An Explanation of the Discrepancy between Direct and Indirect Platelet Counts

By HARRY J. FITCH

THERE ARE two standard methods for counting platelets, the direct and the indirect. For the direct method the blood is accurately diluted with an anticoagulant solution, usually in a hemocytometer pipet, and the platelets are enumerated in a counting chamber.[1] For the indirect method the blood is diluted and a drop is placed on an ordinary microscope slide. It may then be spread and dried[3] or, as is more usual, a coverslip is placed on the drop to make a wet preparation.[2] Platelets and red cells are then counted field by field and the ratio is established. The red cells are also counted directly in a counting chamber to determine their concentration in the blood. The platelet concentration is then derived by referring the platelet-red cell ratio to this direct red cell count. When platelets in the same sample of blood are counted by these two methods the results are not the same. A normal count by the direct method is about 250,000 per cu.mm.; by the indirect method about 500,000. This discrepancy has been known since the beginning of the century.[6, 9] It has also been known that when the proportion of platelets to red cells is established in a counting chamber the ratio is about 1:20, while between a slide and coverslip it is about 1:10.[4] When an indirect count is carried out in a counting chamber[8] or on a dried smear[3], the platelet count of normal human blood is about 250,000, which is no different from that obtained by the direct method.

The difference between results usually obtained by the two methods, while often a subject for speculation or debate, has not been explained.[7] The purpose of this report is to demonstrate the reason for the difference in these counts.

MATERIALS AND METHODS

Direct counts were performed by phase-contrast microscopy using the method of Brecher and Cronkite with a one per cent solution of ammonium oxalate as diluent.[1] Indirect counts were performed by mixing blood and diluent in a small wax cup with a wax stirring rod according to the method of Olef.[5] Dameshek's solution was used as the diluent.[2] Coverslips were ringed with vaseline to prevent drying. Siliconized glassware was prepared with General Electric Dri-Film SC-87. All counts were done on normal human blood. This study involved the testing of several earlier theories of the difference between the two methods and whenever the two methods were compared experimentally, blind counts were done.

The crucial experiment with the indirect method consisted of counting the red cells and platelets in every field from one edge of the coverslip to the opposite edge. The total number of cells counted was divided by the number of fields to derive the average cells per field across the coverslip. The counts of each field were then compared with the average and these values were plotted on a graph similar to those in figures 2 and 3. However, each point in the figures represents an average ten consecutive fields, which simplifies the figures without changing their essential characteristics. Ten such experiments were performed.

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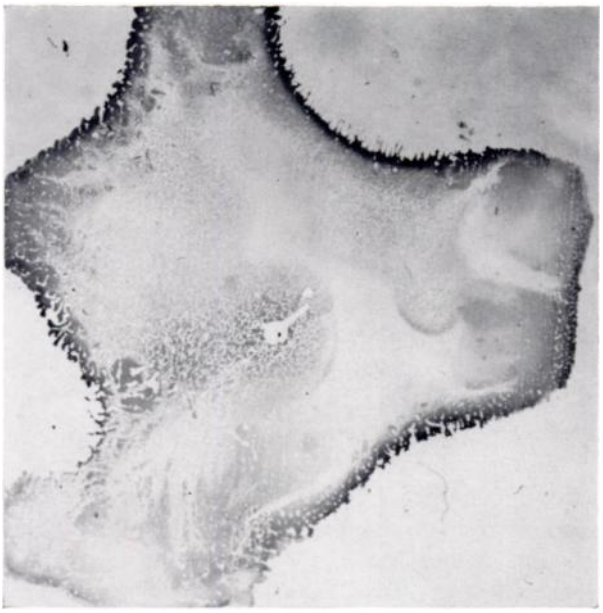

FIG. 1.—A small drop of blood beneath a thin, uncoated coverslip. The concentration of red cells at the edge of the drop is apparent. Granularity is due to rouleaux. The concentration of red cells at the edge occurs also with platelet-counting fluid and destroys the accuracy of the indirect platelet count.

