

# Blood Platelet Response to Plasma from Patients with Ischemic Heart Disease

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**Blood platelets change shape (from small round spheres to larger spread forms) as they participate in thrombosis. Using an electron microscopic technique, we surveyed 14 patients with both acute and chronic ischemic heart disease; each had increased spread platelet forms ( $69 \pm 22.2$  [standard deviation] percent) when compared with 14 asymptomatic control subjects ( $P < 0.001$ ). When platelets from these 14 control subjects were exposed to plasma from the patients with ischemic heart disease, spread forms increased from  $13.4 \pm 9.1$  to  $44.5 \pm 15.5$  percent ( $P < 0.001$ ). There was no significant increase in spread platelets in these control subjects when their blood was mixed with plasma from another control group. Similar studies were performed in seriously ill noncardiac patients; 9 of 13 had increased spread platelet forms when compared with control subjects, but plasma from only 5 of these 9 subjects caused increased spread forms when mixed with platelets from normal subjects ( $P < 0.05$ ).**

**Thus a factor existed in the plasma of these patients with ischemic heart disease that caused normal platelets to become spread. Similarly the plasma of some patients with serious noncardiac disease had a comparable effect on normal platelets. Although the identity of this factor is unknown, it is probably unrelated to hormonal or therapeutic influences occurring either during acute infarction or during the stress of serious illness because (1) the effect of the plasma from patients with acute ischemic heart disease was identical to that of patients with chronic ischemic heart disease, and (2) the effect was not present in all patients with serious noncardiac disease.**

Blood platelets change shape as they aggregate. This fact has been confirmed by several investigators utilizing transmission<sup>1,2</sup> and scanning<sup>3</sup> electron microscopes. Such changes in configuration, from round dense disks that develop extrusions or pseudopods to large less dense spread forms, may be assessed accurately using an electron microscopic technique<sup>1</sup>

To explore further this platelet response, we used this method to study the effect of platelet-poor plasma removed from patients with ischemic heart disease on platelets harvested from asymptomatic control subjects. Similar studies were performed using platelet-poor plasma from 13 patients with serious or life-threatening illnesses of noncardiac origin.

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## Materials and Methods

### Patient Selection

**Acute myocardial infarction:** Seven patients admitted consecutively to the Cardiac Care Unit of the University of Michigan Hospital were studied. Acute myocardial infarction had been confirmed by appropriate electrocardiographic and serum enzyme changes. Platelet studies were performed within 48 hours of admission. The age range was 40 to 71 years (mean 56 years). There were five men and two women.

**Chronic ischemic heart disease:** Seven patients with either healed myocardial infarction (occurring more than 3 months previously) or chronic angina pectoris were studied. The age range was 43 to 76 years (mean 54 years). There were six men and one woman.

### Control Subjects

Volunteers from hospital personnel without symptoms of cardiovascular illness were selected during the study of each patient. These control subjects were excluded if they had ingested aspirin-containing drugs or antihistaminic agents within the preceding 7 days. In addition, women using oral contraceptive agents were exempted. These subjects were not age-matched with patients and base-line electrocardiograms were not obtained from them.

### Whole Platelet Morphologic Studies

Peripheral blood samples were obtained by venipuncture and collected into sterile disposable syringes. The first 2 ml was withdrawn and the syringe detached. A second syringe, containing 1 ml of 3.8 percent sterile sodium citrate, was immediately attached to the tubing and 9 ml of whole blood was withdrawn. The anticoagulant agent was mixed gently and briefly with the whole blood sample. Finally, the mixture was transferred to a 6 oz. polyethylene wide-mouthed bottle. A nonfrosted microscope slide, coated previously with a thin film of Formvar® (1 percent polyvinyl Formvar in ethylene dichloride), was introduced into the bottle, which was placed horizontally in a 37° C oven for exactly 8 minutes. The slide was then immersed in cold 1 percent buffered osmium fixative for 15 minutes at room temperature to stabilize the cellular elements that had adhered to the Formvar film. After fixation, the slide was rinsed in several changes of distilled water and then dried. Next, the edges of the slide were scraped with a no. 22 Bard-Parker blade to free the Formvar film. The platelet population adhering to the surface of the film was surveyed with a phase microscope, and stainless, 200 mesh specimen grids were placed over representative areas. Specimen grids were placed so that their concave surfaces contacted the Formvar film. A thin film of condensed moisture was formed over the film by vigorous exhaling at close range. Immediately, a piece of plastic tape was stretched over the preparation and rubbed firmly. When the tape was removed, the Formvar film became detached from the surface of the slide and covered the concave surface of the specimen grids. Adherent platelets on the surface of the Formvar film were in contact with the surface of the grids, which were removed by rimming their circumference with a blade and then lifting with a pair of tweezers. Preparations were then viewed with an electron microscope.

