medium were used. Compounds were added as indicated at the following final concentrations; thrombin, 0·33 units/ml; oligomycin, 3·0 μM; and TTFB, 2·0 μM.

gated of preincubating suspensions with metabolic inhibitors prior to adding thrombin (Fig. 5). Preincubation of platelet suspensions in the presence of 2 deoxy-D-glucose (inhibitor of glycolysis) or in the presence of antimycin A (inhibitor of mitochondrial respiration) caused a significant inhibition of thrombin-induced aggregation. Combination of these inhibitors during preincubation resulted in a synergistic inhibition of aggregation. Adding glucose during the period of incubation with either 2 deoxy-D-glucose or antimycin A reduced the inhibition of aggregation otherwise

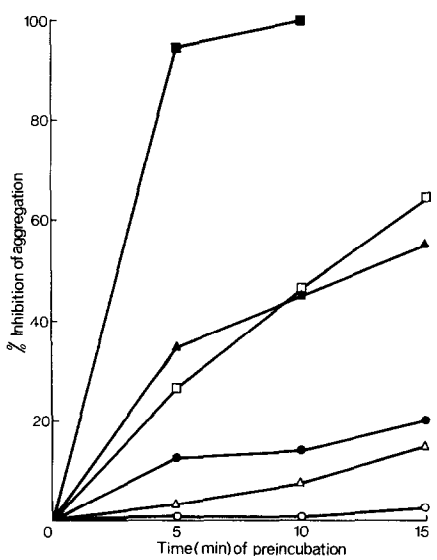

FIG. 5. Effect of glucose on thrombin-induced aggregation in the presence and absence of metabolic inhibitors. The medium contained 2·0 mg platelet protein/ml. Aggregation was induced by adding thrombin at a final concentration of 0·2 units/ml. Prior to addition of thrombin, platelet suspensions were preincubated for the times shown with the following: 2 deoxy-D-glucose, final concentration 3 mM (△, ▲); antimycin A, 0·5 μg, (○, ●); 2 deoxy-D-glucose (3·0 mM final concentration) plus antimycin A (0·5 μg) □, ■. Preincubations were carried out in the absence (▲, ●, ■) or in the presence (△, ○, □) of glucose at a final concentration of 6 mM.

observed. Nevertheless, a synergistic inhibition of aggregation by a combination of the inhibitors was retained in the presence of glucose. Glucose itself caused a small but significant increase in the amount of aggregation observed after 10 and 15 min. of preincubation. Consequently, the results in Fig. 5 are calculated as the per cent inhibition of aggregation of corresponding controls, all experiments where glucose was added together with metabolic inhibitors being referred to controls preincubated for equal periods with glucose alone.

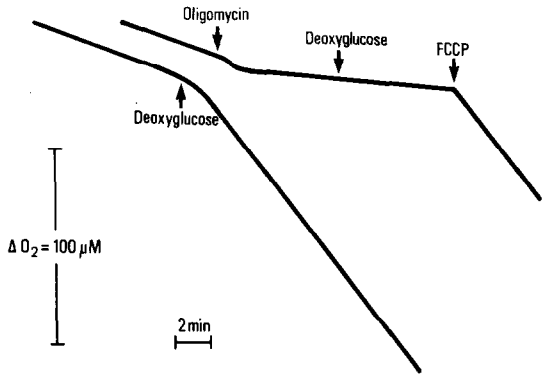

FIG. 6. Effect of 2 deoxy-D-glucose in the presence and absence of oligomycin on platelet respiration. Incubations were carried out in medium at 30° containing glucose (6 mM final concentration). Additions were made as indicated at the following final concentrations: 2 deoxy-D-glucose, 3 mM; oligomycin, 3·0 μM; FCCP 1·8 μM.

The effects of 2 deoxyglucose on respiration were similar to those caused by thrombin. Thus, at 37° and in the absence of glucose, addition of deoxyglucose to platelet suspensions caused an immediate stimulation of respiration to a rate equivalent to that observed in the presence of uncouplers of oxidative phosphorylation. A more marked increase in respiratory rate was observed on addition of deoxyglucose to suspensions respiring in the presence of glucose (Fig. 6). However, in the presence of oligomycin under both sets of conditions, addition of deoxyglucose was without effect on respiratory rate. Demonstration of a stimulation of respiration by deoxyglucose was very dependent on the age of the platelet suspension used. In aged preparations, addition of deoxyglucose typically resulted in a short stimulation of respiration followed by a return to a respiratory rate which was inhibited with respect to that measured before addition of the sugar; in some cases, no stimulation was observed but only inhibition of respiration on addition of deoxyglucose. In preparations which had been aged on ice for periods of about 1·5 hr, addition of deoxyglucose together with thrombin caused an inhibition of respiration under conditions where addition of either compound alone resulted in a respiratory stimulation. The inhibited rate of respiration observed in such aged preparations in the presence of deoxyglucose plus thrombin could however be dramatically stimulated by addition of uncouplers of oxidative phosphorylation.