RESULTS

It was found in the indirect method that when the drop of fluid spreads beneath the coverslip, the red cells become concentrated at the periphery (fig. 1). Two thirds of all the red cells may be found beneath the outer 25 per cent of the coverslip's area (figs. 2, 3). Platelets, on the other hand, are almost randomly dispersed beneath the coverslip (figs. 2, 4). The concentration of red cells is not related to the edge of the coverslip coinciding with the edge of the drop. The same thing occurred with drops too small to reach the edge of the coverslip (fig. 4). Both red cells and platelets were almost randomly dispersed when the coverslip and slide were coated with silicone to make the glass non wettable (fig. 5). When this was done the indirect count almost equaled the direct. Example: Direct count (average of four chambers) 199,000 per cu.mm. Indirect count (blind count): 96 platelets per 2700 red cells × 5.17 million RBC = 184,000 platelets; (author count) 101 platelets per 2727 red cells × 5.17 million RBC = 191,000 platelets per cu.mm.

DISCUSSION

The indirect platelet count, as it is usually done, is inaccurate because the red cells are not randomly distributed beneath the coverslip. When the proportion of platelets to red cells is established by counting in areas away from the edge, the ratio is artificially high because the red cells are not adequately represented, and therefore the final platelet count by the indirect method is artificially high.

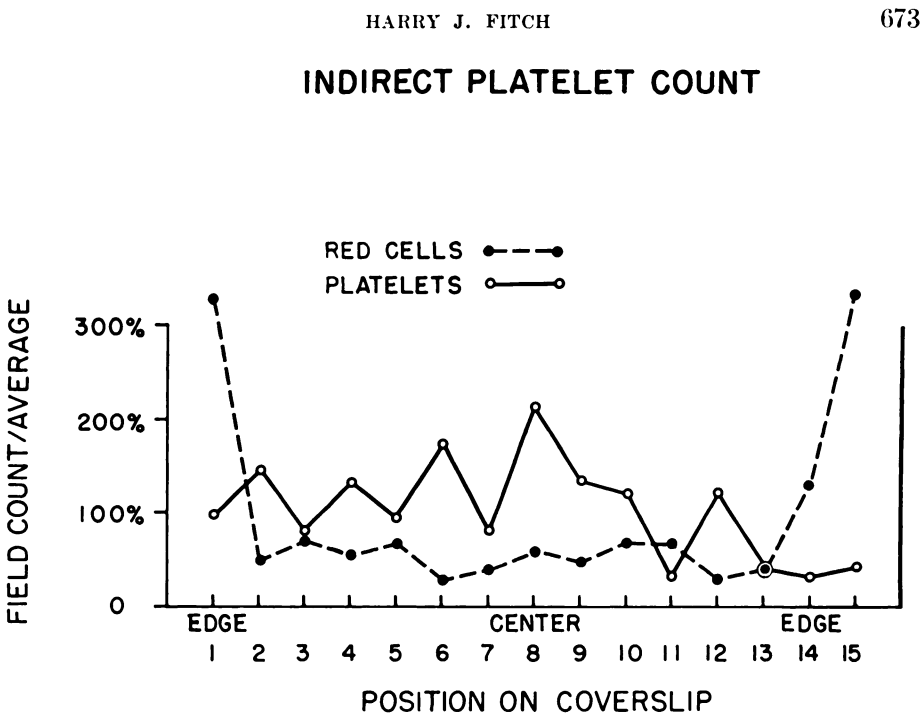

FIG. 2.—Distribution of red cells and platelets in 153 fields, from edge to edge of the coverslip. Red cells averaged 11.6 per field, the platelets 0.75. Each point in the graph represents ten fields. The red cells are concentrated at the edge of the coverslip, but from point 3 to point 13 the distribution is fairly uniform. It is in this area that indirect platelet counts would ordinarily be made. The indirect platelet count based on these fields is 775,000; the indirect count based on all fields is 359,000. Distribution of the platelets is fairly random with perhaps a tendency to concentrate in the central area.

