—was an absolute and permanent preventive of smallpox, and he continued to maintain this view until the end. But it is possible that this indomitable assurance acted as a sheet-anchor to steady the bark in seas which were sometimes rough.

Of Jenner the man any country could be proud. To him fame came, at the age of 49, almost overnight, and he preserved a calmness and an equanimity which are much to be praised. Even in a country at war with Britain his name acted as a charm. At the end of the Treaty of Amiens two Englishmen were held in France, and were suffering from the effects of confinement. It was suggested that Jenner should write a letter to Napoleon asking for their release. This petition was read by Napoleon, who was about to refuse the request, when the name of the sender was pointed out to him. "Jenner!" said Napoleon. "We can refuse nothing to that man."

Certain celebrations were held on May 17 in connexion with this centenary. In the afternoon Sir Edward Mellanby gave an address at the Royal College of Surgeons on Jenner and his influence on research. In the late afternoon Sir Henry Dale opened an exhibition of manuscripts and relics of Jenner at the Wellcome Historical Medical Museum at 28, Portman Square; and in the evening a reception was held at the Royal College of Physicians.

EHRlich's ALDEHYDE TEST FOR UROBILINOGEN

BY

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AND

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Since Neubauer discovered in 1903 that the "mysterious substance" in urine described by Ehrlich as reacting with his aldehyde reagent was urobilinogen, the aldehyde test has come into routine use as an aid to diagnosis and prognosis in haemolytic anaemias and liver disease.

One of us (L. S. P. D.) has used this test for 25 years and has repeatedly stressed its value in lectures to undergraduate and postgraduate students. The investigation described below and a detailed study of the literature on tests for urobilinogen were undertaken as a direct result of a recent experience which gravely shook his confidence in the value of Ehrlich's aldehyde test.

A colleague in London, who was under his care for severe and prolonged infective hepatitis, had been instructed to test his urine with Ehrlich's aldehyde reagent at regular intervals and to keep a record of the findings. This record, together with other information, was sent to Edinburgh to enable progress to be assessed between consultations in London. The reaction for urobilinogen was strongly positive during the early stages of the disease, becoming weaker as the severity of the hepatitis steadily increased, and later becoming strongly positive when improvement began after three months' illness. Coincident with clinical recovery, the reaction again became weaker and finally negative. After a further three months, when the patient appeared to have made a complete clinical and biochemical return to normal, he reported that his urine again showed a red colour when tested with Ehrlich's aldehyde reagent. This made him doubt the

value of the test, so he proceeded to repeat it on samples of urine from various members of his household. In each case a positive reaction was obtained, the colour appearing rapidly and being indistinguishable from that obtained for urobilinogen. One of us (L. S. P. D.), having personally checked these findings, decided to investigate the matter further. The firm from which the patient had obtained his bottle of reagent was Savory and Moore, who have co-operated with us in every way by supplying information and samples of reagent.

It is right to point out at this stage that the reagent supplied by Savory and Moore to us and subsequently tested repeatedly was entirely satisfactory. The false positive reactions obtained by our patient were undoubtedly due to the presence of some oxidizing agent which had developed spontaneously or had been introduced accidentally into the bottle of reagent. The fact that this could occur was in itself an indication of the need for investigation into Ehrlich's aldehyde test.

Ehrlich's aldehyde reagent, which consists of a solution of paradimethylaminobenzaldehyde in hydrochloric acid, has been used by many workers, but, as may be seen from Table I, there have been considerable differences in the

TABLE I.—The Composition of Various Aldehyde Reagents and the Quantities of Reagent and Urine Employed