Addition of antimycin A to platelet suspensions caused an immediate inhibition of respiration which at suitable concentrations of the inhibitor (15–20 ng/mg platelet protein) was total (Fig. 7). Antimycin-inhibited respiration was not stimulated by addition of thrombin or 2 deoxyglucose at either 37° or 30°.

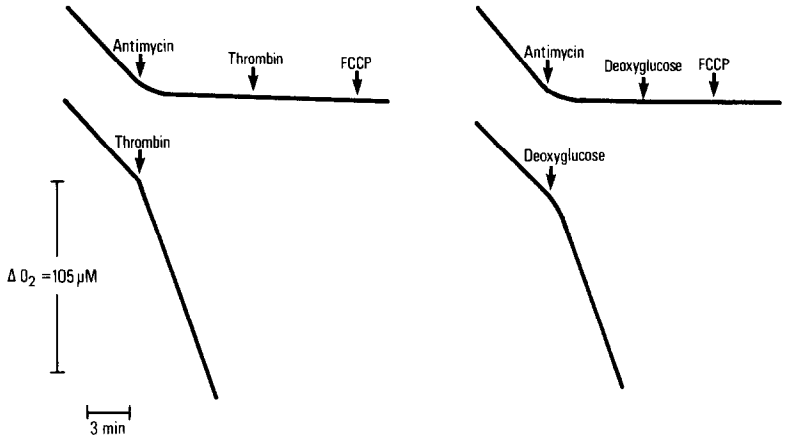

FIG. 7. Effect of antimycin A on the response of respiration to thrombin or 2 deoxy-D-glucose. Incubations were conducted at 37° in the absence of glucose. Additions were made as indicated at the following final concentrations: antimycin A, 0·2 μg/ml; thrombin, 0·33 units/ml; 2 deoxy-D-glucose, 3 mM; and FCCP, 1·8 μM.

DISCUSSION

Control respiration. Respiration of platelet suspensions typically increased on incubation in the polarograph until a limiting respiratory rate was attained. This respiratory rate was established without incubation upon addition of uncouplers of oxidative phosphorylation and in this case was uninfluenced by the prior or subsequent addition of several respiratory substrates including pyruvate/malate and succinate. It can be concluded that neither the respiratory rate measured in the presence of uncouplers, nor the limiting rate of respiration attained in the absence of uncouplers, was substrate limited and that these rates of respiration represented a maximum respiratory response.

Addition of oligomycin caused a pronounced inhibition of respiration indicative of a tight coupling between oxidation and phosphorylation. The respiratory rate observed in the presence of oligomycin was independent of the steady increase in respiration that occurred on incubation of platelet suspensions in the polarograph, indicating[21] that this increase represented an increased demand for ATP and not an uncoupling or structural loosening of respiratory control or the onset of a work performance directly linked to respiration (e.g. aerobic calcium accumulation). That this demand for ATP was not generated by a tendency to undergo a spontaneous release reaction was clear from the finding that where respiration had attained its limiting value, no significant release of adenine nucleotides into the extracellular medium had occurred; significant that is in comparison with the amounts found extracellularly after addition of thrombin. As judged by the ability of platelets to aggregate (on addition of thrombin) after preincubation with glucose in the turbidimeter, glycolysis under these conditions was sufficient to maintain adequate platelet ATP levels even in the absence of oxidative phosphorylation (i.e. in the presence of antimycin). Addition of glucose to the suspending medium also prevented the small decline in control aggregation observed on preincubation of samples in the turbidimeter. The above effects of glucose were most easily interpreted as the result of an increased contribution of glycolysis to total ATP production. It was therefore argued that addition of

glucose to the suspending medium might help to reimpose respiratory control in the cells. Indeed, Detwiler and Zivkovic[8] have reported that platelet ATP/ADP ratios, under aerobic or anaerobic conditions, were higher after incubation in the presence of glucose than it its absence. However, in the present work, while glucose usually depressed respiration and in some preparations supported a linear respiration, these effects were not consistently observed between different preparations of platelets. It was considered that this variability might reflect differences in the availability of preferred endogenous substrates or different extents of endogenous ATP utilisation, resulting from the exclusion of glucose and respiratory substrates from the media used to wash and resuspend the cells. Experiments were therefore conducted in which the respiratory substrates, pyruvate/malate and succinate and glucose were included together in the washing and resuspending media in conjunction with the use of either EDTA or acid-citrate-dextrose as anticoagulants. These modifications of the isolation procedure did not affect the characteristics of the response of respiration to incubation alone, or the variability of the response between different platelet preparations. It is therefore unlikely that the apparent demand for ATP in these cells arose solely as a result of substrate deficiency during the isolation procedure.

