

equipment, makes the search for an alternative procedure an overdue medical obligation.

CONCLUSION

The features of the ideal method of bowel preparation may be enumerated as follows:—

(1) It should produce complete and consistent bowel emptying within a predictable period of time.

(2) After emptying, the bowel should be left in an otherwise unaltered condition.

(3) From the patients' point of view the period of preparation should be short and the method should cause neither discomfort nor apprehension.

(4) From the nurses' point of view the method should be simple and quick.

(5) From the radiologists' point of view the procedure should result in a clear, unobstructed field of vision.

(6) There should be no side-effects or complications.

The conclusion to be drawn from this enquiry is that bisacodyl is an effective substitute for traditional methods of preparing the bowel and has the additional advantages of being simpler and quicker to administer and of being less unpleasant for the patient.

In view of the increasing use in hospitals of disposable enemata, it may be of interest to mention the following relative costs:—

| | Basic Cost to N.H.S. |
|---|----------------------|
| Disposal enema | 1s. 8d. |
| Dulcolax régime (2 tabs. + 2 suppositories) | 7d. |

My thanks are due to Dr. L. J. Rae, Director of the Radiodiagnostic Department, for his help and advice, and to the registrars, radiographers, and staff who have cooperated in this project.

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GASTRO-INTESTINAL BLOOD LOSS MEASURED BY RADIOACTIVE CHROMIUM

BY

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A new technique is described for the measurement of blood loss in the faeces of patients labelled with radioactive chromium (^{51}Cr). The method is simple and is probably more accurate at low levels of faecal radioactivity than those previously described. The method will measure as little as $0.02\mu\text{C}$ of ^{51}Cr in whole blood in a 24-hour stool.

The apparent average daily blood loss in a series of 10 normal people averaged 0.6 ml., with a range of 0.3 to 1.3 ml.

Estimations of plasma and salivary radioactivity have been made in an attempt to assess the importance of contamination from eluted ^{51}Cr . Minor radioactivity in plasma but none in saliva was recorded. Stool contamination from such sources is thought to be insignificant.

No significant correlation existed between chemical occult blood tests and isotope measurements, where there was less than 10 ml. of whole blood in a 24-hour stool.

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Since the turn of the century numerous chemical tests, of varying sensitivity, have been available for the detection of occult blood in the stools, but it is only in the last 10 years that methods have become available for its precise measurement.

The isotope of chromium, ^{51}Cr , when prepared as $\text{Na}_2^{51}\text{CrO}_4$, has proved a satisfactory label for measuring the survival time of human red cells. Owen, Bollman, and Grindlay (1954) showed that it could be used to measure the quantity of blood passed in the faeces of dogs. Their methods have been applied to man by Roche, Perez-Gimenez, Layrisse, and Di Prisco (1957), Ebaugh, Clemens, Rodnan, and Peterson (1958), and Bannerman (1957). All these workers calculate the amount of blood in the faeces by measuring the radioactivity in a weighed aliquot representing 3-4% of a fluid homogenate of the stool. The method is both malodorous and cumbersome. Hughes Jones (1958) compared this rather tedious technique with that described by Booth and Mollin (1956) in which the radioactivity of the whole stool in its collection carton is measured in a ring counter containing 32 Geiger-Muller tubes in parallel. Unfortunately this simple method did not prove very sensitive. Sensitivity is improved by the use of the turret type counter in which the collection carton is placed between two scintillation crystals sensitive to γ rays. This equipment is very expensive, and has the disadvantage that the stool is not completely surrounded by the crystal, thus reducing its sensitivity at low levels of faecal radioactivity; furthermore, considerable care has to be taken to position the stool at the geometric centre of the counter (Lewis, 1959).

The purpose of the present communication is to describe a simple, inexpensive, relatively odourless technique which is sensitive at low levels of faecal radioactivity. The daily blood loss has been measured by this method in normal adults. The possibility of reabsorption of ^{51}Cr from the gut and of leakage of eluted ^{51}Cr from the plasma has been investigated. The daily blood loss has also been measured in patients with positive chemical tests for occult blood and the results compared.

METHODS

A. TECHNIQUE FOR LABELLING BLOOD WITH ^{51}Cr .—The patient's cells are labelled with ^{51}Cr , using the method described by Mollison and Veall (1955). About 20 ml. of venous blood is withdrawn into a sterile universal container, containing 5 ml. of acid citrate dextrose solution. The sample is then centrifuged and the supernatant plasma removed. Approximately $100\ \mu\text{C}$ of $\text{Na}_2^{51}\text{CrO}_4$ with a specific activity of 10 to $30\ \mu\text{C}$ per mg. of chromium is added to the cells, with rapid and thorough mixing, to ensure an even coating of chromium

over the cells. The suspension is then allowed to stand for 30 minutes at room temperature, and is gently rotated every five minutes. The packed cells are then washed three times in sterile saline, and are then resuspended in saline to a volume of approximately 25 ml. By means of a U-shaped needle (Lewis, 1959), 20 ml. is carefully withdrawn into a calibrated syringe and injected intravenously into the patient. Blood samples are taken from the patient 15 minutes later, after 24 hours, and subsequently every three days. The samples are haemolysed by the addition of a knife point of "saponin". Samples, each of 3 ml., are counted, and after correction for background their radioactivity can be plotted. The dosage of administered radioactivity is calculated by the use of a standard prepared from the residue of the original saline suspension of red cells diluted 100 times in water.

