form the medial and lateral stria. The uncauterized remains of the olfactory tracts were seen as "stubs" with free, slightly curled, ends where the cautery had severed the tracts.

The results of this experiment strongly support the view that the virus normally passes from nasal mucous membranes to the central nervous system by the way of the olfactory nerve, olfactory bulb, and olfactory tracts. Only in so far as the cauterization may have destroyed possible vascular communications does the evidence lack finality.

7297 C

A Simplified Method for Measurement of Creatinine Clearance.

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The multitude of substances whose "clearances", or rate of excretion in relation to blood concentration, have been proposed as tests of kidney function indicates that a satisfactory clinical procedure is yet to be devised. The use of urea, as proposed by Möller, McIntosh and Van Slyke,[1] is in our experience the simplest of the more sensitive tests, since it involves only one blood sample and does not require the ingestion of the test substance. But since the rate of urea excretion is less with low urine volume than with high, function tests can be compared only as a percentage of an average empirical normal for urine volumes above or below 2 cc. per minute. There are, therefore, certain advantages in using a test substance whose excretion is independent of urine volume. Creatinine, the use of which was proposed by Rehberg,[2] is such a substance. This method as described[3] has the disadvantage of requiring ingestion of creatinine, and analysis of urine and 2 samples of blood plasma.

The development by Van Slyke and Cope[4] of a clinical method for the determination of urea clearances suggested that a similar

1 Möller, E., McIntosh, J. F., and Van Slyke, D. D., *J. Clin. Invest.*, 1928, **6**, 427.

2 Rehberg, P. B., *Biochem. J.*, 1926, **20**, 447.

3 Rehberg, P. B., *Zentr. f. Inner. Med.*, 1929, **50**, 367.

4 Van Slyke, D. D., and Cope, C. L., PROC. SOC. EXP. BIOL. AND MED., 1932, **29**, 1169.

procedure might be devised for the creatinine clearance which, if practical, would considerably simplify the test.

In the method of Van Slyke and Cope, the ratio between urine and blood urea concentrations is determined by a direct comparison, without determining the actual concentration in either. In the method here described, the same principle is applied except that creatinine is used. In order to increase the concentration of creatinine in the blood and urine, creatinine is administered to the subject by mouth, and water given at hourly intervals during the test if there is any question of the subject's ability to urinate.

The amount of creatinine ingested depends upon the number of hourly urine collections to be made. For a single collection 3 gm. were found sufficient; for 2 periods 5 gm. were better, but if 6 or 7 collections are to be made as much as 10 gm. will be necessary. Following the ingestion of 3 gm. of creatinine on an empty stomach, the blood concentration reaches a maximum in 1 hour, but with larger amounts the highest concentration is not attained until about 1½ hours. At the end of the 1 or 1½ hours, the bladder is emptied and urine discarded. Urine is then collected as accurately as possible for a measured interval of time, approximately 1 hour. The actual time is recorded to the nearest minute. A sample of venous blood is drawn at the middle of each period. Coagulation is prevented by the use of potassium oxalate.

Only 2 reagents are needed in this method: solid picric acid which has been purified to meet the requirements of Folin and Doisy,[5] and a 10% solution of sodium hydroxide. The urine is diluted (in round numbers) as many times as is indicated by the curve of Figure 1, which has been constructed similarly to that of Van Slyke and Cope.[4] Two cubic centimeters of plasma are added to 18 cc. of water in a 50 cc. centrifuge tube and mixed. Twenty to 25 cc. of the diluted urine are placed in a similar centrifuge tube and approximately a gram of picric acid added to both. The mixture is thoroughly stirred with a glass rod until it is uniformly yellow and then allowed to stand with occasional stirring for 20 to 30 minutes after which it is centrifuged and filtered. It is essential that a clear filtrate be obtained.* Ten cc. portions of the picric acid filtrates are placed in test tubes and 0.5 cc. of the 10% sodium hydroxide added to each. The test solutions are then mixed and allowed to stand for 10 minutes before comparison is made in a colorimeter. The cup containing the blood test solution is set at

[5] Folin, O., and Doisy, F. A., *J. Biol. Chem.*, 1917, **28**, 349.

* Schleicher and Schüll filter paper number 597 has been found satisfactory.

