6-8 cm. below the sapheno-femoral junction. At the lower end of the adductor canal tributaries of the highest genicular veins often connect with the saphenous veins (Daseler *et al.*, 1946; de Takats and Quillin, 1933). In addition, deep perforating veins are not infrequently present in the region of the fossa ovalis. If any one of those connexions is over-developed the internal saphenous vein will terminate short of the fossa ovalis (Kopsch, 1955) and the usual upper segment entering the femoral vein 4 cm. below the pubic tubercle will be missing (see Fig 2).

It is thought that the risk of ligating the femoral vein in cases where such an abnormality is present would be minimized by adopting the following measures as a routine: (1) Operations to be performed on in-patients under general anaesthesia, thus facilitating unhurried dissection and exposure of the vessels. (2) To realize the existence of the above anatomical variation from normal. (3) Dissection to be carried out of all the expected tributaries of the internal saphenous vein to make sure of their mode of termination before dividing and ligating the main vein. Although one or more of these tributaries may enter the femoral vein above the level of the internal saphenous, some of these tributaries always enter the internal saphenous vein. (4) In cases of suspected abnormality there should be no hesitation in extending the incision distally to expose the highest existing communicating vein between femoral and internal saphenous systems. (5) When the supposed internal saphenous is dissected free, palpation of the femoral artery lateral to it prior to ligation. The artery should be on a plane deep to the veins. (This measure saved the femoral vein from ligation in Case 4.)

Three of these four patients were aged 15-23. When patients of such a young age group present with varicose veins causing symptoms, an anatomical abnormality such as low termination of the internal saphenous vein should be considered.

Summary

Four cases of entrance of the internal saphenous into the femoral vein at an abnormally low level are recorded.

In two of these cases the femoral vein was ligated. The anatomical abnormality was considered to be mainly responsible for the error.

Measures are suggested as an aid to preserve the femoral vein in similar cases.

We should like to thank Mr. A. J. H. Rains for supplying details of Case 3.

REFERENCES

- Allan, J. C. (1951). *S. Afr. J. med. Sci.*, **16**, 105.
 Anson, B. J., and McVay, C. B. (1938). *Anat. Rec.*, **72**, 399.
 Cathro, A. J. M. (1954). *British Medical Journal*, **1**, 918.
 Daseler, E. H., Anson, B. J., Reimann, A. F., and Beaton, L. E. (1946). *Surg. Gynec. Obstet.*, **82**, 53.
 de Takats, G., and Quillin, L. (1933). *Arch. Surg. (Chicago)*, **26**, 72.
 Edwards, E. A. (1934). *Surg. Gynec. Obstet.*, **59**, 916.
 Foote, R. R. (1954). *Varicose Veins*, 2nd ed., p. 142. Butterworth, London.
 Glasser, S. T. (1943). *Arch. Surg. (Chicago)*, **46**, 289.
 Homans, J. (1916). *Surg. Gynec. Obstet.*, **22**, 143.
 — (1949). *Surgery*, **26**, 8.
 Kopsch, Fr. (1955). *Rauber-Kopsch Lehrbuch und Atlas der Anatomie des Menschen*, 19th ed. Thieme, Stuttgart.
 Luke, J. C., and Miller, G. G. (1948). *Ann. Surg.*, **127**, 426.
 McPheeters, H. O. (1945). *Surg. Gynec. Obstet.*, **81**, 355.
 Mansberger, A. R., jun., Yeager, G. H., Smelser, R. M., and Brumback, F. M. (1950). *Ibid.*, **91**, 533.
 Sherman, R. S. (1944). *Ann. Surg.*, **120**, 772.
 Trendelenburg, F. (1890). *Beitr. klin. Chir.*, **7**, 195.
 Trueta, J. (1947). *Studies of the Renal Circulation*. Blackwell, Oxford.
 Wells, C. (1948). *Brit. J. Surg.*, **36**, 97.

