Hydrogen Peroxide Lowers ATP Levels in Platelets without Altering Adenylate Energy Charge and Platelet Function*  
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H$_2$O$_2$ irreversibly reduced metabolic platelet ATP levels with a corresponding accumulation of hypoxanthine. This process was enhanced by sodium azide or potassium cyanide and by increasing H$_2$O$_2$ concentrations. The adenylate energy charge was unaltered when less than two-thirds of the metabolic ATP had disappeared but decreased markedly when more ATP disappeared. Platelet shape change, primary aggregation, dense granule and $\alpha$-granule secretion were unaffected by H$_2$O$_2$-induced lowering of ATP provided that the adenylate energy charge did not fall by more than 5%; at greater adenylate energy charge reduction, platelet functions were inhibited. These results indicate that cell functions depend more on adenylate energy charge than on the ATP level and expands the applicability of this view from bacterial systems to a mammalian cell, the human platelet.

Secretion, aggregation, and shape change in platelets are inhibited when their ATP content and adenylate energy charge are decreased by incubation with antimycin A and 2-deoxyglucose in combination (1, 2). Since these inhibitors block mitochondrial and glycolytic ATP production, a reduction in the rate of ATP turnover undoubtedly also occurs. The exact ATP-requiring steps for these platelet functions are unknown, but the functions can be separated by their different sensitivities to inhibition of energy metabolism as measured by the parameters of ATP level, adenylate energy charge (2), and probably also the ATP turnover rate. The interrelationships of these parameters have not allowed changes in any of the three parameters by the use of inhibitors of energy metabolism without a corresponding change in the others. It is therefore not known whether or not platelet functions are dependent on adequate levels of all three.

In Escherichia coli in which it has been possible to lower the ATP level while maintaining a constant adenylate energy charge (3-5), typical cell functions such as growth (5) and protein synthesis (6) were shown to depend more on the adenylate energy charge than on the actual ATP level. However, a similar separation in regulation of the ATP level and energy charge has not been reported for cell systems of vertebrates, and the dependence of cell function of these parameters independently has not been explored.

In the course of an investigation of the inhibitory effects of  

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MATERIALS AND METHODS

Human platelet-rich and platelet-poor plasma were prepared and the platelet metabolic pool of adenine nucleotides were labeled with [¹⁴C]adenine as described previously (11) except that [¹²⁵I]adenine from Amersham/Searle (Arlington Heights, Ill., code IPA 436) was used.

Suspensions of gel-filtered platelets were prepared from labeled platelet-rich plasma with Sr$^{2+}$/Tyrode elution fluid (12). Platelet rich plasma and the suspensions of gel-filtered platelets were incubated at 37°C with 25 µl/ml of H$_2$O$_2$ in various concentrations in 0.15 M NaCl in the presence or absence of 10 µg of potassium cyanide or sodium azide (various concentrations) as catalase inhibitors. Samples were removed at noted times for the analyses below. H$_2$O$_2$ (30%) was from J. T. Baker (Phillipsburg, N. J.) and was stored at +4°C. Dilutions were made in 0.15 m NaCl immediately before the experiment and were kept on ice during the experiments. The exact concentration of H$_2$O$_2$ was determined by volumetric titrations with 0.01 M potassium permanganate and found to be 96% of the stated value.

Platelet shape change was measured as the increase in optical density of stirred platelet-rich plasma at 37°C upon addition of a mixture of ADP and EDTA (2). Platelet aggregation with ADP and epinephrine was determined by aggregometry at 37°C as described elsewhere (2, 14).

Thrombin-induced Secretion — Dense granule secretion (previously referred to as "Release II," Ref. 13) and $\alpha$-granule secretion (previously referred to as "Release I," Ref. 13) were measured as the appearance extracellularly of nonlabeled ATP + ADP and $\beta$-N-acetylglycosaminidase, respectively (14).

Collagen-induced secretion of previously absorbed radioactive serotonin was performed as described elsewhere (15). Radioactivity of [¹⁴C]adenine metabolites was determined after their separation with high voltage paper electrophoresis as described earlier (2, 11). This method does not separate inosine and hypoxanthine.

