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HIGH SPEED AUTOMATIC BLOOD CELL COUNTER AND CELL SIZE ANALYZER

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Abstract.—The instrument employs a non-optical scanning system providing a counting rate in excess of 6,000 individual cells per second with a counting interval of 15 seconds. A suspension of blood cells is passed through a small orifice simulataneously with an electric current. The individual blood cells passing through the orifice introduce an impedance change in the orifice determined by the size of the cell. The system will count the individual cells and provides cell size distribution. The number of cells counted per sample is approximately 100 times greater than the usual microscope count to reduce the satistical sampling error by a factor of approximately 10 times.

I. INTRODUCTION

It is difficult to overstate the need for instrumentation to reduce the tedium of visual cell counting and to increase the accuracy of the counts. Best practice limits the number of sample counts per day per average technician to no more than 20 or 30 counts. Probably as a result of the tedium more than any other factor the number of cells counted for a typical sample is limited to about 500. A sampling of only 500 cells to determine concentration is far too low to provide data for any but quite gross changes in the true count. The random error associated with a sampling of 500 corresponds to a standard deviation of slightly more than 4%. This means that while two thirds of a large number of counts should be within this figure about one third of the counts will be in error by more than 4%; 95% should be within plus or minus 8%. One count in 20 may be in error by more than plus or minus 8%. To the random error of the visual count must be added the human errors of judgment. These include failure to count all cells in the counting area, counting some cells twice and in the application of the counting rules regarding boundary cells. A failure to observe the proscribed technique of filling the counting chamber is recognized as an additional source of error. It is interesting that in the course of evaluating the new counting method[1] a group at the National Institutes of Health uncovered another and heretofore unsuspected chamber error. Even when following the proscribed filling technique it was found that the cell concentration in the sample reaching the ruled area of the chamber was appreciably higher than the true concentration. As a result of the liquid flow profile the cell concentration at a distance from the point where the sample is introduced into the chamber is increased at the expense of the concentration near the point of introduction. The magnitude of this error will come as quite a shock to those who have come to consider the counting chamber as a standard. When all the errors are considered it is evident that the primitive state of the cell counting art rules out the possibility of using blood cell counts to closely follow small or even moderate changes of a patient's blood count when successive counts on the same sample can easily differ by 10 or 20 percent.

It is evident that the factors limiting accuracy, in the present day methods of visual counting, are the relatively small number of cells counted and the un-

