imposed at the time of the clinic and in the subsequent few weeks.

The sharpest criticism of multiphasic screening is that the patient, having successfully completed the tests, emerges with what he imagines is a clean bill of health. Though we tried hard to dispel this attitude, it is difficult to impress on each individual that a negative result does not necessarily imply the absence of other disease, either at the time of the test or in the future.

The next clinic is being planned on broadly similar lines but with the inclusion of tests for visual acuity and glaucoma. The clinic will be held for two weeks, mainly to deal with unsatisfied demand but also to enable women who may be menstruating to be examined for cervical carcinoma. Instruction will be given in self-examination of the female breast. In the case of the anaemia test, venous blood will be obtained from those failing the initial drop-test, and this will be submitted for full haematological examination. Pre-loading with glucose should raise the number of diabetics detected to a more acceptable level. The administrative arrangements will also be improved.

Summary

At a multiple screening clinic held in Rotherham for one week the attendance was 3,753. Eight positive cervical smears were reported, and seven new diabetics were diagnosed. We discovered 178 cases of anaemia, 99 of chest diseases, 30 of significant deafness, and many minor gynaecological conditions. Most of these cases were unknown to the family doctor.

The organization and aims of such clinics are discussed, and it is emphasized that close co-operation between the three branches of the Service is required.

The local general practitioners were most helpful and encouraging, and willingly undertook the additional work. We are grateful to the following consultants who freely gave advice and help and to whom many of the patients were referred for further investigation: Mr. D. Ballantine, Miss R. D. Dunsmore, Drs. H. R. Colquitt, A. C. Morrison, H. Richmond, E. Travers, and R. S. Weetch, and especially to Dr. A. MacFarlane, who undertook the examination of cervical smears. We are also indebted to Dr. W. J. Wilson, of the Sheffield Mass Radiography Centre; Dr. C. C. Bowley, of the Sheffield Regional Transfusion Centre; and to Dr. S. Shone, Senior Administrative Medical Officer, Sheffield Regional Hospital Board.

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Plasma Erythropoietin in Chronic Uraemia*

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Haemorrhage and haemolysis may contribute to the anaemia of chronic renal failure, but reduced erythropoiesis seems to be the major factor (Joske *et al.*, 1956; Desforges and Dawson, 1958; Kaye, 1958; Loge *et al.*, 1958; Verel *et al.*, 1959; Ragen *et al.*, 1960; Kurtides *et al.*, 1964). Although the cause of the deficient erythropoiesis is not fully understood there appears to be a disturbance of the mechanism regulating red-cell production. Present theory of the control of erythropoiesis is reviewed by Gordon (1959) and Stohlman (1962). This maintains that under conditions of prolonged tissue anoxia increased amounts of the hormone erythropoietin, which stimulates the bone-marrow, are released into the circulation. Experiments by Jacobson *et al.* (1956), Osnes (1958), and Kuratowska *et al.* (1960) suggest that erythropoietin is produced (or activated) by the kidneys. In accordance with this concept increases in plasma erythropoietin have been demonstrated in patients with most types of anaemia, the single exception being azotaemic anaemia (Gallagher *et al.*, 1959, 1960; Penington, 1961; Naets and Heuse, 1962).

It is tempting to suggest in the light of these findings that the root cause of this anaemia is the inability of the diseased kidneys to produce erythropoietin. Before this can be accepted it would be necessary to show that plasma erythropoietin was lower than normal in patients with renal failure (Penington, 1962a). The evidence on this point is inconclusive. Gurney *et al.* (1957) obtained an erythropoietic response in hypophysectomized rats with normal plasma, but with this technique pituitary, thyroid, or adrenal hormones may have been responsible. Reichlin and Harrington (1960) also claim to have demonstrated erythropoietin in normal plasma, but their assays did not include an erythropoietin standard, so that "false-positive" results cannot be excluded. Other workers have been unable to detect erythropoietin in normal plasma (Gallagher *et al.*, 1959; Penington, 1961; Naets and Heuse, 1962). On the present evidence, therefore, it is equally possible that the low erythropoietin levels in uraemia reflect failure of the compensatory mechanism to some other cause for the anaemia—for example, toxic inhibition of the marrow (Markson and Rennie, 1956; Saito, 1963). The demonstration of transient improvement in erythropoiesis after haemodialysis (Kurtides *et al.*, 1964) and peritoneal dialysis (Berry *et al.*, 1964) suggests that toxic effects may well be important.

Lack of sensitivity and specificity of the erythropoietin bioassays are responsible for the present difficulties. All the workers mentioned used starved or polycythaemic rats as assay animals, but Jacobson *et al.* (1959) and DeGowin *et al.* (1962) have shown that polycythaemic mice are more sensitive. Greater specificity can be obtained by including a standard erythropoietin in at least two dose levels so that dose-response lines with specific slope can be constructed; test plasma given in similar dose increments will, if erythropoietin is present, produce a dose-response line parallel to that of the standard (Bangham, 1962). Non-specific effects can be recognized by the lack of parallelism.