The Philips organization at Eindhoven, Netherlands, has begun publication of a quarterly journal, *Medicamundi*, devoted to material on radiology, "electromedicine," electrotherapy, and related subjects. The first three issues have already appeared; these include papers on radioisotopes, colposcopy, x-ray film, "small-volume irradiation," ultra-violet treatment of psoriasis, etc. *Medicamundi* is obtainable in Britain from Cleaver-Hume Press, Ltd., 31, Wright's Lane, London, W.8, price 21s. per annum.

A QUANTITATIVE CHEMICAL TEST FOR "MICROSCOPIC HAEMATURIA"

BY

H. WYKEHAM BALME, M.D., M.R.C.P.

A. ERIC DORMER, M.B., M.R.C.P.

AND

LIONEL RAWLINGS

From the Professorial Medical Unit, St. Bartholomew's Hospital, London

The detection of very small quantities of blood in urine is best performed by microscopical examination of the centrifuged deposit, from which a rough estimate of the amount of blood present can be made. This is usually expressed in such terms as "5-10 red cells per 1/6-in. field," but often there is no consistent usage of descriptive terminology. Unfortunately, great inaccuracies arise unless a highly standardized technique is used (Addis, 1925, 1926; Goldring and Wyckoff, 1930; Lyttle, 1933), and these techniques are inevitably complex, with the major discrepancy occurring in the estimation of the red blood cells. One of the principal difficulties, apart from errors of centrifugation and sampling, is the variable appearance of phosphates, round uric acid crystals and other particles which may be difficult to distinguish from red cells or by diluting the red-cell content of the deposit lead to very large errors in estimating the amount of blood present.

Chemical methods are largely immune from this difficulty, and it is the purpose of this paper to show that if applied to centrifuged urine they are not only delicate enough for most purposes but can at the same time give a satisfactorily quantitative answer very simply.

The method used was the amidopyrine test for blood, in which iron-containing blood derivatives catalyse the oxidation of amidopyrine by hydrogen peroxide to produce a lilac colour. As a result of a number of preliminary experiments in which varying amounts of blood of known characteristics were added to normal saline or normal urine and tested as described below, it was found that a urine containing 5,000 red blood cells per millilitre would just give a positive reaction.

Details of Method

Five and 10 ml. of urine are pipetted respectively into separate conical centrifuge tubes and spun. Centrifugation is carried out for 10 minutes at 3,000 r.p.m. From each specimen all but 0.2 ml. of the supernatant fluid is carefully removed and the volume made up to 1 ml. with distilled water. Then 0.2 ml. of 30% acetic acid is added, followed by 1 ml. of alcoholic amidopyrine, and, finally, by 3 drops of 20-volume hydrogen peroxide.

The tubes are well shaken and the production of a lilac or mauve colour is noted up to three minutes. If neither is positive the urine is reported as containing fewer than 5,000 red cells per ml. If the precipitate from the 10-ml. tube is positive and that from the 5 ml. negative, report as approximately 5,000 red cells per ml. If both are positive, proceed by making serial dilutions as follows.

Five-millilitre samples of normal urine—that is, urine containing no detectable red cells—or normal saline if this is preferred, are pipetted into each of six graduated conical centrifuge tubes, and into the first tube is also pipetted 5 ml. of the urine to be tested. Mix well, and from this tube pipette 5 ml. into the next tube and so on, the final 5 ml. being discarded. These tubes now contain serial dilutions from 1:2

to 1:64 of the original urine. All tubes are spun and the reaction is noted as described. The tube with the greatest dilution that just gives a positive reaction is noted and the calculation made as follows:

$$\text{Dilution factor} \times 50,000 \text{ r.b.c./ml.}$$

for example, if tubes 1:2 to 1:8 are positive, the specimen contains

$$\frac{8 \times 50,000}{5} = 80,000 \text{ r.b.c./ml.}$$

If all tubes are positive the urine should be approaching macroscopic blood level and should be reported as containing more than 1,000,000 r.b.c./ml.