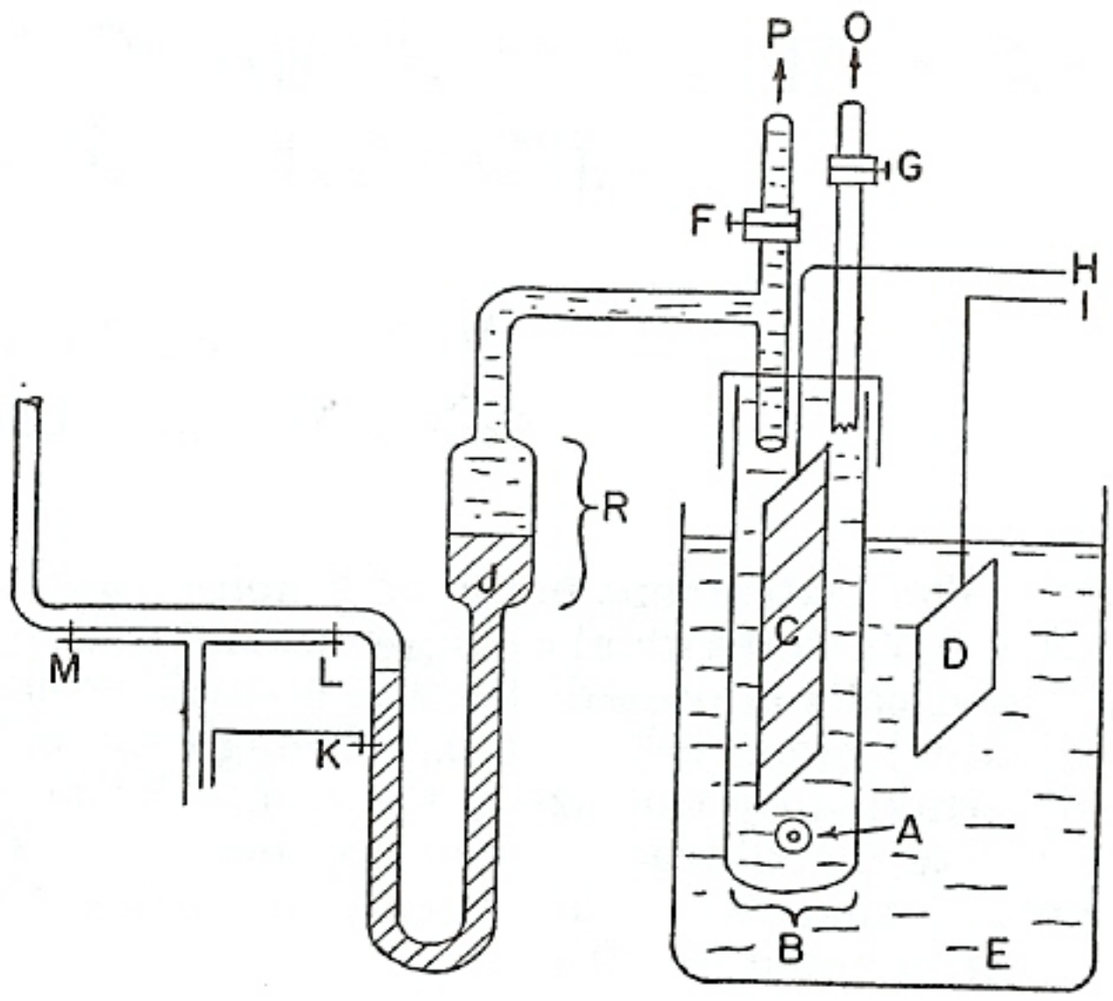

Fig. 1—Simplified drawing showing aperture tube, sample beaker, electrodes and flow control elements.

avoidable human errors. Increasing the number of cells counted by a factor of 100 times significantly reduces the value of mean deviation. Providing automatic instrumentation reduces human errors. A new instrument is described which automatically counts about 50,000 cells for a normal sample in 15 seconds or less. Over 90% of the mean of a large number of counts on the sample is within 1% of the mean.[1]

II. AUTOMATIC HIGH SPEED COUNTER

The new counter provides a means of obtaining complete cell size distribution data of an accuracy not heretofore possible and in such a short interval of time that the procedure could be used routinely should the need become established. The difficulties of making cell size distribution studies by classical methods are so much greater than making a simple count that the procedure is seldom used even in research.

Up to the present time efforts at automatic blood cell counting have generally been based upon optical principles. Individual cells, in various scanning systems, are caused to modulate the amount of light falling upon a photoelectric cell to produce electrical pulses for counting. Detection of a cell depends upon the difference in optical characteristics between the cell and the surrounding medium. In the new counter individual cells are caused to move through a small constricted electric current path in the suspending fluid and detection is based upon differences in electrical conductivity between the cell and the suspending fluid. The constricted current path is analogous to a light beam of small dimensions in an optical system. In passing through the small current path in the fluid the individual blood cell changes the electrical resistance in the circuit, and causes a change in the voltage drop appearing across the current path. The electric current path of small dimensions and the flow of cell bearing fluid through the path is provided for with a very simple structure. The boundary of the current path is the bore of a submerged orifice of small dimensions in the wall of an insulated vessel. Figure 1, to be described later, is a simplified drawing of the sample unit. A suspension of blood cells of known dilution is caused to flow through the orifice. By means of a current source and a pair of relatively

large area electrodes placed in the liquid volumes on each side of the orifice an electric current is also caused to flow through the orifice. The liquid in one of the volumes carries the cells to be counted through the orifice while the liquid in the other serves to complete the electric circuit to its electrode. A typical orifice is 1/10th millimeter diameter and approximately 1/15th milliameter in length. In comparison blood cells may range in size from 1/20th to 1/5th the diameter of the orifice. The orifice or aperture may be considered as a minute conductivity cell with the liquid volumes at each end of the aperture serving as electrodes to make contact with the contents of the bore.

The blood cells may be suspended in any of the commonly used diluting fluids. The diluents, being electrolytes, are relatively good conductors while all body cells are very poor conductors. As the suspending fluid carries a cell through the orifice the cell displaces a volume of the conducting fluid to slightly raise the electrical resitance of the total contents of the orifice. Time of passage through the orifice may be 1/50,000 or 1/100,000 second depending on the length of the orifice and the pressure differential. Since the passage time is of such short duration very high current densities may be employed without vaporizing the liquid. The slight increase in resistance during passage increases the voltage appearing across the orifice for the corresponding interval. The voltage pulses produced are amplified for display on an oscilloscope screen and for feeding a pulse height discriminator followed by decade counters.

