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Research Article

THE DETERMINATION OF THE GLOMERULAR FILTRATION BY THE ENDOGENOUS CREATININE CLEARANCE[1]

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Since Smith (34) and his collaborator (31) and Miller and Winkler (20) have shown that exogenous creatinine is partially excreted by the renal tubules in man, Rehberg's method (28) for the determination of the glomerular filtration has to be questioned. The interest should have been directed towards the determination of the endogenous creatinine, but the discussion as to whether creatinine as such is normally present in plasma, and the absence of an adequate method for the determination of creatinine, delayed investigations upon this subject.

The doubts as to whether plasma creatinine exists or not were finally refuted by the work of Miller and Dubos (19), who developed a specific enzymatic method for the determination of creatinine. They found that in normal individuals creatinine constitutes 80 to 100 per cent of the chromogenic material in tungstic acid filtrates of plasma. In tungstic acid filtrates of plasma from uremic patients there may be large amounts of non-creatinine chromogenic material. This method is very important for scientific research but is unfortunately not applicable in clinical practice.

From another point of view Popper, Mandel, and Meyer (26) developed a method for the determination of creatinine based on Jaffe's principle which is said to be perfectly specific and very sensitive. The normal values obtained in plasma by this method are between 0.5 and 1.0 mgm. per cent, while those found by Folin's method (10) are 1 to 2 mgm. per cent. There is reason to believe that this difference is caused by the fact that the picric acid Popper uses for the precipitation of the proteins precipitates the other chromogenic substances as well. Ferro-Luzzi (8) found that the values of plasma creatinine obtained by Somogyi's (37) zinc precipitation of the proteins were below those obtained by Folin and Wu's (11) tungstic acid precipitation. Another reason for the difference between the results is that Folin used the colorimeter, which shows these slight differences of color only imperfectly, while Popper found his values by the photometer.

Popper and Mandel (25) were the first to apply this method for the determination of glomerular filtration extensively. Only comparatively few previous publications have appeared on the determination of the endogenous creatinine clearance (9, 12, 13, 18). As Popper and Mandel's values, on the average, are below those of the authors who employed creatinine administration, they conclude that this method shows the real filtration. They made some experiments with phlorizin and with xylose to prove that no secretion of creatinine takes place in the tubules. These few experiments, however, do not prove much, as the doses of phlorizin were far too small to inhibit an eventual secretion of creatinine. On the other hand, the experiments with xylose are very doubtful since, according to Shannon and Smith (33), the xylose clearance lies 25 per cent below the glomerular filtration, so that a comparison of the excretion of xylose and creatinine can only be made if this fact is taken into consideration.

A method which employs endogenous creatinine has the following advantages: Without the application of the expensive creatinine the determination can be performed more easily and at considerably lower costs. During the time of the test the level of the plasma creatinine remains constant and only one blood sample has to be drawn. The level of creatinine in plasma is very constant and varies only within narrow limits. Therefore it is very important for clinical purposes to know definitely whether Popper's simple and easily applicable method allows an exact determination of the glomerular filtration. The scope of our observations has been to throw light on this question.

[1] A previous report of this work was given in the Bulletin de la Faculté de Médecine d'Istanbul (40).

THE DETERMINATION OF THE ENDOGENOUS CREATININE CLEARANCE BY POPPER AND MANDEL'S METHOD (25)

1. Preliminary remarks on the method

The determination of the plasma creatinine is performed by a macromethod for which 4 cc. oxalated plasma are used. The protein is precipitated directly by picric acid such as that employed for Jaffe's reaction, so that the dilution of the blood is kept within rather narrow limits. The comparison of color after the alkalinization is made in a photometer at as great a depth of layer as possible. With this method it is possible to get exact double determinations even at very low concentrations of plasma creatinine. In normal persons the values are between 0.5 and 1.0 mgm. per cent. Values between 1.0 and 1.15 mgm. per cent are dubious; those above 1.15 mgm. per cent are certainly pathological.

After preservation for from 1 to 2 days in the refrigerator the result does not change, whereas if kept in storage for a longer time, the content of creatinine decreases by about 10 to 40 per cent. The addition of as much as 400 mgm. per cent glucose has no influence on the level of creatinine; higher concentrations of glucose were not added. Acetone and acetoacetic acid, however, increase the depth of color considerably and interfere with the determination of creatinine. β-hydroxybutyric acid was not examined. For this reason patients with acetonuria were excluded from the tests. Some drugs seem also to produce the Jaffe reaction, *e.g.* salicylic acid given in great quantities. A detailed examination of this point was not made.

In order to avoid such interference and also the probable influence of ingestion of food, all tests are made in the morning on fasting subjects. To get control of regular diuresis it proved practical to collect the urine in two separate hourly specimens in the manner which Möller, McIntosh, and Van Slyke (21) recommended for the urea clearance. If there is a great difference between the quantities of the specimens collected during the two measured periods of about the same length, the test is discontinued. The urines of the two periods, having been measured exactly and mixed, are analyzed and so is the blood sample drawn after the first hour. The analytical methods are briefly described at the end of this article.

2. Comparison between the endogenous creatinine clearance and the inulin clearance

The real answer to the question as to whether a substance fulfills the conditions for the determination of glomerular filtration or not will result from the comparison of the clearance of this substance with the inulin clearance. Inulin alone appears to measure glomerular filtration in man as well as in all the animals examined so far (14, 29, 34). A comparison of the endogenous creatinine clearance with the inulin clearance has only been made in a few cases by Miller and Winkler (20) with the reliable enzymatic method for the determination of creatinine. They found that in most normal persons the clearance of endogenous creatinine equals the inulin clearance. To determine whether the results of Popper's simple method coincided with the inulin clearance we examined 17 cases in forty-two separate periods.

According to Smith, Goldring and Chasis (36) and Miller and Winkler (20) the inulin was introduced by intravenous infusion in order to obtain a constant plasma level. The collection of urine by an inlying catheter, washing the bladder with sterile saline and limiting the periods, is done as described by Smith, Goldring, and Chasis (36).