From our studies we have chosen results which are displayed in the Table and in the Chart; these allow comparison between the two methods of recording haematuria and also show the pattern of haematuria observed in two cases of acute nephritis and a case of subacute bacterial endocarditis.

Discussion of Method

The correct interpretation of any test involves awareness both of factors affecting its sensitivity and of the sensitivity in relation to requirements. The sensitivity of chemical tests for blood in urine have been reviewed by Caplan and Discombe (1951), who report the amidopyrine test (Harrison, 1947) as capable of detecting 10^6 red cells per ml., but by preliminary centrifugation and slight modifications of the usual technique a suitable sensitivity, detecting down to 5,000 red cells per ml., has been found.

Results in a Case of Acute Nephritis

Date	Chemical Test R.B.C./ml.	Microscopy Centrifuged Deposit R.B.C./1/6-in. Field
15/8	5,000	10-14
18/8	—	20-30
22/8	10,000	—
1/9	15,000	10-15
4/9	10,000	—
9/9	20,000	15-30
11/9	20,000	—
15/9	20,000	5-10. Deposit difficult to examine owing to large amounts amorphous phosphate
18/9	5,000	Moderate numbers
22/9	5,000	Moderate numbers
25/9	5,000	—
30/9	10,000	6-8
2/10	5,000	Occasional, less than 1
5/10	10,000	—
9/10	10,000	8-16. Occasional clumps of red cells seen
14/10	80,000	—
16/10	80,000	Large numbers
20/10	80,000	—
23/10	40,000	5-10
27/10	30,000	—
30/10	—	7-14
3/11	40,000	Moderate numbers (4-6 approx.)
6/11	40,000	Moderate numbers
10/11	40,000	Large
13/11	40,000	Moderate
17/11	40,000	"
20/11	40,000	"
23/11	10,000	"
1/12	40,000	"
4/12	10,000	"
8/12	10,000	"
22/12	40,000	Obscured by heavy deposit of amorphous urates; red cells seen in supernatant liquid

Initial experiments showed that, so long as the diluent used in making the serial dilutions is normal urine or normal saline and not tap-water, which causes excessive haemolysis in the higher dilutions, centrifugation leads to no detectable loss of red blood cells. Centrifugation of more than 10 ml. is apt to be incomplete and so leads to less reliable results.

If the urine is highly pigmented and there is any subsequent difficulty with colours, normal urine may be used as diluent rather than normal saline, with corresponding slight reduction in the sensitivity of the test. This result may in part be due to the inhibiting effect of ascorbic acid on the reaction, but this is slight when the ascorbic acid content is reasonably near to the average normal concentration of some 2 mg. per 100 ml.

If the urine contains iodides false positives will be encountered owing to the liberation of iodine.

Although it has been found convenient to report the results in terms of numbers of red blood cells, the test is more strictly related to the haemoglobin content, and this should be remembered in the event of there being marked changes in the mean corpuscular haemoglobin content in the patient's red cells.

We have found that the test, capable of detecting down to 5,000 red cells per ml., covers a useful range, and accords with information concerning the output of red cells in urine which may be regarded as normal (Larcom and Carter, 1948; Lytle, 1933).

Clinical Application

It is not suggested that chemical examination of urine should supersede microscopy of the centrifuged deposit, for this latter method of study is needed to determine the presence of formed elements in addition to that of blood. The chemical test described by Watson-Williams (1955), using a compound tablet, has the advantage of portability and has a place as a "screening test" for haematuria. But where there is microscopic haematuria and where quantitative changes are of significance the method we have described has proved in over two years' experience to be of value, particularly in the management of cases of acute nephritis and of subacute bacterial endocarditis, and in following cases on anticoagulant therapy.

Summary

Slight modifications have been made in the amidopyrine test for blood so that a quantitative estimate of the degree of haematuria may be made. It has been found of value in cases of acute nephritis and of subacute bacterial endocarditis and in cases on anticoagulant therapy, and it may be recommended for routine laboratory use.

