NOTE ON THE SODIUM NITRO-PRUSSIDE REACTION FOR ACETONE.

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LEGAL first showed that Weyl's reaction for creatinin was also given by acetone. The reaction consists in the addition of a few drops of a 5 % solution of sodium nitro-prusside, followed by 1 % sodium hydrate. A red colour is produced both with acetone and creatinin solutions.

Le Nobel[1] elaborated the test by showing in the case of acetone that acidifying with acetic acid changed the red to magenta, and that acetone solutions even when too dilute to produce a red with alkali and nitro-prusside yield a red purple colour when acidified with acetic acid.

He also describes the reaction with ammonia and mentions that adding mineral acid in insufficient amount to neutralize the ammonia improves the delicacy of the test.

Bitto[2] applied the reaction to a vast number of aldehydes and ketones. He preferred the use of potassium hydrate as alkali, and he describes the colour changes on subsequent acidification with phosphoric acid.

His general conclusion is, that bodies with the group—CH_2—CO give the reaction.

In testing for acetone in urine, the presence of creatinin prevents the direct application of the sodium nitro-prusside test when a fixed alkali is used.

If however ammonia be used as alkali the creatinin does not react, at all events not in the concentrations normally present in the urine.

But in testing the delicacy of the ammonia nitro-prusside reaction it was found that it was limited by an acetone concentration of 1 in 600.

The following description indicates how a strikingly delicate and characteristic acetone reaction may be obtained.

The solution of acetone or urine containing the same is taken in a quantity of 5—10 c.c. A solid ammonium salt is added, then 2—3

[1] Le Nobel. *Archiv f. Exp. Pathol.* XVIII. p. 9. 1884.

[2] Bitto. *Liebig's Annalen*, CCLXVII. p. 374.

drops of a fresh 5 % solution of sodium nitro-prusside, and 1—2 c.c. of strong ammonia. A positive reaction is indicated by the development of a most characteristic permanganate colour.

The choice of ammonium salt is not wholly immaterial. The sulphate should be used for preference, or the chloride, bromide, acetate, or carbonate may be selected.

The nitrate is not equally effective, and for use with urine may be less serviceable on account of a possible interaction with urea, and formation of urea nitrate. Ammonium oxalate is the one ammonium salt which is in no way comparable with the others as of service to the reaction. It should not be employed.

The influence of ammonium salts on the reaction is in great part explained by a comparative study of the test. I quote a few substances showing their reactions with ammonia alone, and with ammonia in presence of a solid ammonium salt. For the sake of comparison the results obtained by Bitto with the use of potash solution, and subsequently acidification by phosphoric acid, are placed beside them.

TABLE.

	3 drops 5 % Sodium Nitro Prusside Solution		5 % Sodium Nitro Prusside (Bitto)	
Substance	Ammonia	Ammonia and solid Ammonium Salt	Potash Solution	Acidification by Phosphoric Acid
Aldehyde	Carmine red (reaction of poor delicacy)	Carmine red (reaction of poor delicacy)	Carmine red (delicate reaction)	Carmine red * Colour much weakened
Acetone	Red (not delicate, 1 in 1000)	Permanganate (very delicate, 1 in 20,000)	Red (delicate)	Magenta (more delicate)
Acetyl Acetone	Red (delicate, 1 in 20,000)	Carmine (very delicate, 1 in 400,000)	Red	Carmine
Aceto Phenone	Red (not delicate)	Blue (delicate, 1 in 10,000)	Red	Blue
Aceto Acetic Ether	Orange red	Orange red	Orange red	Orange red
Aceto Acetic Acid	Orange red	Orange red	Orange red	Orange red
Creatinin	Red	No colour	Red	No colour

* The much poorer "acid" colouration applies to all the aldehydes examined by Bitto.

It will be seen that the colour reactions with sodium nitro-prusside, and ammonia in presence of a solid ammonium salt, resemble the colour reactions on acidification, described by Bitto.

The colour change occurring on acidification is a true indicator change. It occurs very closely at the neutral point as indicated by litmus.

For example the reaction of acetophenone with potash and nitro-prusside gives a red colour. On careful titration of this with acid it becomes magenta at the neutral point, and blue with a further drop of acid $\left(\frac{N}{10}\right.$ sulphuric was used$\left.\right)$. If now 2 drops of $\frac{N}{10}$ alkali are added the colour once more changes to red.

Assuming that the reaction with ammonia and ammonium sulphate is related to the acid condition of the pigment 1 drop of a saturated solution of ammonium sulphate was added to the above red. At once it went back to blue, and other tests of a similar nature are convincing in showing that ammonium salts do in fact in all cases quoted in the table cause the development of that colour of pigment which corresponds to the acid condition.

This same action of ammonium salts is well known in connection with phenolphthalein. An alkaline magenta solution of this indicator is decolourized by the addition of ammonium salts, and hence arises its uselessness as an indicator in presence of ammonium salts.

The merits of the test as performed by ammonia in presence of an ammonium salt are:

(1) That it allows of the direct development of the "acid" colouration, which for acetone is more delicate and characteristic.

(2) That whereas the ketones show more characteristic and intenser "acid" colourations, aldehydes show very poor "acid" colourations, and creatinin none.

(3) Creatinin, which reacts red with fixed alkalis and ammonia, has a colourless "acid" condition, and so its presence in urine does not interfere with a direct test for acetone, when performed in the above manner.

(4) The test is very sensitive, as it is performed in a medium of comparatively low OH' concentration. The presence of a high OH' concentration causes a more rapid rise of colour, but at the same time a rapid fading, so that with low acetone values the amount of colour developed at any one time never reaches the same intensity, as with lower OH' concentrations.

(5) The delicacy of the reaction for acetone in urine reaches 1 in 20,000. Foulness of the urine and the presence of dextrose do not interfere with this delicacy.

A reaction however must not be declared negative until after half an hour has been allowed for the development of colour. In using ammonium sulphate a ring of colour is often beautifully shown at a slight height above the undissolved salt, and in order that this may fully develope the tube should not be shaken.

It must be remembered that the colour after its maximum development (half an hour after the reagents have been added) slowly fades.

ERRATA.

VOL. XXXVI.

In the Paper by Prof. Cash:

P. 501, 17 lines from foot, *for* ·9, *read* 9.
P. 502, 17 lines from top, *for* ·4 and ·5, *read* 4 and 5.
P. 506, 22 lines from foot, *for* ·9, *read* 9.

VOL. XXXVII.

In the Paper by Prof. Osborne and Dr Kilvington:

P. 10, last line, *for* 'Weigert-Pal,' *read* 'Marchi.'

CAMBRIDGE: PRINTED BY JOHN CLAY, M.A. AT THE UNIVERSITY PRESS.