protozoa were prevented from growing in dilutions of protoanemonin ranging from 1-200,000 to 1-1,600,000. 5. No inhibition by protoanemonin of the growth of the two bacteriophages and the influenza virus was demonstrable by the techniques employed. 6. A dilution of 1-1,000,000 protoanemonin was toxic for chicken tissue culture epithelial and fibroblastic cells.

15982

Determination of O_2 Capacity on 39.3 Cubic Millimeters of Blood.

WILSON C. GRANT. (Introduced by Walter S. Root.)

From the Department of Physiology, College of Physicians and Surgeons, Columbia University, New York City.

In a recent study of bone marrow blood gases[1] in which the use of minimal sample volumes was imperative, estimation of O_2 capacity was necessary. The current procedure used for such determinations involves aeration of blood after which the O_2 content is determined. This technique has been used by Roughton and Scholander[2] and by Lilienthal and Riley.[3] Preliminary aeration was carried out in a separate vessel with 0.5 or more cc of blood although only 39.3 cmm were used for the O_2 determination. When blood is aerated in a flask or syringe, a certain amount of plasma is lost by evaporation as well as by adherence to the sides of the vessel. Should quantities of blood smaller than 0.5 cc be used, concentration of the red cells becomes greater. To avoid the errors inherent in separate aeration and to reduce the amount of blood required, the micro method of Roughton and Scholander has been modified so that the O_2 capacity can be determined on a total blood sample of only 39.3 cmm.

Apparatus. The Roughton-Scholander syringe and pipette (Roughton and Scholander[2]) are employed. A second mark (designated "upper mark") is scratched on the syringe cup at a distance above the existing one such that the volume of the cup as measured between the two marks is approximately 25 cmm. Since the diameter of most cups is 2.5 mm, the upper mark may be made 5 mm above the first. In the estimation of O_2 capacities exceeding 16 vol % the syringe with the 50 unit capillary is necessary.

Reagents. In addition to those listed by Roughton and Scholander 0.9% NaCl is required.

Sampling blood. From a finger prick or a needle inserted in a vessel, blood is sucked directly into the 39.3 cmm pipette which has been previously flushed with anti-coagulant solution (heparin) and dried in a current of air.

Procedure. 1. The syringe is flushed 3 times with separate portions of saline, emptied, and the cup filled to the lower mark with saline.

2. The pipette is filled to its mark with blood; its tip is passed carefully into the cup containing saline and pressed firmly against the bottom. 3. Blood is sucked into the syringe by a smooth withdrawal of the plunger. Once the pipette is emptied it is quickly removed and the saline in the cup is drawn in after the blood.

4. The blood-saline mixture is lowered far enough into the syringe to permit entry of an air volume of approximately 1.0 cc.

5. The syringe is then rotated for 5 minutes on its long axis with an occasional rotation at right angles to spread a small layer of blood over the inside of the syringe and thus ensure

1 Grant, W. C., and Root, W. S., *Fed. Proc.*, 1947, **6**, 114.

2 Roughton, F. J. W., and Scholander, P. F., *J. Biol. Chem.*, 1943, **148**, 541.

3 Lilienthal, J. L., Jr., and Riley, R. L., *J. Clin. Invest.*, 1944, **23**, 904.

TABLE I.
Comparison of Oxygen Capacities of Blood by Sendroy Method (1.0 cc) and Micromethod (39.3 cmm).

Sendroy, Vol. %	Micro, Vol. %	Difference, Vol. %	Hct. (Van Allen), %
20.3	20.2	—0.1	45.9
19.6	19.8	+0.2	44.5
15.2	15.1	—0.1	35.9
16.7	16.8	+0.1	37.3
17.1	17.2	+0.1	40.8
14.5	14.2	—0.3	35.9
12.5	12.8	+0.3	30.8
10.5	10.4	—0.1	24.1
15.8	15.7	—0.1	38.0

uniform exposure of blood to the air. Once a minute during this procedure the air is renewed by alternately running the blood up to the capillary and lowering to the 1.0 cc mark. In certain syringes the formation of transverse blood films makes the expulsion of air without loss of blood difficult. The addition of 2 capillary divisions of caprylic alcohol alleviates this condition.

6. Air is expelled and blood-saline pushed up to the bottom of the cup. The cup is filled to the upper mark with ferricyanide solution, and the solution is then lowered to the bottom of the cup.

7. Two divisions of caprylic alcohol are drawn into the top of the capillary and the cup emptied.

8. Acetate buffer is added to the upper mark and the original Roughton-Scholander[2] (page 545) procedure for O_2 content is then followed.

Blank determination. To obtain a blank value for reagents as well as for physically dissolved oxygen of the blood, an analysis is performed exactly as indicated above; but instead of delivering the usual 39.3 cmm of blood, 43.3 cmm of 0.9% NaCl is substituted. For this purpose a mark indicating 43.3 cmm volume, or 110% of the usual 39.3 cmm volume, is scratched on the pipette. Sendroy *et al.*[4] have shown that for practical purposes 1.1 cc of aerated saline solution contains the same volume of physically dissolved O_2 as 1.0 cc of blood.