Figure 1 shows a simple arrangement for holding the sample, establishing sample flow and "metering" the flow so that an electronic counter can be activated as a selected sample volume is drawn through and scanned by the orifice. A dilute suspensions of cells E is contained in a sample beaker. The tube B carries the aperture A through which the sample is drawn. C and D are the electrodes. When stopcock F is opened an external vacuum source (and waste discharge) P initiates flow through the orifice and causes the mercury J in the manometer to assume the position shown with the mercury in the open leg of the manometer drawn slightly below the horizontal branch. When stopcock F is closed the unbalanced manometer functions as a syphon to continue the sample flow through the orifice. As the mercury in the open leg rises into the horizontal branch it makes contact with a wire electrode L sealed in the manometer wall and energizes a high speed decade counter which begins counting all pulses which reach or exceed the threshold level. A few seconds later the mercury column makes contact with a second wire electrode M which stops the counter. The syphoning action continues until the mercury column comes to rest at a level near that of the mercury in the reservoir. Contact K provides a ground return path for the start and stop electrodes. The contacts L and M are very carefully located so that the volume contained in the tube between the contacts is 1/2 milliliter. As a consequence of the arrangement the counter is actuated as 1/2 milliliter is drawn into the system. In practice the horizontal section is a U tube in the horizontal plane so that contacts are near together. By this means the vacuum in the system at the start and stop contacts is kept substantially equal so that any elasticity in the system due to bubbles etc., will introduce less than 1/10th percent error in the syphoned volume under the worst conditions.

The function of inlet 0 and stopcock G which is normally left in the closed position is to allow rapid filling of the system when setting it up instead of depending upon the relatively slow flow through the orifice.

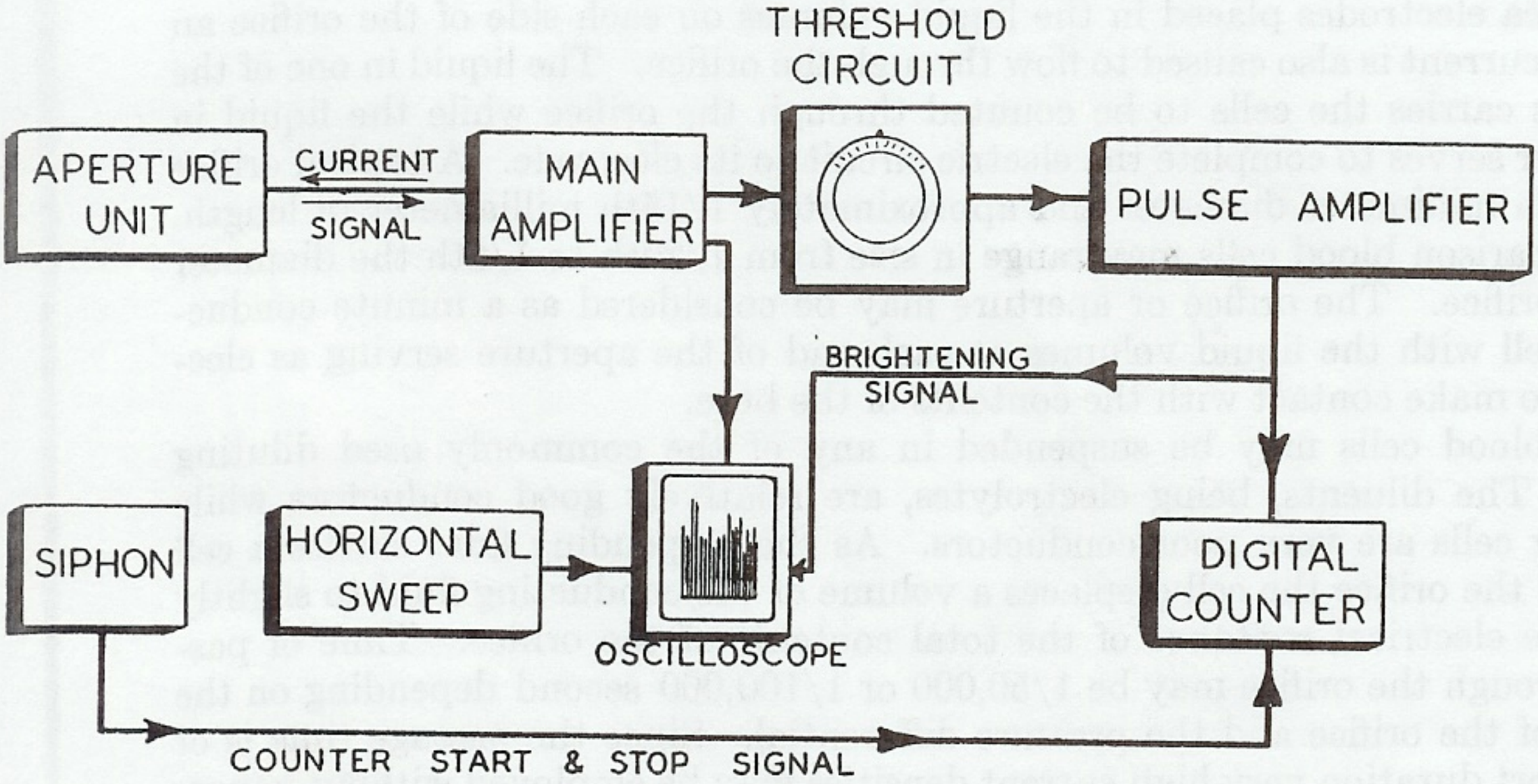

Fig. 2—Block diagraming of electronic system.

