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A NEW TABLET TEST FOR BILIRUBIN IN URINE

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This paper describes a simple method for the detection of small amounts of bilirubin in the urine. Bilirubin is first concentrated by adsorption on a special test mat and then allowed to react with a stable diazonium compound incorporated in a tablet. Results obtained with urine from normal and jaundiced subjects have been compared with those using two standard routine methods for urinary bilirubin, Fouchet's test and the iodine ring test.

Methods

The *tablet test* is performed as follows: (1) Place 5 drops of urine on the test mat, which is 19 mm. square and 2 mm. deep, and is composed of a mixture of asbestos and cellulose fibres. (2) The tablet is placed on the centre of the moistened area of the mat. It contains a stable diazo dye (*p*-nitrobenzene diazonium *p*-toluene sulphonate) and also sulphosalicylic acid, sodium bicarbonate, and boric acid. (3) Two drops of water are allowed to flow on the tablet. (4) The colour developing on the mat is recorded within 30 seconds. The bilirubin is concentrated on the test mat. The diazo dye is taken into solution by the water, aided by the effervescence caused by the presence of sulphosalicylic acid and sodium bicarbonate in the tablet; the boric acid is a vehicle. If bilirubin is present in the urine the mat around the tablet turns purple and the amount of bilirubin is roughly proportional to the speed of development and intensity of the colour. Any colour developing after 30 seconds is ignored. It is found that the most satisfactory colour reaction is given if the first drop of water is allowed to remain on the tablet for a few seconds before the second drop is put on to wash the fluid on to the test mat.

Fouchet's test is performed by adding 2 ml. of 10% barium chloride to 10 ml. of urine, filtering and adding Fouchet's reagent to the filter paper. A positive result is indicated by a green colour.

The *iodine ring test* is performed by layering 2 ml. of tincture of iodine diluted 1 in 4 with water on to 10 ml. of urine in a test-tube. A positive result is indicated by a green ring at the interface.

A *modification of the tablet test* has been employed, using 5 drops of urine on the test mat as before, but replacing the tablet with 1 drop of Fouchet's reagent. A positive result is indicated by the development of a green colour on the mat.

Quantitative urinary bilirubin estimations were performed by the method of Golden and Snively (1948). Urinary urobilin and urobilinogen were tested qualitatively by Schlesinger's test with alcoholic zinc acetate and by Ehrlich's aldehyde reagent. Serum bilirubin levels were estimated by the method of King and Coxon (1950).

Materials

Samples of urine from 100 non-jaundiced patients picked at random from the wards of a general hospital and 200 urines from jaundiced patients were tested. Thirty-six urines containing urobilin or an excess of urobilinogen but no bilirubin were also studied. In 92 instances the serum bilirubin level was estimated at the time of testing the urine.

Specificity

The 100 urines from non-jaundiced patients were tested to determine whether false-positive reactions might result from the administration of drugs or from the presence of other abnormal substances in the urine. The patients were receiving many varied forms of treatment and suffering from a wide range of disorders. In no instance did the urine give a positive tablet reaction, and it therefore seems unlikely that false-positive reactions occur owing to the presence of commonly administered drugs in the urine. Sometimes there was seen on the mat a faint orange-brown colour which became more conspicuous after the mat had been standing for some time. This colour did not interfere with the reading of the test, and was presumably due to the presence in the urine of diazotizable non-bilirubin chromogenic material.

The presence of urobilin or an excess of urobilinogen in the urine does not give a false-positive result, for 36 urines with a positive Ehrlich or Schlesinger reaction were negative both by the tablet test and by Fouchet's method.

These results agree with those previously reported with the tablet test. Free and Free (1953) tested approximately 2,000 urine samples from general-hospital patients and failed to find any substance giving false-positive reactions. They also tested 100 urine samples from normally healthy adults; 99 gave negative reactions and one an unexplained trace reaction. Klatskin and Bungards (1953) found two false-positive tablet reactions in a series of 478 urines, and in both these instances there might have been some hepatic dysfunction. Giordano and Winstead (1953) found that the presence of increased amounts of urobilinogen in the urine did not mask the colour reaction as it did in the Harrison spot test, in which Fouchet's reagent is used.