### Platelet Types

Blood platelets that adhered to the surface of a Formvar film during the 8 minutes of incubation exhibited structural alterations including the extrusion of pseudopodia, hy-



**FIGURE 1.** Electron micrograph of three dendritic platelets, indicating only minimal platelet activation. ( $\times 5,100$ , reduced by 35 percent.)



**FIGURE 2.** Electron micrograph of five platelets. A round form, labeled R, is superimposed over one spread platelet (S), and a dendritic form, labeled D, is contiguous with a spread platelet form. Spread electron-dense central area from which were extruded either a few short blunt pseudopodia or several long pseudopodia (Fig. 1). The spread type was increased in size with only a few of the pseudopodial tips obvious. The central area was less dense or was entirely electron-translucent (Fig. 2).

alomic fusion and hyalomic spreading. Three distinct types of platelets were observed. The round type was compact, had a smooth contour and was uniformly electron-dense. Dendritic types were characterized by a compact electron-dense central area from which were extruded either a few short blunt pseudopodia or several long pseudopodia (Fig. 1). The spread type was increased in size with only a few of the pseudopodial tips obvious. The central area was less dense or was entirely electron-translucent (Fig. 2).

**TABLE I**  
**Clinical Data and Percent Spread Platelets from 13**  
**Seriously Ill Patients Without Underlying Cardiac Disease**

Case no.	Age (yr) & Sex	Condition	Spread Platelets (%)
1	42F	Chronic renal insufficiency	48
2	60F	Septic shock	87
3	61M	Amyloidosis	35
4	37M	Ulcerative colitis	39
5	62M	Abdominal abscess	74
6	19M	Suicide attempt (coma)	48
7	57M	Tracheotomy	63
8	39M	Pneumonitis	38
9	51M	Possible brain tumor	57
Mean ± SD			(54.2 ± 17.7)
10	23F	Respiratory insufficiency	24
11	57M	Chronic renal insufficiency	19
12	68M	Severe metabolic acidosis	22
13	22M	Hepatic pre-coma	9
Mean ± SD			18.5 ± 5.8

SD = standard deviation.

One hundred successive platelet types were categorized. A platelet differential count was performed twice on each blood sample, and the mean of two observations was obtained. To avoid the possibility of bias, each grid containing platelets for examination was coded so that the person surveying the grid was unaware of the source of the sample. Such labeling was not performed by those making the electron microscopic observations.

**Plasma Transfer Experiments**

After selection of the patient, blood was removed to determine ABO blood group and Rh type. Two asymptomatic subjects with the same blood type were then selected. An ABO and Rh cross-match was made between each of these three subjects to avoid the potential of platelet activation due to the formation of antigen-antibody complexes.

*The following procedures were then performed:*

- I. In each of the three subjects to be studied (patient, control subject 1, control subject 2):
  - A. 3 ml of blood was removed for platelet count.
  - B. 9 ml of blood was prepared for whole mount platelet study as described.
- II. From the patient and control subject 2:
  - A. 9 ml of blood was drawn into a syringe to provide 3 ml of platelet-poor plasma, which was subsequently maintained at room temperature.
- III. The following studies were then performed:
  - A. From the patient, 6 ml of blood was drawn into a syringe preloaded with 1 ml of sodium citrate and with 3 ml of platelet-poor plasma from control subject 2. The sample was then prepared for electron microscopic examination of platelets.

**B. From control subject 1:**

- 1. 6 ml of blood was drawn into a syringe preloaded with 1 ml of sodium citrate and 3 ml of platelet-poor plasma derived from the patient. This sample was subsequently prepared for electron microscopic examination of platelets.
- 2. 6 ml of blood was drawn into a syringe preloaded with 1 ml of sodium citrate and 3 ml of platelet-poor plasma harvested from control subject B. This sample was subsequently prepared for examination in the electron microscope.

Volunteers designated control subject 1 were separated at random into control groups A and C. Those designated control subject 2 were separated into control groups B and D (see Results).