Figure 2 is a block diagram of the electrical functions and Fig. 3 is a photograph of a model now in use in a number of laboratories. The pulses produced at the orifice are amplified and displayed on the oscilloscope screen and appear as vertical lines or spikes as shown in Fig. 4. The height of an individual pulse spike from the baseline is a measure of relative size of the cell producing the

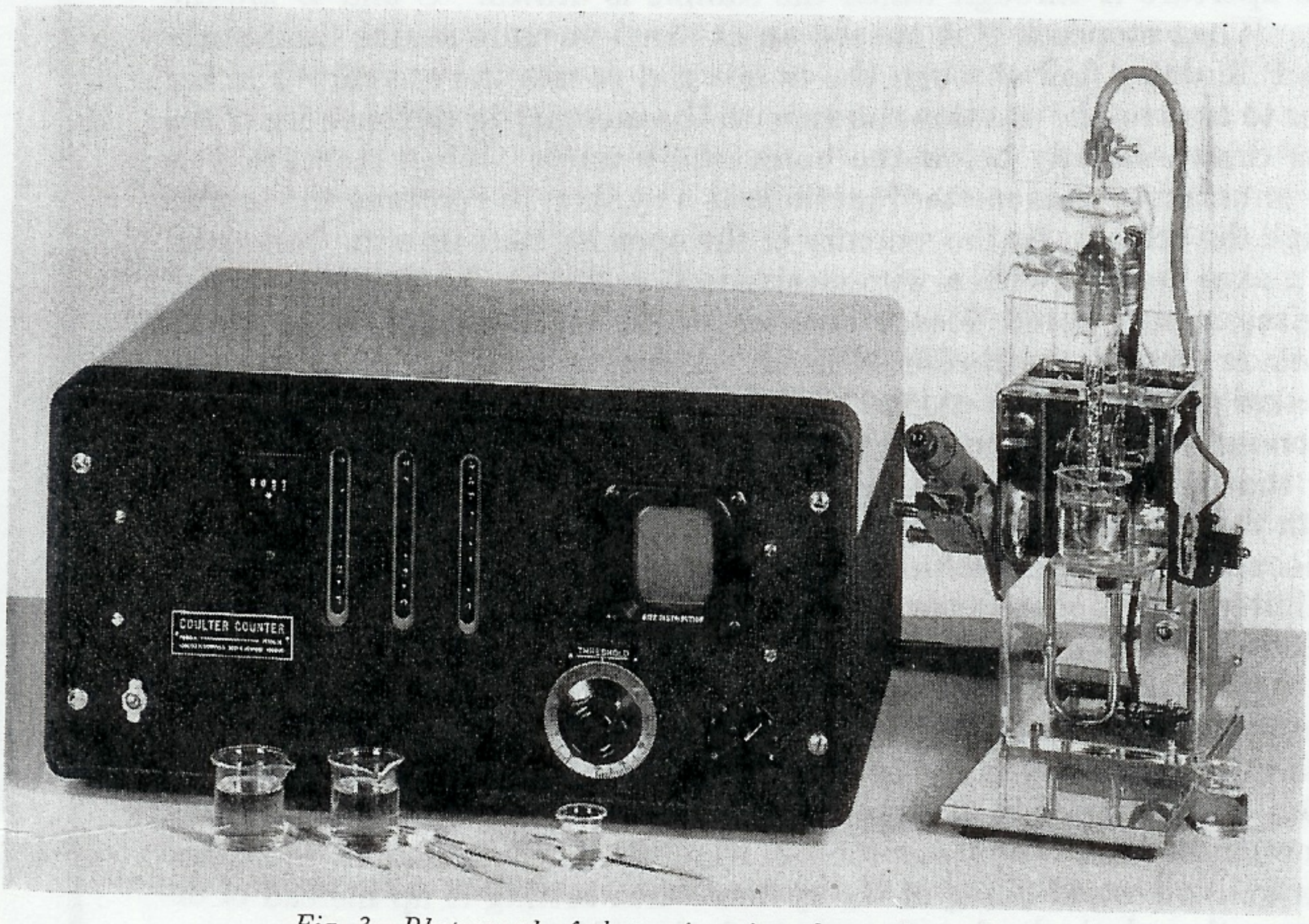

Fig. 3—Photograph of electronic unit and sample stand.

