A Test For Impending Myocardial Infarction

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According to recent evidence, thrombosis is generally initiated by the spontaneous rupture of the endothelial lining of blood vessels. (7, 10). Bleeding comes to a halt as a result of adherence of platelets to each other, to the endothelium of the damaged area, and to the surrounding vessel walls. The adhesiveness of the blood platelets is therefore a critical determinant in the formation of a thrombus. The biochemical basis of adhesiveness has been reviewed by Born (1-3) and Gaarder et al (8).

The role of divalent metal ions in platelets aggregation was studied by Bramble (4). He reported that divalent metal ions are necessary for platelet aggregation. EDTA**, which chelates alkalin earth metals, prevents this aggregation. Aggregation was restored by addition of Sr^{++}, Ca^{++}, or Mg^{++}. These metal ions induced aggregation in blood samples drawn from patients suffering from myocardial infarction, but has minimal effects on blood platelets from normal individuals, and from patients with other diseases. The adhesiveness of blood platelets may therefore be used as a diagnostic tool for assessing the likelihood of myocardial infarction. In practice Sr^{++} is the most convenient ion because, it gives the cleanest slides.

In order to study the interaction of blood platelets with divalent metal ions, the presence of heparin in the incubation mixture is essential(4).

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** Etylene Diamino Tetracetic Acid.

Heparin prevents macroscopic fibrin formation and gross blood clotting(9).

Bull and Zuker (5), have reported that blood platelets become sphe rical and increase in volume following addition of EDTA. However, th does not appear to interfere with the clinical usefulness of this test.

The present paper reports the utilization of Bramble's procedure (4 to study platelet–leucocyte aggregation in order to predict the incidence myocardial infarction.

Materials and Methods

Procedure:

The procedure employed here was that described by Bramble (1961 Experimental details are reproduced here for convenience of access. Briefl the test consists in observing platelet–leucocyte aggregation in the presenc of heparin, EDTA, and Sr^{++} ion (4.5×10^{-2} to 4.5×10^{-4} Molar concer tration).

In order to control metal ion concentration, the water used for p paration of EDTA and $Srcl_2$ was triply distilled and deionized.

Twenty ml of blood were collected as rapidly as possible in a chill plastic syringe by gentle venipuncture. The blood was transfered to d heparinized tubes sealed with parafilm, and mixed. Nine ml of hepariniz blood were transfered to each of two 12 ml conical centrifuge tubes, cont ining one ml of Na_2 EDTA (1g EDTA per 100 ml of 0.75% Nacl The tubes were sealed with parafilm, placed on a vertical rotating tal and rotated for 5 minutes at a speed of nine revolution per minute. T tubes were then allowed to stand for 35 minutes at room temperature. T contents of both centrifuge tubes were transfered to one Erlenmeyer fla and mixed thorougly. One ml aliquots of heparinized–EDTA blood from t main pool were transfered to small (1.0 mm $\times$ 70 mm) tubes. A total of such tubes were prepared. To each tube was added 0.1 ml of the appro

riate $SrCl_2$ solution, to make final concentrations varying from 4.5×10^{-2} to 4.5×10^{-4} Molar. The last tube received 0.1 ml of saline for control purposes. The tubes were removed from the rotating table and allowed to stand at room temperature for 55 minutes, in order to permit cellular aggregation to occur. They were then rotated for another 5 minutes, to assure resuspension of cell elements and aggregates. By means of a capillary tubes, 4–5 microlitres of blood from each sample were placed on a standard slide and air dried. Three such slides were prepared from each individual tube and stained with Wright's stain, for microscopic examination. Total numbers of aggregates were plotted against the concentration of $SrCl_2$ (micro mole/ ml) in the final incubation mixture.

CELLULAR AGGREGATION AND THEIR STRUCTURAL CHARACTERISTICS.

Microscopic studies of stained slides revealed three types of cellular aggregates according to the following morphological variation.

1– Comapct clumps of platelet surrounding by incomplete shells of leucocytes (Fig. 1). This arrangement is designated as incomplete platelet– leucocyte aggregation.

2– A compact clump of platelets encircled by one or more shells of leucocytes, resembling a flower (Fig. 2). This configuration is referred to as complete aggregation.