**Results**

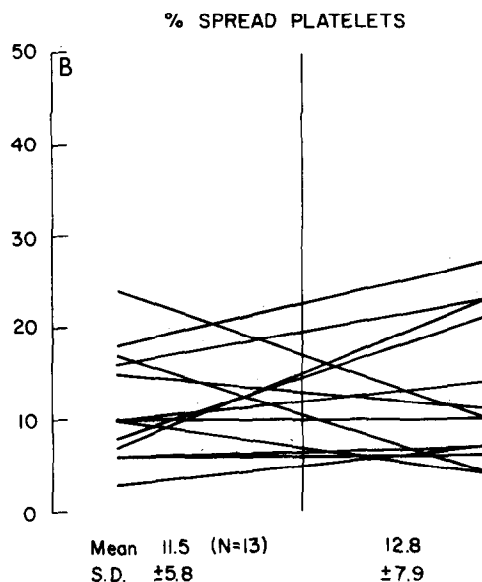
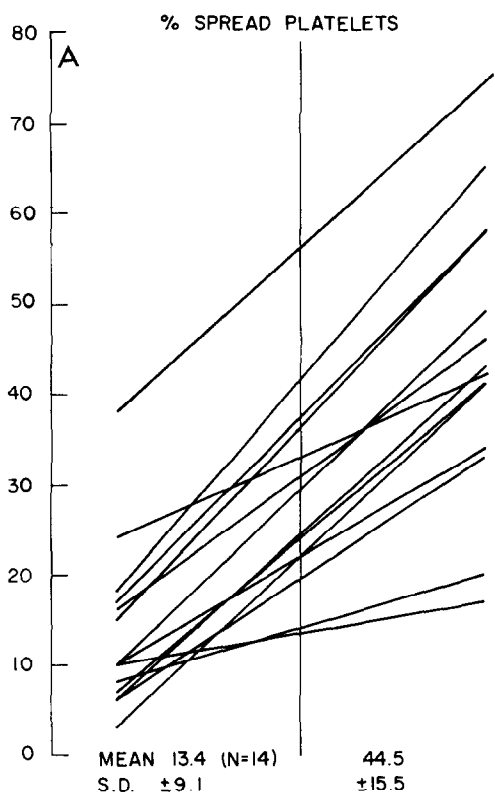
The seven patients with acute myocardial infarction had a preponderance of spread platelet forms (71.2 ± 19.3 [standard deviation] percent), as did the seven patients with chronic ischemic heart disease (68.6 ± 16.4 percent) (four with chronic angina pectoris, three with healed infarction and one with both). No significant correlations were observed between the magnitude of platelet activation (numbers of spread forms) and the clinical and laboratory status (electrocardiograms and serum enzyme, cholesterol and triglyceride determinations).