For the repeated drawing of the blood an Ainit needle was employed according to Ottenstein (22). After one puncture blood may be drawn as often as necessary during the whole period. The production of pure inulin was very difficult at first. The first samples of a preparation of Merck were very well tolerated, but another preparation of the same origin caused a serious febrile reaction. After repeated recrystallization from hot water and treatment with charcoal we succeeded in getting a preparation which caused a slight febrile reaction only in half of the cases. Since the filtration is always made through a Seitz E. K. filter (35), the inulin is always tolerated without any reaction. The solutions for the infusion are freshly prepared with 0.9 per cent NaCl in double distilled water and are boiled once more for a minute after the filtration through the sterile Seitz filter. With a new colorimetric method for the determination described by one of the authors (38), which still exactly indicates values of 5 mgm. per cent, it is possible to reduce the quantities of inulin to the fifth part of those given by other authors.

During the first 10 minutes we infuse 60 cc. of 5 per cent inulin solution, then 4 cc. of 1 per cent inulin per minute continuously to the end. The rate of the infusion is regulated by a uniformly compressing tunnel clamp of 8 cm. in length. The quantity is measured by a burette communicating with the infusion flask. As the infusion flask is closed by a cock, the emptying time of the burette

TABLE I

Data on patient Shin

Period	Urine flow	Plasma level			Clearance			Clearance ratio	
		Inulin	Creatinine	Urea	Inulin	Creatinine	Urea	Cr/I	U/I
	cc. per minute	*mgm. per cent*	*mgm. per cent*	*mgm. per cent*	*cc. per minute*	*cc. per minute*	*cc. per minute*		
I	2.0		1.34	34.8		83.6	40.4		
II	2.0	26.1	1.34	33.5		84.4	40.8		
III	2.7	26.1	1.34	33.5	90.1	92.7	51.0	1.03	0.56
IV	2.11	25.8	1.34	33.5	74.4	78.8	42.2	1.06	0.51
V	2.78	25.8	1.34	33.5	88.8	91.7	46.0	1.03	0.51

is measured with a stop watch. Twenty minutes after the beginning of the infusion of the 1 per cent solution, the plasma inulin has reached a constant value of 25 to 35 mgm. per cent. At this moment the first period begins. The urine and blood samples are drawn as described. A typical example of such an experiment is shown in Table I.

The results of the first 11 cases (Table II) show that the endogenous creatinine clearance corresponds very well to the inulin clearance

TABLE II

Inulin and endogenous creatinine clearances in normal subjects and in patients with slightly diminished renal function

Number	Subject	Diagnosis	Urine flow	Clearance		Clearance ratio Cr/I
				Inulin	Creatinine	
			cc. per minute	*cc. per minute*	*cc. per minute*	
1	St.	Normal	3.48	124	116.7	0.94
2	Hü.	Normal	1.80	139	159	1.14
3	H. Y.	Normal	0.75	107.8	108.5	1.00
			0.85	112.6	105	0.93
4	Ep.	Normal	5.45	116	123	1.06
			4.85	116.8	117.6	1.00
			2.29	130	149	1.15
5	Sh. S.	Bronchial asthma	2.2	138.6	139	1.00
			3.5	151	159	1.05
			3.75	144.6	159.5	1.10
6	Shin.	Hypertension	2.7	90.1	92.7	1.03
			2.11	74.4	78.8	1.06
			2.78	88.8	91.7	1.03
7	Ab.	Pulmonary abscess	1.25	165.2	172.5	1.04
			1.0	160	172.2	1.08
			1.0	159	157	0.99
8	Al.	Hypertension	5.32	71	79.5	1.12
			6.92	79.8	84	1.05
			10.85	96	98	1.02
9	Os.	Rheumatism, normal	3.4	96.6	111.2	1.15
			7.8	113	132.8	1.17
10	Y. Y.		3.0	129.8	99.9	0.77
			2.16	105	83.7	0.80
			1.21	92.3	76.4	0.83
11	K. A.	Hypertension	7.2	116	85	0.73
			4.2	106	85.2	0.80
			5.0	108	95	0.88

Average = 1.03

TABLE III

Inulin and creatinine clearances in subjects with glomerulonephritis

Number	Subject	Diagnosis	Urine flow	Clearance		Clearance ratio Cr/I
				Inulin	Creatinine	
			cc. per minute	*cc. per minute*	*cc. per minute*	
1	Ke.	Chronic nephritis	0.90	5.1	8.5	1.67
			0.95	5.6	8.4	1.50
			0.87	4.9	7.9	1.61
2	Shab.	Nephrosis	0.59	85.5	114	1.33
			0.75	80.6	106	1.32
			1.28	67.3	95.6	1.42
3	Shaz.	Chronic nephritis	1.22	4.9	5.6	1.14
4	Must.	Acute nephritis	3.70	121.5	126	1.04
			3.72	118.5	126.5	1.07
5	Ri.	Chronic nephritis	1.52	3.7	5.3	1.44
			0.75	2.0	3.45	1.73
			1.12	3.0	3.75	1.23
6	Is.	Acute nephritis	2.91	47.3	61.1	1.39
			3.20	53.4	67.8	1.27
			3.0	44.4	63.5	1.43

within the limits of error of the clearance determinations. On the average, the creatinine/inulin ratio of the twenty-seven separate periods is 1.03. This result justifies the opinion that the endogenous creatinine clearance, performed after Popper's method, exactly indicates the filtration rate in normal persons or in patients with slightly diminished renal function (Cases 6 and 8).

So far, we have compared the excretion of these substances in only 6 patients with kidney diseases (Table III). In the first 2 cases, a chronic nephritis in the terminal stage and a nephrosis with renal function still preserved, the creatinine clearance was distinctly higher than the inulin clearance in all the periods. In the third case, a chronic nephritis in the terminal stage, the creatinine clearance was scarcely higher.