3– Cluster of two or more complete platelet–leucocyte aggregation, arranged like a multicentric flower. However, the central masses of platelet clumps were entirely amorphous (Fig. 3). This pattern is referred to as multiple rosettes.

The structural characteristics of aggregates observed by phase microscopy were identical to those seen in stained preparations. Since the stained slides provide a permanent record, this procedure was used throughout.
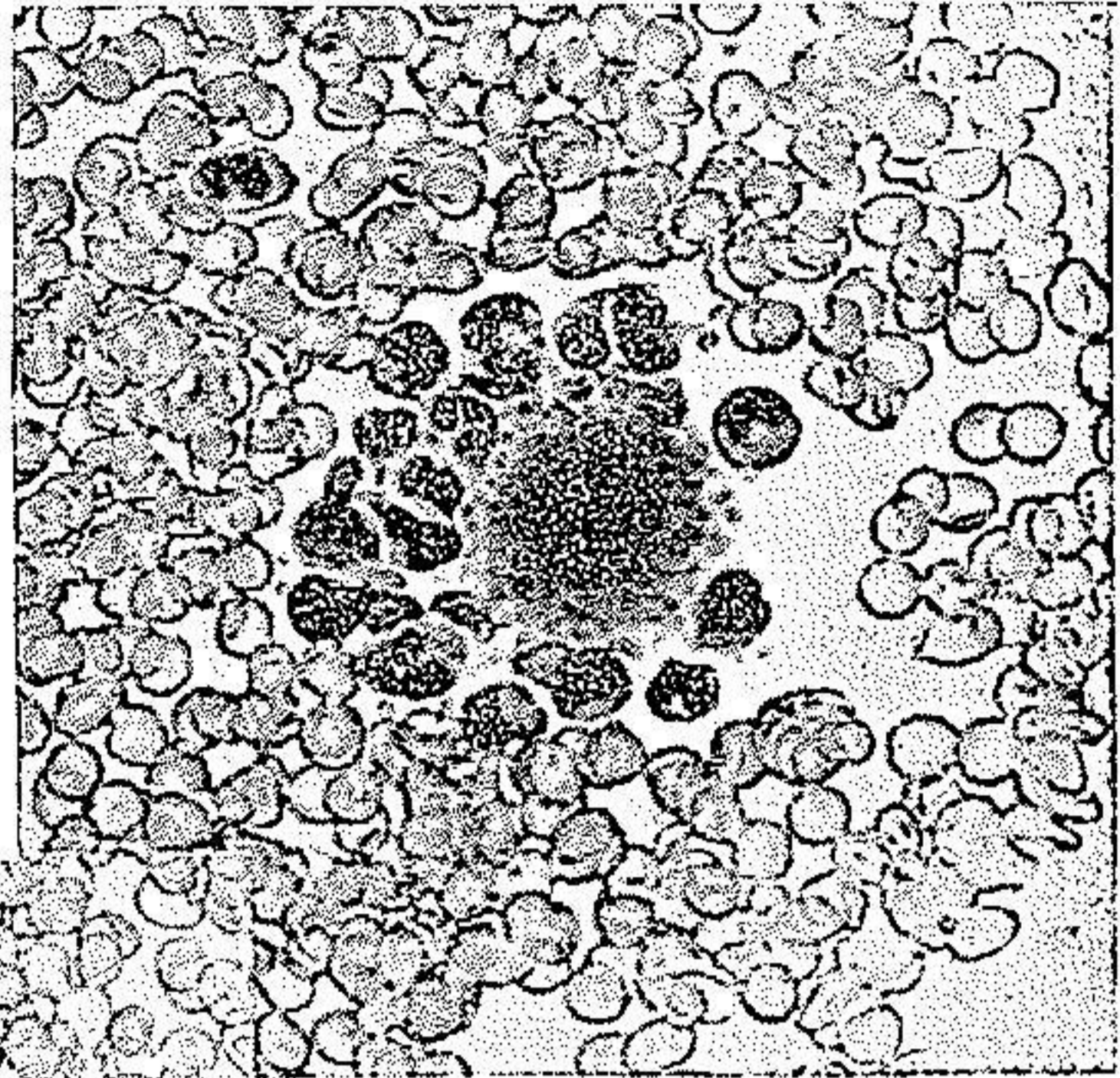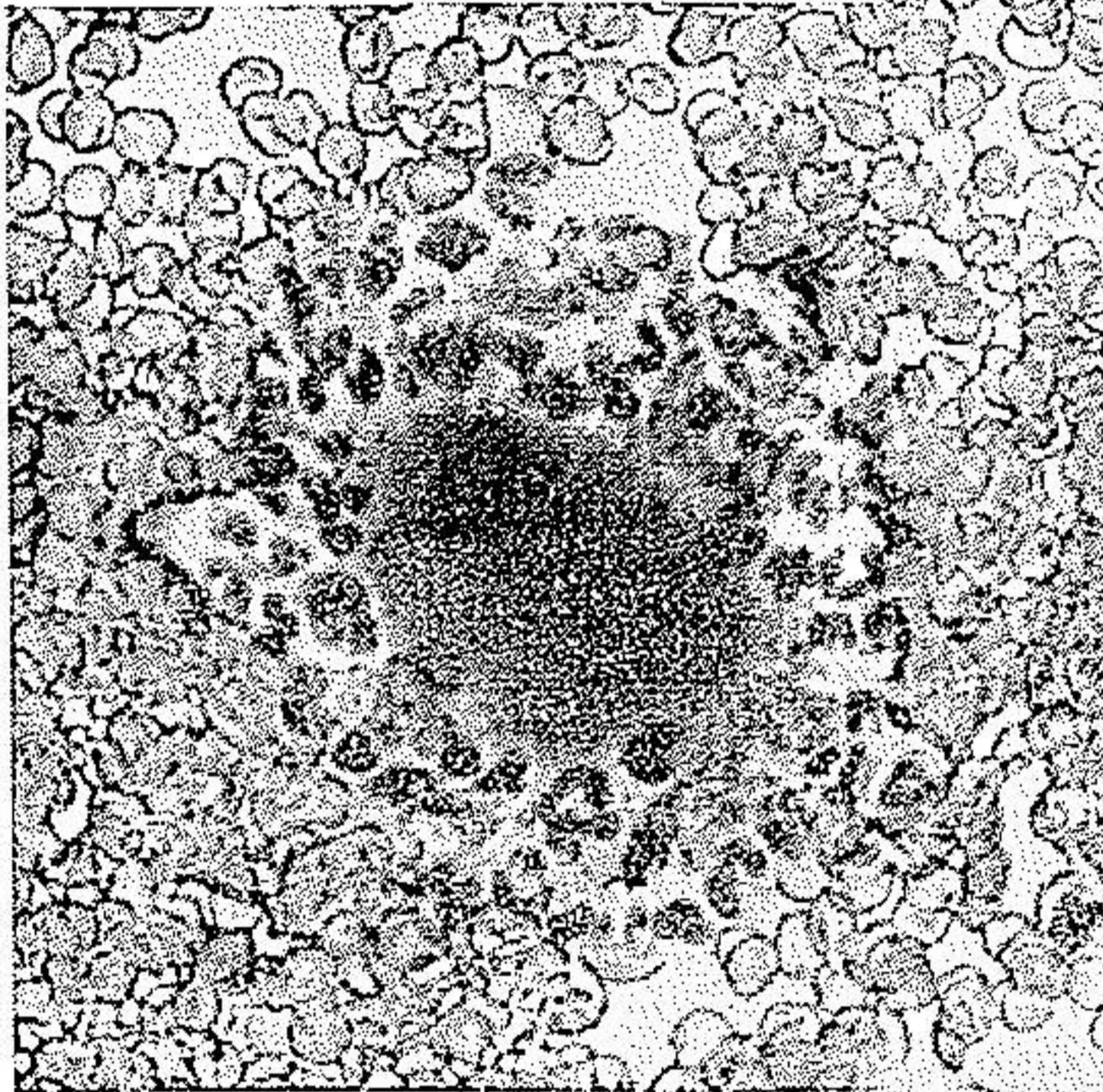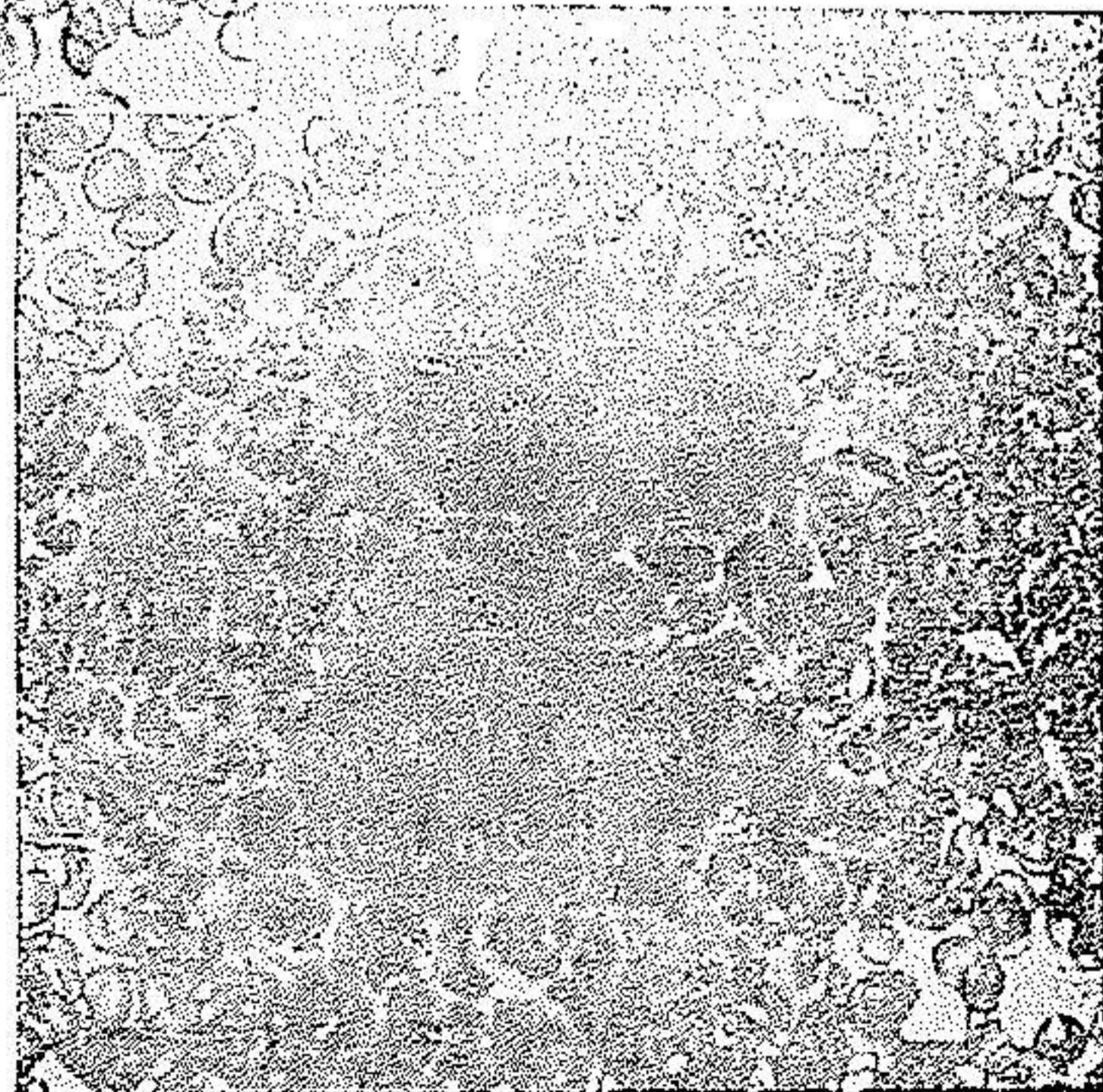
Fig. 1