Calculations. Calculation of results follows that described by Roughton and Scholander.[2]

Aeration of blood. Quite unexpectedly complete saturation of the blood mixture is obtained in only 5 minutes. This is accomplished by the vigorous rotation of the blood with air which is expelled and replaced with fresh air at intervals of a minute, as described above. The ratio of total air to liquid volume is approximately 100 to 1. That a longer time of aeration does not increase the O_2 content was demonstrated by noting that, on the same blood, two determinations after 5 minutes aeration showed O_2 capacities of 17.0 and 16.8 vol % and two others after 10 minutes of aeration, 16.9 and 16.9 vol %.

Accuracy of method. The O_2 capacity as determined on 1.0 cc of blood with the standard method of Sendroy[5] was compared with that measured upon 39.3 cmm of the same blood with the micromethod. These analyses were performed by 2 individuals, one using the Sendroy method and the other the micromethod. The results obtained on 6 dogs are shown in Table I. Hematocrit values measured with Van Allen[6] tubes are included for reference. The agreement between the 2 procedures was good, the maximum difference being 0.3 vol %.

Successive measurements performed on the same sample by the micromethod yielded variations of similar magnitude as demonstrated in the following 2 samples: (a) 15.5, 15.6 vol % and (b) 11.2, 11.4, 11.2 vol %.

Discussion. The Roughton-Scholander micromethod for the measurement of the O_2 content of blood has been modified so that O_2 capacity can be determined on a total blood sample of 39.3 cmm. Since the blood is both aerated and analyzed in the same apparatus the transfer from aeration vessel to Roughton-Scholander syringe is avoided. The elimination of this step which is required when aeration is carried out in a separate vessel avoids the possibility of analyzing blood which contains a concentration of red cells unlike that obtained from the subject.

The method permits the estimation of O_2

4 Sendroy, J., Jr., Dillon, R. T., and Van Slyke, D. D., *J. Biol. Chem.*, 1934, **105**, 597.

5 Sendroy, J., Jr., *J. Biol. Chem.*, 1931, **91**, 307.

6 Van Allen, C. M., *J. Lab. and Clin. Med.*, 1925, **10**, 1027.

capacity on blood obtained from a finger prick. It should prove useful where measurements are needed on small animals such as mice or rats or under conditions where venipuncture is difficult or undesirable, as in premature infants.

Summary. The Roughton-Scholander microgasometric procedure has been modified to provide a simple and accurate micromethod for the determination of the O_2 capacity of blood. Only 39.3 cmm are required and an analysis may be completed in 10 to 12 minutes. O_2 capacities obtained by the micromethod agree well with those measured by the Sendroy macromethod, the average difference being ± 0.16 vol %.

15983

Renal Excretion of Mannitol.*

EUGENE Y. BERGER, SAUL J. FARBER, AND DAVID P. EARLE, JR.
(With the technical assistance of Roslyn Jackenthal.)

From the Research Service, Third (NYU) Medical Division, Goldwater Memorial Hospital, Welfare Island, and Department of Medicine, New York University College of Medicine, New York City.

The evidence that the clearance of inulin in man is a measure of the rate of glomerular filtration, although necessarily indirect, seems fairly conclusive.[1] Other studies by Smith and his colleagues[2] have suggested that a number of hexitols (including mannitol) are excreted solely by glomerular filtration. Finally, Gilman has reported[3] that the clearance of thiosulfate is also a measure of the filtration rate.

Recently, a patient was observed in this laboratory whose inulin clearance was found to be 144 ml per minute, but whose mannitol clearance, 3 months later, was 94 ml per minute. Since this patient had no demonstrable kidney disorder and since there was no reason to expect any significant change in renal function during the elapsed period, it appeared likely that inulin and mannitol clearances are not always identical. Further observations on this point, therefore, were undertaken and form the basis of this report.

Some support for the belief that the mannitol clearance is less than that of inulin is obtained from published reports. The most extensive series of observations on the inulin clearance in normals is that of Smith and his colleagues[4] who found the average value of 131 ml per minute with a standard deviation of 21.5 ml per minute in 67 males, and an average of 117, with a standard deviation of 15.6, in 21 females. The findings of most other investigators are in line with those of Smith and are summarized in Table I. Also included are previously unpublished data from this laboratory. The average inulin clearance for the entire group of 133 males is 128 ml per minute. In contrast, the average mannitol clearance in 52 males is 116 ml per minute. The difference between the mean mannitol and inulin clearances among the male patients is statistically significant. There is little difference between the mannitol and inulin clearances among the female patients.

There are but few published data on direct comparisons between mannitol and inulin

* The work described in this paper was supported by a grant from the Carnegie Corporation of New York.

1 Smith, H. W., *Physiology of the Kidney,* Oxford University Press, New York, 1937.

2 Smith, W. W., Finkelstein, N., and Smith, H. W., *J. Biol. Chem.,* 1940, **135**, 231.

3 Newman, E. V., Gilman, A., and Philips, F. S., *Bull. Johns Hopkins Hosp.,* 1946, **79**, 229.

4 Smith, H. W., *Lectures on the Kidney,* University of Kansas, Extension Division, 1943.