
PLASMA CHROMOGEN AND THE ENDOGENOUS CREATININE CLEARANCE

BY

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One of the troubles of using the endogenous creatinine clearance to measure the filtration rate is that plasma and serum contain chromogens, other than creatinine, which give a colour with the Jaffé reaction. The creatinine can, however, be isolated by adsorption on Fuller's earth (Gaebler and Keltch, 1928) and subsequently eluted with the alkaline picrate used for colour production (Borsook, 1935). This method has been used by a number of workers and its reliability established. Thus Hare and Hare (1949) found with radioactive carbon that recovery is complete, and Ralston (1955) with chromatography that no extraneous chromogen remains after treatment. But there is very little evidence as to the amounts of non-creatinine chromogen in the plasma or as to how neglect of its presence influences the creatinine clearance, and it is these points which will be considered here.

Method

Chromogens in Serum.—The extraneous chromogen is taken as the difference between total chromogen in untreated serum filtrates and true creatinine in the same filtrates treated with Fuller's earth. The total and extraneous chromogen are expressed as the amount of creatinine giving the same colour.

Reagents.—The following were used:

- **Standard creatinine solution**, 0.5 g. in 1 litre N HCl
- **and dilutions with water to suitable strengths.**
- 5% (w/v) sodium tungstate.
- 0.33 N H$_2$SO$_4$.
- Fuller's earth (B.D.H.), 10 g. in 100 ml. of water.
- Oxalic acid, saturated solution (9.5 g. in 100 ml. of water).
- 0.75 N NaOH (3%).
- Picric acid, saturated solution.

Protein Precipitation.—Take two 2.5 ml. samples of serum in centrifuge tubes; to one (X) add 2.5 ml. of water and to the other (S) 2.5 ml. of 20 mg./l. creatinine solution and mix. To each add 2.5 ml. sodium tungstate and 2.5 ml. H$_2$SO$_4$. Mix and stand 10 minutes. Centrifuge and filter. When estimations are made both with and without adsorption double quantities must be used.

Total Chromogens.—To 5 ml. each of X, S, and water, add 3 ml. of a fresh mixture of equal parts of picric acid and NaOH. The colour of X is read against the H$_2$O blank and that of S against X, readings being made after 25 to 30 minutes at room temperature with a Spekker absorptiometer and blue-green filters, No. 603.

Creatinine.—To 5 ml. each of X, S, and water, in conical centrifuge tubes, add 1 ml. of Fuller's earth suspension and 0.5 ml. of oxalic acid. Stopper with plastic bungs and shake intermittently for 10 minutes. Remove bungs and wash them and the sides of the tube with a few drops of water. Centrifuge at 3,000 r.p.m. for 20 minutes. Decant the supernatant fluid and drain in reverse.

To the packed deposit add 8 ml. alkaline picrate solution made from 3 parts each of picric acid and NaOH and 10 parts of water. Stir, stopper, and shake intermittently for 10 minutes. Centrifuge for 10 minutes. The supernatant fluid is then poured off into the absorptiometer cuvettes and the colour of X read against the water blank, that of S against X. Readings are made at room temperature, the colour being fully developed at 30 minutes and remaining stable for several hours. Plastic bungs are better than rubber ones, since alkaline picrate removes a coloured compound from the latter if they are at all worn.

The concentrations of either total chromogen or creatinine are then given by the calculation:

\[
\left( \frac{\text{Reading of X}}{\text{Reading of S}} \times 20 \right) \text{mg./l. creatinine.}
\]

When the creatinine content of the serum is two to three times higher than normal, dilution is necessary. This is carried out after protein precipitation. The amount of standard creatinine added to the serum must then be increased so as to give a final concentration of 5 mg./l.

In addition to the internal standard, which is all that is needed for routine estimations, when total chromogen and true creatinine are being compared, standards in water of 2.5 to 10 mg./l. are used, both with and without treatment with Fuller's earth. The value of the serum is then read from the standard
curve in water and corrected for the colour depression which occurs in serum.

