PHENOTHIAZINES IN URINE

By

S. GOLD

P. D. GRIFFITHS

and

R. G. HUNTSMAN

PATIENTS in the care of the Department of Psychological Medicine, Guy's Hospital, who are receiving moderate and large doses of phenothiazine drugs are regularly tested for evidence of hepatic or haematological disturbance. In the course of such an investigation, when the urine of a schizophrenic patient was tested with modified Ehrlich's reagent for urobilinogen, it was remarked that a deep blue colour was produced. The patient had been receiving 600 mg. of chloropromazine daily in divided doses for the previous 10 days, and as no other cause for this unusual result could be found, the phenothiazine was considered to be responsible.

Other workers have also noted the production of colour reactions with phenothiazines, especially chlorpromazine, in various circumstances, and a review of the available literature revealed that such reactions have been used as the basis of tests for the presence of these compounds or their metabolites in urine (Forrest and Forrest, 1957; Forrest, Forrest and Mason, 1958; Neve, 1958; Nellhaus, 1959; Hayman, Almudevar and Merlis, 1959; O'Toole, 1960; Hayman, Bayne and Merlis, 1960). Forrest and his co-workers have been particularly active in this field, and with the advent of the newer and perhaps more efficacious phenothiazine derivatives, they have developed a series of tests for the detection of these substances in urine claiming both qualitative and quantitative accuracy for them (Forrest, Forrest and Mason, 1958, 1959, 1960; Forrest and Forrest, 1960).

The clinical application of these tests becomes apparent when it is recalled that the types of patient receiving phenothiazine drugs are difficult to manage, and it is imperative that the practitioner should be able to evaluate the real intake of the drug so that he can check whether a given patient is, in fact, refractory to the drugs and also to eliminate wastage of drugs (Forrest, Forrest and Mason, 1958, 1960). To date, these problems have been considered largely in the context of the mental hospital environment. Perhaps of greater significance in this country is the out-patient control of remitting or mild schizophrenic patients. Following the recent trends in the management of psychotic patients, it is likely that many more of these patients will be cared for in the community with, of course, the assistance of the local practitioner. Many of the current methods for estimating and detecting chlorpromazine and other phenothiazines (Dubost and Pascal, 1955; Leach and Crimmin, 1956; Cavanaugh and Ervin, 1957; Kinberger, 1959) require extensive laboratory facilities, so that a reliable and simple method of checking on the drug intake of these patients would be a valuable aid to their management.

These considerations prompted us to undertake a survey of the available rapid procedures for the detection and estimation of phenothiazines and to test their validity. The phenothiazine derivatives concerned in this work were

https://doi.org/10.1192/bjp.108.452.88 Published online by Cambridge University Press

chlorpromazine, promazine, perphenazine, trifluoperazine, thioridazine and perchlorperazine. A rapid test for one of the newer anti-depressant drugs, imipramine, which bears chemical similarities to the phenothiazines (Forrest, Forrest and Mason, 1960; Hegarty, 1960) was also included as similar considerations apply to its use.

METHODS

The specimens of urine were obtained from patients in the psychiatric wards of a teaching hospital and the wards of a large mental hospital. Specimens were usually collected 2 hours after the administration of the morning dose of the drugs. There were 329 patients in the series, 37 of whom were receiving 2 drugs and 5 who were on no drugs. The drug and dose being given to each patient was known to one author, all the tests being carried out by the other two. Following a pilot study, in which numerous tests and modifications were used, it was finally decided to subject each urine specimen to a battery of 7 tests. These were carried out within 6 hours of receipt of the specimens.

1. *FPN Group Test for Phenothiazines*

One ml. of urine is treated with 1 ml. of FPN reagent consisting of 5 parts of 5 per cent. ferric chloride, 45 parts of 20 per cent. perchloric acid and 50 parts of 50 per cent. nitric acid (Forrest and Forrest, 1960). The colour produced, even where high doses of phenothiazines (125 mg. daily or more) are being taken by the patient, is unstable and the colour must be noted almost immediately after adding the reagent. The colour produced varies from a light pinkish-orange with doses of 5–20 mg. daily through various shades of pink to increasingly intense shades of purple with increasing amounts of phenothiazines, an ink-like appearance occurring with very large amounts.

