A CLINICAL METHOD OF ESTIMATING THE AMOUNT OF CALCIUM IN THE URINE AND OTHER PHYSIOLOGICAL FLUIDS

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INTRODUCTION

To gauge the condition of the general metabolism of the calcium salts, an accurate knowledge of the amount at the disposal of the tissues is necessary, and this knowledge has been difficult to obtain.

For instance, some workers have estimated—chiefly experimentally—the amount of calcium taken in with the food, and have compared this with the amount recovered from the faeces.

This method cannot give reliable results. For although we know the amount taken in by the mouth, we are unable to find out how much of this quantity is absorbed and how much passes through the alimentary canal without being absorbed. Consequently, it is evident that although we may know the amount taken into the alimentary canal and the amount passed out, calculations based on such data must err, because we do not know how much has simply passed through. Recognising this difficulty, in 1907 I published a method of estimating the quantity of ionizable calcium in the blood¹. By this method one is able to determine at any given time the relative amount of ionizable calcium in the blood, and so to get a good indication of the amount at the disposal of the tissues. But as I then, and have since, emphasised, even this method requires careful interpretation. For instance, a low calcium content in the blood may mean either that very little was being absorbed or that too much was being excreted. I devised the method for a specific experimental purpose in regard to menstruation, and it served that purpose; but when it was used for routine clinical work, unless careful consideration were given to the findings, wrong deductions—as I have just pointed out—might be drawn from them.

If, however, the blood estimation method be used in conjunction with a method which will indicate the amount excreted, a perfectly reliable

1. *Brit. Med. Journ.*, April 20, 1907.

knowledge of the condition of the general metabolism in regard to the calcium salts may be obtained.

Since the excretions from the intestinal canal are, as I have just shown, useless for the purpose, we must fall back on the urine, in which it is safe to say that, provided the urogenital apparatus be healthy, the calcium found must represent a true index of the excretory ratio. If the blood index be low, and the amount excreted in the urine also be small, it is fair to assume that not enough calcium is being absorbed. If, on the other hand, the blood index be low and the urinary excretion high, then probably too much is being excreted; and conversely a high blood index and low excretory ratio might indicate that an excessive amount was being retained in the tissues.

PRELIMINARY INVESTIGATIONS

1. The precipitates obtained with oxalic and acetic acid from many specimens of urine were pooled, and on chemical examination were found to consist of pure calcium oxalate. It may be noted here that practically all the calcium present in urine is in an inorganic form, consequently it is precipitated. When the proper measures, to be described directly, are adopted, no phosphates were found in the many precipitates examined.

2. It was found that a rough calculation of the amount of precipitate was not accurate, except in so far as a very large quantity could be distinguished from a small one. Our attention was therefore directed to devising some clinical method of estimating accurately the amount of the precipitate which we had proved to consist of pure oxalate of calcium. Our experiments consisted in centrifuging and measuring the deposit in calibrated tubes.

Our first difficulty was to prevent the deposit sticking to the sides of the tube, and to get it to lie fairly evenly to the calibrated portion. After trying many things, we finally overcame what threatened seriously to interfere with the perfection of our method by adding alcohol or methylated spirit to our urine and reagent.

Next, we found, as we anticipated, that with different rates of revolution of the centrifuge, and with different times spent on the operation we obtained different results; that is to say, urines and solutions containing the same quantity of calcium gave deposits which stood at different levels according to the circumstances mentioned.

However, we eventually got over this difficulty by centrifuging a

standard solution of artificial urine containing a known quantity of calcium in one tube and the urine to be examined in the opposite one. In this way we were able always to get an absolutely accurate relation between the two deposits whatever the rate of revolution or the time employed. So that if the urine showed a deposit of calcium oxalate standing at half the height of the precipitate in the tube containing the standard solution, that urine was found on chemical analysis to contain a quantity of calcium just half in amount of the known quantity in the standard solution. All our results were, of course, verified chemically.

DESCRIPTION OF THE METHOD

A sample from a twenty-four hours' specimen of urine is made faintly acid with hydrochloric acid to dissolve any insoluble phosphate present. It is then made faintly alkaline with ammonia, and filtered. Next 5 c.cm. of the filtrate are placed with a pipette in the special centrifuge tube, which is of the usual size and shape in the upper portion, but tapers at the lower end into a cylindrical extremity of even bore (1.25 mm.) and calibrated into 1 mm. divisions (Fig. 1). A line, with 'urine' marked below it, encircles the upper part of the tube at the 5 c.cm. level. Any air bubbles which may collect in the calibrated portion are got rid of with a fine wire or strand of silkworm gut. Then 1 c.cm. of the reagent, consisting of a saturated solution of oxalic acid in a 5 per cent. solution of acetic acid, is added. The correct quantity of reagent (1 c.cm.) is also indicated by a line round the tube which is marked 'reagent.' Finally 2 c.cm. of alcohol or methylated spirit, as indicated by the line marked 'alcohol,' are added, and the contents of the tube are thoroughly mixed by shaking.

The second tube is then taken, and 5 c.cm. of the standard solution¹ is run into it with a pipette that is up to the line marked 'solution' (Fig. 2), and any air bubbles removed as before. Next the reagent and alcohol are added as in the case of the first tube, and the whole is thoroughly shaken. Both tubes with their calibrated ends packed in wool are then carefully placed in the opposite buckets of a centrifuge, and are centrifuged for about a quarter of an hour. On removing the tubes the precipitate will be found to stand at a certain height, say 10 mm. in the 'standard solution' tube, while it may stand at 7 mm. in the other

1. *Standard Solutions*.—0.05 gram. of calcium phosphate [$\text{Ca}_3(\text{PO}_4)_2$] is dissolved in a little hydrochloric acid. This is made alkaline with ammonia, and finally acid with acetic acid. Finally, 2 grams of urea are added to the solution, and the whole is diluted up to 100 c.cm. with distilled water. Specific gravity = 1015.

which contains the urine to be examined. As a rule, there is a slight slant on the surface of the deposit. This can be obviated by stopping the machine at the end of one or two minutes and turning the tubes through half a circle.

But if this be not done—and it is not absolutely necessary, although it makes for greater accuracy—the height of the *middle* of the meniscus or slant in each tube should be read off and compared. In the above instance the deposit in the tube containing the standard solution was stated to stand at 10 mm., and that in tube containing the urine at 7 mm.,

FIG. 1

FIG. 2

consequently the urine contains 7-10th or 0.7 of the quantity of calcium in the standard solution, which is known to be 0.02 per cent; in other words, the urine contains $0.02 \times 0.7 = 0.014$ per cent. of calcium. This gives a general equation $\frac{U}{S} \times \frac{1}{50} = \text{percentage of calcium in the urine examined}$, in which U = height in millimetres of precipitate in the urine

to be examined, and S = height in millimetres of precipitate in the standard solution.

If any specimen of urine be found to contain an unusually large amount of calcium, so that the precipitate more than fills the calibrated portion of the tube, it should first be diluted with an equal quantity of distilled water, and the final result obtained in regard to the calcium content doubled.

In our experiments to test the accuracy of the method we found that the difference between the percentage of calcium obtained in this way and that obtained by chemical analysis never amounted to more than 1 per cent. of the quantity present.