**Plasma Transfer Experiments**

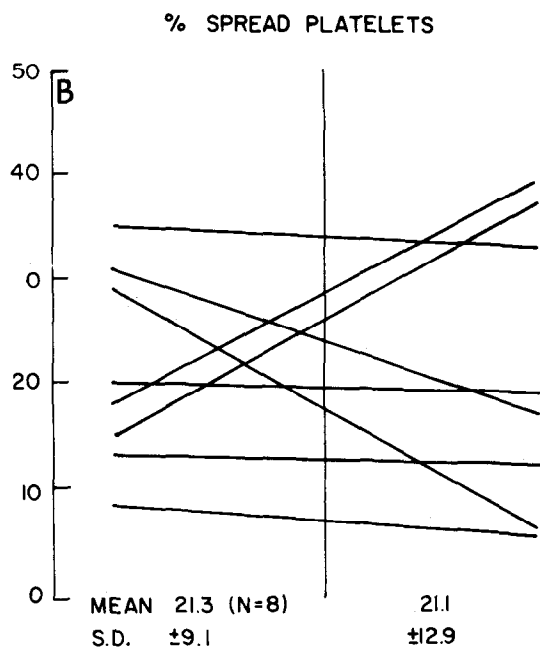
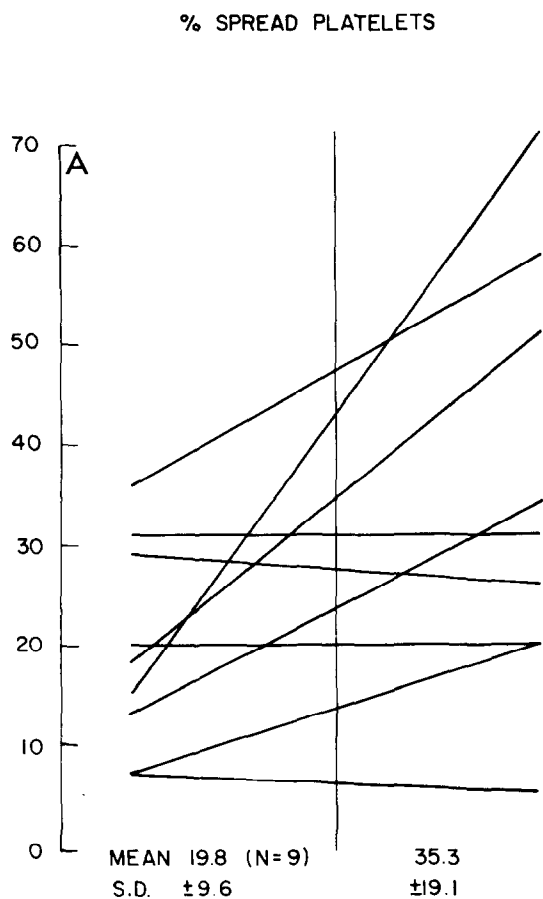
**Patients with ischemic heart disease:** A significant increase in the number of spread platelet forms was observed when blood from asymptomatic subjects (control group A) was exposed to the platelet-poor plasma of patients with ischemic heart disease (from 13.4 ± 9.1 to 44.5 ± 15.5 percent spread forms, *P* <0.001) (Fig. 3A). When blood samples from these asymptomatic subjects were mixed with platelet-poor plasma of another group of asymptomatic subjects (control group B), there was no significant change in the number of spread platelet forms (Fig. 3B). However, when plasma from asymptomatic control subjects was mixed with platelets from patients with ischemic heart disease, no significant decrease in the number of spread forms was observed (from 60.9 ± 22.2 percent to 49.3 ± 26.6 percent).

**Studies in seriously ill patients without heart disease:** Table I lists the age, sex, condition and platelet differential counts for each of the 13 subjects with serious illness of noncardiac origin. Patients 1 to 9 demonstrated a significant increase in number of spread forms (54.2 ± 17.7 percent), but patients 10 to 13 had a normal number of spread forms (18.5 ± 5.8 percent).

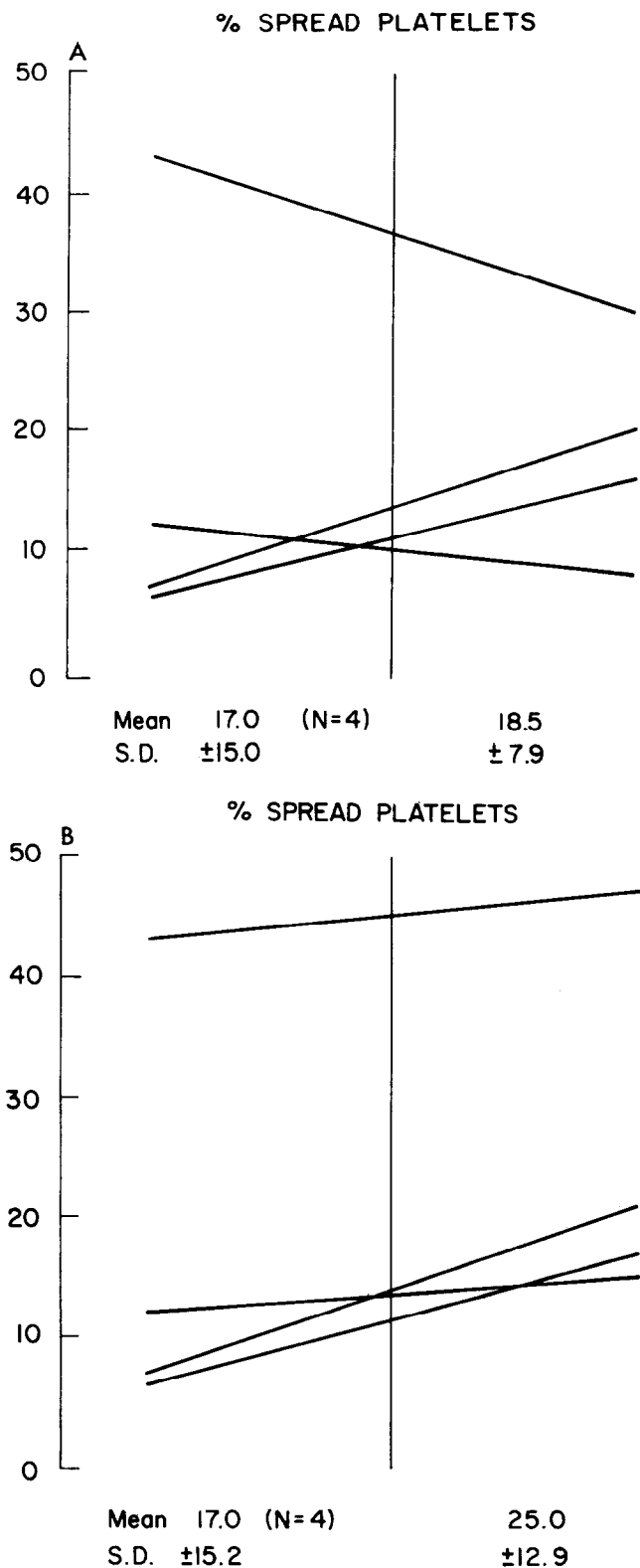
The plasma from the patients without cardiac disease was mixed with the blood of asymptomatic control subjects (group C) and a variability in platelet response was observed. When platelet-poor plasma from Patients 1 to 9 was mixed with the platelets from control subjects (control group C) there was a generalized increase in percent of spread forms (from 19.8 ± 9.6 percent to 35.3 ± 19.1 percent). This



