THE CLINICAL VALUE OF ANDREWES'S DIAZO TEST IN RENAL INEFFICIENCY.

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IN 1924 C. H. Andrewes¹ described an unexplained diazo colour reaction which he found only in uraemic serums. He was working on van den Bergh's test, and noted this peculiar colour reaction incidentally. His procedure was as follows:

To one volume of serum two volumes of alcohol were added. The mixture was centrifuged and the alcoholic supernatant layer removed. To four volumes of this alcoholic extract were added two volumes of alcohol and one volume of freshly prepared diazo reagent, as in van den Bergh's test. In uraemia the mixture slowly developed a brown-buff colour. It was often necessary to let this mixture stand for twenty-four hours at room temperature to obtain full colour development. At the end of this time an addition of a slight excess of strong soda solution (10 to 40 per cent.) produced a cherry-red colour. This latter was the characteristic part of the test. The colour was fleeting. It lasted for a few seconds up to half an hour or so, depending on the concentration of the unknown substance responsible for the colour.

Andrewes obtained a positive reaction in eight cases of uraemia with blood ureas ranging from 220 to 650 mg. per 100 c.cm. Five other cases of nephritis with blood ureas of 40 to 275 mg. gave negative reactions. Twenty-two other patients suffering from various diseases gave negative reactions. Normal serums were negative (Tables I and II). Andrewes made a number of experiments to try to discover the cause of his reaction. He excluded urea, uric acid, creatinine, and proteins. Normal urine, he stated, did not give the reaction. In only one out of three instances in which the cerebro-spinal fluid was examined was a positive reaction obtained, and in this there was slight contamination with blood. In three rabbits with blood ureas of 100 to 300 mg. per 100 c.cm. the reaction was negative. Two of these had experimental nephritis.

L. F. Hewitt⁴ confirmed Andrewes's work. He found the reaction limited to uraemia. He simplified the method as follows:

After adding the diazo reagent to the alcoholic extract of serum or plasma the mixture was boiled thoroughly for about half a minute. The maximum brown-buff colour was thus obtained in a few seconds instead of twenty-four hours. The soda was added at once while the solution was still hot. The reaction thus became very simple and a serviceable clinical test.

Hewitt's tentative suggestion, after performing a large number of chemical experiments to ascertain the cause of the reaction, was that a cyclic amine such as histamine or tyramine might be responsible. Contrary to Andrewes's experience, Hewitt found that alcoholic extracts of urines sometimes gave a reaction which appeared very similar to that in the blood. He noted negative reactions in two infants with uraemia and high blood ureas. In each of these cases there was hyperbilirubinaemia instead of the usual hypobilirubinaemia.

Rabinowitch⁵ in Canada confirmed the value of Andrewes's reaction in patients with marked retention of waste products due to kidney damage. He found positive reactions before the blood creatinine reached 5 mg. per 100 c.cm. One of his cases with an initial positive reaction recovered. He suggested that defective elimination was probably not the only factor responsible for the reaction. Becher² in Germany published a paper on the diazo reaction in alkaline solution shortly after Andrewes, but his technique was essentially different from that of Andrewes, and from our own work we are satisfied that Becher's reaction could not have been given by the substance responsible for Andrewes's reaction.

Blotner and Fitz³ in the United States of America obtained positive reactions in cases with much lower blood

ureas than has been experienced by other workers (Tables I and II). They made the following additional points.

(a) The reaction was of prognostic value in clinical work. A high blood urea was not so significant when accompanied by a negative, as when accompanied by a positive, reaction. The test was of value in differentiating renal coma from other types of coma.

(b) The reaction was positive in two nephrectomized rabbits one or two days after removal of the kidneys.

(c) A dog was nephrectomized, and when Andrewes's reaction was positive an extract of about 33 c.cm. serum was made, partially purified, dissolved in physiological saline, and injected intravenously into a rabbit. The rabbit showed no toxic symptoms.

(d) The ascitic fluid of a human patient gave a positive reaction. The cerebro-spinal fluid did not.

The following tables summarize most of the findings of the above workers, and include our own subsequent work.

TABLE I.—Andrewes's Reaction in Plasma or Serum.

Author.	Reaction Positive.	Reaction Negative.
Andrewes, 1924 ...	8 uraemics	5 nephritics. 22 miscellaneous diseases. 2 rabbits with nephritis. Normal human serums.
Hewitt, 1925 ...	12 uraemics	Many miscellaneous diseases in children. 2 uraemic infants with hyperbilirubinaemia.
Rabinowitch, 1925	8 uraemics	Many miscellaneous diseases. 4 cases of typhoid. 12 cases of arterio-sclerosis or nephritis.
Blotner and Fitz, 1927	35 cases with marked renal insufficiency 2 nephrectomized rabbits 1 nephrectomized dog	Several hundreds of cases with miscellaneous diseases. 100 cardio-renal cases.
Harrison ...	13 uraemics	17 kidney diseases. 25 miscellaneous diseases. 4 normal.
Hewitt ...	22 uraemics	18 kidney diseases. Many miscellaneous diseases in adults.
Total: 99 human cases.		

