LXXXII. A NOTE ON THE RELATIVE SENSITIVITY OF THE BENZIDINE AND PHENOLPHTHALEIN TESTS FOR BLOOD.

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HITHERTO, the benzidine test for blood in urine and faeces has been regarded as the most sensitive and satisfactory for clinical use. The writer's attention having recently been directed to Johannessen's test [1921], which embodies the use of reduced alkaline phenolphthalein solution, the following notes of the relative sensitivity of these tests may be of interest.

The reagents were made up in the following way:

Benzidine reagent. 5 g. of benzidine were shaken until dissolved with 25 cc. of glacial acetic acid, the same volume of hydrogen peroxide being added just prior to use.

Phenolphthalein reagent. 1 g. of phenolphthalein and 25 g. of KOH were dissolved in 100 cc. of distilled water. The resulting red solution was boiled until decolorised with 10 g. zinc dust, filtered and an equal volume of 96 % alcohol added. Before use 10 cc. of hydrogen peroxide were added to 90 cc. of the reagent.

The first series of tests was carried out on aqueous haemoglobin solutions of varying dilutions and the results are summarised in the following table.

In each case 5 cc. of solution were used with 5 cc. of reagent:

Reagent	Reaction	Dilution of Hb soln.	Equivalent in mm. ³ blood
Benzidine	Very slight	1/ 100,000	0·08
	Definite	1/ 71,000	0·14
	Strong	1/ 50,000	0·20
Phenolphthalein	Slight	1/4,000,000	0·0025
	Definite	1/2,000,000	0·005
	Strong	1/ 500,000	0·020

The phenolphthalein reagent, when used with blood diluted with water alone is thus from 10–30 times as sensitive as the benzidine reagent.

The second series of tests was carried out on urine, to which was added a 1/5000 solution of blood in water in quantities appropriate to the dilutions required.

The following results were obtained:

Reagent	Reaction	Dilution of Hb soln.	Equivalent in mm. ³ blood
Benzidine	Very slight (after 3-4 mins.)	1/12,500	0.8
	Slight but definite (at once)	1/10,000	1.0
	Strong (at once)	1/ 6,600	1.5
Phenolphthalein	Very slight	1/50,000	0.2
	Definite	1/33,000	0.3
	Strong	1/25,000	0.4

The phenolphthalein reagent, when used with urine containing blood, is three to four times as sensitive as the benzidine reagent.

With watery solutions of haemoglobin, the colour given by the phenolphthalein reagent is pink-red. It takes a short time to develop with very dilute solutions and fades in 15-25 minutes, according to the original depth of colour. With urine, owing to interference by the urinary pigment, the colour appears orange-pink or orange-red and is also transitory, fading with dilute solutions in 3-4 minutes.

The reagent also gives satisfactory results when used for the detection of occult blood in faeces.

REFERENCE.

Johannessen (1921). *Ugeskrift. Laeger*, **83**, 1613.