**FIGURE 3.** Change in percent of spread platelets from control subjects (group A). **A**, before and after mixing with plasma from patients with ischemic heart disease. Control values are to left, and results after mixing are to the right of the vertical axis. There is a significant increase in spread platelets after exposure to patient plasma ( $P < 0.001$ ). **B**, before and after mixing with plasma from other control subjects (group B). S.D. = standard deviation. These data demonstrate no significant change in percent of spread platelets.



**FIGURE 4.** Change in percent of spread platelets. **A**, from control subjects (group C1) before and after mixing with plasma from nine seriously ill patients without underlying cardiac illness. Each of these nine patients had an increased percent of spread platelets (mean  $54.2 \pm 17.7$  percent). The change in total number of spread forms approaches statistical significance ( $P < 0.05$ ), but there is greater variability for each individual experiment (in four subjects there was no change or a slight decrease in percent spread forms). **B**, from the same control subjects before and after mixing with plasma from other control subjects (group D1). The change is not significant.



**FIGURE 5.** Change in percent of spread platelets. **A**, from control subjects (group C2) before and after mixing with plasma from four seriously ill patients without cardiac disease and a normal number of spread platelets (18.5 ± 5.8 percent). **B**, from the same control subjects before and after mixing with plasma from other control subjects (group D2). There is no significant change after mixing in either study.

change appeared to be statistically significant at the 0.05 level, using the Wilcoxon Signed Rank test<sup>4</sup> (Fig. 4A). A control experiment in which platelets from control group C were exposed to plasma from another control group resulted in no significant change in percent of spread platelets (Fig. 4B). Patients 10 to 13 had no indication of platelet activation and there was no detectable difference in spread platelet forms from control group blood when exposed to plasma from these patients (Fig. 5, A and B).

**Discussion**

**Platelet Function and Vascular Disease**

Clumping and fusion of blood platelets are the earliest visible manifestations of arterial thrombosis. Accordingly, investigations into the function and structure of platelets have been pursued actively for at least two practical reasons: first, because inhibition of platelet aggregation may be a possible means of preventing arterial thrombosis and, second, because it is possible that the continual laying down of microthrombi on the intimal surface of arteries represents an important and fundamental cause of atherosclerosis.<sup>5</sup>