Fig. 2

Fig .3

Results

Various degrees of cellular aggregation were classified into three groups: incomplete, complete and multiple rosettes (Fig. 1–3).

Minimal numbers of aggregates were seen in blood drawn from 23 males and 7 females volunteers (age range 21–30). Under the condition used here the number of aggregates did not exceed 20 per slides (Fig. 4-A).

Samples from (11 males and 4 females) slightly older persons (age range 31–55) showed about the same number of aggregates (Fig. 4–B).
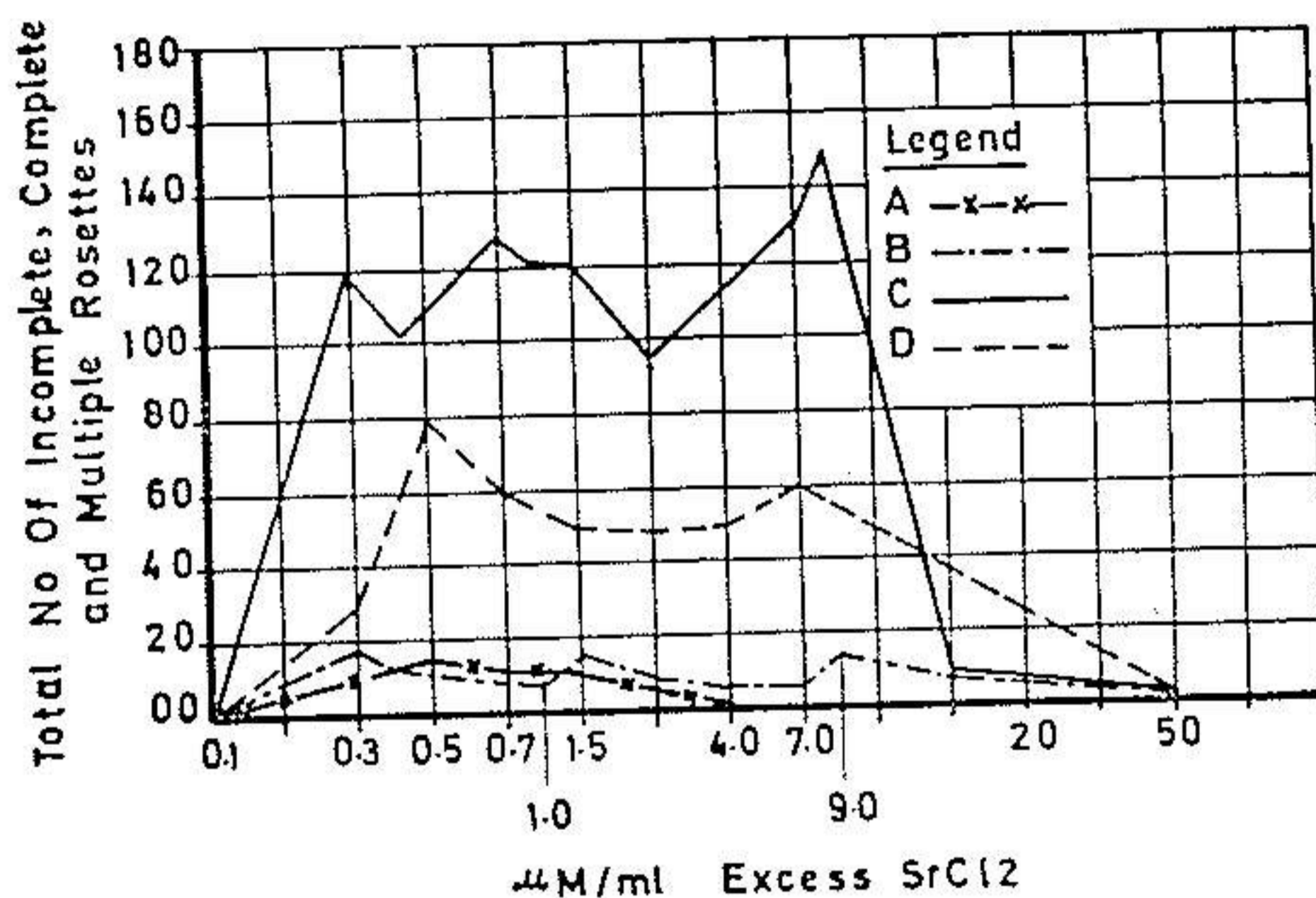

Fig. 4

In six patients (4 males and 2 females) who died within 24 hours of the test as many as 150 aggregates per slides were found (Fig. 4–C). Patients (5 males and 3 females) suffering from coronary insufficiency also exhibited increased platelet–leucocyte aggregation, but the numbers were smaller than those seen in thrombotic patients (Fig. 4–D).