B. COLLECTION AND PREPARATION OF FAECAL SAMPLES.—Because of the relatively long half life—27.8 days—of ^{51}Cr , each patient can be studied for four to six weeks after his red cells have been labelled. All stools passed during the period of investigation are collected in successive numbered cartons. Each stool is passed onto a square of cellophane paper placed in a bed pan under a portable commode. The cellophane-wrapped stool is then transferred to a 20-oz. waxed screw-topped carton. In the laboratory a small faecal sample is removed for chemical occult blood estimation. The stool is then dried in an electric oven, thermostatically controlled at 170°F . so that spattering does not occur; drying takes about 15 hours. The odour is eliminated by housing the oven in a fume cupboard containing an extractor fan.

The dried stool is then placed in a glazed glass mortar, the cellophane ashed by ignition, and the whole ground to a fine powder. Thorough mixing occurs during the grinding process. The powdered stool is weighed and a 10 g. aliquot packed in glass test tubes and counted in a well-type scintillation crystal. The aliquot averages 20% of a normal dried stool, and is packed into three or four tubes, so that each tube contains about 3 ml. of ground stool which is the optimal volume for the counter.

C. MEASUREMENT OF RADIOACTIVITY.—The most convenient and sensitive method for estimating the faecal radioactivity is by the gamma ray emission from ^{51}Cr measured in a well-type thallium-activated sodium iodide crystal. An "echo" scintillation counter type N.550A was used in these experiments. A minimum of 2,500 counts was made so that the coefficient of variation was about 2%. The recorded activity of the faecal aliquot was corrected for background, and the blood content of the 24-hour faecal collection calculated from the formula:—

$$\frac{\text{Total C.P.M. in aliquot}}{\text{C.P.M./ml. of venous blood standard}} \times \frac{\text{Wt. of 24-hr. stool}}{\text{Wt. of aliquot}}$$

Because it is impossible to measure accurately intestinal transit time the blood samples used as the standards in the formula were those taken two or three days before passage of the stool.

RESULTS

PERCENTAGE RECOVERY OF INGESTED ^{51}Cr -LABELLED RED CELLS.—Labelled red cells, 1.5 ml., containing 0.02 to 0.03 microcuries/ml. of ^{51}Cr , were given orally in a gelatin capsule to 10 patients, none of whom had previously received any radioactive blood. An average of 97.4% of the administered blood was recovered in faecal collections over the succeeding five days. This suggests that loss of ^{51}Cr by absorption from the alimentary canal does not constitute an important source of error in the method. It also shows that drying the stools at 170°F. does not apparently lead to any significant loss of radioactivity.

The results are shown in Table I.

TABLE I
PERCENTAGE RECOVERY OF INGESTED ^{51}Cr -LABELLED BLOOD

| Case | Sex | Age | Blood Ingested (ml.) | Blood Recovered from Four-day Collection (ml.) | % Recovery |
|------|-----|-----|----------------------|--|------------|
| 1 | F | 65 | 1 | 0.9 | 90 |
| 2 | M | 47 | 1 | 1.02 | 102 |
| 3 | M | 32 | 1 | 1.04 | 104 |
| 4 | M | 43 | 2 | 2.18 | 109 |
| 5 | M | 34 | 2 | 1.92 | 95 |
| 6 | F | 24 | 2 | 2.1 | 105 |
| 7 | M | 43 | 3 | 2.7 | 90 |
| 8 | M | 34 | 3 | 2.8 | 93 |
| 9 | M | 34 | 5 | 4.6 | 92 |
| 10 | F | 24 | 5 | 4.7 | 94 |

Mean = 97.4%, S.D. = ± 6.6 , S.E. = 1.9.

VALIDITY OF RESULTS OBTAINED FROM A 10G. ALIQUOT.—To test the validity of results obtained from a 10 g. aliquot, stools were collected from six patients who were on a small dose of aspirin and whose red cells had been labelled with ^{51}Cr . The radioactivity in the whole of each stool was measured, after drying and powdering. An aliquot of 10 g. was then taken from each stool and counted

TABLE II
RECOVERY FROM TOTAL STOOL COUNT WITH ESTIMATED RECOVERY FROM 10g. ALIQUOT COMPARED

| Case | Sex | Age | Blood Recovered from Whole Stool (ml.) | Blood Estimated from 10g. Aliquot (ml.) | % Recovery by Aliquot Method |
|------|-----|-----|--|---|------------------------------|
| 1 | M | 72 | 3.7 | 3.9 | 105.5 |
| 2 | M | 73 | 3.5 | 3.8 | 102.5 |
| 3 | M | 70 | 4.7 | 3.6 | 102.5 |
| 4 | M | 19 | 2.3 | 3.5 | 100.0 |
| 5 | M | 70 | 2.6 | 4.7 | 100.0 |
| 6 | M | 19 | 7.6 | 2.3 | 100.0 |
| | | | | 2.6 | 100.0 |
| | | | | 7.8 | 102.5 |

Mean = 101.6%, S.D. = ± 1.99 , S.E. = 0.7.

and the total radioactivity was calculated. Table II compares the results obtained by counting the whole stool with the results obtained from a 10 g. aliquot, and shows that the aliquot technique is both reliable and satisfactory.