Origin or Type of Reagent	Quantity of Benzaldehyde in Reagent (%)	Quantity of HCl in Reagent (%)	Volume of Reagent Used (ml.)	Volume of Urine Used (ml.)	Final Concentration of Benzaldehyde (%)	Final Concentration of HCl (%)
(a) Reagents used by other workers:						
Watson (1944)	0.28	22	2.5	2.5	0.14	11*
Naumann (1936)	1.0	36	0.15	2.0	0.07	2.5
Wallace and Diamond (1925)	2.0	20	1.0	10.0	0.18	1.8
Farmer (1937)	2.0	18	1.0	10.0	0.18	1.6
Sparkman (1939)	6.6	18	1.0	10.0	0.60	1.6
Hutchison and Hunter (1940)	2.0	5	1.0†	3.0†	0.50†	1.1†
Chamberlain (1947)	3.0	18	0.1†	5.0	0.06†	0.35†
(b) Reagents tested in present investigation:						
Savory and Moore	2.0	18	0.5	5.0	0.18	1.6
Ehrlich's rosindole	0.87	0.6	1.0	5.0	0.14	1.1

* Immediately neutralized by sodium acetate.

† Approximate figures only, as authors stated quantity in "drops."

composition of the reagents used and in the relative quantities of reagent and urine employed. With the various reagents in current use the final concentration of hydrochloric acid varies between 0.35 and 2.5% (excluding Watson's reagent, in which the hydrochloric acid is immediately neutralized by sodium acetate).^{*} The final concentration of paradimethylaminobenzaldehyde varies between 0.06 and 0.6%. Terwen (1925) in his studies of urobilinogen used an ethereal solution of benzaldehyde. In the Royal Infirmary, Edinburgh, Ehrlich's rosindole reagent was issued many years ago for the urobilinogen test, and the wards have continued to use it with apparent satisfaction. This reagent was originally designed for testing indole formation by *Bacterium coli* and other organisms, and its composition is as follows: paradimethylaminobenzaldehyde, 4 g.; absolute alcohol, 380 ml.; and concentrated hydrochloric acid, 80 ml.

^{*}The percentages for hydrochloric acid concentration in Table I refer to the concentrations of hydrogen chloride calculated on the basis that concentrated hydrochloric acid represents a saturated aqueous solution (approximately 36% w/w) of hydrogen chloride.

In current British textbooks of clinical chemistry the aldehyde test for urobilinogen is not discussed in detail. Some authors prefer spectroscopy of the urine or the Jaffé-Schlesinger test for urobilin.

A study of the specialized literature on the subject reveals references to the lack of specificity of the aldehyde test, there being numerous substances other than urobilinogen which can give colour reactions with paradimethylaminobenzaldehyde. In addition, the detection of urobilinogen may be inhibited or masked by normal or abnormal constituents of urine. Interfering substances are listed in Tables II and III.

TABLE II.—Substances which are said to give rise to False Positive Results with Ehrlich's Aldehyde Reagent

Detectable at Room Temperature	Detectable Only After Heating	Positive Reaction with HCl Alone
Porphobilinogen Protein derivatives Phenazone Indole	Phenol Tryptophan Phenacetin Morphine	Urorosein Urofuscine Pyridium Phenylhydrazine Oxidizing agents added to urine

TABLE III.—Substances which are said to be Capable of Inhibiting or Masking Ehrlich's Aldehyde Reaction

Inhibitors	Masking Substances
Other aldehydes (e.g., formaldehyde, acetaldehyde) Hexamethylenetetramine Albumin Serum Mucus Excess alkali or acid Unknown substances in normal urine	Indican Urea Bilirubin Sulphonamides Extract of senna Rhubarb extract

Since the finding of urobilinogen in the urine is of definite clinical importance it is essential that the test for its detection should be simple and reasonably specific. It should not be so sensitive that it detects the amount of urobilinogen normally present in urine, nor so insensitive that it fails to show the presence of urobilinogen in amounts greater than normal. In view of the considerable variation in the reagents used by other workers and of the possible importance of substances interfering with the reaction, it was decided to investigate Ehrlich's aldehyde test.

The following points were studied: (1) The optimum conditions for the detection of urobilinogen in the urine with regard to temperature, age of the specimen, and composition of the reagents used. (2) The importance of substances in urine other than urobilinogen which may give rise to a red or pink colour with Ehrlich's aldehyde reagent. (3) The significance of factors likely to mask or inhibit the aldehyde reaction. (This aspect is not dealt with in the present communication.)

