On Counting the Blood Cells of the Rat with an Electronic Counter

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A new method of counting blood cells by an electronic particle counter has been reported by Mattern *et al.* (4). In this counter the cells, suspended in an electrolyte, flow through an aperture 0.1 mm in diameter. Since the cells are poor conductors, they introduce a resistance into the electrical circuit with each cell passage. This resistance produces increased voltage pulses which are amplified and visualized by means of an oscilloscope. A calibrated threshold circuit ensures that only pulses greater than a selected magnitude are counted. Taking successive counts at increasing thresholds, the cell size distribution is measured. The instrument used in this laboratory is the Coulter (2) counter which counts the cells in 0.5 ml of cell suspension in 15 seconds at a rate of 6000 individual cells per second. Mattern *et al.* evaluated the reliability of the method and have found excellent reproducibility. An evaluation of this method for the routine blood cell counting of the rat is presented in this paper.

The human error is high in the hematological technique with laboratory rodents because of the difficulties encountered in sample taking. This would explain in part the variation in normal values obtained in different laboratories.

The hemocytometer counts were found by Mattern *et al.*, to be consistently higher than counts obtained by the electronic counter. The results from our laboratory support this finding.

The electronic count may however, eventually supplant the hemocytometer method, because of better reproducibility and the speed with which the count is obtained.

Red Cell Counts: Animals used were Albino rats of the Wistar strain from 1 week to 6 months old.

Using a micropipette, 20 mm^3 of blood, obtained by puncture of the middle caudal vein, was diluted with 10 ml 0.9 per cent filtered saline (coarse sintered glass filter). An amount of 0.1 ml of this suspension (which is set aside for the leucocyte count) is placed in 10 ml saline to give a 1:50,000 dilution. Since the sample scanned is 0.5 ml, a multiplication by 100 is required to obtain the cell count in mm^3. The occasional simultaneous passage of more than one cell through the aperture necessitates a correction of this count. Charts are available for making necessary corrections. In order to investigate the reproducibility of the technique using a significantly smaller amount of blood and diluent than did previous investigators, three successive samples were taken from six rats. Three subsamples of each sample were examined by multiple successive instrument counts. The standard deviations derived from the analysis of variances of the red cell counts from these samples are presented in Table I.

The cell size distributions of both normal and pathologic blood samples were determined using the electronic counter. These values were compared with the results of the ocular micrometric determination of cell diameters (measuring 200 cells of a sample) as well as with the calculated mean corpuscular volumes (taking microhematocrit determination). The results are summarized in Table II.

The erythrocyte count is read at threshhold 10 where the total cell population is counted. Reading at higher thresholds (15, 20) only a part of the cell population is counted i.e., the cells of greater diameter. In normocytic blood the count drops significantly between threshold settings 10 and 20. In a microcytic blood sample this drop is observed between threshold settings 10 and 15 and in a macrocytic sample the drop is indistinct until above threshold 20. Taking successive counts at thresholds 10, 15 and 20 the relative cell size distribution is obtained. Figure No. I shows the characteristic curves of the samples.

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TABLE I

Standard Deviation of Cell Counts

Cells	STANDARD DEVIATIONS Between determinations on same sample	Between Samples	On single count
Erythrocytes	± 105,600	± 363,000	± 378,000
Leucocytes – solution			
(a) Saponin	± 268	± 2,114	± 2,131
(b) Triton	± 219	± 1,008	± 1,032

White Cell Counts. The counting of leucocytes is performed in a dilution of 1:500. Two hemolysing agents, Saponin (5) and Triton X-100 (1), were compared for reproducibility. Three blood samples of 10 rats were taken in both studies.

The Saponin treated samples (1 drop of 5 per cent Saponin) were read 15 minutes after treatment, and the Triton treated, (1:200 solution) within 5 minutes. The readings were taken at threshold 20. The standard deviations derived from the analysis of variances of the white cell counts from these samples are presented in Table I.

Discussion

Because of the difficulties involved in sample taking in the rat, the difference between samples and thus the possible error on a single count, is higher in the rat than it is in humans. In the red blood cell counts the main error arises from pipetting and diluting of the sample. This error may decrease when automatic pipettes are used. The error in the leucocyte counts is decreased by using Triton in preference to Saponin as a hemolysing agent. The determination of the size distribution by the counter gives a highly reliable method in the assessment of the type of anemia which concerns cell size. The mean corpuscular volume may be directly obtained if the relation of the threshold scale to cell volume is known (3).