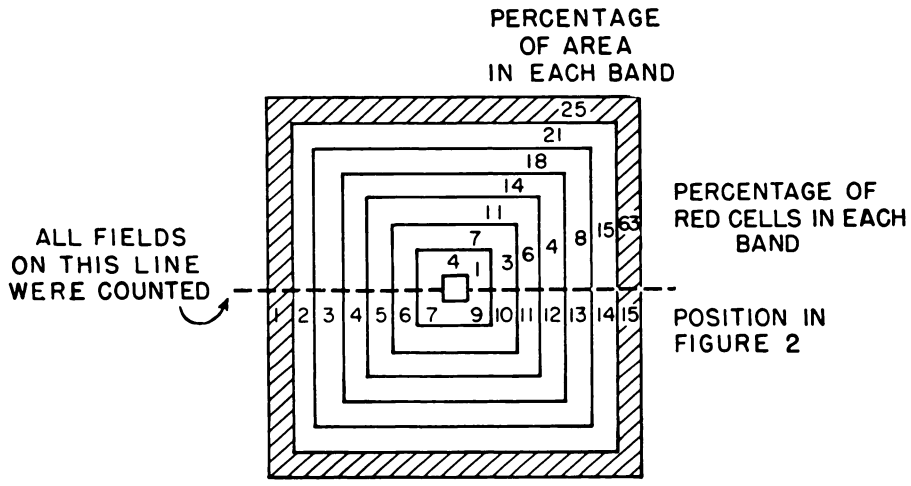

FIG. 3.—Diagram of a coverslip representing the experiment in figure 2. Each band in the diagram corresponds to two points in figure 2 and the average of the two points gives a value which represents the concentration of red cells in the particular band. Each band represents a different amount of the coverslip's area. The percentage of red cells in each band was established by multiplying the concentration of red cells by the area. Almost two-thirds of the red cells are concentrated in the peripheral 25 per cent of the area.

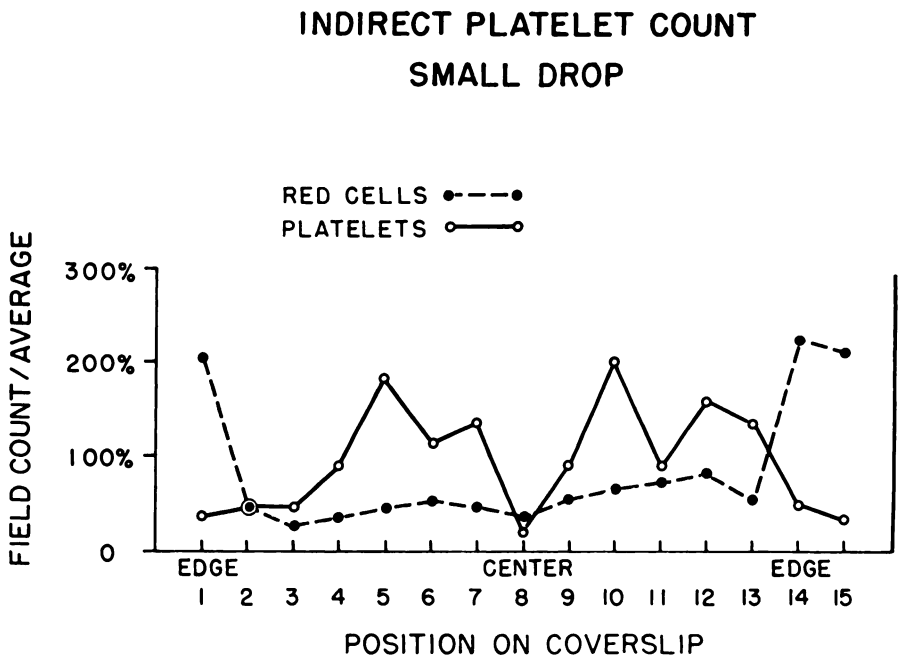

FIG. 4.—Distribution of red cells and platelets in a drop so small that it could not reach the edge of the coverslip. Red cells averaged 17.1 per field, the platelets 0.88. Seventy-eight fields counted. Each point represents 10 fields. The red cells are concentrated at the edge of the drop even though the edge of the drop is not at the edge of the coverslip. The indirect platelet count based on all fields is 290,000 per cu. mm.; based on the fields represented by points 4 to 12 inclusive (areas that would be scrutinized in ordinary platelet counting) the indirect platelet count is 420,000.