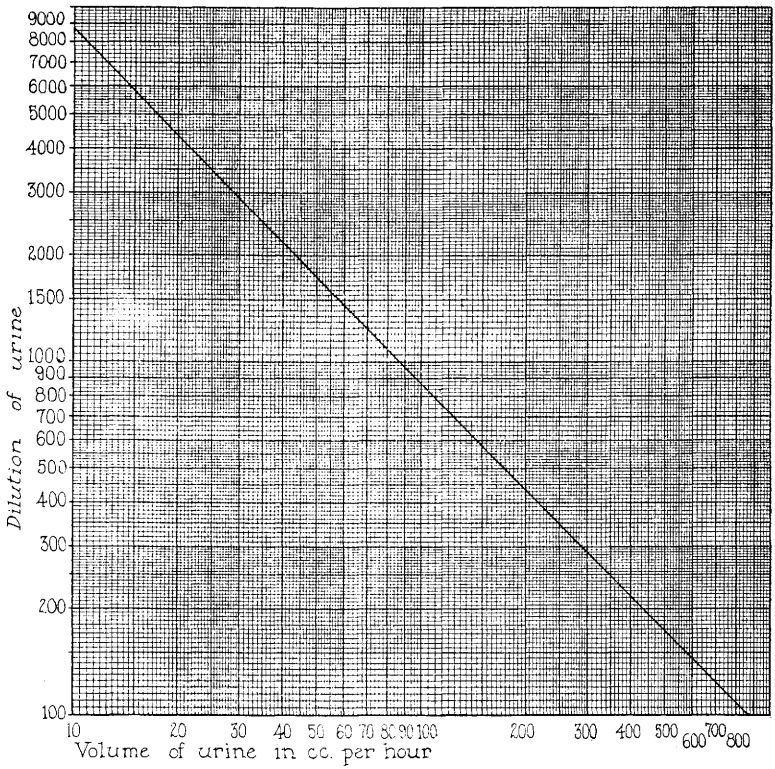

FIG. 1.

20.0 mm. and the cup containing the urine test solution is adjusted so that the two match.

Calculation. The equation for calculation of results is as follows:

$\frac{Rb}{Ru} \times \frac{Du}{Db} \times V =$ creatinine clearance in cc. per minute where Rb and Ru represent the colorimeter readings of the blood and urine test solutions respectively, Du and Db are the dilutions made of the urine and blood, and V is the number of cc. of urine excreted per minute. To express the clearance in percent of the average normal value the equation becomes:

$\frac{Rb}{Ru} \times \frac{Du \times V \times 100}{Db \times 148} = \%$ of average normal creatinine clearance, when 148 cc. is taken as the average normal for adults.[6]

Table I shows the results obtained by 2 observers working independently. One used Rehberg's modification of Folin's method,

6 Hayman, J. M., Jr., Halsted, J. A., and Seyler, L. E., *J. Clin. Invest.*, 1933, **12**, 861.

TABLE I.
Comparison of Results Obtained in Present Method and Rehberg Method.

Subject No.	Observed Clearance This Method cc.	Observed Clearance Rehberg cc.	% Average Normal Clearance This Method	% Average Normal Clearance Rehberg	Difference in % Average Normal
1	128.9	125.0	87.1	84.5	+ 2.6
2	132.6	123.5	89.5	83.4	+ 6.1
	167.9	185.0	113.4	125.0	—11.6
	161.7	162.5	109.3	109.7	— 0.4
	163.1	167.0	110.2	113.6	— 3.4
3	103.6	107.7	69.9	72.7	— 2.8
	91.0	101.8	61.5	68.8	— 7.3
	81.7	85.4	55.1	57.7	— 2.6
	85.7	83.0	57.8	56.0	+ 1.8
	96.7	84.0	65.3	56.7	+ 8.6
	109.4	96.1	74.0	65.0	+ 9.0
4	35.1	33.8	23.7	22.8	+ 0.9
	41.1	45.0	27.8	30.4	— 2.6
5	143.1	153.0	96.8	103.4	— 6.6
	114.4	115.0	77.3	77.7	— 0.4
6	106.0	103.0	71.6	69.6	+ 2.0
	93.1	99.5	62.9	67.2	— 4.3
	90.1	96.5	60.9	65.2	— 4.3
	92.9	93.5	62.7	63.1	— 0.4
	101.7	96.5	68.9	65.2	+ 3.3
	87.8	86.5	59.3	58.4	+ 0.9
	98.5	101.0	66.5	68.2	— 1.7
7	10.5	11.6	7.1	7.8	— 0.6
	10.9	11.5	7.3	7.8	— 0.5
8	20.9	18.4	14.1	12.4	+ 1.7
9	138.5	136.5	93.5	92.2	+ 1.3
10	51.4	49.5	34.7	33.4	+ 1.3

employing a colorimeter with Burker optical system, and the other the present method.

Summary. A simplified method of obtaining the ratio of concentration of creatinine in urine and blood plasma for calculation of the creatinine clearance is described.

7298 C

Effect of Chlorides upon Distribution Ratio of Bromides in Blood and Cerebrospinal Fluid.

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It was found[1] that the quantitative determination of bromides by the Walter method was influenced by the variations in the chloride

[1] Malamud, W., Mullins, B. M., and Brown, J. R., PROC. SOC. EXP. BIOL. AND MED., 1933, **30**, 1084.