The present results showed that respiration (even when maximal, after prolonged incubation) was always tightly coupled to phosphorylation (i.e. was inhibited by oligomycin) and was responsive to alterations in the intracellular ATP/ADP ratio effected by glycolysis (i.e. was inhibited in the presence of glucose). Thus, neither route of ATP regeneration appeared to be functionally impaired. Nevertheless, the energy demand for aggregation in platelet suspensions, isolated in the cold and rewarmed and incubated at 37° could not, in the absence of glycolysis, be accommodated by oxidative phosphorylation. These results are compatible with those of Rock and Nemerson,[22] who found that the ATP content of platelets declined on storage and that this decline could be largely prevented by the addition of glucose, but not by the addition of pyruvate or succinate (substrates that were aerobically metabolised). Rock and Nemerson concluded that the isolation of platelets from plasma resulted in uncoupling of oxidative phosphorylation. However, the present results and those of other investigations[11,23,24] have indicated that platelet respiration is tightly coupled to phosphorylation. The occurrence of reactions leading to an energy dissipation in excess of the platelets' capacity to resynthesise ATP by oxidative phosphorylation alone would appear a reasonable explanation of the present results and indeed those of previous studies.

The nature of the endogenous energy dissipating reactions in these cells was not definitely established. At 30° in the presence of glucose, the respiratory rate remained constant on incubation and was well below the respiratory rate obtainable with this resuspension on addition of uncouplers or thrombin. The latter effect, in contrast to the variable effect of incubation of platelet suspensions at 37° in the presence of glucose was consistently observed among different preparations of platelets. This suggests that the endogenous energy dissipating reactions are more sensitive to lowering of temperature than are the pathways of ATP regeneration.

Running equatorially beneath the outer membrane of platelets is located a bundle of microtubules.[25] Chilling the cells to 0° is associated with the dissociation of these elements; and rewarming and incubation at 37° with their reassociation.[25] Born[26] has studied the change in shape of platelets from disks to spheres, a phenomenon

that precedes certain types of aggregation. This shape change is believed to be mediated by an alteration in function of these microtubules and one of its characteristics is a marked temperature dependence. It is possible that the apparent loss of respiratory control observed here, that attended the rewarming of cells and their incubation at 37°, might reflect an alteration in microtubular function.

Effects of thrombin. Addition of thrombin caused an immediate stimulation of respiration with the attendant release of adenine nucleotides. Inhibition of the thrombin-induced stimulation of respiration by oligomycin indicates that this stimulation does not, as has been suggested,[6] represent either an uncoupling of oxidative phosphorylation or a requirement for the respiratory–linked accumulation of calcium by mitochondria. Indeed, the oligomycin sensitivity of the thrombin-induced increase in respiratory rate provides a direct indication that the latter reflects an increased demand for the rephosphorylation of ADP (i.e. a fall in the intramitochondrial ATP/ADP ratio). This can be stated with some confidence since, although oligomycin can impose a certain degree of "pseudo-respiratory control" on partially damaged sub-mitochondrial particles,[27] all effects of oligomycin in intact mitochondria relate directly to the inhibition of the ATP–synthetase complex, which is functionally coupled to the respiratory chain.[21]

The catabolism during the release reaction of the platelets' metabolically active adenine nucleotide pool has been well documented.[2 4] Addition of thrombin results in a rapid breakdown of ATP concomitant with a small rise in ADP and a more dramatic increase in the formation of adenosine and inosine monophosphates, inosine and hypoxanthine. An immediate effect of thrombin is thus to decrease the ATP/ADP ratio in the metabolically active nucleotide pool. The oligomycin-sensitive increase in respiration observed on addition of thrombin in the present study is thus entirely consistent with the known changes in metabolism of adenine nucleotides occurring under these conditions.