The amounts of ATP and ADP were determined in ethanolic extracts by a luciferase method (16). H$_2$O$_2$ concentrations in the incubation mixtures were determined after extraction with 10% trichloroacetic acid by a ferrithiocyanate method (17). Lactate was determined fluorometrically in the ethanol extracts used for adenine nucleotide determination (2, 11, 16) according to Hohorst (18) except that hydrazine hydrate was substituted for hydrazine sulfate.

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Inorganic phosphate was measured in suspensions of gel-filtered platelets as previously described for platelet-rich plasma (22). ATP Levels, Adenylate Energy Charge, and Platelet Function

RESULTS AND DISCUSSION

Effects of \( \text{H}_2\text{O}_2 \) on Adenine Nucleotides and Inducers of Platelet Functions – \( \text{H}_2\text{O}_2 \) is a powerful oxidizing agent, and the possibility that it could interact directly with adenine nucleotides and the inducers of platelet functions used was examined. \([\text{\textsuperscript{14}C}]\text{ATP} \) and \([\text{\textsuperscript{3}H}]\text{ADP} \) were incubated in 0.05 and 5 mM concentrations (solvent: 0.15 M NaCl) at 37°C with the highest \( \text{H}_2\text{O}_2 \) concentration used. Samples were prepared at noted times for ATP and ADP estimation with the luciferin-luciferase system and with paper electrophoresis and scintillation counting. No changes in the ATP and ADP concentrations were found after 30 min of incubation. Furthermore, we confirmed the findings of Canoso et al. (7) that \( \text{H}_2\text{O}_2 \) (300 \( \mu \text{M} \)) did not alter the platelet aggregating activity of ADP (50 \( \mu \text{M} \)) when incubated at 37°C for 5 min. \( \text{H}_2\text{O}_2 \) also did not alter the functional activities of epinephrine (50 \( \mu \text{M} \)) or thrombin (50 units/ml).

Effects of Catalase Inhibitors on \( \text{H}_2\text{O}_2 \)-induced Conversions of \([\text{\textsuperscript{14}C}]\text{Adenine Nucleotides in Platelets} \) – The effects of \( \text{H}_2\text{O}_2 \) on platelets were studied in two systems, platelet-rich plasma (3 to 5 \( \times 10^9 \) cells/ml) and suspensions of gel-filtered platelets in calcium-free Tyrode’s solutions containing albumin (2 to 4 \( \times 10^9 \) cells/ml). The platelet system is the most physiological one, but the presence of enzymes and substances in plasma itself makes certain studies difficult. In the studies below, catalase in plasma interfered with the measurements. Its activity is assessed directly in platelet-poor plasma (<0.06 \( \times 10^9 \) cells/ml), and the platelet contribution to the \( \text{H}_2\text{O}_2 \)-removing capacity of platelet-rich plasma can be computed. However, some parameters measured here, i.e., lactate and glucose (in the determination of glycogen), are present in large amounts in plasma, and study of the effects of thrombin on platelets are complicated by the presence of fibrinogen and antithrombin in plasma. For these studies, gel-filtered platelets were used, but since transfer of platelets from plasma to other media often causes changes in platelet behavior (23), direct comparisons between platelet-rich plasma and gel-filtered platelets have been performed when possible.

\( \text{H}_2\text{O}_2 \) caused conversion of \([\text{\textsuperscript{14}C}]\text{ATP} \) to \([\text{\textsuperscript{14}C}]\text{inosine} + \text{hypoxanthine} \) in platelet-rich plasma (Fig. 1), and a qualitatively similar pattern was obtained with suspensions of gel-filtered platelets. The extent of this conversion was, however, greater with gel-filtered platelets than in platelet-rich plasma (Table I, Fig. 2). The catalase inhibitors cyanide and azide markedly increased both the rate and the extent of the \( \text{H}_2\text{O}_2 \)-induced reduction in the \([\text{\textsuperscript{14}C}]\text{ATP} \) level. Incubation of gel-filtered platelets or platelet-rich plasma with azide alone did not cause any changes in the ATP level or in the adenylate energy charge (Table I). In contrast, cyanide caused a slight, slow decrease in the ATP level of platelets in both systems, especially in gel-filtered platelets, but had no noticeable effect on the adenylate energy charge (Figs. 1 and 5, Table I).