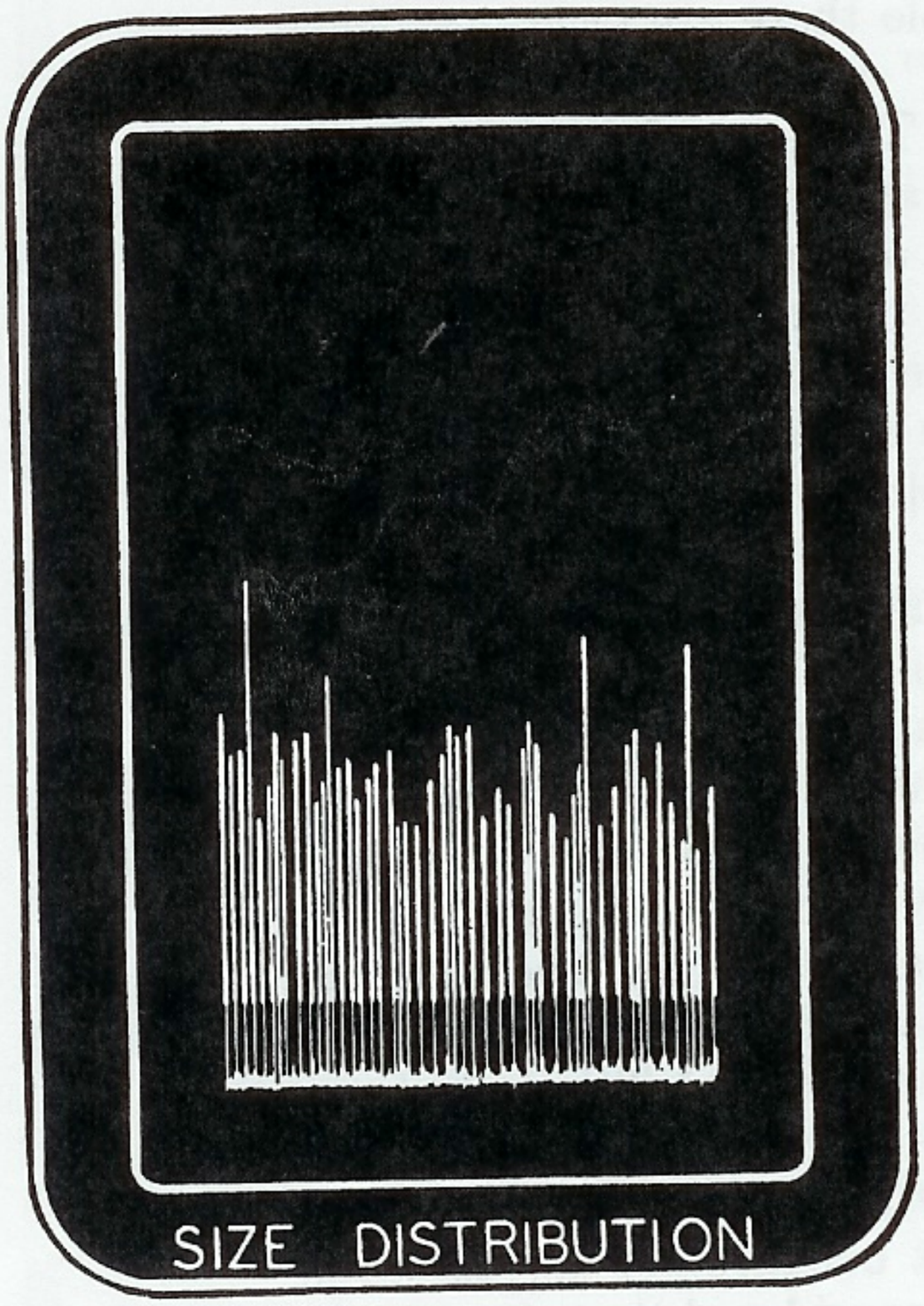

Fig. 4—Oscilloscope pattern showing relative cell size and cell size distribution of a typical sample.

pulse (except for coincident passage to be discussed) and since the rate at which they are produced is several thousand per second the viewer obtains an immediate impression of the averge cell size and cell size distribution. The threshold control dial located below the oscilloscope screen enables the operator to select the height or level above the baseline which if reached or exceeded by a pulse will result in the pulse being counted. The height or level corresponds to a particular cell size. When the syphon activates the counter, all cells of this particular size or larger present in the half milliliter drawn through the orifice will be counted. Cells below this minimum size will not be counted. In addition to a display of relative cell size the oscilloscope also indicates the effective level or setting of the threshold control by brightening that portion above the threshold level of any pulse which reaches or exceeds it and which of course would be counted. In Fig. 4 the threshold level is shown intermediate between the height of the smallest of the desired pulses and the small irregularities on the baseline which represent the passage of very small bits of debris and any extraneous electrical disturbances which may be present. The screen indicates, at a glance, a wide discrimination against undesired debris and electrical background noise and correct functioning of the threshold circuit in relation to the particles to be counted. For the particular sample represented the threshold control setting could be varied considerably without affecting the count. For routine cell counting the threshold control is left at a sufficiently low setting to count the smallest cells likely to be encountered and need not be reset for different samples. The minimum function of the oscilloscope display is to provide a check of overall instrument performance in a manner which requires only an instant's observation and is readily understood by the average medical laboratory technician.