We may conclude from Table I that in all probability the reaction is confined to uraemia, or at any rate to severe renal inefficiency. A negative reaction, however, does not absolutely exclude the diagnosis of uraemia.

TABLE II.—Relation of Andrewes's Reaction to Urea Retention.

Author.	Positive Diazo.			Negative Diazo.		
	Cases.	Blood Urea, mg. per 100 c.cm.		Cases.	Blood Urea, mg. per 100 c.cm.	
		Max.	Min.		Max.	Min.
Andrewes, 1924 ...	8	650	220	5 3 rabbits	275 300	40 100
Hewitt, 1925 ...	12	692	240	—	—	—
Rabinowitch, 1925	8	450	152	12	225	32
Blotner and Fitz, 1927	7	107	60			
	8	171	108			
	10	223	172			
	7	321	224			
	4	536	322			
	36	536	60			
Harrison	2 rabbits	725	311			
	13	633	154			
Hewitt	22	725	114	18	300	50

The results in Table II confirm Andrewes's conclusion that nitrogenous retention always accompanies retention of the substance responsible for his reaction, but that urea retention and his reaction do not run strictly in parallel. We have noted that the reaction is not confined to any one of the different clinical types of uraemia. In general the reaction becomes more marked as the uraemia becomes more severe clinically. The following findings in one case are given in illustration (Table III).

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TABLE III.

Date.	Clinical Notes.	Andrewes's Reaction.	Blood Urea, mg. per 100 c.cm.
July 24th, 1926 ...	In coma	Negative	144
August 16th, 1926 ...	—	Negative	117
October 26th, 1926 ...	First convulsion on October 29th	+	128
December 7th, 1926...	Second convulsion on December 2nd	++	242

TABLE IV.—Findings in Cerebro-spinal Fluid when Andrewes's Reaction is Positive in Blood.

Author.	Andrewes's Reaction Positive. No. of Cases.	Andrewes's Reaction Negative. No. of Cases.
Andrewes, 1924	1*	2
Hewitt, 1925	0	1
Blotner and Fitz, 1927	0	1
Harrison	0	2
Hewitt	0	3
<i>In Ascitic Fluid:</i>		
Blotner and Fitz, 1927	1	0

* Slightly contaminated with blood.

It appears probable that the substance responsible for Andrewes's reaction does not pass into the cerebro-spinal fluid. It seems to have gone into the ascitic fluid in the one case in which the point has been tested.³ In this connexion we would add that we have been unable to obtain a positive reaction with an alcoholic extract of the corpuscles separated from serums which give strongly positive Andrewes's reactions. The substance responsible for the reaction, therefore, probably does not diffuse into the red corpuscles, or only to a slight degree.

In a paper one of us (L. F. H.) published in 1925⁴ it was noted that in two cases of uraemia in infants with negative Andrewes's reactions there was hyperbilirubinaemia instead of the usual hypobilirubinaemia. We have since encountered a third case. The patient, an adult, was clinically uraemic, and the blood urea was 320 mg. per 100 c.cm. There was in addition severe jaundice with marked hyperbilirubinaemia and bile in the urine. Andrewes's reaction was, however, negative. We thought at first that there might be some connexion between the hyperbilirubinaemia and the negative Andrewes's reactions, but subsequently four cases have been encountered in which hyperbilirubinaemia was associated with positive reactions.

In conclusion, Andrewes's reaction is so little known that a brief outline of the simplified method may be of value.

Simplified Method.

Reagents (identical with those used in van den Bergh's test).

Solution A:		
Sulphanilic acid	...	1 gram
Concentrated hydrochloric acid	...	15 c.cm.
Distilled water	...	to 1,000 c.cm.
Solution B:		
Sodium nitrite	...	0.5 gram
Distilled water	...	to 100 c.cm.

The diazo reagent is prepared by mixing 25 c.cm. of Solution A with 0.75 c.cm. of Solution B. The mixture may be kept for at least a week at room temperature without loss of sensitivity so far as Andrewes's reaction is concerned, although, of course, the Solutions A and B must be mixed freshly for use in van den Bergh's test. The mixture often turns slightly yellow after keeping for a day or two, but this does not matter in Andrewes's test.