2. "*Phenistix*"

This is a cellulose strip impregnated with ferric and magnesium ions and cyclohexylsulphanilic acid which was designed for the detection of phenylpyruvic acid in urine (Ames and Co., London). The treated end of the reagent strip is dipped into the urine and the colour read after 30 seconds. When a colour change occurred only a definite purple was accepted as a positive result for the presence of phenothiazines.

3. *Forrest's Test for Chlorpromazine and Promazine*

One ml. of a reagent consisting of 4 parts of 10 per cent. sulphuric acid to 1 part of 5 per cent. ferric chloride is added to 1 ml. of urine and the colour noted immediately after mixing. A violet colour, pale with amounts of the order of 100 mg. daily and inky with amounts greater than 900 mg. daily, is taken as indicating chlorpromazine, and a coral pink through crimson to a muddy and finally deep pinky-violet as indicating similar amounts of promazine (Forrest, Forrest and Mason, 1958).

4. *Neve's Test for Chlorpromazine*

In this test, 0·5 ml. of a freshly-prepared reagent consisting of 10 parts of 27 per cent. nitric acid and 1 part of 2·5 per cent. sodium nitrite is added to 5 ml. of urine. As originally described, a red or reddish-violet colour with 150–175 mg. daily and a dark blue or bluish-violet with larger amounts are accepted as positive results for chlorpromazine, other phenothiazines not being discussed

https://doi.org/10.1192/bjp.108.452.88 Published online by Cambridge University Press

(Neve, 1958). However, it was noted that on the whole chlorpromazine gave blue colours, whereas when reddish colours were obtained this was often due to promazine, and an attempt at differentiating between the two drugs was made using this observation.

5. *Test for Piperazine-linked Phenothiazines*

One ml. of 10 per cent. trichloracetic acid is added to 1 ml. of urine. After shaking 1 ml. of mercuric nitrate reagent (M/1,000 mercuric nitrate in concentrated hydrochloric acid) is added. The colour, which is pale pink with 10–30 mg. daily of the drugs, progressing through pink and pink-violet to violet with 120 mg. daily or more, is read after 30 seconds (Forrest, Forrest and Mason, 1959).

6. *Test for Piperidine-linked Phenothiazines*

The reagent used is 5 per cent. ferric chloride (2 parts) and 30 per cent. sulphuric acid (98 parts). One ml. of this is added to 1 ml. of urine and the colour read 20–30 seconds after mixing. Urines from patients on 75–150 mg. of the drugs daily give a pale purplish-pink colour which deepens becoming an opaque deep violet and finally a definite blue with 800 mg. or more of the drug. (Forrest, Forrest and Mason, 1960b.)

7. *Test for Imipramine*

To 1 ml. of urine is added 1 ml. of a reagent consisting of equal parts of 0·2 per cent. potassium dichromate, 30 per cent. sulphuric acid, 20 per cent. perchloric acid and 50 per cent. nitric acid. Doses of 25–50 mg. daily lead to the development of a transitory pale olive colour, larger amounts a light green, then an emerald, and 150–250 mg. daily a deep turquoise. (Forrest, Forrest and Mason, 1960.)