Remarks

In routine examination in our laboratory it was noted that the standard deviation of both the erythrocyte and leucocyte counts were increased in samples where a free flow of blood was not obtained.

The data presented in this paper included both good and poor bleeders.

Summary

An evaluation of the electronic blood cell

TABLE II

Comparison of the results of cell size determinations

Sample	Number of rats	R.B.C. in million per mm³ (corrected count)	Micro hematocrit values %	M.C.V. u³	Red cell diameter u	Thresholds 5	10	15	20 (count in millions)	Remarks
Normocytic	8	7.2	50	69	6.7	6.3	6.3	6.1	4.0	Drops at the threshold 20.
Microcytic	8	5.6	32	57	6.2	5.4	5.0	3.2	1.7	Drops at the threshold 15.
Macrocytic	8	5.2	55	105	8.9	4.7	4.7	4.7	4.4	No drop.

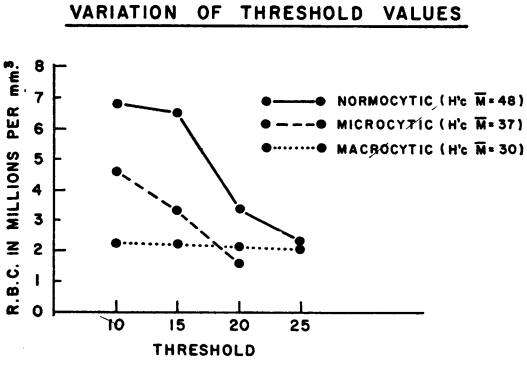

Fig. 1. Characteristic pattern of three types of blood samples at increasing thresholds.

counter for routine use in laboratory rats is given. The evaluation was based on studies of normocytic, macrocytic and microcytic red blood cells from albino rats.

The electronic counter made it possible to carry out routine hematological examination in rats with increased speed and accuracy.

References

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Ecology and Epizootiology of Coccidioidomycosis

Coccidioidomycosis is a disease geographically confined to the low elevation, warm desert areas of the southwest in the United States. The disease is produced by a fungus, Coccidioides immitis, that grows in the soil. Mammals are infected by inhalation of spores. Very low rainfall decreases prevalence of the fungus, as does rainfall in excess of 20 inches a year. Reproduction is halted during hot, dry periods. The fungus does not tolerate cold weather in nature. Probably all animals living in an area where the fungus grows can become infected. Serious disease has been observed in man, the monkey, gorilla, llama, horse, burro, chinchilla, sheep, desert rodents and the dog. The dog is apparently much more susceptible to the disease than man, as evidenced by a high rate of disseminated infection. The first signs of infection in the dog are fever, slight cough, increased lacrimation and loose stool. As the disease progresses the fever fluctuates, the cough becomes harsh and persistent, the hair coat becomes dry, and the dog loses weight. After several weeks to several months of struggle in this stage of the disease, the dog may recover, or it may continue to lose ground as the organism spreads from tissue to tissue. In Arizona's two largest cities, Phoenix and Tucson, a total of 201 canine coccidioidal infections were reported in 1957 and 123 in 1958. Many thousands of cattle are infected each year but the disease in these and many other animals is apparently benign.

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Prevention of Muscular Distrophy in Lambs

Recent research has indicated that selenium as well as vitamin E is involved in preventing muscular dystrophy in lambs. Research at Cornell on the effect of oral administration and maternal transfer of selenium and vitamin E on the incidence of muscular dystrophy in lambs indicates that the administration of 100 I.U. dl-alpha-tocopherol acetate per ewe per day for one month pre partum was effective in drastically reducing the incidence of m.d. in their lambs. Equally effective was the inclusion of 1 p.p.m. selenium as Na_2SeO_3 in the basal ration of the ewes commencing one month prepartum. A zero incidence of the disease was observed when lambs were given orally every second day either 50 I.U. dl-alphatocopherol or 0.5 mg. selenium as an aqueous solution of sodium solenite (Na_2SeO_3). All ewes in the experiment were maintained on a basal dystrogenic diet.

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