The increased respiratory rate observed on addition of thrombin was maintained for periods in excess of those required for the release reaction to go to completion. This sustained response of respiration is not unexpected, since as stated above the addition of thrombin to platelets results in a significant intracellular breakdown of adenine nucleotides to adenosine monophosphate and to inosine monophosphate, inosine and finally to hypoxanthine.[3] Breakdown of ADP to these metabolites is an irreversible process and probably reflects the inability of the ATP regenerating pathways to accommodate immediately the energy demand associated with the release reaction.[4]

The stimulation of oxygen uptake occurring on addition of thrombin was prevented by the prior addition of antimycin A. This inhibition by antimycin confirms that the increase in oxygen uptake described is of mitochondrial origin. Mürer has reported[14] on addition of thrombin to suspensions of human platelets, a burst in oxygen uptake which was associated with the release reaction but which was insensitive to addition of antimycin. In all but the species used, the present experiments were conducted under conditions very similar to those described by Mürer. The species difference would seem the most probable reason for these different results.

In contrast to the thrombin-induced "burst" in oxygen uptake described by Mürer,[14] other workers[28] have reported a more sustained increase in oxygen uptake on addition of thrombin to platelet suspensions. However these latter workers pro-

vided no evidence as to whether or not this increased respiration was coupled or indeed that it was of mitochondrial origin.

The energy demand generated by addition of thrombin may represent an extension of the activity of the endogenous energy dissipating reactions, which it was suggested arose from an alteration in the function of platelet contractile elements. It has been suggested that the release reaction might be effected by an intracellular contraction initiated by an increase in the intracellular concentration of free calcium ions.[29,30] It is reasonable to suggest that ATP hydrolysis by contractile elements may be responsible for the observed energy demand but other alternatives or the occurrence of other endergonic reactions occurring simultaneously with contraction cannot be excluded by the present results.

Effects of deoxyglucose. In contrast to a report by Solomon and Gaut,[31] other workers[32,33] have found evidence for a rapid rate of uptake of deoxyglucose into platelets. The extensive inhibition of aggregation observed in the present work after 5 min of preincubation of platelet suspensions with deoxyglucose plus antimycin would also suggest a rapid entry of deoxyglucose into platelets, since the inhibitory effect of antimycin on respiration was established instantly on its addition and antimycin alone caused little inhibition of aggregation. Detwiler[32] found deoxyglucose to be without effect on platelet respiration at 37° in the absence of glucose. This result is not inconsistent with those reported here since, under these conditions, and in some preparations of platelets, respiration was already maximal and could not be further stimulated by uncouplers. However, Detwiler[32] also observed an inhibitory effect of glucose on respiration (Crabtree effect) which was not reversed by addition of the deoxysugar. The stimulation of glucose inhibited respiration by deoxyglucose observed in the present account was inhibited by oligomycin. The effect of deoxyglucose on respiration would thus depend on the availability of ADP and P_i for rephosphorylation. In ascites tumour cells,[34] addition of deoxyglucose results in a transitory stimulation of respiration followed by an inhibition; inhibition of respiration being associated with a fall in the intracellular P_i concentration to almost zero and a decrease in phosphorylation. Similar changes in respiration were observed on addition of 2-deoxyglucose to aged preparations of platelets in the present study. It is thus possible that inhibition of platelet function by deoxyglucose might be due to the removal of P_i from metabolism as suggested by Mürer.[23] Other results[32] indicate that addition of 2-deoxyglucose results in a rapid decline in ATP to a level compatible with that present in the platelets' non-metabolic pool of nucleotides. Since the breakdown of ATP on addition of 2-deoxyglucose is not associated with a corresponding increase in ADP,[23] it is also important to consider the possibility that the removal of ADP from the metabolic pool by virtue of its degradation to AMP, inosine and hypoxanthine would result in an inhibition of respiration. It was found in the present study that addition of thrombin together with deoxyglucose resulted, in aged platelet preparations, in an immediate inhibition of respiration under conditions where addition of either compound alone caused a respiratory stimulation. This observation might support the suggestion that respiration can become ADP limited; addition of thrombin might be expected to result in a greater conversion of adenine nucleotides to hypoxanthine in the presence of deoxyglucose than in its absence.[4] Certainly, respiration inhibited by thrombin plus deoxyglucose was stimulated by uncouplers indicating that it was not substrate limited.

The absence of an antimycin-insensitive burst in oxygen uptake in these cells suggest that such suspensions would provide a convenient system in which to measure polarographically the response of platelet mitochondria to addition of other inducers of the release reaction and to inhibitors of this process.

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