Maximal effect of \( \text{H}_2\text{O}_2 \) on platelet \([\text{\textsuperscript{14}C}]\text{ATP} \) was observed with 0.1 mM azide or 1 mM cyanide both in platelet-rich plasma and in gel-filtered platelets (Table I). In the presence of the catalase inhibitors the adenylate energy charge decreased while the level of \([\text{\textsuperscript{14}C}]\text{ATP} \) decreased, but returned to almost normal levels (0.89 to 0.92) after \( \text{H}_2\text{O}_2 \) had reached its lowest value (Figs. 1 and 3). The \([\text{\textsuperscript{14}C}]\text{ATP} \) level increased slowly but steadily after the initial fall with a corresponding decrease in the level of \([\text{\textsuperscript{14}C}]\text{inosine} + \text{hypoxanthine} \) (Fig. 1). This confirms the findings of Rivard et al. (24) that platelets are able to synthesize adenine nucleotides from hypoxanthine. Furthermore, it follows from Fig. 1 (KCN present) that the ATP radioactivity increases at a rate of 14% (of total radioac-

![Fig. 1. Effect of KCN on metabolic adenine nucleotides in platelet-rich plasma during incubation with 150 \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \). \([\text{\textsuperscript{14}C}]\text{Adenine-labeled platelet-rich plasma} (4.2 \times 10^9 \text{cells/ml}) \) was incubated in the presence (solid lines) or absence (broken lines) of 150 \( \mu \text{M} \) \( \text{H}_2\text{O}_2 \) with and without 2 mM KCN at 37°C. Samples were removed at noted times for determinations of the adenylate energy charge (\( \gamma \)) and of the radioactivity of ATP (\( \bullet \)), ADP, AMP, IMP (\( \blacksquare \)), and hypoxanthine + inosine (\( \Delta \)), all of which are expressed in percentage of total radioactivity. In the presence of \( \text{H}_2\text{O}_2 \) the radioactivity of ADP and AMP decreased almost in parallel to that of ATP.](http://www.jbc.org/)
ATP Levels, Adenylate Energy Charge, and Platelet Function

Effect of varying concentrations of cyanide and azide on H₂O₂-induced ATP breakdown in platelet-rich plasma and gel-filtered platelets

(14C)Adenine-labeled platelet-rich plasma and gel-filtered platelets were incubated with or without 300 μM H₂O₂ in the absence or presence of various concentrations of cyanide or azide for 10 min at 37° and the levels of radioactive ATP (in percentage of total radioactivity) and the adenylate energy charge were determined.

| Additions | Platelet-rich plasma | Gel-filtered platelets |
|-----------|----------------------|------------------------|
|           | ATP | AEC | ATP | AEC | ATP | AEC |
| None      | 79  | 0.93| 72  | 0.92| 79  | 0.92|
| CN⁻ or N₃⁻ (C₁) | 77  | 0.92| 69  | 0.89| 72  | 0.90|
| H₂O₂      | 50  | 0.90| 55  | 0.85| 59  | 0.91|
| H₂O₂ + CN⁻ or N₃⁻ (C₂) | 24  | 0.88| 20  | 0.77| 44  | 0.89|
| H₂O₂ + CN⁻ or N₃⁻ (C₃) | 13  | 0.81| 18  | 0.79| 21  | 0.87|
| H₂O₂ + CN⁻ or N₃⁻ (C₄) | 13  | 0.72| 16  | 0.72| 23  | 0.88|

* Platelet-rich plasma: 3 x 10⁸ cells/ml; gel-filtered platelets: 2.4 x 10⁸ cells/ml.

** Platelet-rich plasma: 3.4 x 10⁶ cells/ml; gel-filtered platelets: 2.7 x 10⁶ cells/ml.

AEC, adenylate energy charge.

FIG. 2. Effect of varying the H₂O₂ concentration with and without 2 mM KCN. [14C]Adenine-labeled platelet-rich plasma (3.7 x 10⁸ cells/ml) was incubated with the concentrations of H₂O₂ given on the abscissa in the absence of (open symbols) and presence (closed symbols) for 10 min at 37° and the radioactivities of the [14C]adenine metabolites were determined. Only the percentage of radioactivity of ATP is shown. AEC, adenylate energy charge.