In the fourth case, that of a patient who had recovered from an acute nephritis, the clearances of creatinine and inulin were equal within the limit of error. In the fifth and sixth cases however, one a uremia and the other an acute nephritis, the excretion of creatinine exceeded the excretion of inulin.

Although the number of cases examined is too small to draw general conclusions, it may be supposed that in some patients with kidney diseases there exists a difference between the endogenous creatinine clearance and the inulin clearance. This fact may be explained in two ways. Either there exists in some patients an additional secretion of creatinine in the tubules—which, however, is not very probable just in the terminal stages of nephritis—or the pathologically changed glomerular membrane does not allow inulin to filter freely and in such cases the values of the inulin clearance are too low. The following consideration will show how doubtful these questions are.

The laws for the tubular secretion have been clearly formulated by Smith (34) and his collaborators (36). The tubular secretion is limited by the fact that the tubular mass can only transport a limited quantity of the substance from the blood to the urine in a unit of time. If the concentration of the substance in plasma is low, under a certain limit, the tubular mechanism can excrete the substance in a sufficient quantity. With increasing concentration, the capacity of secretion of the tubules reaches the limit, the "threshold," where this capacity is fully utilized. If the plasma concentration increases still more, only a quantity which corresponds to the threshold value can be secreted by tubular activity. The surplus quantity of the substance can only be excreted by filtration. Therefore, the clearance of the substance decreases with increasing plasma level and slowly approaches the level of the filtration. Smith (34) calls this process the "self depression" of the clearance. The existence of this self depression is an important proof of the tubular secretion of a substance.

Shannon (31) also demonstrated these facts for exogenous creatinine. In low plasma concentrations (7 to 13 mgm. per cent) the creatinine/inulin ratio is 1.4, *i.e.*, creatinine is distinctly secreted. In high plasma concentrations (96 to 127 mgm. per cent) this ratio decreases to 1.12. Therefore, Shannon has proved the existence of the self depression for exogenous creatinine.

What is the explanation of the fact that endogenous creatinine, which certainly has such a low plasma level that it ought to be within the supposed secretory capacity of the tubules, is not secreted but concentrated at the same rate as inulin in normal persons? And how can one explain that, as shown by Miller and Winkler (20) and our own experiments in pathologically increased plasma creatinine, there seems to be a secretion in some cases instead of the self depression which one would expect? We cannot yet answer these questions. In a personal interview (1938) Shannon remarked that perhaps there exists a chemical difference between endogenous and exogenous creatinine, and that this may account for the different behavior of the kidneys. As quoted above, we could also assume a restriction of inulin filtration by very serious glomerular alterations. In Case 5, Table III, for instance, a uremia in the terminal stage, albuminuria had also ceased.

However, if we have not yet found a complete explanation of the secretion of endogenous creatinine in kidney diseases, the conformity of the clearance of creatinine and inulin is sufficient for practical diagnosis. According to our opinion, the endogenous creatinine is, except for inulin, the most suitable substance for the determination of the filtration rate in man. The great advantages of such an endogenous substance for the function test of the kidneys will be described later.

3. Comparison between the endogenous creatinine clearance and the exogenous creatinine clearance

The additional secretion of intravenously administered creatinine in man has been tested by Shannon (31) and by Miller and Winkler (20) by comparison with the inulin clearance. If the endogenous creatinine clearance really corresponds to the filtration rate, the determination of the filtration after administration of creatinine (according to Rehberg (28)) ought to show a higher value than the endogenous creatinine clearance. This question was examined in 22 cases which are presented in Table IV.

TABLE IV

Endogenous and exogenous creatinine clearances

Number	Endogenous clearance			Creatinine by mouth	Exogenous clearance			Clearance ratio exogenous/endogenous
	Urine flow	Plasma creatinine	Clearance		Urine flow	Plasma creatinine	Clearance	
	cc. per minute	*mgm. per cent*	*cc. per minute*	*grams*	*cc. per minute*	*mgm. per cent*	*cc. per minute*	
1	1.43	1.04	110.2	6	1.35	11.68	122.2	1.11
2	2.08	2.34	46.2	4	1.92	8.80	56.2	1.22
3	0.70	0.52	125.2	4	0.64	8.06	140.5	1.12
4	0.58	1.79	44.2	4	0.66	7.42	40.5	0.92
5	0.57	1.65	56.6	4	0.52	7.76	88.4	1.56
6	0.32	1.93	51.3	4	0.35	7.72	51.2	1.00
7	0.94	0.65	147	4	1.06	7.08	159	1.08
8	0.85	1.22	122.6	4	0.83	8.44	124.8	1.02
9	0.86	1.10	120.5	4	0.86	8.50	160.8	1.33
10	1.16	1.12	55.9	4	1.16	5.28	83.4	1.49
11	2.42	0.97	99.8	4	1.83	9.54	104	1.04
12	1.05	1.09	121.3	4	0.82	7.74	215	1.77
13	0.77		84.7	2	0.60		93	1.11
14	1.83	0.65	127	6	1.10	10.64	121.2	0.98
15	0.83	0.87	129	3	1.01	5.95	110	0.85
16	0.88	1.04	92.5	4	1.31	7.27	111.7	1.21
17	1.0	0.94	77.6	4	2.83	9.8	76.2	0.98
18	0.58	1.09	101.5	4	0.90	7.94	86.5	0.85
19	0.40	0.77	104	4	0.80	9.78	138	1.33
20	0.15	1.22	54.8	4	0.35	10.04	95.6	1.77
21	0.83	1.22	61.1	4	1.07	5.76	114.7	1.87
22	0.96	1.02	73.7	6	1.15	8.56	86.9	1.18

Average = 1.22

In the first period, lasting 1 hour, the endogenous creatinine clearance was determined. After the end of this period the patients were given the creatinine dissolved in 150 cc. of water. One hour later the bladder was voided, and then the period of the exogenous creatinine clearance began. The average value of the blood samples drawn at the beginning and at the end of this period was used for the determination.