Sensitivity

Of the 200 urines tested which gave either a positive Fouchet or a positive tablet reaction, 185 were positive for both methods; 192 gave a positive Fouchet and 193 a positive tablet reaction, while only 135 were positive with the iodine method.

Ninety urines giving a positive tablet reaction were also tested with the modification using the test mat and replacing the tablet by Fouchet's reagent: 58 gave a positive result and 32 a negative one.

These results suggest that the tablet test and Fouchet's method are of about equal sensitivity and are considerably superior to the iodine ring test and to the modification for the detection of bilirubin in the urine. Other workers found that the tablet test was more sensitive than methods using Fouchet's reagent (Klatskin and Bungards, 1953; Giordano and Winstead, 1953).

Quantitative analysis of bilirubin in urine is made difficult by the presence of interfering substances. However, an attempt was made to compare the quantitative urinary bilirubin values with the results for the qualitative tests. Eighteen urines were analysed and then diluted serially, and the lowest concentration giving a positive qualitative reaction was recorded (Table I). Although the results varied, the tablet test could usually detect bilirubin between 0.1

and 0.15 mg. per 100 ml. The Fouchet method was only slightly less sensitive, usually detecting concentrations between 0.15 and 0.20 mg. per 100 ml. The iodine method was always less sensitive than the other tests, and usually over 1 mg. per 100 ml. was needed for a positive result. The sensitivity did not alter with the cause of the bilirubinuria, whether due to obstruction of the bile passages, to infective hepatitis, or to portal or biliary cirrhosis.

These results agree well with those of Sobotka and his co-workers (1953), who found that the lowest concentration of urinary bilirubin giving a colour varied from 0.04 to

TABLE I.—Comparison of the Test Results with the Quantitative Analysis of Urinary Bilirubin

Diagnosis	Undiluted Urine Bilirubin (mg./100 ml.)	Lowest concentration of bilirubin (mg./100 ml.) in diluted urine giving positive test		
		Tablet	Fouchet	Iodine
Obstructive jaundice	8.5	0.13	0.27	4.25
Infective hepatitis	8.2	0.06	0.06	2.05
"	7.6	0.05	0.11	1.90
Cirrhosis	6.2	0.10	0.10	3.10
Obstructive jaundice	5.8	0.09	0.18	Neg.
Infective hepatitis	3.2	0.40	0.40	3.20
"	3.0	0.09	0.18	0.75
Obstructive jaundice	2.5	0.31	0.62	1.25
Subacute hepatitis	2.4	0.15	0.15	1.20
Cirrhosis	2.3	0.07	0.14	2.30
Obstructive jaundice	1.4	0.17	0.17	Neg.
Infective hepatitis	1.1	0.14	0.07	1.1
Obstructive jaundice	1.0	0.50	Neg.	Neg.
Cirrhosis	1.0	0.12	0.06	0.50
Obstructive jaundice	0.9	0.06	0.11	Neg.
Cirrhosis	0.9	0.22	Neg.	Neg.
Obstructive jaundice	0.7	0.02	0.04	"
Cirrhosis	0.13	Neg.	Neg.	"

0.1 mg. per 100 ml. Free and Free (1953) stated that 0.1 mg. of bilirubin per 100 ml. of urine usually gave a positive result. Klatskin and Bungards (1953) found that the first definite colour change was at 0.05 mg. per 100 ml., but 0.9 mg. was needed for a definite positive. These workers found that albumin in the urine would interfere with the sensitivity of the tablet test. This finding could not be confirmed in the present study.

Relation to Serum Bilirubin Level

Simultaneous serum bilirubin estimations and urine tests were performed on 92 occasions. In every instance the serum bilirubin level was greater than 1 mg. per 100 ml. (Table II).