FIG. 3. H₂O₂ levels, adenylate energy charge, and ATP concentration in platelet-rich plasma (■, ▼, ▲) and gel-filtered platelets (□, ▼, ◆). [14C]Adenine-labeled platelet-rich plasma (3.53 x 10⁸ cells/ml) and a suspension of gel-filtered autologous platelets (2.91 x 10⁸ cells/ml) were incubated with 170 μM H₂O₂ in the presence of 0.2 mM NaN₃. Samples were prepared at the times noted for determination of the adenylate energy charge (AEC), ATP radioactivity (expressed in percentage of total radioactivity), and [H₂O₂].
moved from the systems (Fig. 3). The rate of H₂O₂ disappearance was proportional to the number of platelets in the suspensions of gel-filtered platelets, which had lower platelet concentration than platelet-rich plasma. Platelet-poor plasma contained a cyanide- and azide-insensitive H₂O₂-removing system (k₂ = 0.022 s⁻¹ without and 0.0042 s⁻¹ with cyanide or azide). In platelet-rich plasma the corresponding values were 0.057 and 0.0096 s⁻¹. The slower disappearance of H₂O₂ in the suspensions of gel-filtered platelets compared to platelet-rich plasma was probably due to both the absence of plasma and to the lower platelet count in gel-filtered suspensions. H₂O₂ is thus removed in platelet-rich plasma by at least three different systems: (a) catalase which is inhibited by cyanide and azide; (b) a cyanide- and azide-insensitive system (both in plasma and platelets); and (c) platelet-bound system that appears to involve utilization of ATP. The very high H₂O₂ removal capacity of platelets (and plasma) is of special interest since it has recently been shown that platelets may be exposed in vivo to H₂O₂ in concentrations comparable to those used here (8).

Effects of H₂O₂ on Platelet Lactate, Glycogen, and Inorganic Phosphate—The level of inorganic phosphate did either not change or decreased slightly in glucose-free suspensions of gel-filtered platelets during H₂O₂-induced ATP breakdown. A pronounced increase in glycogen breakdown took place during ATP breakdown but the rate of glycogen disappearance was the same as in control platelets after the ATP level had stabilized. The increase in glycogen breakdown was not associated with a sustained increase in the lactate production (Table III). Usually there was a small increase in lactate production during the first 6 min of incubation of platelets with H₂O₂; after this period lactate was produced at the same rate as in control platelets incubated with cyanide alone.

These results suggest that the H₂O₂-induced ATP disappearance did not take place by simple hydrolysis. It is tempting to speculate that ATP may be consumed in phosphorylation processes, possibly of glycolytic intermediates, since the increased glycogen breakdown occurred without an increase in lactate production when cyanide was present. It has been shown that perfusion of rat liver with substrates for glutathione peroxidase (H₂O₂, t-butyl, and cumene hydroperoxides) increases glycogenolysis (27) and causes lowering of the adenylate pool, however, with a lowering of the adenylate energy charge (28). Platelets contain large amounts of glutathione, glutathione peroxidase, and glutathione reductase (29), which makes it possible that there is a link between H₂O₂ removal, glycogen degradation, and reduction in the steady state levels of metabolic ATP. The transient fall in adenylate energy charge during H₂O₂ consumption indicates that ATP consumption is not balanced by ATP regeneration, which results in a fall in the ATP level.

Functional Properties of Platelets Treated with H₂O₂—It has been reported (7) that the inhibitory effect of H₂O₂ on platelet aggregation varied much among platelet-rich plasma from different donors. We have observed the same and have also seen that the H₂O₂-removing capacity (without catalase inhibitor) varied much. However, the variation in inhibitory effect and removing capacity was diminished when catalase inhibitors were used, indicating that the differences seen were partly due to variations in the catalase level. Azide was found to be a powerful inhibitor of platelet aggregation induced by ADP, epinephrine, or collagen and could not be used in the aggregation studies. Cyanide, on the other hand, had only a small inhibitory effect on aggregation (see KCN control, Fig. 4) and was used in the subsequent studies.