As a matter of course it can be objected that, during the delay between the two determinations, the filtration rate might change uncontrollably. Only those tests in which the inulin clearance is made simultaneously to establish a control ought to be used as proof. In view of this objection these determinations cannot be considered as absolutely convincing. The cases have been divided into 3 groups: In the first one the diuresis during the first and the second period is the same. In the second one there is a decrease of the diuresis in the second period; in the third, an increase. In spite of large fluctuations in the individual cases, on the average, in each of the 3 groups the exogenous creatinine is more concentrated than the endogenous. The ratio between exogenous and endogenous clearance, on an average 1.22, together with the results of the other tests, indirectly contributes to the opinion that the endogenous creatinine clearance can be regarded as the real measure of the filtration rate.

4. Comparison between the endogenous creatinine clearance and the urea clearance

From their experience, Möller, McIntosh, and Van Slyke (21) introduced the terms of maximum and standard clearance and thus gave expression to the fact that the urea excretion decreases with decreasing quantities of urine. Rehberg (28) and Holten and Rehberg (15) explained this relation between urea clearance and the quantity of urine on the basis of a passive reabsorption of urea by the tubules according to the concentration gradient resulting from the reabsorption of water. Depending on the space of time in which the filtrate is in contact with the epithelium of the tubule, more or less urea will be reabsorbed. If this simple explanation were right, we ought to be able to formulate a mathematical relation between the concentration of the urine, measured by the U/P ratio of inulin, and the quantity of the reabsorbed urea. According to this, the reabsorption of urea had to decrease with increasing dilution of the urine until, in the extreme case when no more water is reabsorbed, the reabsorption of urea also ceases and urea and inulin clearances are equal. This conception is rather contradictory to the maximum clearance, which does not take into consideration the influence of the quantity of urine on the reabsorption of urea in extreme dilutions.

Recent investigations by Shannon (32) and by Chasis and Smith (5), however, demonstrated that Rehberg's simple idea alone is not sufficient for the explanation of the urea reabsorption. In fact it has been ascertained in normal and pathological cases, in man as well as in the dog, that no more than 10 to 15 per cent of urea is reabsorbed at extremely low concentrations of the urine and the urea clearance approaches the filtration rate. But as soon as the urine/plasma ratio of inulin has risen to 10, 35 to 40 per cent of the urea is reabsorbed. The reabsorbed share of the urea, however, increases much more slowly

with increasing concentration of the urine. Therefore, in the range of the concentration ratio between 10 and 200, the urea reabsorption slowly increases from 40 to 70 per cent. So it seems that in this range other mechanisms influence urea absorption than those active at extreme dilutions.

Trying to explain this fact Smith (34) introduced the hypothesis of at least a double-sided water and urea reabsorption. During the "obligatory isoosmotic water reabsorption" up to 40 per cent of the urea is reabsorbed in the proximal segment of the tubule, while the urea absorption exceeding 40 per cent occurs in the distal tubule, together with the "facultative water reabsorption."

For purely diagnostic purposes and ignorant of these opinions, we compared the clearances of urea and endogenous creatinine in 111 cases. The results are arranged according to the concentration of the urine, expressed by the creatinine U/P ratio, in Table V. It is evident that each

TABLE V

Creatinine U/P ratio and urea reabsorption (111 cases)

Creatinine U/P ratio	Number of cases	Filtration rate	Urea reabsorption
		cc. per minute	*per cent*
2– 10	8	1.6– 7	29
10– 20	11	11 –103	32
20– 40	14	25 –128	47
40– 60	19	21 –145	55
60– 80	11	38 –163	57
80–100	9	70 –140	62
100–120	13	46 –166	52
120–140	5	68 –128	66
140–160	7	55 –142	62
160–180	4	55 –163	54
180–200	4	17 –160	69
200–340	6	87 –142	75

column, except the first one, includes normal and pathological filtration values. The results agree very well with those of Chasis and Smith (5). In the extremely low concentration of pathological cases the urea reabsorption is on the average 29 per cent; in particular cases it is even as low as 10 per cent. When the diuresis of normal kidneys is great (column 2 and 3), the reabsorption amounts to about 40 per cent. With increasing concentration the reabsorption slowly increases until, at a creatinine U/P ratio of 40 to 180, it attains an average value of 57 per cent. In high concentrations of more than 200, an average of 75 per cent of urea is absorbed; in particular cases even as high as 82 per cent. The conformity of these values with those of Chasis and Smith (5) can be considered as a further indirect proof of the fact that the endogenous creatinine clearance of Popper must approach the glomerular filtration.

As mentioned above, the results are to a certain extent contradictory to the idea of maximum and standard clearance, *i.e.*, at extremely low concentrations of urine. In clinical practice the glomerular filtration agrees rather well with the urea clearance, expressed as percentage of the normal value.

The results of the three comparisons are as follows:

1. The comparatively high conformity between the clearances of inulin and creatinine supports the hypothesis that the endogenous creatinine clearance expresses the filtration rate (Tables I and II). The obviously higher concentration of creatinine in some cases (Table III), however, is not in accordance with Shannon's laws of secretion in the tubules. Therefore, the problem of secretion of creatinine cannot yet be considered as definitely solved.

2. The fact that creatinine orally administered is more concentrated than endogenous creatinine renders the sole filtration of endogenous creatinine very probable (Table IV).

3. The conformity of the values for urea reabsorption with those found by Chasis and Smith further confirms the correctness of the determination of the filtration rate by endogenous creatinine.

These results do not prove with absolute evidence that the endogenous creatinine clearance and the filtration rate agree perfectly in all cases. For all clinical purposes, however, this evidence is sufficient and the determination of the filtration by the endogenous creatinine clearance is much easier and more convenient than by any other method.

