Discussion

There are reports of successful treatment of subacute bacterial endocarditis with phenoxymethylpenicillin. Quinn *et al.* (1956) describe four cases, in two of which a *Strep. viridans* infection was treated successfully with 7.2 g. daily for six weeks. In the third patient the infection was due to a species of *Neisseria*, and treatment comprised oral phenoxymethylpenicillin together with parenteral streptomycin and dihydrostreptomycin. The fourth case was less successful owing to a penicillin-resistant *Micrococcus pyogenes*. Martin *et al.* (1956) treated two cases successfully with doses of 12,000,000 units daily, supplemented with intramuscular streptomycin.

In the two cases reported here the use of oral penicillin was indicated by the difficulties associated with parenteral therapy. The first patient was beginning to respond favourably when intramuscular penicillin had to be abandoned owing to local reactions following the mechanical trauma of injections. The persistence of pyrexia in this patient, when the infecting organisms showed such a marked sensitivity to penicillin and the penicillin blood levels seemed quite adequate therapeutically, was thought to be due to the extensive tissue destruction associated with these local reactions. The rapid fall of temperature on withdrawal of injections tended to confirm this. In Case 2 parenteral therapy was not used at all, as the severe emaciation and loss of muscle tissue, especially of the buttocks, contra-indicated a prolonged course of frequent injections. The high sensitivity of the infecting organism to penicillin and the therapeutic blood levels attainable with phenoxymethylpenicillin (see Table) were additional indications for this form of therapy. Though the use of phenoxymethylpenicillin may be associated with gastro-intestinal side-effects, they did not occur in these two patients, even with a prolonged course of treatment.

The two important considerations in the treatment of subacute bacterial endocarditis are the production of frequent high peaks of penicillin concentration in the blood (Finland, 1954; *Lancet*, 1954) and a prolonged course of therapy lasting at least four to six weeks (Bloomfield, 1950; Cates and Christie, 1951; Hunter, 1951), although satisfactory results have been claimed with shorter courses of penicillin (Hamburger and Stein, 1950). Among the factors predisposing to relapse is a short course of treatment (Cates and Christie, 1951). Whereas phenoxymethylpenicillin cannot produce penicillin blood concentrations comparable to those of parenteral penicillin, it does offer an easy and effective method of continuing therapy after discharge from hospital, and consequently of reducing the risk of relapse. With parenteral penicillin the difficulties associated with a prolonged course of out-patient treatment are self-evident.

Whereas the immediate results of therapy in both these patients have been completely satisfactory, long-term observation is required to see that these results are maintained. The first patient has been under observation for six months with no recrudescence of infection while on a prophylactic dose of 240 mg. four-hourly. The second patient is on the same prophylactic dose and has been followed up for two months after discharge from hospital.

Summary

The use of oral phenoxymethylpenicillin in two cases of subacute bacterial endocarditis due to a penicillin-sensitive *Streptococcus viridans* is described. In one case treatment was initiated with parenteral crystalline penicillin and maintained with phenoxymethylpenicillin for nine months, and in the other the oral preparation was used alone for three months. No gastro-intestinal or other side-effects occurred. The importance of penicillin blood concentrations and duration of treatment is considered.

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DETERMINATION OF PEROXIDASE IN URINE

SIMPLE METHOD FOR ROUTINE EXAMINATION OF URINE FOR ERYTHROCYTES AND LEUCOCYTES

BY

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In diseases characterized by the presence of pathological amounts of erythrocytes and leucocytes in the urine (infections of urinary tract, tumours, calculi, nephritis, renal infarction, etc.) the correct diagnosis is generally easy if these cells or other protein are found in such quantity that a routine protein test is positive, indicating need for further examinations. If the number of cells is so small that the routine test is negative, microscopy of the centrifuged deposit will prove necessary for the diagnosis. When the symptoms do not suggest the diseases in question, however, this examination is often overlooked in routine consultation work, to the detriment of correct diagnosis. Unfortunately the local symptoms indicating the answer are fairly often absent. Infections of the urinary tract constitute an example in point, and they rank first among the diseases in question in regard to incidence.