The reason for this phenomenon has not been established, but several possibilities come to mind. The red cells may be impelled toward the edge because wettable glass is hydrophilic while the red cells are hydrophobic and, repelled by the glass, they tend to stream in the direction of the air-water interface. There may also be some slight warping of the flexible coverslip: if a small drop of whole blood is placed between a coverslip and slide and examined immediately, the concentrated red cells at the periphery form rouleaux and stand on edge indicating that the two glass surfaces are at least 8 μ apart. But in the center of the drop the few red cells lie flat and as they move about they do not turn over presumably because they cannot, the slide and slip being too close together. The platelets are small and they are probably protected from the squeeze by the presence of larger red cells. The red cells themselves can be so protected by using a drop so large that the coverslip floats above the slide. Coating the surfaces of the slide and coverslip with silicone reduces their capillary attraction for one another, and also makes the surfaces hydrophobic so they would no longer tend to repel the red cells.

The lack of randomness of red cell distribution can be demonstrated to the naked eye by placing a small drop of whole blood beneath a thin coverslip. As the drop spreads the red cells immediately become concentrated at the edge of

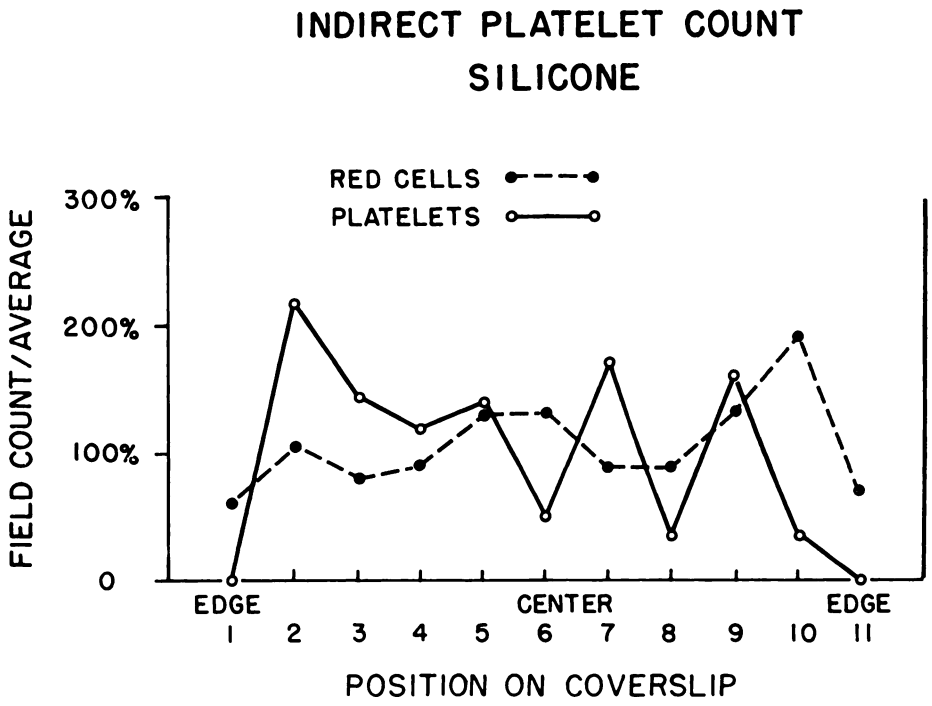

FIG. 5.—Distribution of red cells and platelets in a drop suspended between siliconized surfaces. The experiment was performed on a small drop. Forty-four fields were counted; each point represents four fields. There were 441 red cells and 26 platelets. There is a fairly random distribution of the red cells.

the drop. But if the surfaces are coated with silicone, or if the drop of blood is a large one, the red cells appear evenly dispersed. The phenomenon is less apparent if the coverslip is thick and rigid, of the sort used on a counting chamber.

SUMMARY

The indirect platelet count is higher than the direct count because the red cells, which are used as a point of reference in the indirect method, are not randomly distributed beneath the coverslip. The red cells are concentrated at the edge of the coverslip so that the true ratio of red cells to platelets cannot be accurately established. Indirect platelet counts based on the ratio in the central areas of the coverslip are too high.

SUMMARIO IN INTERLINGUA

Le numeration indirecte de plachettas es plus alte que le numeration directe proque le erythrocytos, que es usate como base de referentia in le methodo indirecte, non es distribuite al hasardo infra le lamella coperi-objectos. Le erythrocytos es concentrate al margine del lamella de maniera que le ver proportion inter erythrocytos e plachettas non es determinabile. Numerationes indirecte de plachettas, basate super le proportion al centro del area del lamella es troppo alte.

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