THE DIAGNOSTIC VALUE OF PLASMA CREATININE AND ENDOGENOUS CREATININE CLEARANCE

In all the cases in which the size of the filtering surface of the kidney is reduced, be it as a con-

sequence of inflammation, by sclerosis, or by any other kind of reduction of the vascular caliber, the filtration rate will be reduced. So the filtration rate is a clear measure of the part of the kidney still in function. It is evident that the quantitative determination of the part of the kidney still functioning must be considered as the essential measurement of renal function. All other examinations of partial function must be of secondary importance. One must take into consideration, of course, the possibility that some extrarenal factors may cause a temporary decrease of filtration. For instance, in cardiac decompensation the filtration may decrease to 20 cc., and a sudden loss of blood or a shock may depress the filtration almost to zero. Also, in various conditions accompanied by hypochloremic azotemia, caused by extrarenal factors, and in certain cases of acute infection and jaundice, temporary reductions of filtration rate may often be found. Back pressure, caused by various urological conditions, may also considerably reduce the filtration. All these conditions must be kept in mind when a reduced filtration rate is to be interpreted. Nor must it be forgotten that a normal filtration rate need not exclude pathological changes in the kidney.

1. Comparison between the endogenous creatinine clearance and the plasma creatinine

What are the laws for the excretion of an endogenous substance which is always supplied at a constant rate, and therefore shows a constant plasma level, and which is only filtered but neither

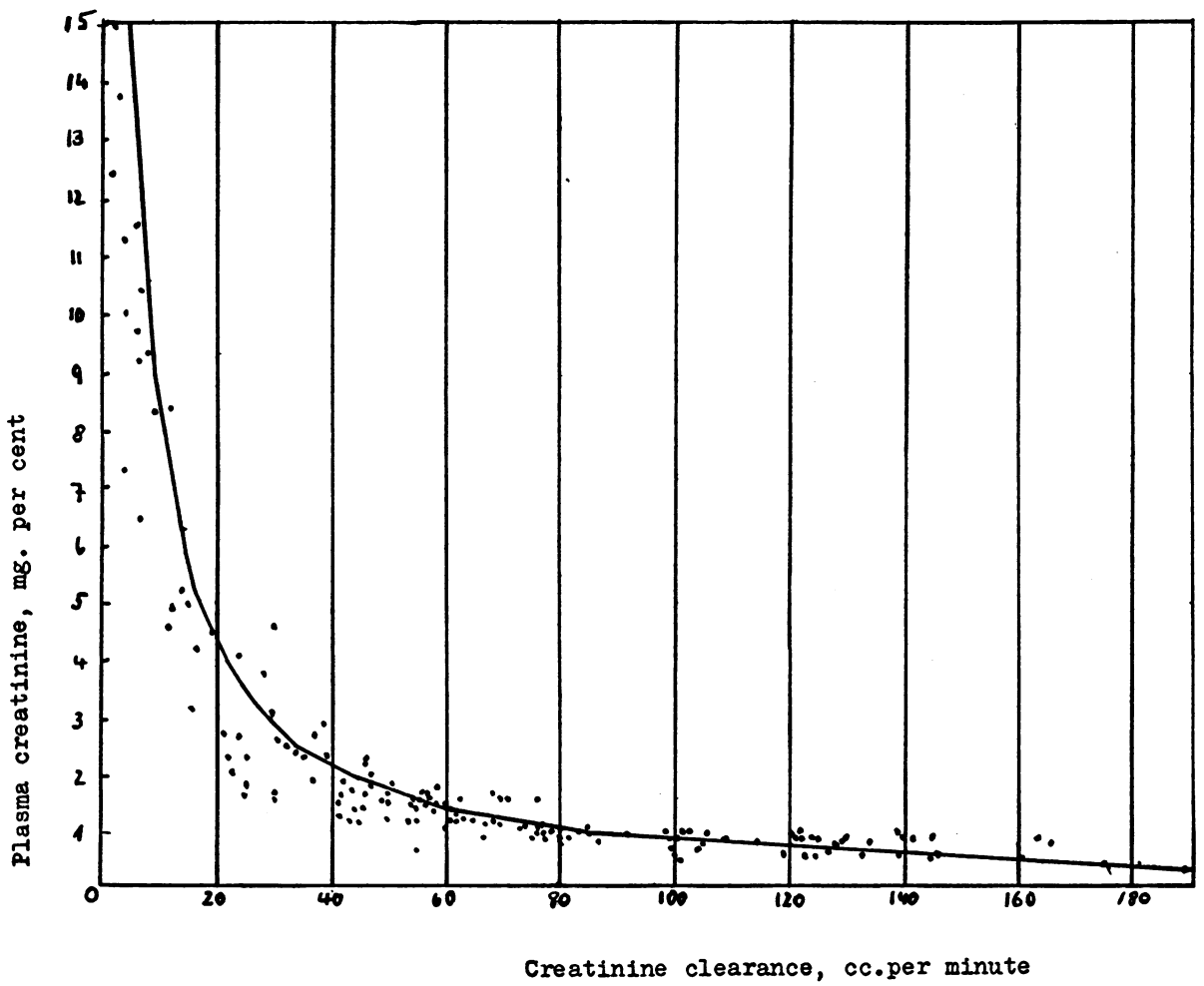

FIG. 1. RELATIONSHIP OF PLASMA CREATININE CONCENTRATION TO ENDOGENOUS CREATININE CLEARANCE (160 DETERMINATIONS)

reabsorbed nor secreted? Suppose that in a unit of time 100 cc. of glomerular filtrate are formed at a plasma level of 1 mgm. per cent and 1 mgm. of the substance is excreted. If, the substance being supplied at a constant rate, the filtration decreases to 50 cc., the plasma or filtrate concentration must increase to 2 mgm. per cent so that the excreted quantity remains the same. That means that the product of the plasma concentration and the filtration rate must be a constant value. If we could find out that this simple relation is valid for an endogenous substance, we shall have proved that this substance has been only excreted by filtration and neither reabsorbed nor secreted. Moreover, with the aid of this constant value we should be able to deduce the filtration rate only from the plasma value of this substance.