Serum or oxalated plasma is obtained from the patient in the usual way. *Post-mortem* blood from a case of uraemia will often give the reaction, though our impression is that the serum obtained after death does not give so strong a reaction as the serum obtained from the same patient shortly before death.

To one volume of serum two volumes of alcohol (absolute or 96 per cent.) are added. The precipitated proteins are separated by centrifuging or filtering. (A slightly improved extraction may be obtained by heating the mixture of serum and alcohol to the boiling point.) To four volumes of the filtrate one volume of the diazo reagent is added. The mixture is boiled thoroughly for half to one minute, and a solution of 40 per cent. soda is added drop by drop, shaking after each addition. The soda may be added immediately after the boiling.

The colour change on making alkaline with soda is the essential part of the reaction. The test should be called positive only when a definite pink or cherry-red colour is seen. This pink colour is fleeting. It may last for a few seconds only, and the mixture must therefore be observed carefully whilst adding the soda. In a few very severe cases of uraemia the pink colour may persist for longer periods, even up to half an hour. The test in its present form is only qualitative. We have found that the volumes recommended may be judged sufficiently well by eye without greatly lessening the sensitivity of the reaction.

On boiling the mixture of protein-free filtrate and diazo reagent, a yellow-brown colour always results in positive cases, but a similar colour may also be obtained in negative cases. We have found that substances other than that responsible for Andrewes's reaction may give a yellow-brown colour at this stage (in acid solution), but that these other substances do not yield a red colour on alkalization.

One of us (G. A. H.) has established a clue as to the nature of the substance responsible for Andrewes's reaction, but the work is not so advanced as to allow of a definite statement here. It may be mentioned, however, that the substance is certainly present in normal urine.

Summary.

1. A positive Andrewes's diazo test is only found in uraemia or severe renal inefficiency. A negative reaction, however, does not exclude uraemia.

2. The unknown substance responsible for the reaction does not appear to pass into the cerebro-spinal fluid, and is either absent from, or present only in minute quantities in, the red blood corpuscles. The blood of uraemics obtained *post mortem* often gives the reaction.

3. Nitrogenous retention always accompanies retention of the substance responsible for the reaction, but urea retention and Andrewes's test do not run strictly in parallel.

4. An outline of the simplified method is given. It is claimed that the reaction thereby becomes a simple clinical test.

REFERENCES.

- Andrewes, C. H.: *Lancet*, 1924, i, 590.
- Becher, E.: *Deut. Arch. f. Klin. Med.*, 1925, 148, 10.
- Blotner, H., and Fitz, R.: *Journ. Amer. Med. Assoc.*, 1927, 88, 985.
- Hewitt, L. F.: *Biochem. Journ.*, 1925, 19, 171.
- Rabinowitch, I. M.: *Canadian Med. Assoc. Journ.*, 1925, 15, 725.

Memoranda:

MEDICAL, SURGICAL, OBSTETRICAL.

A CASE OF THALLIUM POISONING.

THE following case is worth recording as one of the few in which serious toxic symptoms have followed the therapeutic use of thallium.

F. and M., sisters, aged respectively 11 and 8, brunettes of excellent physique, were given thallium acetate, one 8.5 and the other 8.75 mg. per kilo. On the fourteenth day the hair of F. had begun to loosen, and both children were stated to have complained of some slight pains in the knees since the twelfth day. On the sixteenth day F. was seen by one of us in the casualty department. The lower extremities were swollen, hot, and tender from the lower third of the thigh downwards. The skin of the legs was red and shiny with irregular livid blotches. There was fluid in the knee-joints, which were exceedingly tender and painful. Sodium salicylate and methyl salicylate ointment were prescribed, and the child was sent home. On the eighteenth day the child had a fit and, according to the parent, remained only partly conscious for two hours. The fit began with clonic movements in the right arm and leg, which later involved both sides. The face was pale and the pupils dilated; there was foaming at the mouth, which was partly open; the tongue was not bitten. Faeces were passed involuntarily. The child was given some bromide, but was restless during the night and micturated every half-hour for several hours. The patient has no recollection of the fit. There is no personal or family history of epilepsy.

On the nineteenth morning she was admitted, quite conscious, but slow in her replies to questions and unable to lie still. Choreiform movements were present, the face was flushed, and there was a faint rash on the trunk marking the meshes of the vascular network. The temperature was 101°, and the blood pressure 120 mm. Hg. The hair was loose, but depilation incomplete. The urine, which was acid, did not contain albumin, sugar, or abnormal pigment, but acetone bodies were present.

The second sound of the heart was accentuated at the apex; otherwise there was nothing abnormal in the chest. In the abdomen there was vague tenderness in both lumbar regions. The tendon-jerks were all obtained except the knee-jerks, the knees being too tender. The pupils, which were dilated, reacted