Thus if

- Reading of serum +
- Reading of serum +
- Expected reading of
- Corrected serum concentration

\[
\text{Corrected serum concentration} = 15.6 \times \frac{0.363}{0.345} = 16.4 \text{ mg./l.}
\]

**Creatinine Excretion Rates and Clearances.**—The excretion rates are obtained from the means of three to 16 samples of urine. Creatinine is estimated in the urine without adsorption with Fuller's earth, since it has been shown that little if any extraneous chromogen is present (Hare and Hare, 1949; Owen, Iggo, Scandrett, and Stewart, 1954).

The mean minute excretion of creatinine divided by the total chromogen or creatinine in 1 ml. of urine gives the uncorrected creatinine clearance.

**Results**

The colours of alkaline creatinine picrate and alkaline picrate are influenced by the medium in which the creatinine is present and the temperature. In serum, creatinine gives less colour than in water (Roscoe, 1953) so that a standard in serum should be used, but the colour depression is less when the serum filtrates are treated with Fuller's earth than when they are untreated. Thus the colour given by creatinine added to 20 serum samples is 5.5% (S.D. 3.5) less than the expected without adsorption, only 1.6% (S.D. 2.3) less with adsorption. With adsorption there will, therefore, only be a small error if standards in water are used instead of the internal one, but these must be treated with Fuller's earth, because after treatment there is about 10% reduction in colour in all concentrations.

The colours of both alkaline picrate and alkaline creatinine picrate increase as the temperature rises and decrease again as it falls (Owen et al., 1954). The increase in this laboratory is as much as 14% for alkaline picrate and 12% for alkaline creatinine picrate, between 15° and 25° C. It is therefore essential that the colours should be read with the blank, standards, and unknowns all at the same temperature. Here the readings were made at room temperature, since if, as has been recommended, the samples are brought to 25° C., they may cool unequally in cold weather, before all the readings are made.

**Creatinine and Extraneous Chromogen in Serum.—**Table I shows the true creatinine concen-

**Table I**

<table>
<thead>
<tr>
<th>Subject</th>
<th>Sex</th>
<th>Surface Area (sq. m.)</th>
<th>Plasma Creatinine (mg./l.)</th>
<th>Extraneous Chromogen (mg./l.)</th>
<th>Creatinine Excretion (µg./min./173 sq. m.)</th>
<th>Creatinine Clearance</th>
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trations in 33 sera from 30 subjects and the extraneous chromogen. The extraneous chromogen varies from 0 to 4.5 mg./litre. It does not increase as the true creatinine concentration rises, but rather tends to be less. The mean concentration in 14 subjects with serum creatinines below 15.0 mg./l. (mean 11.15 mg./l.) is 3.0 mg./l., while in 10 subjects with serum creatinines of 15 to 33 mg./l. (mean 21.9 mg./l.) it is 1.8 mg./litre. These estimations were all carried out with 1 in 4 serum dilutions and may be compared; but with still higher serum creatinines, when higher dilutions are used, a constant amount of extraneous chromogen gives a decreased colour response, soon falling within the error of estimation, so that it is not possible to say whether it remains constant or decreases.

The proportion of the total chromogen provided by extraneous chromogen thus decreases as the creatinine rises. It is in this series, 21.4% in 14 samples with a mean creatinine concentration of 11.15 mg./l., 7.6% in 10 samples with a mean creatinine concentration of 21.9 mg./l., and 2.2% in five samples with a mean creatinine concentration of 55.1 mg./litre.

Creatinine Clearance.—Since the creatinine clearance is given by the minute excretion of creatinine divided by the plasma creatinine, if the total chromogen is used in its calculation it will be too low. The depression will be greater the higher the proportion of extraneous chromogen, and, since this is most in normal subjects with low plasma creatinines, it follows that the error will be greater in these, and will decrease as the plasma creatinine rises. The uncorrected and corrected clearances are shown in Table I and the difference is seen to be as much as 43 ml./min. in one case with a corrected clearance of 128 ml./min., while it is never more than 6 ml./min. when the corrected clearance is less than 50 ml./min. The corrected and uncorrected clearances are also compared in Fig. 1, where the decreasing difference at lower values is clearly seen. The curve shows the depression which will be produced if the creatinine excretion is 1,100 μg./min. and the extraneous chromogen 3.0 ml./litre.