We have made an attempt to prove this relation for the endogenous creatinine by 160 determinations (Figure 1). Only adults of medium body surface have been examined. The average product of plasma creatinine and filtration rate has been found to be 86, *i.e.*, if the plasma contains 1 mgm. per cent creatinine, an average of 86 cc. of glomerular filtrate is formed. The average value of this product shows sufficient uniformity for different values of plasma creatinine (Table VI). For higher creatinine values (more than 6 mgm. per cent) we obtained a lower average value in the 9 cases examined. For the moment we cannot draw theoretical conclusions from this small number of cases. Table VII demonstrates the distribution of the values of the product of plasma creatinine and creatinine clearance. It is evident that the product lies between 60 and 120 in 74 per cent of the 160 cases.

TABLE VI

Product of plasma creatinine and filtration rate

Plasma creatinine	Number of determinations	Average product
mgm. per cent		
0.4– 1.0	60	89.8
1.0– 1.5	46	89.7
1.5– 2.0	27	85.0
2.0– 3.0	11	81.4
3.0– 4.0	3	84.2
4.0– 5.0	5	83.7
5.0– 6.0	3	93.3
6.0–15.0	9	50

A serious objection to this conclusion has been made by Van Slyke (42). The rate of creatinine formation per square meter of body surface is not the same in all subjects. Prolonged illness, with loss of muscular ability, and some other uncontrollable circumstances may reduce the creatinine output to less than one-half of the normal amount. Therefore, it is impossible to deduce the filtration rate from the plasma creatinine in every case. In the recent reviews on the excretion of creatinine (1, 41), no data concerning the reduction of the excretion of preformed creatinine are given. We therefore examined another series of 74 cases and found that in the majority of these the creatinine formation was comparatively constant. In 12 of 74 cases, *i.e.*, in 16 per cent, the formation was reduced one-half to three-fourths, in perfect accordance with the rates summarized in Table VII. These cases include cachexias, women with diabetes after a long fasting period, a woman with hypertension, and patients with kidney diseases in bad condition.

TABLE VII

Distribution of the product

Product	Percentage of determinations	Product	Percentage of determinations
30–40	2.4	90–100	18.1
40–50	6.2	100–110	10.0
50–60	6.9	110–120	6.2
60–70	13.8	120–130	2.5
70–80	14.4	130–140	3.1
80–90	11.5	140–150	3.1
		150–160	1.8

For the present, disregarding the fact that in 16 per cent of the cases the creatinine formation is reduced, we may conclude from the other cases that the endogenous creatinine is only excreted by filtration, as plasma creatinine and filtration rate are inversely proportional. The quantity of filtered and excreted creatinine remains constant within certain limits, independent of the plasma level. This fact is very important for clinical diagnosis.

As shown in Figure 1, at a reduction of the filtration rate below 80 cc. the plasma creatinine is forced to increase to pathological values. This retention occurs at first in creatinine which in normal persons shows a very constant level, and which of all the components of the blood has the greatest U/P ratio. Filtration rate multiplied by plasma creatinine equals 86; 86 divided by the

plasma creatinine gives the filtration rate corrected for the normal body surface (1.73 sq.m.). So it is possible, with an accuracy sufficient for clinical purposes, to determine the rate of the glomerular filtration by the determination of one constituent of the blood, and to obtain results which include the correction for the body surface. Holten and Rehberg (15) would have employed the endogenous creatinine for the determination of the filtration rate had they known an exact method for the determination of creatinine.

We estimate the filtration rate by deduction from the plasma creatinine chiefly in outside patients in whom the determination of the clearances would be difficult. In the hospital we continue estimating the creatinine clearance. After forming an opinion about the rate of creatinine formation in a patient by means of several preliminary tests and putting the obtained product into the calculation, it is possible to deduce the filtration rate for months solely from the plasma creatinine level.

2. Plasma creatinine and urea

If the renal function, as measured by the urea clearance, has already decreased to 20 to 40 per cent of normal, the blood urea is still within the normal range of 50 mgm. per cent in more than half of the cases. These results demonstrate the uncertainty of the diagnostic value of normal blood urea contents in kidney diseases. In Figure 2 the relation of blood urea to creatinine is represented in 171 cases. In a great number of cases in which the urea content is found within the normal range of 50 mgm. per cent, we can observe a distinct increase of plasma creatinine, often to twice the normal value or more. As the two lines limiting the area indicate, no fixed relation exists between urea and creatinine. The content of urea in blood is greatly influenced by extrarenal factors. The level of creatinine in blood, however, can indicate a considerable reduction of the filtration rate at a still normal urea value. Even Popper, Mandel, and Meyer's method (27) for the rapid estimation of renal deficiency, which is a qualitative determination of creatinine in blood performable within 3 minutes at the bedside, often indicates pathological results when the urea level of the blood is still normal. Popper and Brod (24) emphasize the superiority of the simple determination of creatinine in plasma over the determination of the filtration. Physiological fluctuations of the filtration are of no importance in the estimation of the results.

It must be mentioned once more that the quantitative results represented here have only been found with Popper's method (26). The insufficiency of the original method must be the reason for the lack of conformity between the early investigators on the diagnostic value of plasma creatinine, and especially on the sequence of the retention of urea, non-protein nitrogen, uric acid, creatinine, and indican. (See discussion by Peters and Van Slyke (23)).

3. Filtration and urea clearance

As already quoted above, filtration and urea clearance were compared in many cases. The findings of previous investigators (3, 13) have shown that in kidney diseases the decrease of the urea clearance, expressed as percentage of normal, was roughly parallel to that of the filtration. This is true chiefly for the field between 10 and 50 per cent of urea clearance, where both values nearly coincide.

In uremia, with a filtration of less than 10 cc., the value of the urea clearance is sometimes a little higher. But that is of no practical importance. As has been stated, this fact is to be explained by the extremely low capacity of concentration of these kidneys and by the reduced reabsorption combined with it.