Such a standard is now available (standard "B" M.R.C.), and a simple reliable method for producing polycythaemia in mice has been devised by Cotes and Bangham (1961) with a decompression chamber (Wright, 1964). With these improve-

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ments available it seemed worth while to undertake a further study of the erythropoietin levels in normal and anaemic subjects with and without renal failure in the hope, as Gordon and Weintraub (1962) have suggested, that hitherto undetected small differences might be revealed.

Patients Studied

Of the 21 patients studied, 14 were suffering from chronic renal failure due to various causes and anaemia. Many of these cases were at an advanced stage and under consideration for long-term treatment with repeated haemodialysis. None of these patients showed evidence of iron deficiency; plasma iron levels were within the normal range or slightly raised. Reticulocyte counts in all these patients were less than 1%. Diagnosis was confirmed by renal biopsy in many cases or at necropsy. The remaining seven patients had anaemia due to a variety of causes other than renal failure; one of these, however (Case 16), showed evidence of renal impairment, but myeloma was considered the principal factor in his anaemia. Details of all the cases are shown in Tables I and II.

Plasma erythropoietin levels were also measured in six normal subjects—five male and one female. Their ages ranged from 21 to 32 years and the haemoglobin in all was between 95 and 107%.

Erythropoietin Assay

Blood was collected into heparinized syringes, the plasma being separated and stored at −20° C. until used.

The anoxia-induced polycythaemic mouse technique as described by Cotes and Bangham (1961) was used to assay the plasma erythropoietin, the only modification being to keep the animals at one-half atmosphere pressure for three weeks rather than two. This was found to produce a more constant level of polycythaemia (Cotes, 1964—personal communication). The packed cell volumes of the mice at the end of the assay ranged from 65 to 70%.

On days 4 and 5 after their return to atmospheric pressure the mice were injected intraperitoneally with test plasma, erythropoietin standard "B," or saline. Two dose levels of test plasma and standard erythropoietin were employed with five mice for each level, the dose being given in two equal portions. The total dosages used were as follows: plasma, 1 and 2 ml., or 2 and 4 ml.; erythropoietin, 0.5 and 1 unit. On day 7 0.1 μc. of ^{59}Fe as ferric iron in 0.1 ml. of isotonic sodium citrate was given intravenously. Erythropoietic response to the test substances was measured in terms of the percentage uptake of this radio-iron into the mouse red cells 20 hours after the intravenous injection. Animals with packed cell volumes of less than 55% were excluded. Radioactivity in 0.5 ml. of whole blood was counted by means of a well-type scintillation counter and an Ekco N5309 Scaler. Male mice of a Webster Swiss strain were used, their total blood-volume after three weeks' hypoxia having been previously estimated by radioactive chromium-labelled mouse red cells to be 9.6 ml./100 g. body weight. Results were plotted as log. dose-response lines.

Results

Before any assay can be accepted as valid there must be a significant difference between the responses to the low and high doses of the standard. Wide variations in the results for individual animals within one dose group will obscure the difference between the two groups; this is particularly likely to occur as the responses approach the upper physiological limit. To avoid this, doses of standard erythropoietin producing a percentage uptake of radio-iron within the normal range for this strain of mice (12–30%) were used. Statistically significant log. dose-response regression lines ($P<0.05$) were obtained in all but one assay (Case 15) with the doubling doses mentioned.

Results are summarized in Tables I and II. Erythropoietic activity was not detected in the plasma of 12 out of the 14 patients with uraemia and anaemia; in Cases 2 and 8, however, titres of 0.1 unit/ml. and 0.03 unit/ml. were found. In the negative assays there was no significant difference between the effects produced by the plasma injections and the saline controls. In Case 8 the titre was very low, but the responses were significantly above those of the saline controls.

TABLE I.—*Details of Patients with Uraemia Together with Plasma Erythropoietin Levels*

Case No.	Age	Sex	Diagnosis	Blood Urea (mg./100 ml.)	Hb (%)	Erythropoietin Level*
1	40	M	Chronic glomerulonephritis	351	41	None detected
2	43	F	„ „	270	50	0·1 unit/ml.
3	52	F	Polycystic kidneys	440	50	None detected
4	28	M	Chronic glomerulonephritis	488	40	„ „
5	52	F	„ „	460	36	„ „
6	37	F	Chronic pylonephritis	492	54	„ „
7	40	M	Chronic glomerulonephritis	272	52	„ „
8	70	F	Hypertensive renal disease	210	64	0·03 unit/ml.
9	22	F	Renal cortical necrosis	200	52	None detected
10	40	M	Chronic glomerulonephritis	480	48	„ „
11	47	F	Hypertensive renal disease	188	50	„ „
12	40	F	Chronic glomerulonephritis	330	36	„ „
13	46	M	Chronic pyelonephritis	200	48	„ „
14	54	F	Polycystic kidneys	278	46	„ „

* Units of standard "B" per ml. of plasma.