Results

The results of all the qualitative tests are summarized in Table I. These show that the FPN, Forrest's and Neve's tests were reasonably satisfactory for

Table I

Number of correct qualitative results with tests for Phenothiazines

Drug	Chlorpro-mazine	Pro-mazine	Perphen-azine	Trifluo-perazine	Perchlor-perazine	Thiorid-azine	Imi-pramine
No. of patients	101	42	50	50	2	66	50
Test							
F.P.N.	90	40	8	15	0	42	(3)
Phenistix	5	30	0	0	0	8	0
Forrest's	86	40	(6)	(3)	0	(2)	0
Neve's	80	40	4	4	0	6	0
Piperazine-linked	+	+	10	10	1	+	0
Piperadine-linked	+	+	+	+	+	34	0
Impramine	+	(2)	+	+	0	+	37

( ) Positive result with drugs other than those for which test was designed.
+ Production of colours other than those regarded as a positive for the particular test.

https://doi.org/10.1192/bjp.108.452.88 Published online by Cambridge University Press

the detection of chlorpromazine and promazine, but the results with the other tests are not impressive. The FPN test was apparently better for the detection of thioridazine than its special test. The piperazine-linked group was not detected readily with any of the tests. The test for imipramine was only moderately satisfactory but rarely gave false positive results. As other phenothiazines will produce interfering colours with the tests for the piperazine and piperidine-linked groups, the results obtained with the appropriate phenothiazines only are considered.

Table II summarizes the results of the quantitative assessments. These were disappointing, the best being Forrest's test in which 41 per cent. correct results were obtained.

TABLE II

Number of correct quantitative results with tests for Phenothiazines

Drug	Chlorpro-mazine	Pro-mazine	Perphen-nazine	Trifluo-perazine	Perchlor-perazine	Thiorid-azine	Imi-pramine
No. of patients	101	42	50	50	2	66	50
Test							
F.P.N.	18	12	3	7	0	3	—
Forrest's	42	15	—	—	—	—	—
Piperazine-linked	—	—	all in lowest dosage range			—	—
Piperadine-linked	—	—	—	—	—	20	—
Imipramine	—	—	—	—	—	—	2

Figures are given only for the drug where the appropriate test was employed.

The attempts to distinguish chlorpromazine from promazine were similar in both Forrest's and Neve's tests there being 75 per cent. correct results in cases in which the phenothiazines had been detected.

DISCUSSION

Among the difficulties encountered in the management of many disturbed patients is the attempt of some to conceal from their medical advisers that they are failing to take such drugs as are prescribed for them. When such patients do not respond as well as might be expected, it is vital to know if they really are taking their medicines and if so, whether they are taking the full amount. Although there are many adequate methods for detecting and measuring the excretion of phenothiazines in the urine, most of these are unsuitable for use in the ward, side room or the general practitioner's surgery. The various tests that have been considered in this paper have the advantages that they require the minimum of apparatus, they are simple to carry out and take very little time.

The first of the tests examined was the FPN test which is a general test for the whole group of the phenothiazines. It might be argued that this test is of limited use in that the phenothiazine drug being taken by the patient is on the whole known and it is perhaps better to use a more specific test. On the other hand, it would be considerably simpler to be able to use only one test, instead of being equipped for the specific tests as well. Furthermore, in cases of suspected poisoning with phenothiazines this might serve as a useful and rapid

https://doi.org/10.1192/bjp.108.452.88 Published online by Cambridge University Press

screening test for the presence of one of these drugs. In our hands, however, this test was only successful with chlorpromazine and promazine. One difficulty lies in the fact that with the lower dosage ranges the colour produced is only transitory and is easily missed, especially with concentrated urines, in that one is trying to detect a light pinkish-orange colour which appears briefly often while one is still adding the reagent.

The use of "phenistix" for detecting chlorporomazine has been described by Nellhaus (1959). Although in 15 per cent. of the patients the grey to grey-green colour of the 15–40 mg. range of phenylpyruvic acid was obtained, it was decided only to record as positive those specimens in which a definite purple coloration appeared. In these conditions, promazine and not chlorpromazine was the only drug to be detected with any regularity, but chlorpromazine and thioridazine were detected occasionally. There were, however, no false positive results. None of the patients was also taking para-aminosalicylic acid, which may produce the purple coloration of "phenistix" (Luntz and Austin, 1960).