We can often observe a divergence of urea clearance and filtration at a urea clearance of more than 70 per cent of normal. The filtration rate is normal, but often not higher than the percentage of the urea clearance.

As a whole, we must state that the determination of the filtration rate by means of the endogenous creatinine and the urea clearance are of the same diagnostic value. Since the determination of plasma creatinine alone allows one to estimate the rate of the filtration, this simple method is sufficient for the diagnosis of the still functioning part of the kidneys. The only disadvantage of this method is the fact that 4 cc. plasma, *i.e.*, 10 cc. blood, are needed, whereas an exact determination of urea can be made with 0.2 cc. blood.

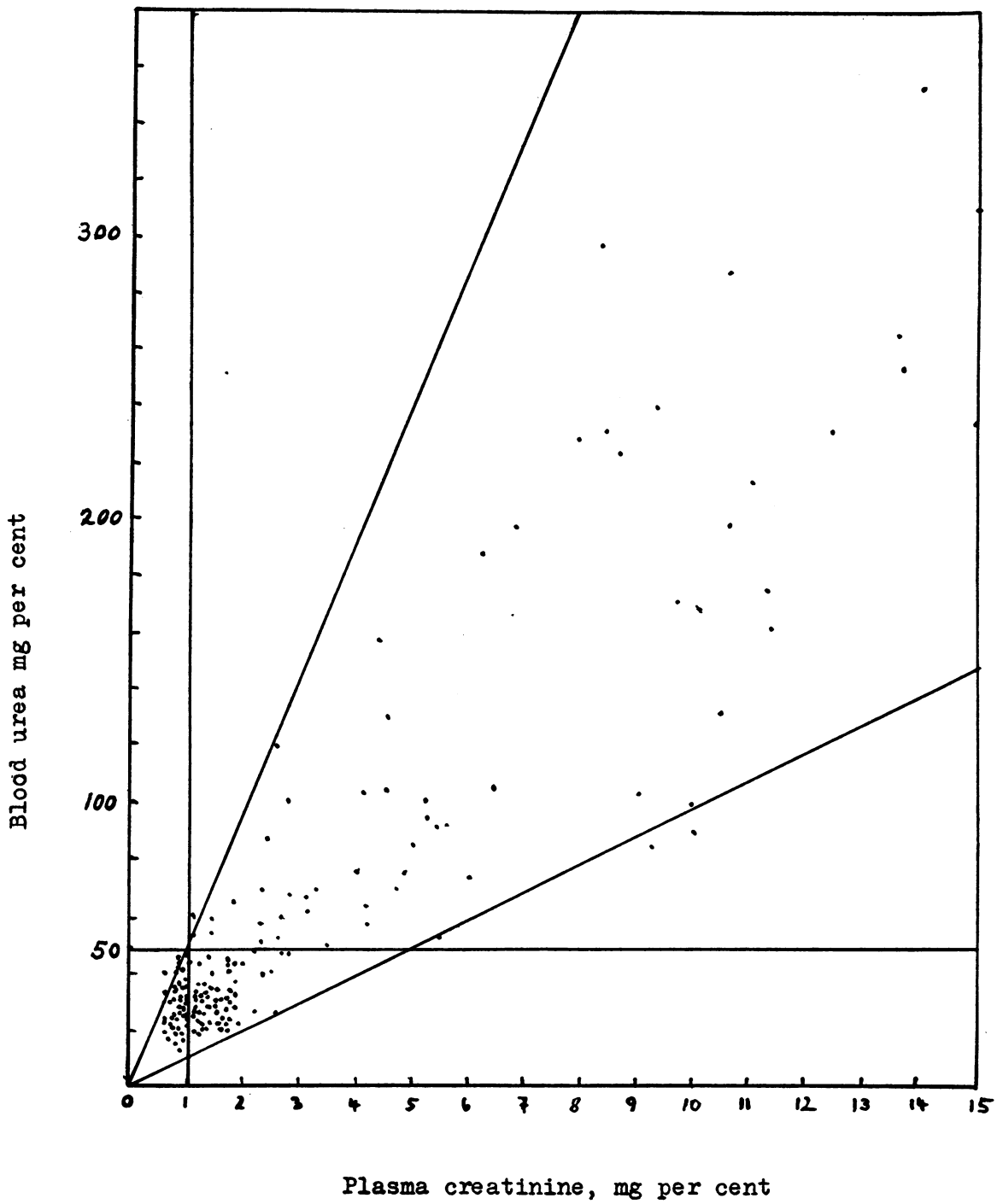

FIG. 2. BLOOD UREA AND PLASMA CREATININE IN 171 CASES

The simple determination of plasma creatinine is a further step in the simplification of the diagnosis of renal function. It can, in practice, replace all determinations of glomerular function such as those of non-protein nitrogen, urea, urea clearance, the test of orally administered urea, the administration of other substances, and clearance determinations with other substances.

We call attention to the fact that the urine of urological patients with an inlying catheter is often decomposed by bacterial action in the bladder, and therefore the determination of urea in urine results in values which are too low and which give the illusion of too low urea clearances and of a more than normal urea reabsorption. In the cases of some of these patients we were of the erroneous opinion that we had to deal with an extreme urea reabsorption. In such cases only the creatinine clearance gives the right result.

4. Compensation and reabsorption uremia

Before we finish the discussion about the excretion of creatinine and urea we still have to deal with the ideas of " compensation " and " reabsorption uremia." Compensation means that, at a slightly reduced filtration together with an increased plasma creatinine, the retention of urea can be compensated by an actively reduced reabsorption. Thus a moderate reduction of filtration would not necessarily influence the actual urea excretion. In our patients we never observed this but, on the contrary, sometimes found a normal filtration rate and a slightly reduced urea clearance.