TABLE II.—Comparison of Urinary Test Results with the Simultaneous Serum Bilirubin Concentration

	Serum Bilirubin (mg./100 ml.)		
	1-3.0	3.1-5.9	>6.0
No. of urines tested	23	19	50
Tablet positive	10	15	49
Fouchet	10	14	49
Iodine	2	7	47

Clinical icterus is apparent at 2 to 3 mg. of serum bilirubin per 100 ml., and at this level the tablet and Fouchet reactions may or may not be positive while the iodine reaction is usually negative. Even at values of 3 to 6 mg. per 100 ml. all these methods may give negative results. At serum bilirubin levels above 6 mg. per 100 ml. the reactions were usually positive.

The failure to detect bile pigment in the urine at low serum bilirubin levels can be attributed to the small number of patients with early infective hepatitis included in the series. In these subjects bilirubin occurs in the urine at low serum bilirubin levels. Cases of obstructive jaundice with low serum bilirubin values were also few in number. The majority of the patients studied when their serum bilirubin value was low were recovering from infective hepatitis, in which disease bilirubin is known to disappear from the urine in the presence of a raised serum bilirubin level.

For the early detection of bilirubin in the urine in the jaundiced subject the tablet test is at least as good as

Fouchet's method. The iodine method is not sensitive enough to justify its continued routine performance in ward side-rooms. The modification with the mat and Fouchet's reagent but no tablet is superior to the iodine ring method although inferior to the usual Fouchet's method and to the tablet test.

Summary

A new tablet test for urinary bilirubin is described which is specific for bilirubin, and of at least equal sensitivity to the Fouchet test while being quicker and easier to perform. The iodine ring test is of little value as a method of detecting small amounts of bilirubin in the urine.

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Medical Memoranda

Scrotal Pneumocele Complicating Induction of Artificial Pneumothorax

Although several cases of scrotal pneumocele complicating artificial pneumonoperitoneum have been reported it has not been possible to find any report of this occurring as a complication of artificial pneumothorax treatment. The following case is therefore recorded.

CASE REPORT

A man aged 40 was admitted to the London Hospital Annexe on March 17, 1952, with advanced bilateral pulmonary tuberculosis, and a previous history of a right inguinal herniorrhaphy some 25 years before. After treatment with bed rest and full courses of chemotherapy it was decided to attempt to induce a right artificial pneumothorax, although at the time it was realized that this might not be possible as extensive adhesions were to be expected.

Induction of a right artificial pneumothorax was carried out at 2 p.m. on December 17. The initial intrapleural pressures were -11 and -3 mm. of water, and there was a free rise and fall in the manometer. 350 c.cm. of air was introduced, the final pressures being -11 and 0 mm. of water.

During the course of the induction the patient had an attack of coughing, but did not complain of any difficulty in breathing or of pain, and there was no surgical emphysema at the completion of the induction. Shortly afterwards another bout of coughing began, again with no distress, but this time it was accompanied by the appearance of minimal surgical emphysema around the site of the injection. The cough was settled by the administration of linct. terp. heroin co., 1 dr. (3.5 ml.), and no more surgical emphysema developed.

However, at 9 p.m. another bout of coughing began, accompanied by some slight breathlessness. The patient went to sleep after this, but awoke at 11 p.m. to find that his scrotum was distended but not painful. On examination he was not distressed or dyspnoeic. Other than a small collection in the right axilla, surgical emphysema was minimal down the right side of the trunk and along the line of the right inguinal canal to the right side of the scrotum, which was distended to about 5 by 3 by 2 in. (12.5 by 7.5 by 5 cm.).

The patient was sedated and slept well, but by 10 a.m. the next morning surgical emphysema was extensive in the right axilla and the right side of the chest, pitting up to about 1 in. (2.5 cm.). It was minimal in the neck, but