We wish to thank Dr. H. E. Archer for his help in the initial experiments and Professor R. V. Christie and Dr. G. W. Hayward for their encouragement.

REFERENCES

- Addis, T. (1925). *J. Amer. med. Ass.*, 85, 163.
 — (1926). *J. clin. Invest.*, 2, 409.
 Caplan, H., and Discombe, G. (1951). *British Medical Journal*, 2, 774.
 Goldring, W., and Wyckoff, J. (1930). *J. clin. Invest.*, 8, 569.
 Harrison, G. A. (1947). *Chemical Methods in Clinical Medicine*, 3rd ed. Churchill, London.
 Larcom, R. C., and Carter, G. H. (1948). *J. Lab. clin. Med.*, 33, 875.
 Lytle, J. D. (1933). *J. clin. Invest.*, 12, 87.
 Watson-Williams, E. J. (1955). *British Medical Journal*, 1, 1511.

During 1955 the United Nations Children's Fund (Unicef) sold 4,500,000 greeting cards. In terms of help to the children the estimated profits from the sale of the cards, £82,500, would, for instance, help provide milk for 432,600 children for a year. In Great Britain 870,000 cards were sold, almost one-fifth of the world total and nearly double the number in 1954. Many of the schools helped in selling the cards. Prominent among them was Ilford County High School, Essex; in 1954 the boys sold 13,280 cards, and in 1955 reached the remarkable total of 37,720.

Medical Memoranda

Severe Skin Reaction to I.Z.S.

One of the problems of insulin therapy has been the occurrence of skin reactions. These are particularly common when protamine zinc insulin (P.Z.I.) and globulin insulin (G.I.) are used, and it was thought that they are related to the protamine and globin content. The introduction of insulin zinc suspension (I.Z.S.) appeared to herald a new era, as these preparations contained thrice-crystallized insulin, and were free of foreign protein. The *British Medical Journal* (1953) went so far as to state that these suspensions do not cause allergic reactions in patients who are sensitive to P.Z.I. or G.I., and Murray (1954) chose patients for trial who had previously had untoward reactions to P.Z.I. and G.I. In these cases he found little reaction. Nevertheless, with their increasing use, some workers have recorded that local reaction may occur (Mason and Verel, 1953; Murray, 1954; Armstrong and Lloyd, 1954).

The following case showed a very severe reaction to I.Z.S.

CASE REPORT

A married woman, aged 60, a known diabetic for eight years, had been satisfactorily controlled on diet alone without insulin. She was admitted to hospital on November 10, 1954, in diabetic coma. She made a satisfactory recovery from this, with the aid of soluble insulin, and no untoward effects were noted.

On December 7 it was decided to put her on I.Z.S., and 30 units of an English preparation was administered. That evening a little local irritation was noted, but it was not considered significant, and the injections were continued daily. On December 10 there was intense local reaction. The whole arm was oedematous and inflamed, and the oedema and inflammatory reaction spread rapidly to involve the neck and face. By the following day an extensive maculo-papular rash extended over the entire upper part of the trunk, face, and both arms (see Fig.). Soluble insulin replaced the I.Z.S.

Over the next few days the rash became more severe, erythematous, and itching, and there was extensive blotting and oedema. She was treated with antihistamines. The skin gradually subsided and exfoliated, but it was nearly four weeks before the skin had returned to normal.

Photograph of patient, showing the rash.

COMMENT

I have been unable to find in the literature a report of a case with such severe and widespread skin reaction to I.Z.S. Gurling *et al.* (1955) stated that in 479 cases no skin reactions were encountered of sufficient severity to necessitate stopping the suspension. Most reactions appear to be very mild, though Armstrong and Lloyd (1954) quote a case in which the local reaction went on to bulla formation. Nevertheless, Paley (1954) has shown that a reaction to other insulins may also be associated with a reaction to I.Z.S., and evidence is accumulating to suggest that insulin itself may be the offending substance.