The clearances also depend on the minute excretion of creatinine. This is given in Table I for those cases where the surface area is known. The excretion rate, corrected for surface area, is higher in men than in women, the means being 1,076 μg./min. in 17 men and 774 μg./min. in seven women. There are also large variations in rate within the male and female groups. There is no obvious falling off in excretion at lower filtration rates.

Discussion

Miller and Dubos (1937) estimated extraneous chromogen in serum, after destruction of the creatinine with an enzyme, and found it to be responsible for 10% of the total colour in normals, but for up to 50% in cases of nephritis with raised creatinine levels. In a later paper, however, Miller and Miller (1951), using both the enzymatic and the adsorption method, showed that, while 20% of the total chromogen was extraneous in normals, this decreased to 10% when the plasma creatinine was 10 times normal and to 5% when it was 20 times normal. Brod and Kotátko (1949) used adsorption with Lloyd’s reagent and found 1.2–4.0 mg./l. (mean 2.8 mg./l.) of extraneous chromogen in sera from normals, but in renal failure this increased up to 135 mg./l. or 24% of the total chromogen. Hare and Hare (1949), with adsorption, again found approximately 20% of the total chromogen in normals to be extraneous, but only 10% or less in chronic renal insufficiency. Ralston (1955), with adsorption, found 2.8–5.0 mg./l. of extraneous chromogen in sera from 10 normals, or a mean of 36% of the total chromogen.

These results all agree in that in normals approximately 20% of the total chromogen is found to be extraneous and this has also been shown in the present work. When the true creatinine is increased the results of different workers vary widely, but the suggestion that the
extraneous chromogen accumulates and increases in the blood as kidney function fails is not on the whole supported, and this certainly does not occur in the cases studied in this paper where there is no increase or a fall.

Since the extraneous chromogen does not increase as the plasma creatinine rises and creatinine clearance falls, the error introduced by use of the total chromogen instead of the creatinine decreases, and when the plasma creatinine is more than twice normal, or the creatinine clearance less than 50 ml./min., it is usually negligible. On the other hand, with normal plasma creatinines the error may be very considerable, and, since the extraneous chromogen is by no means constant, it is not possible to correct by subtracting a mean value from the total chromogen, when this is the only estimate that has been made.

The clearance also depends directly on the creatinine excretion, and this varies widely. The lower excretion in females than males is related to the naturally lower clearances, but the variation found in one sex is sufficient to give a 50% difference in clearance, with the same plasma creatinine. This finding is not in agreement with that of Steinitz and Türkand (1940) and Effersoe (1957); the latter considered that the excretion is so constant for males or females respectively that the clearance can be calculated from the plasma value to within 20%. He used 24-hour clearances and ensured that the plasma creatinine was not changing, whereas here some clearances are only based on a few hourly samples and may not be representative. But in fact, in Case 2, with such a low excretion that, in spite of a low normal plasma concentration, the clearance is only 47 ml./min./1.73 sq.m., the excretion is the mean over 15 days during which the plasma creatinine did not alter.

A falling off in excretion rate in severe degrees of renal failure has been recorded by Steinitz and Türkand (1940). Here any such fall was obscured by an insufficiency of cases and the large variations even in normal subjects.

Summary

The total chromogenic material in serum filtrates, estimated by the Jaffé reaction, is compared with the true creatinine, separated from serum filtrates with Fuller's earth. Some practical points in the estimations are discussed.

The non-creatinine chromogen varies from 0 to 4.5 mg./l. creatinine equivalents in 33 sera. The amount does not increase and may even fall as the creatinine concentration rises.

If the total chromogen in plasma is used to calculate the endogenous creatinine clearance, this may be falsely depressed, the error being considerable in normal subjects, but decreasing as the filtration rate falls.

REFERENCES

Plasma Chromogen and the Endogenous Creatinine Clearance

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