When ADP- and epinephrine-induced platelet aggregation was measured in platelet-rich plasma following the addition of H₂O₂, primary aggregation was inhibited only during the period when the level of metabolic ATP was falling and the adenylate energy charge was lower than normal. As the level of ATP stabilized and the energy charge returned to normal, the rate and extent of primary platelet aggregation also returned to normal. Fig. 4 shows a typical experiment with ADP as the aggregating agent in which the variation in aggregation, [¹⁴C]adenine metabolites, and adenylate energy charge...
are shown separately for the control (KCN) and the experimental sample (KCN + H₂O₂). Fig. 5 shows a typical experiment with epinephrine where the aggregation rate is given in percentage of control. When various concentrations (0.5, 1, 2, and 5 μM) of ADP or epinephrine were used, the inhibitory period lasted longer for the lower concentrations than for the higher, but when the adenylate energy charge had returned to less than 5% below the control value, no effect on aggregation was seen with any concentration of ADP or epinephrine.

The effect of H₂O₂ on the platelet shape change process was studied with 0.2 to 2 μM ADP (in the presence of 5 mM EDTA) in platelet-rich plasma and with an initial concentration of 300 μM H₂O₂ over a 20-min incubation period. No change in the shape change response was noted during incubation, even at an adenylate energy charge as low as 0.82 (data not shown).

Collagen-induced secretion of [³⁵S]serotonin previously incorporated by platelets in plasma was not inhibited when the adenylate energy charge was less than 5% below control values. Dense granule secretion by thrombin was not inhibited at any stage of incubation of gel-filtered platelets with H₂O₂, whereas α-granule secretion was inhibited at slightly reduced adenylate energy charge, but then was unaffected when the charge had returned to control values (Table IV).

Secretion and secondary aggregation in platelet-rich plasma in response to ADP and epinephrine was, however, abolished by H₂O₂ even when the adenylate energy charge had returned to normal values.

Concluding Remarks — Since cyanide was used in studies on platelet function, oxidative regeneration of ATP did not take place and lactate production could be used as a measure of ATP turnover. Our experiments show that H₂O₂ can be used as a tool to specifically reduce the ATP level in platelets without altering ATP turnover and adenylate energy charge. In addition, the agent is rapidly removed by the cells. Thus, at normal ATP turnover rates shape change, aggregation, dense granule secretion, and α-granule secretion are unaltered by large reductions (up to 60%) in the level of metabolic ATP, provided that the adenylate energy charge is not lowered by more than 5%. When the energy charge is lowered by more than 5% several of these platelet functions are inhibited. Evidently, these platelet functions depend more on the adenylate energy charge rather than ATP. Our results strongly support the view of Atkinson (30) that the adenylate energy charge rather than ATP is the important regulatory parameter for control of cell functions. This has previously been demonstrated for bacteria, and the present results expand the applicability of this view to a mammalian cell, the human platelet.

TABLE IV

Effect of H₂O₂ on thrombin-induced secretion in gel-filtered platelets

A suspension of [³⁵S]labeled gel-filtered platelets was incubated with and without 300 μM H₂O₂ at 37° and samples were removed at noted times for determination of adenosine metabolite radioactivity, adenylate energy charge (AEC), and for the amounts of ATP + ADP and β-N-acetylglucosaminidase (β-N-acet.) that were secreted in 1 min by 4.5 units/ml of thrombin.

| Pre-treatment | Dense gran. | α-Gra. | ATP+ADP | β-N-acet. | ATP | AEC |
|---------------|------------|--------|---------|-----------|-----|-----|
|               | secretion (ATP + ADP) | secretion (β-N-acet.) | [³⁵S]ATP | [³⁵S]ATP | AEC |
| NaCl 3 | 44.6 | 30.2 | 96.0 | 0.919 |
| 20 | 46.6 | 29.9 | 92.7 | 0.905 |
| H₂O₂ 3 | 45.2 | 19.2 | 45.6 | 0.820 |
| 20 | 43.7 | 29.3 | 46.0 | 0.889 |

* Percentage of total level in control (0 time).