At the Third Medical Clinic of the University of Helsinki an accurate routine examination was made of the urine of every female patient admitted. Infection of the urinary tract was diagnosed in 147 (13.1%) of 1,141 patients; symptoms suggestive of the disease were felt by only 35.4% of the patients in whom it was diagnosed (Halonen and Halonen, 1950). However, a routine examination calling for the centrifugation and microscopy of the urine is a fairly laborious task for the practising physician to perform for each patient. There should be a simpler method of determining the pathological erythrocyte and leucocyte count of urine.

Several methods of this type have been suggested. Caplan and Discombe (1951) made an analysis of the methods of estimating urine erythrocytes. Among the most recent methods applied in practice are Baron's (1951) modification of the *o*-tolidine test, Watson-Williams's (1955) tablet test, and the method of Balme *et al.* (1956) working on the same principle and enabling also the quantitative determination of erythrocytes. The last-mentioned method is based on the circumstance that iron-containing blood derivatives catalyse the oxidation of amidopyrine by hydrogen peroxide to produce a lilac colour.

I have found a method, which I believe is simpler than those suggested so far, of determining the presence of pathological quantities of erythrocytes and leucocytes in urine. As the results have proved good in practice, the method is here reported.

Method

This is based on the ability of the peroxidase ferment present in erythrocytes and leucocytes (also haemoglobin) to dissolve hydrogen peroxide. The volume of oxygen given off when hydrogen peroxide decomposes is dependent on the erythrocyte and leucocyte count of the urine sample. Bearing in mind the apparatus ordinarily available to the practitioner, I sought the simplest possible method of determining the quantity of this gas produced. The following procedure was evolved. The only reagent needed is 30% hydrogen peroxide. The apparatus is that employed in the conventional determinations of the erythrocyte sedimentation rate (Westergren).

Two ml. of urine is poured into the small tube. (No pipette is necessary, as it was found that the quantity need not be accurately measured.) It is most helpful if the tube has a 2-ml. graduation mark. Four drops of 30% hydrogen peroxide are then added, and the mixture is immediately put into the E.S.R. tube proper, up to the usual mark. It is advisable to do the stirring first, by drawing the mixture into the tube and expelling it several times. The height to which the liquid or any froth on it rises above the original graduation mark is read after 30 minutes in the usual manner in millimetres. No rise is found in a normal case.

This method I call the peroxidase amount determination (P.A.).

Controlling the Method

Result with Healthy Test Subjects.—The urine samples of 40 healthy persons (20 of both sexes), in which nothing pathological was found by microscopy of the centrifuged deposit, gave a P.A. reading of 0 mm. in 38 cases and 1 mm. in two cases (both females).

Controlling Accuracy of the Method

1. Given amounts of erythrocytes were added to the urine of six different males; the samples had shown a minimum of sediment when subjected to routine examination. A comparison was made between the different erythrocyte dilutions and the P.A. readings obtained. The procedure was repeated, using 0.9% sodium chloride solution as erythrocyte diluent. The results are shown in Table I. The P.A. reading was over 1 mm. (positive) without exception in samples with an erythrocyte count of over 25/c.mm. Samples with 10 and 20/c.mm. gave an uncertain positive result (P.A. 1 mm.) in two out of six cases. Samples with 25/c.mm. likewise gave an uncertain positive result (1 mm.) in one case out of six and a positive result (2 mm.) in one case; in the other cases the result was negative. The results were even clearer in sodium chloride solution. Over 25 erythrocytes per c.mm. always gave a positive result. With this amount and quantities below it the result was without exception negative.

2. The effect of the urine leucocyte content on the P.A. value was examined by preparing dilution series from urine

containing a number of leucocytes but no other cell elements. In addition to the P.A. determination, routine microscopy of the centrifuged sediment was also performed on the samples of these series. The results are shown in Table II. The P.A. values corresponded fairly well with the dilution ratios—even better than the results given by microscopy.

TABLE I

Erythrocytes per c.mm.	Turbidity of Urine	Test for Urine Protein	P.A. Readings (mm./30 min.). Minimum and Maximum Value from Six Different Series
5,000	Turbid	Positive	100 * (100)
2,500	Slightly turbid	Negative	100 † (100)
1,000	Clear	"	100 ‡ (100)
500	"	"	26-39 (33)
250	"	"	12-26 (24)
100	"	"	5-19 (6)
50	"	"	2-12 (2)
25	"	"	0-2 (0)
20	"	"	0-1 (0)
10	"	"	0-1 (0)
8	"	"	0-0 (0)
5	"	"	0-0 (0)

Figures in parentheses are the results with 0.9% NaCl solution instead of urine (mean).

