stored in an incubator at 35.5° C. Several media were used; (1) McLeod's chocolate agar,[8] (2) McLeod's base with heated serum rather than whole blood, (3) Huntoon's semi-solid agar, (4) Difco's proteose peptone No. 3—hemoglobin agar and (5) Difco's dextrose starch agar. All the media gave practically the same results, except that those which remained viable longest were in Huntoon's agar. That, however, may have been because they were hardier and had been altered sufficiently before growth could be sustained in that medium. Monthly transfers were made from the original mineral oil covered slants for from 14-18 months. After 12 months all strains were transferred and then not transferred again for another 12 months. All 30 strains have survived 2 transfers made at 12-month intervals.

Better results were obtained when the tubes were stoppered with both cotton plugs and rubber stoppers than if cotton plugs alone were used.

Since this study was begun over 400 other strains have been isolated and placed under oil the second generation after isolation. Many of them have been transferred after 3- and 6-month intervals and found to be alive.

Conclusions. Strains of *N. gonorrhoeae* preserved by sterile mineral oil seals have been shown to be viable after transfer at 6 and 12 month intervals.

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Cell Opacity Method for Determination of Cell Volume on a Single Drop of Rat Blood.*

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The rat is admirably adapted to the study of certain problems in the physiology and pathology of blood. It has been used extensively in experimental nutritional anemia. For such studies it is desirable to know not only the hemoglobin content and red cell count of the blood but also the volume of the packed red cells. Sufficient blood for the first two determinations is easily obtainable by incision of the tail vein, but not for the third determination, with methods at present available. The red cell volume can be

[8] McLeod, J. W., Coates, J. C., Happold, F. C., Priestley, D. P., and Wheatley, B., *J. Path. and Bact.*, 1934, **39**, 221.

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determined by the widely used hematocrit method. This requires 1 cc of blood and it is necessary to sacrifice the animals by bleeding. This procedure, however, precludes a series of observations on the same animal. A method for determining red cell volume on a small amount of blood is therefore especially desirable.

A method is here proposed for the determination of red cell volume on a single drop of rat blood, similar to that of human blood.[1] Hemoglobin content may be determined on the same sample.[2] Erythrocyte count may be made at the same time. Thus all the information desired may be secured without killing the animal or withdrawing enough blood to cause depletion which would alter its subsequent condition.

The method for cell volume, which we have called the cell opacity method, depends upon the measurement of the transmission of light through a suspension of blood cells in citrate solution. The Evelyn photoelectric colorimeter was used with a filter transmitting light at or near 660 mμ. The light transmitted impinges upon a photoelectric cell and the amount of current developed is measured on a galvanometer. Other types of photoelectric colorimeter may be employed. The impedance to light is directly related to the cell volume. The galvanometer reading can be converted to cell volume from a plot showing the relation of the two values (Fig. 1). Sub-

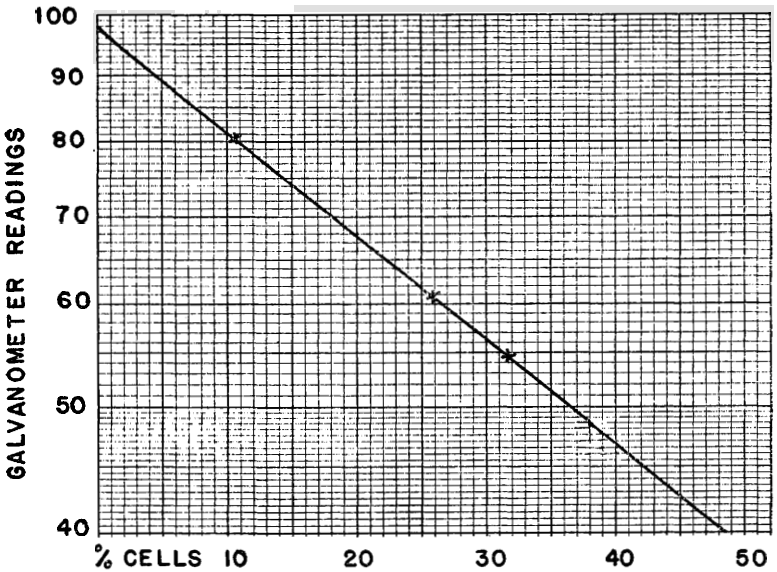

FIG. 1.
See text for description.

1 Shohl, A. T., *J. Lab. and Clin. Med.*, 1940, **25**, 1325.
2 Evelyn, K. A., *J. Biol. Chem.*, 1936, **115**, 63.

sequently the same sample of blood may be hemolyzed by addition of saponin and ammonia, and the hemoglobin may be quantitated by measuring the transmission of light with the substitution of a filter which transmits light in the region of 540 mμ.[2]

The optical properties of rat blood differ slightly from those of human blood. Normal rat blood has approximately 8 million red cells per cm compared to 5 million in human blood, but has nearly the same red cell volume. The individual cells are therefore smaller. The studies of Drabkin and Singer[3] showed that, although the size of the individual particles is a factor in the scattering of light, the total volume and not the number of cells is the main determinant of the transmission of light through a cell suspension.

Experimental. Inasmuch as the correlation between the transmission of light and the volume of the packed cells is empirical, it was necessary to make comparative determinations on aliquots of the same blood by an alternative method, and thus to establish a standard for interpretation of galvanometer readings. Once this standard is established the cell opacity method may be used independently of another method.