Three reagents were compared in this investigation: (a) the preparation in present use at the Royal Infirmary, Edinburgh—Ehrlich's rosindole reagent; (b) an aldehyde reagent supplied by Messrs. Savory and Moore; and (c) Watson's reagent.

As a preliminary to the investigation the amounts of rosindole reagent and Savory and Moore's reagent were determined which would produce the maximum colour reaction with 5 ml. of urine known to contain urobilinogen. The optimal amounts of reagent and urine used were determined from 40 tests carried out on 10 urines containing urobilinogen, and these quantities are shown in Table I. While these estimations were being made the following incidental points were noted:

1. Addition of excessive quantities of Savory and Moore's reagent inhibited the urobilinogen-aldehyde colour reaction. This was presumably due to the destruction of urobilinogen by excessive amounts of hydrochloric acid.

2. When the tests were conducted at room temperature (14–18°C.) the colour change on naked-eye estimation reached maximum intensity in three to five minutes. On the other hand, when the tests were conducted at 35°C. the speed of the reaction was increased, maximum colour change developing in half to one minute, and the colour was more intense than at room temperature.

3. Addition to the urine of varying amounts of a saturated aqueous solution of sodium acetate immediately after the rosindole or Savory and Moore's reagent accelerated the development of the colour in every case but did not increase its final intensity.

Watson's test was carried out according to the author's instructions—namely, the addition of 2.5 ml. of Watson's aldehyde reagent to 2.5 ml. of urine, followed immediately by 5 ml. of a saturated aqueous solution of sodium acetate (Watson *et al.*, 1944). The reader is referred to Watson's notable work on the metabolism of urobilin and particularly to his methods for quantitative estimation of urobilinogen in faeces and in urine. The present communication, however, is concerned only with simple clinical qualitative tests for the detection of urobilinogen in the urine.

The urines of 100 unselected ward patients were investigated in a variety of ways. The specimens were examined (a) within one hour of voiding, (b) about 18 hours after voiding, (c) at room temperature (14–18°C.), and (d) at 35°C. They were subjected to the following tests:

(i) Addition of the optimal amounts of rosindole reagent as shown in Table I.

(ii) Addition of the optimal amounts of Savory and Moore's reagent as shown in Table I.

(iii) Watson's test as described above.

(iv) Addition of Watson's reagent, 2.5 ml. to 2.5 ml. of

TABLE IV.—Number of Urines giving Colour Reactions with Aldehyde Reagents and Hydrochloric Acid

Reagent		" Fresh " Urines						" Old " Urines					
		Room Temperature			35° C.			Room Temperature			35° C.		
		Total	Col. CHCl ₃	Insol. CHCl ₃	Total	Sol. CHCl ₃	Insol. CHCl ₃	Total	Sol. CHCl ₃	Insol. CHCl ₃	Total	Sol. CHCl ₃	Insol. CHCl ₃
(i) Rosindole	A	20	20	Nil	29	28	1	11	11	Nil	14	14	Nil
	B	10	10	Nil	16	15	1	6	6	Nil	7	7	Nil
(ii) Savory and Moore	A	20	20	Nil	28	28	Nil	11	11	Nil	14	14	Nil
	B	10	10	Nil	15	15	Nil	5	5	Nil	7	7	Nil
(iii) Watson's plus acetate immediately	A	11	11	Nil	15	15	Nil	5	5	Nil	5	5	Nil
	B	6	6	Nil	8	8	Nil	3	3	Nil	5	5	Nil
(iv) Watson's plus acetate after 15 min. delay	A	21	15	6	32	18	14	27	15	12	29	16	13
	B	11	6	5	19	8	11	17	6	11	18	7	11
(v) 22% HCl (A.R.)	A	12	0	12	66	3	63	46	2	44	71	9	62
	B	9	0	9	62	1	61	38	1	37	60	1	59
(vi) 22% HCl (B.P.)	A	77	6	71	—	—	—	82	7	75	—	—	—
	B	71	2	69	—	—	—	78	4	74	—	—	—
(vii) 22% HCl (A.R.) plus H ₂ O ₂	A	89	7	82	—	—	—	86	8	78	—	—	—
	B	82	2	80	—	—	—	81	2	79	—	—	—

A = Total colour reactions of all hues. B = Total pink or red colours observed.

urine, with a delay of 15 minutes before adding the acetate solution.