Irrespective of the degree of dilution of the sample there are occasions when more than one cell will pass through the "scanning" volume at a time so that not all pulses produced are caused by a single cell. There is, of course, a count loss due to this effect for which a correction must be applied. The loss increases with cell concentration and aperture volume. For a concentration of 50,000 cells per half milliliter, and for the standard 1/10th millimeter diameter orifice the count loss and required correction is 5250. The coincidence effect for the 1/20th millimeter orifice is approximately 1/8th this amount. For convenience a correction chart is provided for the 1/10th millimeter orifice for obtaining the true count for each instrument reading.

Calibration of the instrument, which is largely a matter of determination of the coincidence count loss, is a special problem because of the lack of any existing independent counting method sufficiently accurate to serve as a reference. A close approximation of the count loss is readily made by making counts of multiple dilutions of a given sample to obtain a series of suspensions having a known ratio of concentrations. In the range where the count loss is only a few percent and the frequency with which more than 2 particles are in the aperture at one time is very low, the count loss will be substantially proportional to cell concentration. When the 1/20th millimeter aperture, which has 1/8th the volume of the larger aperture, is used with large dilutions, the coincidence effect is a small fraction of a percent and can be determined within about 1/10th percent. When this small correction together with the diluent background correction is made to the average of a large number of counts, it is considered that the sample concentration is known to a relatively high degree of accuracy. The background count is the count made of the diluting fluid only and is normally disregarded in routine counting, since its count may be maintained below 100 or even 25 with careful filtering and handling. Any volumetric error of the syphon does not enter in the determination of the count loss correction by this procedure although of course it must be considered in the overall accuracy. Having a relatively accurate count of the high dilution enables one to calculate the relative concentrations of the other dilutions. These other samples are then used for calibration to establish the count loss correction curve at various concentrations for the 1/10th millimeter aperture. The 1/10th millimeter aperture is reproduced with high precision which allows the use of a single correction chart for all 1/10th millimeter apertures. Different orifices should affect the corrected count by less than plus or minus 1/2 percent at an instrument count of 50,000. The chart also includes the count loss due to response time resolution of the amplifier and counter circuits. Fortunately the favorable signal to noise ratio allows the use of relatively wide bandwidth system so that the loss due to circuitry is a small fraction of the aperture count loss and is readily controlled from instrument to instrument.

The sample is diluted so that the cell concentration in the half milliliter metered will fall between 1,000 and 100,000 cells and preferably be between 20,000 and 80,000. For counting red cells this requires a dilution of 50,000 to 1 which may be accomplished with very high reproducibility with a self filling pipette of two cubic millimeters capacity for taking the sample and a 100 milliliter volume of diluent delivered to a sample beaker by an automatic burette. After mixing the components the sample beaker is put in position on the spring portddesu beaker platform so that the aperture and sample electrode are im-

mersed in the sample. This is a one hand operation. The stopcock is opened to initiate flow through the orifice and cock the mercury syphon. The reset toggle on the main panel is actuated to erase any prior count and to reset the decades to zero. A pulse pattern then appears on the screen. The aperture current switch on the panel at the lower right is readjusted if necessary to obtain cells pulses which exceed baseline background noise and fall within the range of the screen. The threshold control dial is readjusted if necessary and the stopcock is closed to allow the syphon to function. After a lapse of about 2 seconds the rising mercury column of the syphon contacts the start electrode and the counting circuits begin to register. After an interval of 10 to 15 seconds, depending upon syphon dimensions, the count is terminated and the count is noted. From 3 to 6 seconds later the mercury in the syphon comes to rest. If desired a duplicate count can be made in about 20 seconds by repeating the sequence with the stopcock and zero reset toggle. To take a count on another sample the prior sample is removed and the new beaker is put in place. The small fraction of a milliliter of the prior sample left on the aperture and electrode is mixed and lost in the 50 or 100 milliliter volume of the new sample by a slight rotation of the new sample beaker as it is left in place. Where greatest accuracy is required the procedure is to hold the prior sample beaker beneath the tube and electrode before removing it completely and pouring a few milliliters from the new sample beaker over the aperture tube and electrode to rinse the prior sample residue away. The rinse water is caught by the lower beaker. For a normal blood sample the instrument might register, for instance, 44,821 individual pulses as having been counted. Disregarding the last 2 digits and referring to the count loss correction chart, a correct count of 50,100 (per 0.5 millileter) is indicated. Since a dilution of 50,000 to 1 was employed and the sample scanned was 0.5 milliliter, a multiplication factor of 100 is required to obtain the concentration per cubic millimeter. In the present example this yields an actual bloodcell count of 5,010,000.

