VII. THE CAUSE OF ANDREWES'S DIAZO-TEST FOR RENAL INEFFICIENCY.

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ANDREWES [1924] noted a peculiar colour reaction in uraemic sera when performing Van den Bergh's diazo reaction. Hewitt [1925] simplified the test, and as a result of his investigations suggested tentatively that a cyclic amine, such as histamine or tyramine, might be responsible. A summary of previous work, including a description and the clinical value of the test, has been reported elsewhere [Harrison and Hewitt, 1927]. In the present paper the steps leading to the identification of the substance responsible for the reaction will be given.

Hewitt's hypothesis not confirmed.

If Hewitt's suggestion were correct, a suitable extract of uraemic plasma giving a positive Andrewes's reaction would probably give Pauly's diazo reaction. In other words, Andrewes's reaction might be merely another way of performing the Pauly test. The latter, however, is inhibited by alcohol, whereas in the former about 50 % of alcohol is present. The following table shows that neither histamine nor tyramine gives Andrewes's reaction, and conversely that extracts of positive sera do not give Pauly's reaction.

Substance tested	Andrewes reaction	Pauly reaction
Alcoholic extracts of uraemic sera...	Strongly positive	Negative
Same, but alcohol removed	Positive	„
Histamine (ergamine phosphate), aqueous 0·01 %	Negative	Positive
Same, with alcohol to 50 %	„	Negative
Tyramine (acid phosphate), aqueous 0·1 % and greater dilutions	„	Positive
Same, with alcohol	„	Negative
Tyrosine, 0·1 to 0·01 %	„	Positive
Histidine, 0·1 to 0·01 %	„	„
Tryptophan	„	—
Indole...	„	—
Skatole	„	—

INVESTIGATION OF POSITIVE SERA.

It was found that *X*, the substance responsible for the reaction, was very sensitive to changes in the hydrogen ion concentration. It was apparently decomposed by heating in either acid or ammoniacal solution. It could safely

be heated to boiling in neutral organic solvents. It could be boiled in neutral aqueous solution without decomposition, and such a solution could be evaporated to dryness. *X*, in a grossly impure state, was soluble in water (very); aqueous hydrochloric acid (slowly decomposed); ethyl alcohol (slightly); amyl alcohol (slightly) whether the reaction was slightly acid, neutral, or alkaline; acetone (fairly); butyl alcohol (slightly), and aqueous pyridine. It was insoluble in ether, chloroform, and light petroleum.

The fact that it was possible to *boil* the diazo reagent with an alcoholic extract of serum was puzzling because most diazo reactions must be carried out in the cold. The presence of alcohol was not essential. After removing it by evaporation, the residue, dissolved in water, also gave the test.

It was possible to concentrate the colour reaction some 20 to 50 times, but all attempts to make even a rough separation of *X* failed.

X in normal urine.

Hewitt [1925] noted that alcoholic extracts of some normal urines gave a reaction very like that of Andrewes. One of us (G.A.H.) remembered that Hewitt's positive observations were mostly obtained from a subject whose urine was often used in class-work for demonstrating a positive indican reaction. This suggested that *X* might be indican.

Potassium indoxyl sulphate reacts with phenyldiazonium chloride, and on making alkaline, the colour change in dilute solution is very similar to that in Andrewes's reaction [see Neubauer-Huppert, 1913]. Moreover, the methods we had found the best for concentrating the colour reaction both in uraemic sera and in normal urines, were very like that recommended by Hoppe-Seyler [1882] for separating indican from urine. Again, indicanaemia is well known to occur in uraemia, and a marked excess of indican in the blood is found in no other condition. We found that all our sera giving a positive Andrewes's reaction also gave a positive Jaffé test for indican, the latter reaction was, however, less sensitive than the former. If *X* were indican it might account for the observation that an alcoholic extract of a positive serum had either to stand for many hours at room temperature, or to be boiled with the diazo reagent. Potassium indoxyl sulphate is hydrolysed by dilute acid slowly at room temperature, but rapidly when boiled, to liberate free indoxyl which then reacts with the diazo reagent.

An attempt was therefore made to isolate indican in a state of purity from urine by the methods of Baumann and Brieger [1879] and of Hoppe-Seyler [1882], and by modifications of these. A very small yield of indican was obtained from horse's urine[1], and from the combined urines of human patients showing marked indicanuria, but the salt was far from pure. It was noted, however, that Andrewes's reaction and Jaffé's test for indican ran

[1] We are greatly indebted to Dr R. A. O'Brien, Director of the Wellcome Research Laboratories, for the supply of horse's urine. Horse's urine is 10 to 25 times as rich in indican as normal human urine.

parallel. *X* was therefore either indican or some substance concentrated along with indican at each stage of the process.

It therefore became necessary to obtain pure indican in some other way and the method of Jolles and Schwenk [1915], with slight modifications[1], was found to be feasible. *N*-Acetylindoxyl and potassium indoxyl sulphate both gave a typical Andrewes's reaction, in either alcoholic or aqueous solution. Moreover, the reactions were positive when solutions of each of these substances in normal human sera were tested, the solutions containing 1 mg. per 100 cc. serum or being even more dilute. This is important because the reported findings for indicanaemia in uraemia [see Wells, 1925] range from 0·2 to 2·2 mg. per 100 cc. serum.

Conclusion.

It is therefore concluded that the substance in uraemic sera responsible for Andrewes's reaction is an indoxyl compound, presumably potassium indoxyl sulphate (indican), or possibly in part indoxyl glycuronate.

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[1] The preparation from *N*-acetylindoxyl, and the isolation of indican from urine will be reported later.