(v) The addition of hydrochloric acid of analytical quality, 2.5 ml. of 22% aqueous solution to 2.5 ml. of urine. Hydrochloric acid of this standard of purity is subsequently referred to in the text as hydrochloric acid A.R.

(vi) The addition of hydrochloric acid, corresponding in quality to the standards laid down in the *British Pharmacopoeia*, in the same concentration and proportions as in test v. Hydrochloric acid of this standard of purity is subsequently referred to in the text as hydrochloric acid B.P.

(vii) The addition of hydrochloric acid of analytical quality as in test v, together with small amounts of an oxidizing agent, hydrogen peroxide.

All the aldehyde reagents used were made up with reagents of analytical quality. In tests v, vi, and vii large amounts of hydrochloric acid were used (actually equal to the amount in Watson's test), as it was thought that colour reactions due to substances other than urobilinogen would be more likely to appear at this high level of acidity. It is well known that the pigment produced by the urobilinogen-aldehyde reaction is soluble in chloroform. Accordingly, all the specimens were extracted with chloroform after submission to the tests described. A total of 5,600 tests were carried out on the 100 urines. The results are shown in Table IV.

A study of Table IV indicates the following points:

1. Out of the 100 urines tested 29 gave colour reactions of varying hues with Ehrlich's rosindole reagent, Savory and Moore's reagent, or Watson's test. A pink or red colour, however, was obtained in only 16 urines.

2. Colour reactions with all aldehyde reagents were more readily demonstrated at 35° C. than at room temperature.

3. Colour reactions with aldehyde reagents were more readily demonstrated in fresh than in old urines.

4. Delay in addition of acetate to specimens tested with Watson's reagent resulted in a considerable increase in the appearance of colouring substances insoluble in chloroform. This phenomenon was noted by Kelly *et al.* (1946). It was obviously due to colour reactions induced by the large amount of hydrochloric acid present in Watson's reagent which had not been neutralized until 15 minutes later.

5. Substances giving colour reactions with hydrochloric acid alone were more readily demonstrated in warm urines than in cold urines, and this applied both to fresh and to old specimens.

6. Of the 100 urines tested at room temperature with 22% hydrochloric acid A.R., 9 specimens gave a pink colour not extracted by chloroform, whereas when the same urines were tested under the same conditions with hydrochloric acid B.P. 69 gave a pink colour not extracted by chloroform. A similar high incidence of colour changes was noted when hydrochloric acid A.R. was added to the urines in the presence of a small quantity of hydrogen peroxide. From this it may be assumed that the principal impurity in hydrochloric acid B.P. leading to colour reactions is an oxidizing agent, and indicates the essential need for using hydrochloric acid A.R. in making up the Ehrlich aldehyde reagent. The substance which gives rise to a pink or red colour following the addition of impure hydrochloric acid to urine, and which is insoluble in chloroform, is believed to be an indole derivative, urorosein (Meiklejohn, 1948—personal communication). The production of urorosein depends both on the presence of an oxidizing agent and on the strength and quantity of hydrochloric acid used in the test. The high incidence of chloroform-insoluble pink substances observed when the addition of acetate was delayed in Watson's aldehyde test can possibly be explained on this basis.

In a previous investigation of the Ehrlich aldehyde test a different series of 45 urines had been tested with the rosindole reagent. Twenty positive colour reactions were recorded, and in six instances the colour observed, although macroscopically indistinguishable from that produced by urobilinogen, was not extracted by chloroform. Thus in

one-third of cases this reagent gave false positive results. On inquiry it was found that hydrochloric acid B.P. had been used in making up the reagent, as no instructions had been given to use analytical reagent. When the tests were repeated with rosindole reagent containing hydrochloric acid A.R. no colouring substances that were insoluble in chloroform were obtained.