The term " reabsorption uremia " was used for the first time by Ferro-Luzzi (7) and was employed in a larger sense by Popper and Mandel (25). Yamaguchi (43) defines the action of the tubules not as active reabsorption but as a selective inhibition of the complete reabsorption. This faculty of selective inhibition of the reabsorption is supposed to be upset in serious deficiencies of the tubuli, so that creatinine and urea pass back into the blood in increased quantity. Contrary to Popper, we believe that it is impossible to apply to cases typical of the course of glomerulonephritis these very interesting impressions which have been derived from experiments with toads. We are of the same opinion as Chasis and Smith (5) who, by comparison of urea and inulin clearance, came to the conclusion that the idea of reabsorption uremia does not conform to the facts. The diffusion coefficient of urea is seven times greater than that of inulin (4). The normal walls of the tubules are impermeable to inulin and only a little permeable to urea. If we suppose that the permeability increases, we ought to conclude that urea passes earlier and more rapidly than inulin, which means that the reabsorbed quantity of urea ought to increase. But the contrary is true. With decreasing capacity of concentration of the kidneys the reabsorbed quantity of urea decreases, as Chasis and Smith (5) and ourselves were able to demonstrate. This fact indicates a reduced and not an increased urea reabsorption. Until a final proof has been established, the term " reabsorption uremia "—which would be suitable for the explanation of some facts otherwise still unexplainable—ought to be abandoned and any retention in cases typical of the course of glomerulonephritis should be explained only by reduction of the filtering surface.

In some other conditions, however, an exceptional behavior must be admitted. In the cases designated by McCance and Widdowson (17) as functional disorganization of the kidney, a temporary decrease of the filtration rate occurs by decrease of the blood pressure or by other extrarenal influences. In absence of any constant anatomical alteration, these conditions are characterized by the functional integrity of the reabsorbing parts of the nephrons. In diabetic coma, severe salt deficiency (16), uncompensated alkalosis, dehydration, and certain other conditions, the glomerular filtration decreases, but the reabsorption on the part of the tubules is unrestrained. The rise in blood urea may be accounted for by an additional urea reabsorption. Observations on chloride reabsorption may be explained in the same manner.

McCance and Widdowson (17) observed in diabetic coma an abnormal fall of the creatinine/inulin clearance ratio, but were not able to explain this fact by the existence of a reabsorptive mechanism. The observation of the low creatinine/inulin ratio may be erroneous by an analytical reason. The values of plasma creatinine given by McCance and Widdowson (17) are perhaps too high, as in diabetic coma great amounts of

acetoacetic acid and acetone are present in plasma. To demonstrate this assumption we selected period 1, patient 1, Table III from McCance and Widdowson (17). The inulin U/P ratio is 38, the creatinine U/P ratio 18.6 at a plasma creatinine level of 25.8 mgm. per cent. If the creatinine U/P ratio were also 38, the plasma creatinine would be 12.6 mgm. per cent. Regarding the great quantity of ketone bodies present in plasma during diabetic coma, we can suppose that the difference between the creatinine value found and the creatinine value deduced is in fact caused by ketone bodies.

METHODS

1. Determination of creatinine in plasma and urine according to Popper, Mandel, and Meyer (26)

In a test tube 4 cc. oxalated plasma are introduced with an Ostwald pipette into 12 cc. saturated picric acid, previously purified according to Benedict (2) and mixed by shaking vigorously. The mixture is immersed in boiling water for 15 seconds. The precipitate, after cooling, is resuspended by shaking and is filtered. To 10 cc. of the filtrate 0.5 cc. 10 per cent NaOH are added and mixed. The photometric reading is made after 20 minutes with a filter of 530 mμ and a depth of layer of 60 mm. As the mixture often tends to become slightly turbid, it is filtered once more 5 minutes before the reading. A blank analysis with 4 cc. water instead of plasma is treated in the same way and used for the compensation in the photometer. As the Beer-Lambert law is not strictly applicable, it is necessary to make an exact comparison curve. In concentrations of more than 4 to 5 mgm. per cent it is necessary to dilute the plasma twice, four, or even ten times.

The urine is diluted accordingly, usually fifty times and 4 cc. of the dilution are added to 12 cc. of the picric acid solution. Twenty minutes after the addition and mixing of 0.8 cc. 10 per cent NaOH, it is read in the photometer. If the urine contains albumin, it is filtered 5 minutes before reading. Absolutely clean tubes and pipettes are necessary to get reliable results.

2. Determination of inulin according to Steinitz (38)

After the desalbumination of 1 cc. oxalated plasma with zinc sulfate and sodium hydroxide, according to Somogyi (37), the quantitative Selivanoff reaction, modified by Roe (30), is performed. The principle of the method consists of the colorimetric determination of the levulose formed from the inulin during acid hydrolysis. The acid hydrolysis and the colorimetric reaction are performed simultaneously.

3. Microdetermination of urea according to Conway (6)

The principle and the technic of this method are described in another paper (39).

SUMMARY

Since the tubular secretion of exogenous creatinine has been demonstrated, the determination of the glomerular filtration by the administration of exogenous creatinine has become problematic. Since that time reliable methods for the determination of endogenous creatinine have been developed.

After a short discussion of Popper's method for the determination of creatinine, the results of 3 series of experiments, performed to show the suitability of this method for the determination of the glomerular filtration, are reported:

1. Comparison between the endogenous creatinine clearance and the inulin clearance.
2. Comparison between the endogenous creatinine clearance and the exogenous creatinine clearance.
3. Comparison between the endogenous creatinine clearance and the urea clearance.

The results seem to prove that with Popper's method the glomerular filtration can be determined much more easily by the endogenous creatinine clearance than with any other method.

The determination of plasma creatinine is of high diagnostic value because it makes possible the estimation of the glomerular filtration rate by one simple blood analysis. This simple method can therefore replace any other test of glomerular function. It is a further step in simplifying the diagnosis of renal function.

The conception of reabsorption uremia is discussed, but cannot be maintained for the explanation of the retention of urea in cases of glomerulonephritis, except for the cases of functional disorganization of the kidney.

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