Although the same measure of success as the authors of Forrest's test for chlorpromazine and promazine was not achieved, the drugs were detected in 91 per cent. of cases. Of the 9 per cent. negative results which were obtained, half gave a negative result with all the other tests, and in fact one patient was proved not to be taking his prescribed medication. At high dosage levels the distinction between chlorpromazine and promazine is made difficult by the occurrence of intermediate shades, this perhaps accounting for the reduced accuracy when an attempt is made to distinguish between the two drugs.

Neve's test for the detection of chlorpromazine was not quite as successful as Forrest's, and as we were unable to confirm Neve's contention that small doses of chlorpromazine gave reddish colours and high doses blue colours, this test did not appear suitable for quantitative purposes. On the other hand, the colours produced were more stable than those in the other tests described. There were 6 per cent. of false positive results but all these patients were receiving other phenothiazines.

The group of patients available were receiving doses of the piperazine-linked phenothiazines (perphenazine, trifluoperazine and prochlorperazine) which were too small to make the investigation of the specific test for this group comprehensive. Certainly where doses of less than 30 mg. per day were being given this test appeared to be of little value, and in this country most out-patients receiving these drugs are on doses of this order. Unlike the preceding tests, this is not one that is very suitable for nurses to carry out, as it involves the pipetting of a reagent which is largely fuming concentrated hydrochloric acid, this being unpleasant and dangerous for the inexperienced to handle.

The piperidine group of phenothiazines was represented in this trial by thioridazine. Despite the fact that all the patients were receiving substantial doses of the drug, it was disappointing to find that only 52 per cent. were detected by means of the specific test, whereas the more general test using the FPN reagent succeeded in detecting 66 per cent.

Although not a phenothiazine, imipramine is a valuable addition to the therapy available in the field of psychotic depression. The test for this drug like those for the phenothiazine group is simple and produces a colour, green, which is observed more readily than some of those produced in the other tests. Although roughly three-quarters of the patients receiving the drug were detected there was little correlation between the drug dosage and depth of colour produced. Of the other patients on the drug, who were not detected by the test, half were receiving phenothiazines as well (usually chlorpromazine). These produce their

https://doi.org/10.1192/bjp.108.452.88 Published online by Cambridge University Press

own colour, masking that of imipramine, so that the test is probably better qualitatively than the results suggest.

It will have been noticed that in all cases except "phenistix" a major part of the reagent used is a strong acid, and this probably accounts for the unusual colour production with modified Ehrlich's reagent in our patient. Unfortunately, this was only an incidental finding and a similar result was only very rarely obtained in other patients on phenothiazines. It is of interest that para-aminosalicylic acid in urine produces a colour both with Ehrlich's reagent (Venkataram *et al.*, 1948; Herold, 1951, Penman and Wraith, 1956, Ruiz, 1957) and "phenistix". The major problem has been the production of a stable colour and the incorporation of ferric chloride, which also proved helpful in the detection of para-aminosalicylic acid (Simpson, 1956, Penman and Wraith, 1956; Dixon *et al.*, 1957), has been a considerable advance.

In our hands the tests have not been as successful as their authors claimed, they having achieved an almost absolute accuracy. It seems unlikely that those who might use these tests in the circumstances discussed in this paper, e.g., nurses, would achieve better results than laboratory workers. Chlorpromazine and promazine can be detected in most cases by the FPN, Forrest's or Neve's test, but as far as the other phenothiazines are concerned the number of false negative results obtained with the specific tests reduces their value considerably. Admittedly more patients may be omitting their drugs than was discovered, so the tests may be a little better than our results suggest. However, since the FPN test detected chlorpromazine and promazine as well as did the specific tests, and was perhaps better than the specific test for the piperazine-linked group and clearly superior for the piperidine-linked group, one is led to conclude that only the FPN test need be retained as a rapid screening test for the presence of phenothiazines in urine. Where appropriate, the test for the related drug imipramine would also be necessary.