Terwen (1925) and Watson (1931, 1936) have pointed out that the rapid conversion of hydrochloric acid to acetic acid by the addition of sodium acetate inhibits colour reactions due to indole. This procedure apparently also inhibits colour reactions due to indirubin. Thus when Watson's test is carried out the only important substances apart from porphobilinogen contributing to colour reactions are unidentified compounds having in general the same clinical significance as urobilinogen. The 15 urines which had given pink or red colour substances soluble in chloroform with rosindole reagent and Savory and Moore's reagent were accordingly submitted to the following procedure: 0.5 ml. of Savory and Moore's reagent was mixed with 5 ml. of urine followed immediately by 1 ml. of a saturated aqueous solution of sodium acetate. If a red or reddish-pink colour developed urobilinogen was considered present in an abnormal amount. In two of the 15 urines the colour reaction was inhibited and therefore the pink colour originally observed in these specimens was due to substances other than urobilinogen. In both instances the colour reactions had been only weakly positive, the colour change in one being perceptible only at 35° C., and in the other after several minutes at room temperature and at 35° C. This would suggest that weak delayed colour reactions with aldehyde reagents should be discarded as negative. The suggested lowest criterion of positivity is the development of a definite reddish-pink colour within five minutes at room temperature or within one minute at 35° C.

TABLE V.—A Comparison of Typical Colour Reactions Obtained from 100 Urines with the three Aldehyde Reagents Tested

Reagent	Temperature	Positive Results	Urobilinogen Proved Present	False Positive Results
Rosindole ..	14–18° C.	10	9	1
	35° C.	15	13	2
Savory and Moore's ..	14–18° C.	10	9	1
	35° C.	15	13	2
Watson's ..	14–18° C.	6	6	0
	35° C.	8	8	0

Table V gives results which enable the sensitivity of the three tests to be compared. It shows that tests with Ehrlich's rosindole reagent and Savory and Moore's reagent are more sensitive, although less specific, than Watson's test when read by the naked eye. The reduced sensitivity in Watson's test must be due in part to the threefold dilution of the urine with reagents.

The substances which are most likely to give rise to a reddish-pink colour when urine is tested with Ehrlich's aldehyde reagent are urobilinogen, indole, the precursors of two indole pigments—indirubin and urorosein—and porphobilinogen. Urorosein and porphobilinogen-aldehyde are insoluble in chloroform. The other pigments produced are soluble in chloroform. If the hydrochloric acid employed in the reagent is pure and free from oxidizing agents, and the reagent is used in small amounts, or if, as in Watson's test, the acid is immediately neutralized with sodium acetate, the reddish-pink colour developing in urine is unlikely to be due to any substance other than urobilinogen. Since, however, it is never possible to be certain that excessive amounts of hydrochloric acid or impure acid have not been used in making up the aldehyde reagent, and since the latter may become contaminated

with oxidizing substances, it is essential that the red or reddish-pink colour developing in the test should invariably be submitted to chloroform extraction. By this means the most important single fallacy—namely, a pink colour due to uroresin—can be excluded. If it is suspected that the substance giving rise to a pink colour soluble in chloroform is not urobilinogen (an exceptional finding if the reagents are properly prepared and if very weak delayed colour reactions are disregarded), then a saturated solution of sodium acetate should be added immediately after the aldehyde reagent to ensure the specificity of the test.

Conclusions

Ehrlich's aldehyde test is a simple and valuable clinical procedure for the detection of abnormal amounts of urobilinogen in the urine. The reaction is more sensitive if urines are tested in the fresh and warm state.

The following precautions should be rigidly observed: (1) The hydrochloric acid in the test reagent should be of analytical standard and used in appropriate amount. (2) The reagent should be put out for use in small drop-bottles, and contamination with pipettes, etc., should be avoided. (3) The reaction should be considered negative unless a definite reddish-pink colour develops within five minutes at room temperature or within one minute at 35° C. (4) Chloroform extraction should be undertaken in every urine giving a red or reddish-pink colour subsequent to the addition of aldehyde reagent. (5) Sodium acetate solution should be added immediately after the addition of Watson's reagent if Watson's test is employed. The addition of sodium acetate is unnecessary if Savory and Moore's reagent is employed in the amount used in these experiments. (6) The reagent should not contain alcohol, as this is unnecessary and more likely to produce false positive results with indole.