One of the requirements of the method is that the diluting fluid must be filtered and carefully handled to minimize debris problems. Occasionally debris may become lodged in the orifice and must be removed. Presence of debris is noted by a change in the character of the oscilloscope pattern or by a change in the clicking rate of the mechanical register. When debris does occur in the orifice it is readily dislodged by merely lowering the sample beaker to allow air to bubble through the orifice as a finger tip is drawn across the orifice. A low power long focal distance microscope is mounted so that visual inspection of the aperture is accomplished by merely looking into the microscope once it has been positioned and focused. Changing sample beakers does not affect the focus provided the beakers are placed in approximately the same place each time. In Fig. 3 the orifice on the aperture tube is facing to the left and the objective of the microscope, which is mounted on the left of the stand, is fitted with a right angle prism for viewing the orifice. The microscope is focused by sliding it to the right or left on carriage rods. A lamp for microscope illumination is mounted on the right of the stand.

With the new instrument it is now possible to obtain cell size distribution data on a sample in less than 4 or 5 minutes. Such data will be based on many thousands of cells and should be capable of indicating relatively small changes in population size distribution. The procedure is to take a number of counts at

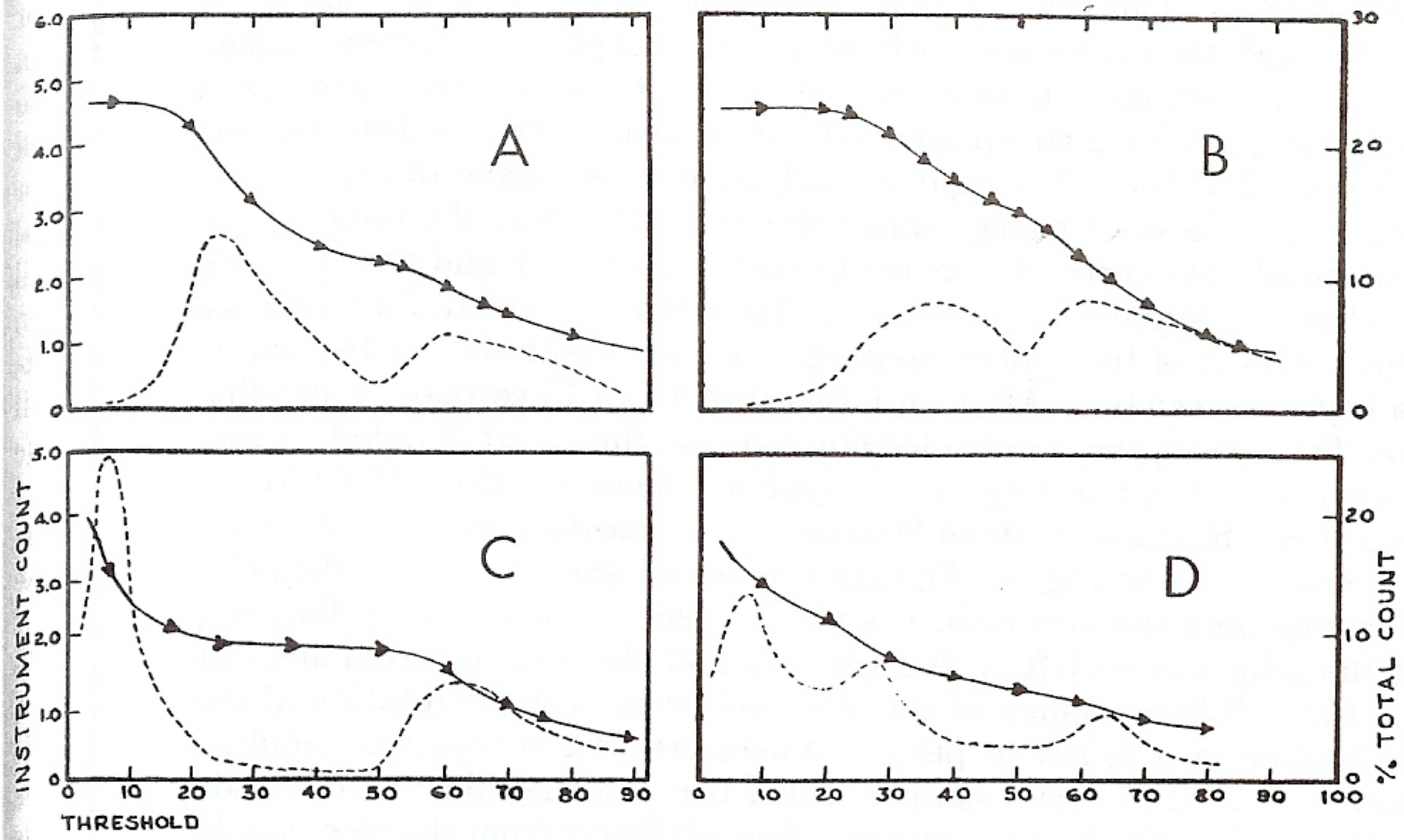

Fig. 5—Threshold curves (solid lines) and relative cell size distribution curves (broken lines) of mixtures of different cell size populations.
A) Sheep and normal human red cells.
B) Dba mouse and normal human red cells.
C) Goat and normal human red cells.
D) Goat, sheep and normal human red cells.

successively higher threshold dial settings. For most studies six or eight equally spaced settings should suffice with the lowest setting just above the baseline background noise. The difference in the count between two successive threshold levels represents the number of cells present which fall in the size range between the cell sizes which correspond to each of the two threshold settings. These values can then be plotted against threshold settings. An initial calibration of the threshold cell size relation must be made with cells of known size and using conventional visual methods. The graphs in Fig. 5 show relative cell size distribution curves of various mixtures of 4 different sized red blood cells. The solid curves are a plot of count versus threshold while the dashed curves are percent total count versus threshold. The curves in A represent a mixture of 4.2 micron sheep cells and 7.8 micron human cells. The curves in B represent 5.9 micron Dba mouse and 7.8 micron human cells. The curves in C are 3.0 micron goat and 7.8 micron human cells. The curves at the lower right represent a mixture of the goat, sheep and human cells.

