control subjects[21]. These findings have led some workers to suggest that increased rates of purine synthesis *de novo* in gout may result from faulty feedback inhibition[22,23]. The results of the present investigation suggest that it may be possible to test this hypothesis using methylthioinosine. The results would be expected to be less ambiguous than those from 4-amino-5-imidazolecarboxylic acid or 6-mercaptopurine because of its potency, specificity of effect, limited metabolism, prolonged effect, and because it does not utilize 5-phosphoribosyl-1-pyrophosphate.

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Effect of Thrombin, Adenosine Diphosphate, Connective Tissue, and Endotoxin on Platelet Glycolysis

THROMBIN, which aggregates platelets, also induces an increase in rate of platelet glycolysis[1,2]. Other substances capable of aggregating platelets include adenosine diphosphate (ADP)[3], connective tissue[4], and (in rabbit platelet systems) endotoxin[5]. I have compared these other aggregating substances with thrombin in their ability to influence platelet glycolysis as measured by the production of lactate.

Citrate plasma rich in platelets and suspensions of three times washed platelets in barbital buffer (*p*H 7·6) were prepared from freshly obtained human blood by methods previously described[2]. Platelets were counted by phase microscopy. Connective tissue suspension was prepared from human breast tissue by the method of Zucker and Borelli[4]. In each experiment portions were used from a single pool of plasma rich in platelets or suspension of washed platelets. For the evaluation of platelets in plasma, the test system consisted of silicone treated tubes containing 0·9 ml. of plasma rich in platelets, to which were added one of the following: 0·1 ml. isotonic saline, 0·1 ml. thrombin (20 N.I.H. units/ml.), 0·1 ml. ADP (100 μg/ml.), 0·1 ml. endotoxin, *Escherichia coli* (1 mg/ml.), or 0·1 ml. connective tissue suspension. For the evaluation of washed platelets, 0·8 ml. of platelet suspension was mixed with 0·1 ml. glucose (55 μmoles/ml.), and then with 0·1 ml. isotonic saline, or 0·1 ml. thrombin (20 N.I.H. units/ml.) or 0·1 ml. thrombin–calcium solution (20 N.I.H. units/ml. 0·015 M calcium chloride).

Table 1. EFFECT OF PLATELET AGGREGATING SUBSTANCES ON LACTATE FORMATION IN PLATELET-RICH PLASMA

Addition	Aggregation*	μMoles lactate/10⁹ platelets† (range in three experiments)
Isotonic saline	0	3·7–4·4
Thrombin‡	+	5·4–6·0
ADP	+	3·6–4·6
Endotoxin	0	3·4–4·6
Connective tissue suspension	+	4·0–4·9

* + Denotes aggregation, 0 denotes no aggregation. Platelets in thrombin system aggregated just before clot formation.
† After incubation for 45 min at 37° C.
‡ Only in this system was the production of lactate significantly different (at 5 per cent level) from that of the saline control as evaluated by the *q* statistic for range.

Table 2. EFFECT OF THROMBIN WITH AND WITHOUT CALCIUM ION ON AGGREGATION AND GLYCOLYSIS OF WASHED PLATELETS IN BUFFER CONTAINING GLUCOSE

Addition	Aggregation*	μMoles lactate/10⁹ platelets† (range in five experiments)
Isotonic saline	0	1·4–3·3
Thrombin‡	0	2·9–5·3
Thrombin-calcium‡	+	2·6–5·8

* + Denotes aggregation, 0 denotes no aggregation.
† After incubation for 45 min at 37° C.
‡ Lactate production in these systems was significantly greater (at the 5 per cent level) than in the saline control as evaluated by the *q* statistic for range.

All tubes were incubated for 45 min at 37° C in a water bath without shaking. Reactions were stopped by the addition of two volumes cold trichloroacetic acid (15 per cent w/v) and the mixtures analysed for lactate by the method of Barker[6]. Aggregation was evaluated by phase microscopy at 10 sec and at 5, 15 and 60 min in a duplicate set of tubes which were shaken for 5 min by hand, then incubated at 37° C. Although thrombin, connective tissue suspension and ADP caused platelet aggregation, only thrombin induced an increase in lactate concentration (Table 1). The effect of thrombin did not require the occurrence of platelet aggregation because increased lactate formation was noted in washed platelet suspensions which did not contain any extra calcium ions (Table 2).

Contact with connective tissue or ADP, rather than with thrombin, may be responsible for some or all instances of platelet aggregation *in vivo*. In fact, the ability of thrombin to aggregate platelets may in part be mediated through ADP, released from platelets after breakdown of adenosine triphosphate[7]. Aggregation alone, however, does not seem to alter the rate of formation of lactate. The effect of thrombin on platelet glycolysis (which is believed to supply energy necessary for clot retraction[1]) can occur without previous aggregation and presumably involves another mechanism—perhaps the conversion of fibrinogen to fibrin on the platelet surface[2].

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