TABLE II.—*Details of the Non-uraemic Anaemic Patients with Their Plasma Erythropoietin Levels*

Case No.	Age	Sex	Diagnosis	Blood Urea (mg./100 ml.)	Hb (%)	Erythropoietin Level
15	60	F	Pernicious anaemia ..	40	22	0·2 unit/ml.
16	54	M	Myeloma	60	50	„
17	62	F	Hypoplastic anaemia ..	38	40	„
18	45	F	Iron deficiency	30	67	0·6 unit/ml.
19	60	F	Folic-acid deficiency ..	30	20	Assay invalid
20	50	M	Iron deficiency	34	58	0·3 unit/ml.
21	24	F	„ „	40	76	0·2 unit/ml.

The plasmas of all the patients who had anaemia without uraemia contained erythropoietin. In six it was possible to estimate the quantity. In Case 19 plasma produced very high radio-iron uptakes, but because of the wide variation of the responses within each dosage group a satisfactory regression line was not obtained. These findings suggest that the erythropoietin content of the plasma was very high, but the assay was not valid as a quantitative estimation.

Erythropoietin was not detected in any of the normal plasmas.

Discussion

In comparing these results with those obtained by other assay techniques it is necessary to consider the dose of test plasma in relation to the sensitivity of the test animal. Mice used in the present study were found to be approximately 15 times more sensitive to the standard erythropoietin than male Wistar rats, so that 2 ml. of plasma would therefore be equivalent to about 30 ml. in rats. The workers who claim to have demonstrated erythropoietin in normal plasma by rat assays used only 6 ml. of plasma (Reichlin and Harrington, 1960), or an extract equivalent to less than 20 ml. of plasma (Gurney *et al.*, 1957). Thus failure to detect erythropoietin in the present assay strongly supports the contention that the effects observed by these workers were not due to erythropoietin. In the hypophysectomized rats used by Gurney *et al.* other hormones in which the test animals were deficient were probably responsible. This explanation does not apply in the polycythaemic rats used by Reichlin and Harrington, but an observation made by Penington (1962b) may account for their

findings. He showed that increased incorporation of radio-iron into rat red cells could be produced by normal plasma taken at intervals before and after a blood transfusion. It persisted until some time after the subject's bone-marrow had become inactive as a result of the transfusion, and he suggests that it could be due to some by-product of erythropoiesis rather than erythropoietin.

The negative results in the majority of cases of azotaemic anaemia in the present investigation are similar to the findings of the other workers mentioned and contrast with the elevated levels found in the non-uraemic patients. Even with this more sensitive assay procedure it has proved impossible to detect differences in erythropoietin titre between normal and most of the uraemic plasmas, so that no conclusion can be reached regarding the possibility of subnormal levels in renal failure. This of course does not imply that erythropoietin is not present, but only that its concentration is less than can be detected. The positive findings in Cases 2 and 8 are important because it is unlikely, with this type of bioassay, that the effects noted were due to non-specific stimulation. It therefore seems reasonable to assume that, at least in these cases, the anaemia is not due solely or primarily to a failure of erythropoietin production. More sensitive assay techniques will have to be developed before the full significance of the apparent disturbance of erythropoietin production in renal disease can be assessed.

Summary

Bioassays of plasma erythropoietin were undertaken in normal subjects and in anaemic patients with and without uraemia. The anoxia-induced polycythaemic mouse technique was used together with standard "B" erythropoietin. In the anaemic patients without uraemia erythropoietin levels were raised. In the normal subject and in 12 of the 14 patients with anaemia and uraemia erythropoietin was not detected, but in two uraemic patients the level was raised.

The results suggest that the anaemia in some uraemic patients is not due primarily to a deficiency of erythropoietin.

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Hookworm Infection in Great Britain: Experimental Observations

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In a previous article (Salem and Truelove, 1964) we described four cases of hookworm disease in Pakistani immigrants resident in Oxford. We mentioned that we had had the opportunity to examine the faeces of nine other Pakistanis, relatives of the patients or workers in our own hospital, and had found hookworm ova in four of them. In view of the large numbers of Pakistanis (as well as other immigrants from the tropics) now resident in Britain we concluded that a public health problem exists for two distinct reasons. First, there are probably many undiagnosed examples of hookworm disease in this country and the subjects require treatment for the sake of their own health. Secondly, there is a potential risk of the disease spreading beyond its original hosts.

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We now present some evidence which bears on the second of these issues—namely, whether the disease can spread in Great Britain.

On 20 June 1964 hookworm ova were found in the faeces of another Pakistani patient with hookworm disease. We decided to see whether the ova would hatch and, if so, whether the young larvae would develop into the infective stage outdoors in the British climate.

Culture in Flower-bed

A portion of the faeces, representing about one-third of a single motion, was placed in a flower-bed in the garden of the Radcliffe Infirmary, Oxford, the site being marked off to prevent accidental infection of the gardeners. The portion of faeces was thinly covered with soil and the bed was watered to represent a fall of rain. A specimen of the faeces was recovered daily from the flower-bed and examined under the microscope.