* Maximal value reached in 1-2 minutes. (In NaCl solution, 32 sec.).

† Reached in 3-6 minutes (NaCl, about 5 minutes).

‡ Reached in 6-10 minutes (NaCl, about 11 minutes).

TABLE II.—Effect of Quantitative Dilution of Urine Containing About 800 Leucocytes per c.mm. on the P.A. Reading

Leucocytes per c.mm.	P.A. Reading	Leucocytes per Field of Vision (Enlargement × 290) in Microscopy of Centrifuged Deposit (Mean Value from 10 Counts)
800	100*	40.1
100	45	12.4
50	25	7.2
25	9	4.2
20	5	2.9
5	0	1.0

* Maximal value reached in 10 minutes.

TABLE III.—Relation of P.A. Readings to Microscopy of the Centrifuged Deposit

Erythrocytes or Leucocytes per Field of Vision (Enlargement × 200) by Microscopy of Centrifuged Deposit	No. of Cases	P.A. Readings (mm./30 Minutes)			
		0	1-2	3-10	11+
0-3	47	35 (1)	6	—	6 (5)
4-9	36	13 (1)	13	6	4 (3)
10-20	11	—	2	6 (2)	3 (3)
21+	24	—	2	2 (1)	10 (10)

Figures in parentheses are numbers of cases with positive urine culture.

3. In 117 urine samples sent to the hospital laboratory the results obtained by microscopy of the centrifuged deposit and the P.A. reading were compared. Bacterial cultures were also made from the same urine samples. Table III shows the results. It brings out the general correspondence between the microscopy and P.A. determinations, and also reveals that in individual cases there was some inconsistency. At the top of the right-hand column are presented the six cases in which microscopy gave a normal result and P.A. a high reading (at least 11 mm.). The positive culture in five out of these six cases (*Escherichia coli* was found in 3, *Proteus mirabilis* in 1, enterococcus in 1) supports the reliability of the P.A. value. It is worthy of note that in three of these cases the microscopist stated that the sediment contained a considerable amount of urate precipitate, which, as is well known, hampers the examination of sediment. Furthermore, the sixth case had suspected nephrolithiasis.

The results with a P.A. of 0 mm. call for comment. Urine culture is stated to have given a positive result in two cases. In one of these the first culture was positive (*Pr. mirabilis*). However, when the culture was repeated the result was negative, although the patient had had no treatment. She was also symptom-free in regard to the

urinary tract. In the other case *E. coli* was found, as had occurred in the earlier sample. Repeated culture gave a negative result. The patient had, however, received sulpha preparations continuously.

Discussion

The first question in the assessment of a test for the determination of urine erythrocytes and leucocytes is whether it is sensitive enough. Normal urine is thought to contain a maximum of some 20 red cells per c.mm. For normal adult males the content is no more than two red cells per c.mm. (Larcom and Carter, 1948). Watson-Williams (1955) estimated the red-cell content to be about 10 times as great in women. The P.A. test was either 0 or 1 mm. if the quantity of red cells in question was 25 or fewer per c.mm. A larger red-cell count invariably gave a higher reading. With regard to erythrocytes, then, the P.A. test can be regarded as sensitive enough to indicate pathological amounts of red cells in urine. The value 0 mm. is negative, 1 mm. is uncertainly positive, 2 mm. and over positive. The reading gives a sufficiently good idea also of the quantity of red cells. As regards leucocytes, the same conclusions are warranted on the basis of the tests performed (Tables II and III). Normally fewer than 10 leucocytes are present per c.mm. of urine (Dukes, 1939). Five leucocytes per c.mm. gave a P.A. reading of 0 mm.; when there were 20 leucocytes per c.mm. the reading was 5 mm.