In blood samples from patients (23 males and 8 females) with definite thrombotic episodes, numerous complete and multiple rosettes were seen (40–50 per slide)(Fig. 5). In patients (3 males and 2 females) who suffered two successive attacks, the number of aggregates seen after the second

attack were slightly higher than after the first, but the results are not statistically significant (Fig. 5-A and B).
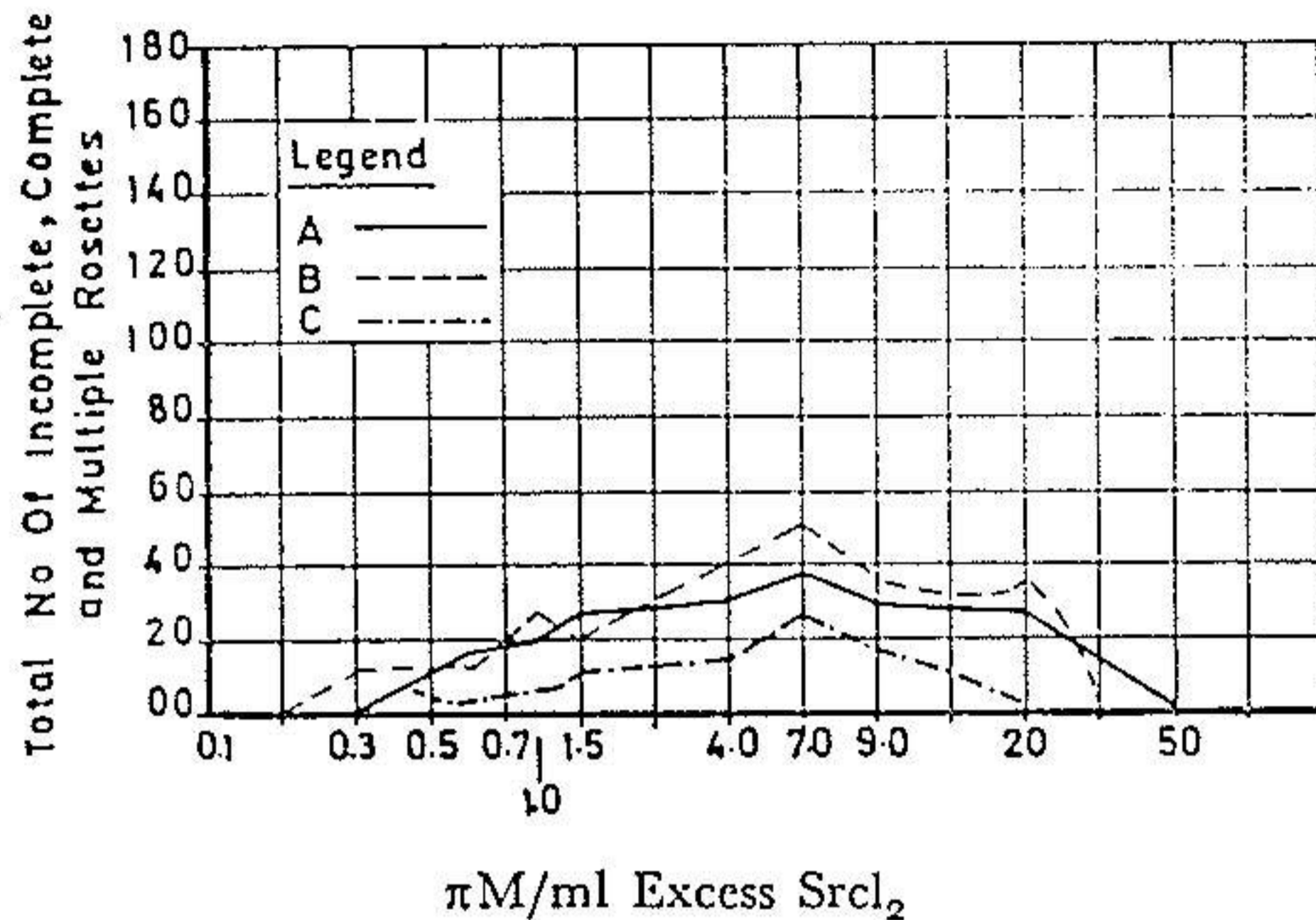

$\pi M/ml$ Excess $Srcl_2$

Fig. 5

The number of aggregates decreased folowing administration of heparin, and dicumarol/or dicumarol alone The patients were discharged 2-3 weeks after treatment (Fig 5-C).