For size distribution studies the pulse height spread seen on the screen is a guide for determining the threshold levels at which counts should be taken. An instance where pulse height discrimination and display has been found useful is in rapidly determining the number of red cells and ascites tumor cells in the same sample. The tumor cells, in this instance, are 5 to 8 times the diameter of the red cells and produce very high amplitude pulses in relation to the red cell pulses. With the aperture current adjusted so that tumor cells appear about as the pulse shown in Fig. 4, the red cell pulses are of very small amplitude and

hardly distinguishable from electrical background noise. A count of the tumor cells is taken at a level about as shown in Fig. 4. The aperture current is then increased by a factor of 8 or 16 times to bring the red cell pulses above the noise level and the threshold level is adjusted downward to include all the red cell population. The count taken at this level is a total of both red cells and tumor cells and the red cell count is the difference between the two. Without the total information regarding relative noise level, red cell pulse height, tumor cell pulse height and threshold level setting as displayed on the oscilloscope screen the required counts would be very difficult to determine. The electronic circuitry has been designed to handle an extreme amplitude range so that the small signal performance is not seriously affected by the presence of high amplitude pulses.

The effect of coincident passage of more than one cell at a time must be considered. In most instances it will be sufficient to reduce the effect to a tolerable level by using higher dilutions or smaller apertures or both. At an instrument count level of 5,000 the coincidence effect amounts to approximately 1.5% with the 1/10th millimeter orifice. With a 1/15th millimeter orifice the effect is below about 1/2% which would probably suffice for the most critical investigations. Exact coincidence passage of two cells results in a single pulse which is equal in height to the sum of the pulses which would be produced by the cells individually. However, since the pulse shape of a single cell is not rectangular but of a peaked form, the single pulse produced in the case where one cell is emerging from the orifice as another is entering is increased in length but not in height. For equal sized particles the pulses produced by coincidence passage are about equally distributed from unity height to twice the height of a single cell pulse.

III. CONCLUSION

The automatic counter and cell size analyzer is now in use in a number of laboratories and a considerable saving in time as well as an increase in accuracy has been demonstrated in routine use by average medical technicians.[2] It is expected that the instrument will find wide usage in biological fields and also in the industrial field where knowledge of cell and particle concentration and size distribution is becoming increasingly important.

ACKNOWLEDGMENT

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REFERENCES

1. C. F. T. Mattern, F. S. Brackett and B. J. Olson, "The Determination of Number and Size of Particles by Electronic Gating," *Journal of Applied Physiology*, January, 1957.
2. G. Brecher, M. Schneiderman, and G. Z. Williams, "Evaluation of an Electronic Blood Cell Counter," *American Journal of Clinical Pathology*, December, 1956.

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