In order to establish this correlation, the blood of each of 44 rats was analyzed by both methods. The blood was obtained from albino rats of Wistar Institute stock, bred in our own laboratory. The animals were anesthetized with ether, bled from the femoral vessels and the blood collected in graduated tubes containing sufficient dry oxalate (60% ammonium oxalate and 40% potassium oxalate) to make a final concentration of 0.2%. Aliquots were taken for the two methods.

The standard of reference used was the hematocrit method described by Wintrobe.[4] By this technic oxalated blood is centrifuged in a tube with 100 marked divisions at 3000 R.P.M. for 30 minutes and the red cell volume read directly in percentage.

The technic for the cell opacity method is as follows: In a hemoglobin pipette calibrated "to contain", 20 cm of the oxalated blood are measured. This is delivered into and the pipette washed with 8 cc of citrate solution contained in a test tube selected to fit the colorimeter.† The diluent consists of 3% anhydrous sodium

[3] Drabkin, D., and Singer, R. B., *J. Biol. Chem.*, 1939, **129**, 739.

[4] Wintrobe, M. M., *J. Lab. and Clin. Med.*, 1929, **15**, 287.

† The new Evelyn colorimeters are equipped to use 6 or 10 cc of solution. The Klett-Summerson colorimeter uses tubes marked at 5 and 10 cc. The same concentration of blood is obtained if 15 cm are added to 6 cc or 25 cm to 10 cc of citrate solution. If other dilutions are used, the readings give corresponding fractional values. Complete directions for the use of the instrument are given in the manual supplied with the photoelectric colorimeter.

citrate (4.15% Na citrate. 5½ H_2O). One cc of formalin (36-38% formaldehyde gas) per liter is added before making the citrate solution up to volume. The tube is placed in the photoelectric colorimeter after the galvanometer has been adjusted to read 100 with a "blank" tube containing the diluent only. The reading is made after 15-30 seconds. The galvanometer reading is then translated into cell volume from the curve given in Fig. 1, which was established from the experimental data. The ordinates represent the logarithm of the galvanometer readings, and the abscissae the volume of the packed red cells by the hematocrit method. The intercept of the galvanometer reading and the cell volume was plotted for each of the 44 determinations. Fifteen determinations were made with 10 cmm of blood and 29 with 20 cm. The two crosses represent the arithmetical mean calculated for each of the 2 groups. The point of 0 cells was taken as 98, the galvanometer reading for plasma alone, and a line drawn from this point through the crosses. This was seen to be a representative line.

The calculation of variations from the line shows approximately equal distribution of + and — values. A plot of the variation between the values obtained by the two methods shows a normal distribution curve of variation. Two-thirds of the values fall within ±1.5 volumes per 100 cc of blood. This agreement is not quite as close as that previously reported for human blood.[1]

A series of 10 normal rats was studied in order to ascertain whether the values obtained by the cell opacity method using blood from the tail vein agreed with the values obtained when the animals were sacrificed by bleeding into tubes containing dry oxalate. In the latter procedure the blood is a mixture of arterial and venous blood. Determinations were made of the cell opacity, hemoglobin and red cell counts, in duplicate, on both cutaneous and mixed blood. Cell volume was determined also by the hematocrit method on mixed blood. All determinations were made on each rat.

The cutaneous blood gave values sometimes higher and sometimes lower than the mixed blood by either the cell opacity method or the hematocrit method. The variations were within the limits given in Fig. 1. Moreover, the variation in cell volume as determined by the hematocrit method and by the cell opacity method, either in the same sample or in comparison between the cutaneous and mixed blood, is less than the variation in the red cell count between the two types of blood or even between duplicate red counts on the same blood.

Summary. A method is presented for determination by the use

of the photoelectric colorimeter of red cell volume of rat blood on a single drop of blood obtained from the tail vein. Hemoglobin content may be determined on the same sample.

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Specific Substances in the Urine of Leucemia Patients.

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Miller, Wearn and Heinle[1] reported specific proliferations of cells in the organs of guinea pigs which had been given daily injections of extracts of urine from patients with chronic myeloid and lymphoid leucemias. In the course of further experiments with the same extracts attempts were made to further concentrate the substance. This was accomplished by reducing the pH of the final water extract to 1.5 or 2.0 with hydrochloric acid and shaking vigorously for 15 minutes with an equal amount of chloroform. The chloroform and water were separated and the chloroform removed by vacuum. The residue was resuspended in alkaline water at a pH of 7.4 to 7.6. Each cc of the extract was computed to equal from 1000 to 1500 cc of urine. These extracts were given to guinea pigs in step doses starting with ¼ cc and increasing every 5 days until 1½ or 2 cc was given daily. Animals on such regimes again presented specific pictures in 4 to 6 weeks.

Because we felt that neither of the preceding methods of extraction seemed to yield as much active material as was present another method has been evolved, and for this we have adapted a modification of McCullagh's method of extracting androgens from urine. The urines are hydrolyzed by boiling at a pH of 2.0. It has been found that such a pH in most instances could be obtained by the addition of 40 to 45 cc of concentrated hydrochloric acid to each 1000 cc of urine. After cooling 100 cc of chloroform is added to each 1000 cc of urine and this is stirred violently with an electric stirrer

[1] Miller, F. R., Wearn, J. T., and Heinle, R. W., PROC. SOC. EXP. BIOL. AND MED., 1939, **41**, 478.