Drug sensitive patients (8 males and 2 females) with definite myocardial infarction did not receive any treatment but were in absolute bed rest for three weeks. Recovery was evident from reduction of the number of
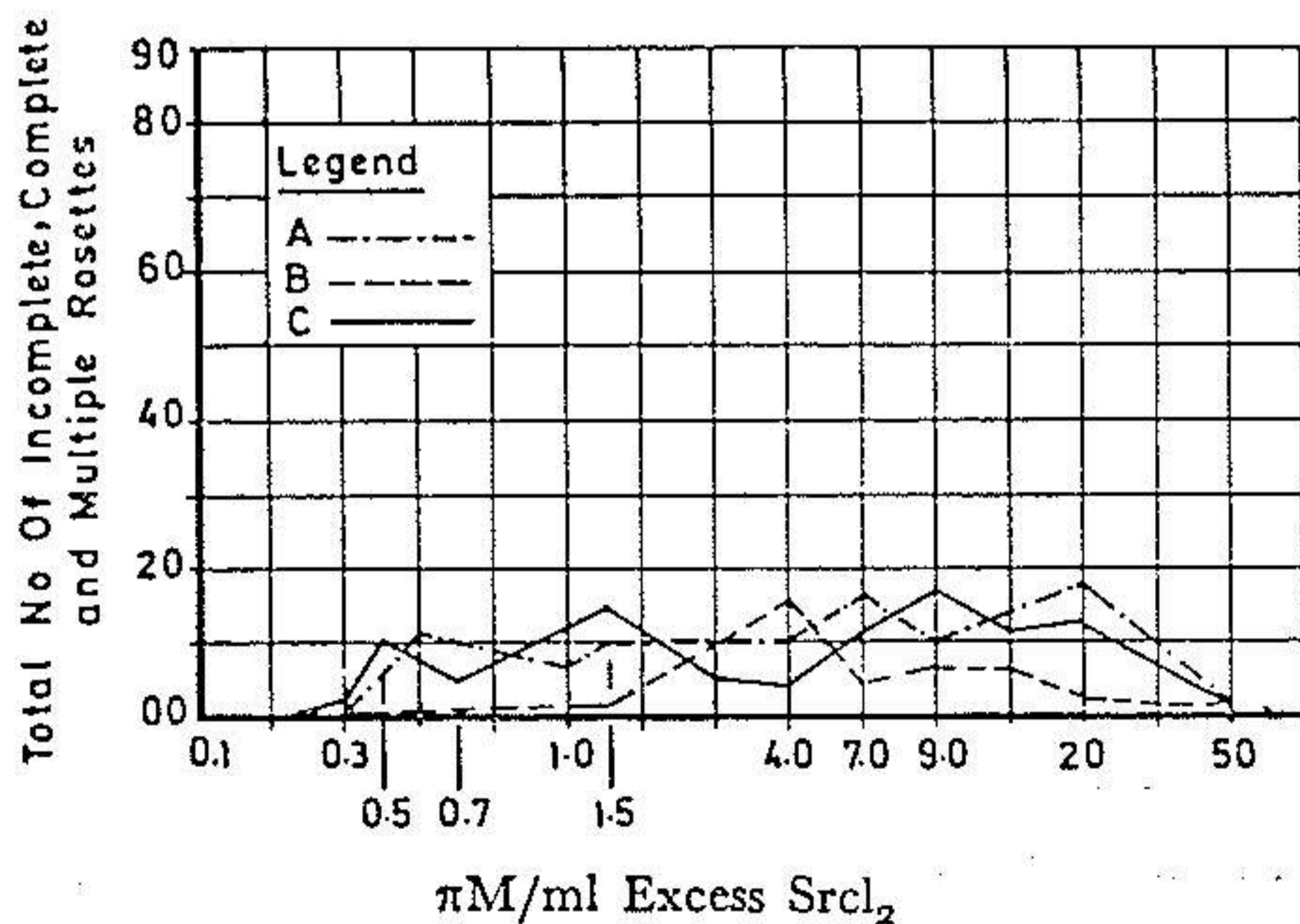

$\pi M/ml$ Excess $Srcl_2$

Fig. 6