A weak point, of course, is that the test does not differentiate between erythrocytes and leucocytes, which still has to be done by microscopy. As already pointed out, haemoglobin also gives a positive reaction, since it contains peroxidase. This weakness notwithstanding, I find it suitable for routine examination on account of its simplicity and reliability. Opinions may naturally differ over the necessity for routine urine examination for erythrocytes and leucocytes. However, infections of the urinary tract alone are so common, especially in women, that some such examination seems necessary. Halonen and Halonen (1950) found these infections in 13.1% of female hospital patients. In two Amsterdam hospitals the figure was 29% (Loopuyt, 1946). Jäämeri (1943) found in 600 pregnant or puerperal women that 29% in pregnancy and 21% in puerperium had an infection of the urinary tract. The early diagnosis of this infection has an added importance to-day, when the facilities for its treatment are better than before. Only thus is it possible to avoid the development of numerous cases of nephrocirrhosis pyelonephritica.

The procedure in the P.A. test was arrived at empirically. Great emphasis was laid on making even moderately pathological amounts of red cells and leucocytes in urine give the strongest possible peroxidase reaction. For instance, hydrogen peroxide solutions were experimented with in 3, 10, and 30% dilutions. The quantities used in the tests varied from 1 drop to 2 ml. Although 4 drops of 30% hydrogen peroxide was finally decided upon, it was observed that 3-7 drops gave nearly the same result. Actually this means that the urine sample taken in the small tube need not be measured with particular accuracy. Thirty minutes was established, also empirically, for the reading of the P.A. value. A further increase in the value often occurred between 30 and 60 minutes, but it was slight. The maximum was often reached before 30 minutes: in fact, the qualitative and to some extent even the quantitative result can be indicated within some 20 seconds of taking the sample into the tube. The formation of gas bubbles in the tube points to the presence of an increased amount of peroxidase, and the rate of bubble formation is in relation to its quantity. In 20 seconds 50 red cells per c.mm. have already produced a distinct bubble formation.

Clearly, a more accurate result would be possible in peroxidase determinations if the volume of gas given off could be ascertained more exactly. Indeed, it is obvious that when the above procedure is used the quality of the urine—for instance, surface tension—affects the result, as was shown in Table I. The amount of gas given off was easy

to determine in the tube apparatus employed in the sugar fermentation test. Greater stress was laid, however, on simplicity of method than on small gains in accuracy.

Summary

A simple method has been evolved for the preliminary determination of the quantity of erythrocytes and leucocytes in urine. The amount of peroxidase contained in erythrocytes and leucocytes (haemoglobin) was estimated with an ordinary E.S.R. apparatus. The only reagent needed was 30% hydrogen peroxide. The accuracy of the method was assessed as satisfactory for the practical requirements of clinical work.

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CARBUTAMIDE IN TREATMENT OF SCHIZOPHRENIA*

BY

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Carbutamide (BZ.55), appearing to act as a euphorizing anti-hallucinogen, has given encouraging results in the treatment of 60 patients with schizophrenic illness, 45 of whom left the mental hospital recovered or relieved. The drug was originally introduced by Achelis and Hardebeck (1955) as an oral hypoglycaemic substitute for insulin in the treatment of diabetes, and was known to produce euphoria in normal healthy subjects (Franke and Fuchs, 1955).

I have already described a tentative application of carbutamide to psychiatric cases (Frost, 1957), and the present communication gives substance to the value of this drug as an anti-hallucinogen in schizophrenia.

Administration.—On the first day—a test day—a fasting blood-sugar is estimated; three 0.5-g. carbutamide tablets are given and the patient takes no food for one hour, when a further blood specimen is withdrawn for glucose estimation. The patient is then given food and glucose. Three 0.5-g. carbutamide tablets are thereafter given for not more than three successive days a week, followed by a drug-free interval of four days. This regime is repeated for four weeks only. Except on the first test day, the tablets are given with $\frac{1}{2}$ –1 oz. (14–28 g.) of glucose in a drink of water or mineral water, and the patients are encouraged to take glucose *ad lib.* throughout every day of the course and for two weeks after. *The hypoglycaemic effect of the carbutamide is to be avoided during treatment.* Patients should be kept under hospital observation for two weeks after the end of the course.

Results

Of 60 patients, 45 were discharged recovered or relieved. Of seven subsequently readmitted, four left hospital again after a second, shorter, course of carbutamide.

*Based on a paper read to the second International Congress for Psychiatry, Zurich, September, 1957.