Further work is required to determine the optimal quantities of paradimethylaminobenzaldehyde and hydrochloric acid to be included in reagents used for qualitative estimation of urobilinogen and the relative amounts of reagent and urine to be employed in the test. Having been determined, these quantities should be strictly adhered to, as by this means the inhibition of specific urobilinogen colour reactions and the production of colour reactions due to indoles and other unknown substances will be reduced to a minimum.

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The Minister of Health has decided, after consultation with the Central National Health Service (Chemist-Contractors) Committee, that the use by contractors of envelopes or other paper containers for tablets, pills, capsules, pastilles, lozenges, and for bulk powders is undesirable, and that they should not be used for this purpose after May 1, 1949. The British Standards Institution is considering minimum standard requirements for cardboard or paperboard containers. Until the inclusion in the Drug Tariff of a reference to these requirements, boxes used for a small number of tablets, pills, etc., must be large enough to bear a label on which the directions can be clearly shown.

RETENTION OF URINE*

BY

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Among the many possible causes of inability to pass urine prostatic obstruction is the most frequent in everyday practice. Paralytic or neurogenic causes occupy second place, and stricture nowadays comes third because its principal cause, gonorrhoea, is more effectively treated. Retention from prostatic obstruction can be taken as the example for description, but the same principles of treatment are applicable to other cases.

Retention implies a full bladder from which either nothing can be passed, as in acute retention, or only a dribbling overflow escapes, leaving a large amount of residual urine, as in chronic retention. It must be distinguished from the more serious condition of suppression of urine in which the bladder is empty. It is therefore necessary at the outset to be able to recognize a full bladder, and to do it without having to pass a catheter, the method recommended in some gynaecological textbooks. It is generally easy by ordinary clinical methods. There is a rounded or irregular swelling arising from the pelvis, fixed below, dull on percussion, cystic, either tense or soft, and sometimes translucent from side to side. In cases of doubt examination with the foot of the bed raised will occasionally make it more evident. Sometimes, however, there are difficulties, especially in a fat patient, and particularly where there are no urinary complaints and the condition is not suspected.

A man of 64 went to his doctor complaining of general ill-health, with loss of weight, morning vomiting, and loss of taste. He had no urinary symptoms and did not have to get up at night to micturate. He was sent for a complete barium-meal examination, and the radiologist discovered a pelvic tumour, outside the bowel but pushing it up. The tumour was an enlarged bladder. The prostate was moderately enlarged and the blood urea was 84 mg. per 100 ml., the urine having a specific gravity of 1002. After the bladder had been gradually decompressed through a small suprapubic catheter his symptoms ceased, the blood urea fell to 30 mg. per 100 ml., and after prostatectomy he recovered.

Clinical Types of Retention

Acute Retention.—There is often no sharp dividing-line between acute and chronic retention, and while the amount of residual urine is generally large in chronic retention this factor alone does not give a complete definition. Acute retention is of sudden onset and is painful. It follows congestion and is brought about by cold, wet, and alcoholic, dietary, or sexual excess. Brodie in 1822 said: "The exciting cause is sometimes cold. One gentleman will be affected after drinking punch, another after drinking more wine than usual. One gentleman I knew was seized after two or three days' costiveness. The cause is often too minute to be detected." Other potent causes of acute retention are enforced holding of urine when the call comes and confinement to bed for some intercurrent disease such as bronchitis, pneumonia, or heart failure. A painful acute retention may be superimposed on a degree of chronic retention. The clinical picture of acute retention is unmistakable: there is a constant urge to micturate, but inability to expel more than a few bloody drops, with severe pain in the penis and lower abdomen, restless changes

*Based on a lecture given to the Leeds Branch of the British Medical Association on Feb. 9.