The evidence that platelet function is altered in patients with atherosclerotic vascular disorders is contradictory. We have observed increased spread platelet formation in all of a group of patients with acute myocardial infarction and in approximately 50 percent of these patients for up to 18 months after infarction.<sup>6,7</sup> Similarly, the majority of a group of patients with chronic angina pectoris had increased spread forms.<sup>8</sup> Recently, Salky and Dugdale<sup>9</sup> observed persistent platelet hyperresponsiveness to collagen in 5 of 47 patients with ischemic heart disease 10 to 16 months after infarction. Other investigators have demonstrated increased platelet adhesiveness or platelet aggregation, or both, in ischemic heart disease by using various methods of measuring platelet function; these include rotating aliquots of platelet-rich plasma at 33 revolutions/min at an angle of 45° in the refrigerator,<sup>10</sup> measuring the length and weight of a thrombus within a closed rotating circular plastic loop,<sup>11</sup> observing the stressed template bleeding time and the amount of platelet retention in a column of glass beads,<sup>12</sup> and tabulating the number of platelet aggregates in the coronary circulation of patients who died suddenly.<sup>13</sup> Other investigators found no detectable change in platelet adhesiveness or platelet function by studying adenosine diphosphate-induced platelet adhesiveness to glass beads,<sup>14,15</sup> measuring platelet survival by the chromium-51 method,<sup>16</sup> or observing platelet aggregometry using the commercially available aggregometer.<sup>16</sup>

Another approach to the problem of defining the relation of platelet function and vascular disease was described by Bolton et al.,<sup>17</sup> who identified in the plasma from patients with arterial disease a "transferable factor" that caused an abnormal sensitivity to

adenosine diphosphate by normal platelets.<sup>17</sup> These investigators concluded that this factor had two components—(1), a low density lipoprotein, the active moiety of which was lecithin, and (2) an enzyme that converted the lecithin to lysolecithin. They speculated that the lysolecithin induced an abnormal platelet sensitivity to adenosine diphosphate as measured by a maximal increase in platelet electrophoretic mobility. A subsequent report has described an “aggregating material” in the plasma of “healthy individuals.”<sup>18</sup>

### Platelet Morphologic Studies

Our observations depend upon two facts: Platelets change shape immediately after blood is removed from the circulation, and these changes are a morphologic expression of platelet surface activation. Clearly, the dimensions of the interaction that occurs between the platelets and the polyvinyl plastic Formvar are unknown; nevertheless, even if this interface provides an artificial stimulus for platelet morphologic change, such provocation is present for blood from asymptomatic subjects as well as from patients.

**A plasma factor causing platelet activation:** Is it valid to conclude that the plasma of patients with ischemic heart disease contains a separate and potentially identifiable factor that causes platelets to become activated? Although the control experiments disclosed no signs of increased platelet activity when plasma from asymptomatic subjects was mixed with normal platelets, there was a discrepancy between the mean age and age range of the control subjects and those patients who were studied. It is thus conceivable that the plasma factor is a normal accompaniment of age; this possibility has not been excluded by appropriate experiments in which platelets from control subjects were exposed to plasma of another group of control subjects, each age-matched for the patients under study. However, this seems highly unlikely to us since in a survey of 18 asymptomatic subjects over age 40 years, platelet configuration was not significantly different from that in 67 asymptomatic subjects under age 40 years (unpublished data).

The presence of a plasma factor causing platelet activation in some but not all of 13 patients with serious noncardiac disease is unexplained. The plasma of each of 14 patients with ischemic heart disease dem-

onstrated this effect, yet it was observed in only 5 of those 9 patients without heart disease who had an increased number of spread platelets. This finding indicates that a preponderance of spread platelet forms is not always accompanied by a transferable platelet-activating plasma factor. Thus, one might speculate that spread platelet formation may be the morphologic reflection of a final common pathway of functional platelet response to a number of different stimuli.

**Effect of hormonal and stress situations during acute infarction:** A further inference from these data would be that platelet activation and the transferable plasma factor are probably not the direct result of hormonal or therapeutic influences during the stress of infarction. No significant difference in the effect of plasma from patients with chronic ischemic heart disease (measured at least 3 months after myocardial infarction in four patients) and from patients with acute infarction was demonstrated. Since higher plasma levels of catecholamines occur during the first days of acute myocardial infarction,<sup>19</sup> we suggest that these substances are probably not responsible for the changes observed. There were no data to suggest that the seven patients with chronic ischemic heart disease were under any particular stress that would have caused increased plasma catecholamines.

Similarly, it is well known that plasma catecholamines increase in various stress situations, including hemorrhage<sup>20</sup> and shock.<sup>21</sup> It is probable that each of the 13 seriously ill patients without cardiac disease in this series was sufficiently stressed to have high levels of plasma catecholamines, yet increased platelet activity, and particularly the plasma factor, was found in only some of these patients.

Clearly, further studies are required to identify and isolate this plasma factor, to determine its relation to the lysolecithin described by Bolton et al.<sup>17</sup> and to examine whether platelets are uniformly responsive to its influence.

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