REFERENCES

1. Kattlove, H. (1974) Am. J. Physiol. 226, 325-329
2. Holmsen, H., Setkowsky, C. A., and Day, H. J. (1974) Biochem. J. 144, 385-396
3. Dietzler, D. N., Laas, C. J., Magnani, J. L., and Leckie, M. (1974) Biochem. Biophys. Res. Commun. 60, 875-881
4. Dietzler, D. N., Leckie, M. P., Magnani, J. L., Sughrue, M. J., and Bergstein, P. E. (1975) J. Biol. Chem. 250, 7194-7202
5. Swedes, J. S., Sedo, R. J., and Atkinson, D. E. (1975) J. Biol. Chem. 250, 6903-6908
6. Chapman, A. G., Fall, L., and Atkinson, D. E. (1971) J. Bacteriol. 106, 1072-1086
7. Canoas, B. T., Rodvien, B., Scoon, K., and Levine, P. H. (1974) Blood 43, 445-456
8. Levine, P. H., Weinger, R. S., Simon, J., Scoon, K. L., and Krinsky, N. I. (1976) J. Clin. Invest. 57, 995-963
9. Rodvien, R., Lindon, J. N., and Levine, P. H. (1976) Br. J. Haematol. 33, 19-38
10. Stuart, M. H., and Holmsen, H. (1977) Am. J. Hematol. 2, 53-62
11. Holmsen, H., Day, H. J., and Setkowsky, C. A. (1972) Biochem. J. 129, 67-82
12. Lagoe, B., Scrutton, M. C., and Holmmon, H. (1975) J. Lab. Clin. Med. 85, 811-825
13. Holmsen, H. (1975) Ciba Found. Symp. 35, 175-196
14. Holmsen, H., Setkowsky, C. A., Lagoa, B., Day, H. J., Wcissa, H. J., and Scrutton, M. C. (1975) Blood 46, 131-142
15. Holmsen, H., Oustvold, A.-C., and Day, H. J. (1973) Biochem. Pharmacol. 22, 2599-2608
16. Holmsen, H., Storm, E., and Day, H. J. (1972) Anal. Biochem. 46, 97-80
17. Thurman, R. G., Ley, H. G., and Scholes, R. (1973) Eur. J. Biochem. 25, 420-430
18. Hohorst, H. J. (1965) in Methods of Enzymatic Analysis (Bergmeyer, H. U., ed) pp. 295-270, Academic Press, New York
19. Lowry, O. H., and Passoneau, J. V. (1972) A Flexible System of Enzymatic Analysis, pp. 189-191, Academic Press, New York
20. Atkinson, D. E., and Walleau, G. M. (1967) J. Biol. Chem. 242, 3235-3241
21. Mills, D. C. B. (1973) Nature New Biol. 243, 220-222
22. Holmsen, H. (1972) Annu. N. Y. Acad. Sci. 201, 109-121
23. Day, H. J., Holmsen, H., and Zacker, M. B. (1975) Thromb. Diath. Haemorrh. 33, 648-654
ATP Levels, Adenylate Energy Charge, and Platelet Function

24. Rivard, G. E., McLaren, J. G., and Brunst, R. F. (1975) Biochim. Biophys. Acta 381, 144-153
25. Mäenpää, P. H., Raivio, K. O., and Kekomäki, M. P. (1968) Science 161, 1253-1254
26. Woods, H. F., Eggleston, L. V., and Krebo, H. A. (1970) Biochem. J. 119, 501-510
27. Sies, H., Gerstenecker, C., Menzel, H., and Flohe, L. (1972) FEBS Lett. 27, 171-175
28. Sies, H., Gerstenecker, C., Summer, K. H., Menzel, H., and Flohe, L. (1974) in Glutathione (Flohe, L., BenoHR, H. Ch., Sies, H., Walter, H. D., and Wendel, A., eds) pp. 261-274, Georg Thieme, Stuttgart
29. Karpatkin, S., and Weiss, H. J. (1972) N. Engl. J. Med. 287, 1062-1066
30. Atkinson, D. E. (1970) in The Enzymes (Boyer, P. D., ed) 3rd Ed, Vol. 1, pp. 461-489, Academic Press, New York
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H Holmsen and L Robkin

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