LXV. NOTE ON KNOOP'S TEST FOR HISTIDINE.

By GEORGE HUNTER, *Strang-Steel Scholar.*

Institute of Physiology, University of Glasgow.

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IN performing this test Knoop [1908] adds bromine water to the solution to be tested until there is just a very slight excess. On heating, the excess bromine disappears and in the presence of histidine a brownish red colour is developed; if the latter is in sufficient concentration a dark precipitate settles out. The solution to be tested should be slightly acid. The only substance other than histidine found to give this test is iminazolyethylamine or histamine. The test, according to Knoop, is given with histidine at a dilution of 1 : 1000.

When using this test the writer found that different intensities of colour were produced from solutions of the same concentration of histidine. This peculiar behaviour was accounted for by slight variations in the bromine excess. The colour produced is very soluble in excess of bromine and in dilute solutions of histidine an initial excess may prevent all colour development. The difficulty of adding just sufficient bromine especially to slightly coloured solutions has been overcome by adding a definite excess followed by washing the solution repeatedly with chloroform in a small separating funnel until the chloroform is no longer coloured. By this procedure even strongly coloured meat extracts and other fluids may be successfully tested by Knoop's method. The excess of bromine appears to oxidise the colouring matters and after washing with chloroform such solutions are almost colourless, so that on heating the brown colour due to histidine is readily detected. Most uniform results have been obtained when the washed solution is transferred to a test tube and set in a boiling water-bath.

That the chloroform does not extract any of the histidine compound was shown by adding to a series of test tubes equal amounts of 0.1 % histidine monohydrochloride and adding increasing small amounts of bromine water from a pipette until definite excess of bromine was present. By heating all the test tubes together on the water-bath it was found that there was first a rise, then a fall in colour values corresponding with insufficiency or excess of bromine. The maximum thus obtained was less than that obtained by the chloroform method.

The maximum colour appears to be developed when the histidine has absorbed just three atoms of bromine per molecule. A dilute solution of bromine water was standardised against $N/100$ sodium thiosulphate using a mixture of potassium iodide and starch as indicator on a tile. The bromine solution was $0.00785 N$.

In one burette was placed 0.1% solution of histidine monohydrochloride and in another the standard bromine water just before using it. At ordinary temperature it was found that 10 cc. of the histidine solution absorbed 18 cc. of the standard bromine water. When the solution was kept cold in ice the same quantity of histidine absorbed 16 cc. of the bromine solution, whilst at about 40° in a water-bath it absorbed 21.8 cc. of the bromine solution. (These amounts are not quite definite as on long standing more bromine is absorbed.)

The histidine-bromine solution was in each case transferred to a 100 cc. measuring flask which was filled up to the mark with water, so that the solution represented 0.01% original histidine. About 10 cc. of each were put into three dry test tubes and heated on the water-bath. The colour developed was maximum when the bromine was absorbed at ordinary temperature.

It follows from this that one molecule of histidine monohydrochloride absorbs approximately three atoms of bromine at ordinary temperature.

To narrow the limits still further five lots of 5 cc. of 0.1% histidine solution were taken and to these respectively were added 8, 8.5, 9, 9.5 and 10 cc. of standard bromine solution. On diluting to 50 cc. and heating portions, the colour was greatest where 9 cc. had been used. This is the same as the above. The maximum colour developed here was not greater than that got from solutions of the same concentration washed with chloroform.

By the modification above suggested Knoop's test can be obtained with certainty in solutions of histidine at a dilution of $1 : 10,000$; a faint colour in pure solutions is still observable at $1 : 20,000$.

The colour developed on heating is very variable according to the reaction of the liquid. In markedly acid solution it is a dull yellow brown. In approximately neutral solution, the shade is dark brown. In solutions made faintly alkaline with sodium hydroxide—after bromine has been added and the excess removed with chloroform—a bright pink colour develops which is less stable than that in neutral or acid solutions. If made very faintly alkaline with ammonia instead of caustic soda, a deep purple colour rapidly develops on heating. The presence of ammonia in just the requisite amount gives a more intense colour than in the other cases, but since it is difficult to regulate this amount, and further, since there is reason to believe that the specific value of the test may suffer—for carnosine gives a faint yellow by this treatment—the use of alkali cannot be recommended.

The colour developed from tryptophan by addition of bromine is readily distinguished from the colour due to histidine. The former develops in the cold and is easily extractable with amyl alcohol. The coloured substance in

Knoop's test is not extracted by any of the ordinary solvents and is peculiarly unaffected by reagents other than bromine.

SUMMARY.

By a modification of Knoop's test for histidine its delicacy is increased at least ten times and the test can be performed with greater certainty especially in coloured fluids. The proportion of three atoms of bromine per molecule of histidine is found to give the maximum colour on heating.

REFERENCE.

Knoop (1908). *Beiträge*, 11, 356.