

THE QUANTITATIVE ESTIMATION OF INDOLE.

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DURING my work in Yucatán (tropical Mexico) I observed that we nearly always found by the current analysis of urine, a well pronounced indican reaction, certainly in more than 80% of the cases examined; and frequently the blue colour obtained by the Obermeyer or Jaffé reaction was so strong that one would have been inclined, if in Europe, to postulate the presence of intestinal obstruction or some serious intestinal disorder. Experience soon taught us however that the reaction had not the same significance in Yucatán and probably in other tropical countries as it possesses elsewhere. With the object of studying the underlying causes I suggested to one of my assistants an investigation of the indole-producing power of *B. coli* isolated from the faeces of different individuals. The results which have been published in a dissertation (Hernandez, 1908) were naturally very incomplete but the work led me to realise the importance of a quantitative indole-determination, and, as I did not find described in the literature any method suitable for our purpose, I proceeded to work out a method for myself. I used the Salkowski test, with nitrite of potassium, sulphuric acid, and extraction with amyl-alcohol, preparing a series of tubes containing determined quantities of indole in progressive concentrations. The indole I distilled from bacterial cultures. The scale kept well, having been preserved for three years in the dark and only brought into the light when in use.

Working at the Lister Institute, I desired to make a similar scale, using the more sensitive indole test with paradimethyldiamidobenzaldehyde and potassium persulphate, known as the Ehrlich test. Considerable difficulties were however encountered, as the colour soon faded, when

the tubes were preserved for a short time, and it was only after many attempts that I found a useful method.

Very little has been published regarding the quantitative estimation of indole. The most exact method is probably Marshall's (1907), but as it necessitates in each case first a distillation and afterwards a rather laborious colorimetric process it is not likely to enter largely into ordinary bacteriological routine. The same may be said of the methods of Peckham (1897), and Herter and Foster (1906). They are too complicated for daily work, and a method is needed which does not involve the use of distillation or other apparatus and which can, if possible, be completed in a few minutes. This object would apparently be attained by Crossonini (1910), who uses a direct colorimetric comparison, his scale consisting simply of a series of tubes in which he has obtained Ehrlich's reaction. Here again it would however be necessary to make a new scale almost every day, as the colour, especially in the weaker indole-solutions, fades very quickly when preserved in this manner, even if the tubes are kept in darkness. Crossonini does not use extraction and does not start with very weak solutions and accordingly the method presently to be described is not only much more reliable but also more sensitive and convenient.

Some few principles may be laid down for a useful reaction and estimation.

The first principle must be that the reaction shall demonstrate small quantities of indole, and for that purpose it is necessary to extract the indole either with amyl-alcohol or with chloroform, as the colouring substance can be concentrated in this manner in a smaller volume of liquid and it will moreover be more easily observable, being transferred from a yellow to a colourless medium. For a qualitative test it may be preferable to use only a very small quantity of 1 or 2 c.c. but in a comparative scale it is evidently necessary to always extract with the same quantity of liquid which must not be too small, because variations would not show well, nor too large, otherwise faint reactions would pass unobserved. I have found that a quantity of 5 c.c. suits the purpose very well. By this method not only a dilution of one to a million gives a distinct reaction, as generally stated, but also the presence of indole in a proportion of one to ten millions may easily be demonstrated.

The second essential must be that the standard tubes may be kept for a very long time without alteration, that is to say without fading of the colour produced. As the fading seems to depend principally or entirely on the progress of the oxidizing process beyond the stage which is

necessary for the reaction, the object must be to exclude the possibility of a continued oxidation.

The continuance of this process is caused by the presence of the potassium persulphate.

Accordingly when we separate the coloured substance, after extraction, from the original liquid in which the reaction is produced, we eliminate the principal source of alteration. Simple contact with the air may also produce a certain amount of fading although this occurs after a much longer lapse of time; this fading might be prevented by keeping the extracts *in vacuo* or by substituting another gas for the atmospheric air, but a much simpler method is to preserve the extract below a liquid, such as sterile physiological salt solution which is indifferent by itself and which excludes the air to a sufficient degree. Amyl-alcohol is therefore unsuited for the purpose, but chloroform may be employed as the extracting liquid. I have not been able to find a liquid of less specific gravity than amyl-alcohol which does not mix with it. Moreover, as in the case of a very weak indole solution, the oxidation may progress very rapidly when the ordinary saturated potassium persulphate solution is employed, I prefer to use only a 1% solution, since it gives more trustworthy results. Using a weak solution the reaction attains its greatest intensity somewhat later, but it occurs nevertheless in the low numbers of the scale within half an hour. With higher concentrations of indole a few hours are required for the colour to attain its full intensity and the colour undergoes no noticeable alteration during several hours.

Thirdly it must be easy to distinguish between the different numbers of the scale and therefore the difference in concentration between any two following numbers must be in proportion to the absolute concentration. However, for various reasons, it would not be practical to use a geometric progression. I have therefore used a scale which runs from 1 to 100 but actually employing continuous numbers from one to ten only and hereafter omitting intermediate tubes as the concentrations mount higher.

Taking into consideration these principles I have developed the following technique: 5 centigrams of pure indole (Merck) are dissolved in a few c.c. of absolute alcohol, adding subsequently distilled water up to 500 c.c., making a 1:10,000 solution. This represents No. 100 of the scale.

Of this solution 1 c.c. is added to 99 c.c. of water, which represents a solution of 1:1,000,000 or No. 1 of the scale and in a corresponding

manner the Nos. 2, 3, 4, 5, 6, 7, 8, 9, 10, 12, 14, 16, 18, 20, 25, 30, 40, 50, 60, 70, 80, 90, are prepared so that the scale consists of 24 numbers. Of each solution 10 c.c. are introduced into a series of test-tubes, all of approximately the same diameter and rather wide. To each tube are added 5 c.c. of the ordinary Ehrlich's solution (paradimethylamidobenzaldehyde 4, alcohol (96 %) 380, conc. hydrochloric acid 80) and 5 c.c. of a 1% solution of potassium persulphate. When the red colour has reached its greatest intensity, 5 c.c. of chloroform are added and the whole is well mixed without shaking violently. The chloroform extracts nearly all the colouring substance and collects in the bottom of the tube so that there remains above only a very faintly stained liquid. By means of a burette the chloroform is separated into other tubes, which subsequently are nearly filled with sterile salt solution (0.85 %) and closed with cotton wool and paraffin or with rubber caps. When not in use the scale should preferably be kept in darkness, although so far as I have observed, exposure to light does not seem to have any deteriorating influence.

The difference between any two tubes in sequence is always sufficiently pronounced, so that it is easy to make a quick comparative colorimetric determination each time an indole reaction is made. For the ordinary reactions the saturated or the 1% potassium persulphate solution may be used.

Several of the standard tubes have been kept for more than a month, being daily exposed to the daylight for some time, without any alteration.

By means of this scale a considerable number of indole determinations in different cultures have been made, which will be published at a later date.

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