Summary

The importance of being able to check quickly the drug intake of patients receiving phenothiazines and allied drugs is considered.

Urine specimens from 329 psychiatric patients were tested by a battery of 6 rapid and simple tests for phenothiazines and 1 for imipramine.

Only chlorpromazine and promazine could be detected consistently. Imipramine was detected in 75 per cent. of cases. The tests for the piperazine- and piperidine-linked phenothiazines were much less successful.

The FPN test appeared to be the most valuable of these simple methods. The need for an improved group reagent is noted.

If these tests are used, whereas a positive result is to be accepted, a negative must be treated with reserve. As the tests are unreliable for quantitative purposes also, an improved form of the tests is clearly desirable.

Acknowledgments

We would like to thank the physicians of the York Clinic, Guy's Hospital, and of Bexley Hospital for access to the patients under their care, the staff of the wards for co-operation in collection of specimens and Drs. E. N. Allott, W. H. H. Merivale and D. Stafford-Clark for help and encouragement.

References

Cavanaugh, D. J., and Ervin, F. R., *Amer. J. Psychiat.*, 1957, **114**, 171.
Dixon, W. M., Stradling, P., and Wootson, I. D. P., *Lancet*, 1957, **2**, 871.
Dubost, P., and Pascal, S., *Ann. Pharm. franc.*, 1955, **11**, 615.

https://doi.org/10.1192/bjp.108.452.88 Published online by Cambridge University Press

FORREST, F. M., and FORREST, I. S., *Amer. J. Psychiat.*, 1957, **113,** 931.
Iidem, ibid, 1960a, **116,** 840.
FORREST, F. M., FORREST, I. S., and MASON, A., *Amer. J. Psychiat.*, 1958, **114,** 931.
Iidem, ibid, 1959, **116,** 549.
Iidem, ibid, 1960b, **116,** 928.
Iidem, ibid, 1960c, **116,** 1,021.
FORREST, I. S., and FORREST, F. M., *Clin. Chem.*, 1960, **6,** 11.
HAYMAN, J. J., ALMUDEVAR, M., and MERLIS, S., *Amer. J. Psychiat.*, 1959, **116,** 259.
HAYMAN, J. J., BAYNE, B., and MERLIS, S., *ibid*, 1960, 1,108.
HEGARTY, J. G., *Brit. med. J.*, 1960, **1,** 1,736.
HEROLD, K., *Klin. Wschr.*, 1951, **29,** 199.
KINBERGER, B., *Nord. psykiat. Tidsskr.*, 1959, **13,** 95.
LEACH, H., and CRIMMIN, W. R. C., *J. clin. Path.*, 1956, **9,** 164.
LUNTZ, G. R. W. N., and AUSTIN, R., *Brit. med. J.*, 1960, **1,** 1,679.
NELLHAUS, G., *J. Amer. med. Ass.*, 1959, **170,** 1,052.
NEVE, H. K., *Ugeskr. Laeg.*, 1958, **120,** 250.
O'TOOLE, C. P. O., *Brit. med. J.*, 1960, **2,** 1,382.
PENMAN, H. G., and WRAITH, D. G., *Lancet*, 1956, **2,** 552.
RUIZ, R. C., *Tubercle* (*Lond.*), 1957, **38,** 152.
SIMPSON, J. McD., *ibid*, 1956, **37,** 333.
VENTKATARAM, A., VENTKATARAM, P. R., and LEWIS, H. B., *J. biol. Chem.*, 1958, **173,** 641.

S. Gold, M.B., B.S., D.P.M., *Registrar in Psychological Medicine*

P. D. Griffiths, M.B., B.S., B.Sc., *Senior Registrar in Clinical Pathology, Guy's Hospital, S.E.1*

R. G. Huntsman, M.A., M.B., B.Ch., M.R.C.P., *Consultant Pathologist, Peterborough Group of Hospitals*

https://doi.org/10.1192/bjp.108.452.88 Published online by Cambridge University Press