

CORRESPONDENCE

Platelet aggregation in asthmatic patients

To the Editor:

A recent report by Harwell, Patterson, Lieberman, and Beachey, in the May, 1973, issue of the JOURNAL, concerning platelet aggregation in asthmatic patients was unable to confirm earlier studies from our laboratory. A review of their article reveals several important differences from our method and may explain the divergent results. Although a larger patient sample was apparently investigated in the preliminary studies, the data of their report are from a sample of 12 atopic patients and 10 normal controls. These small sample sizes are of concern because, in our studies, only about half of the patients demonstrated the defect and, as was subsequently shown, this abnormality was largely dependent upon the concentration of epinephrine used as the aggregating agent.

A second important difference was the later use of heparin as the anticoagulant rather than citrate. Most workers in the field of platelet aggregation employ citrate, as a recent seminar intitled "Platelets and Their Role in Hemostasis"* so indicates. It is true that the authors obtained better results when heparin was used since there was less scatter of the data with this anticoagulant. Nevertheless, examination of their data for both citrate and heparin (Figs. 1 and 2) shows a wide variability at all time intervals after addition of epinephrine in their normal subjects. Thus, in the case of citrate, at least 4 of 10 normal subjects show aggregation patterns that would be considered abnormal in our laboratory. The data are somewhat better when heparin is used, but again it is noted that 4 subjects of the normal group also showed impaired aggregation. Naturally, such wide variations in the normal group preclude valid comparison with patient samples.

Another important difference between citrate and heparin is the fact that the detection of the second wave is much more difficult to determine with the latter anticoagulant. Since most of the changes we observed were related to abolition or changes in the second wave of aggregation, this also makes a comparison between the two studies somewhat tenuous.

From the technical point of view, there are other differences between the two methods. In our study, the most striking changes were observed when epinephrine concentrations were less than those employed by Harwell and colleagues. Thus, abnormalities in some of their cases might have been obscured by failure to employ the same concentrations that we used. In our original paper a miscalculation (under the section entitled "Methods") was made in the epinephrine concentration expressed as molarity. However, milligram concentrations in the final mixtures are correct. The final molar concentrations of epinephrine in each of our dose response studies were 2×10^{-5} , 4.5×10^{-6} , and 2.3×10^{-6} M, respectively. The highest concentration of epinephrine used by Harwell and colleagues, exceeded our highest concentration by a factor of 10. Furthermore, we found the most significant deviations from normal when we used concentrations of epinephrine 100 fold less than that used by Harwell and his group.

Other technical differences should also be mentioned. Their rate of centrifugation, 700 G, is much faster than that employed by us (100 G). Since the number of platelets is critical in determining the effectiveness of an aggregating agent, centrifugal speed may be of significance. Harwell and colleagues gave no figures for the number of platelets in samples of platelet-rich plasma. In our studies the platelet count was always adjusted to 300,000 per cubic millimeter. This may be important because even with "normal" platelet counts, a reduction from 300,000 per cubic millimeter to 200,000 per cubic millimeter will have a marked effect on the final platelet aggregation pattern. Another possible variable is the temperature that is controlled in the aggregometer and uncontrolled in the method described by Harwell and colleagues. Thus, we recently determined that significant differences in aggregation

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patterns with epinephrine were obtained in 3 normal persons at two different temperatures (37° C. and 22° C.).

Inspection of Harwell's data in Figs. 3 and 4 is of interest. Because of the wide variability of the two population samples, no significant statistical difference was noted between atopic and normal groups. Nevertheless, at the 1, 2, 3 minute interval (Fig. 3) and at all intervals in Fig. 4, the mean normals show better aggregation when compared to atopic patients. Had the sample sizes been larger or the normals "more normal," these differences might have been statistically significant.

We agree with the authors and others that the platelet aggregation model is extremely complex. The role of cyclic AMP in this system is even more controversial because both inhibitory and stimulatory aggregating agents have produced increase or depletion in intracellular platelet cyclic AMP. These conflicting reports suggest that seemingly slight changes in in vitro techniques employed by different laboratories had a profound impact on the test system. Although the previously documented inhibitory effects of dibutyl cyclic AMP on platelet aggregation were corroborated by the authors, this effect does not reflect the actual changes in intracellular platelet cyclic AMP after stimulation by various aggregating agents. In any case, until data of intracellular platelet cyclic AMP in asthmatic patients become available, the authors' comments about expected aggregation patterns in these patients are only speculative.

We do not wish to imply that the procedure is infallible. In fact, one of us (H.I.G.) has observed that abnormal responses may be encountered in about 5 per cent of presumably normal individuals. However, our results of abnormal second-wave aggregation in asthmatic patients, as well as the abnormalities conferred on normal platelets by platelet-poor plasma from these same patients, continue to command our interest in pursuing these studies.

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Effect of beta stimulant on eosinopenic responses

To the Editor:

We have previously reported¹ that a beta stimulant (salbutamol, 250 µg, inhaled, and 50 mg., oral) and a phosphodiesterase inhibitor (PDI: diprophylline, 300 mg. intramuscularly) decreased the blood total eosinophil count (TEC) in allergic patients, which has been recently confirmed.²

In addition we found the TEC could be modified merely by skin testing of patients, probably because of emotional release of catecholamines and/or corticosteroids.

Therefore the clinician using the TEC as a method for the diagnosis of allergy must know if the patient has previously taken any beta stimulant or PDI, and must avoid doing TEC after skin tests.

In contrast to these changes in TEC, the skin reaction to allergen was not changed after the intake of beta stimulant and PDI.

TABLE I

	2 hours after allergic skin tests	2 hours after allergic skin tests and intake of salbutamol and diprophylline
Mean decrease of TEC	n:7 21% (p < 0.01)	n:18 39% (p < 0.01)

REFERENCES

- 1 Gayrard, P., et al: Effects des bronchodilatateurs sur les tests cutanes d'allergie, *Rev. Fr. Allergol.* 12: 219, 1972.
- 2 Ohmann, J. L., et al: Effect of propranolol on the eosinopenic responses of cortisol, isoproterenol, and aminophyllin, *J. ALLERGY CLIN. IMMUNOL.* 50: 151, 1972.

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