APPARENT AVERAGE DAILY BLOOD LOSS IN NORMAL ADULTS.—The apparent average daily blood loss was investigated in 10 patients who had no known gastro-intestinal disease and negative occult blood reactions both to the benzidine and "haematest" reaction. The radioactivity in 60 stools was measured after intravenous administration of labelled R.B.C.s. The results in Table III show an average daily blood loss of 0.6 ml. with a range from 0.3 ml. to 1.3 ml.

PLASMA RADIOACTIVITY DUE TO ELUTED ^{51}Cr .—It is not known for certain that the ^{51}Cr in stools represents ^{51}Cr -labelled red cells only, and it has been suggested that a proportion might be due to eluted ^{51}Cr excreted into the gut. It is because of this uncertainty that the figure for normal daily blood loss has been expressed as "apparent" normal daily loss. In order to assess the level of radioactivity in the plasma due to eluted ^{51}Cr , 30 patients

TABLE III
AVERAGE DAILY BLOOD LOSS IN STOOLS IN NORMAL SUBJECTS TAGGED WITH ^{51}Cr

| No. of Case and Diagnosis | Age | Sex | Days Stools Collected after Labelling with ^{51}Cr | No. of Stools | Apparent Average Blood Loss (ml./day) |
|---------------------------|-----|-----|---|---------------|---------------------------------------|
| 1. Normal | 19 | F | 1 to 15 | 15 | 0.3 |
| 2. Sideroblastic anaemia | 58 | M | 12 to 16 | 4 | 0.5 |
| | | | 20 to 23 | 3 | 0.6 |
| 3. Hypochromic anaemia | 70 | F | 1 to 5 | 5 | 1.2 |
| 4. Pulmonary tuberculosis | 25 | F | 1 to 3 | 3 | 0.3 |
| 5. Diabetes | 72 | M | 1 to 4 | 4 | 1.3 |
| | | | 16 to 18 | 3 | 0.6 |
| 6. Normal | 44 | M | 1 to 7 | 7 | 1.0 |
| 7. Pulmonary tuberculosis | 58 | M | 1 to 4 | 4 | 0.3 |
| 8. Pulmonary tuberculosis | 51 | M | 1 to 3 | 3 | 0.3 |
| 9. Arteriosclerosis | 70 | M | 1 to 3 | 3 | 0.5 |
| 10. Cholelithiasis | 45 | F | 1 to 3 | 3 | 0.7 |

Mean = 0.6 ml., S.D. = $\pm .35$ ml., S.E. = 0.1 ml.

TABLE IV
PLASMA LEVEL OF RADIOACTIVITY AS % OF RED CELL
RADIOACTIVITY

| No. of Patients | Time of Measurement after Tagging | Mean Plasma Level of Radioactivity (%) |
|-----------------|-----------------------------------|--|
| 9 | First 24 hours | 0.28 Range 0 - 0.5 |
| 21 | Between one and four weeks | 0.3 Range 0.08-0.45 |

labelled with $\text{Na}_2^{51}\text{CrO}_4$ were investigated. The results are expressed in Table IV. In nine patients blood samples were taken during the first 24-hour period after labelling. The blood was centrifuged and the radioactivity in 3 ml. of the supernatant plasma measured and compared with the radioactivity in 3 ml. of red cells. In a further 21 patients plasma activities were measured from one to four weeks after labelling. The mean result of plasma activity, expressed as a percentage of the red cell activity measured at the same time, was 0.28% in the first 24 hours and 0.3% in any sample taken during a subsequent period.

RADIOACTIVITY OF SALIVA DUE TO ELUTED ^{51}Cr .—The radioactivity of the saliva was measured in 10 patients labelled with ^{51}Cr . Measurements were made during the first 24 hours and at subsequent intervals over the course of four weeks, but in no case was the background count exceeded.

COMPARISON WITH CHEMICAL TESTS FOR OCCULT BLOOD.—The volume of blood was measured in 500 stools from 25 patients, and compared with the results of testing for occult blood by the benzidine and "haematest" methods. The benzidine test was performed using the method of Needham and Simpson (1952), and the criteria accepted were:—

Strong positive (+++) was taken as the immediate appearance of a deep blue colour.

Positive (++) was taken as a deep blue colour appearing within 15 sec.

Weak positive (+) was taken as a blue colour appearing within 30 sec.

Any colour appearing after this period was expressed as a negative (—).

The "haematest" method was performed according to the manufacturers' instructions.

A positive (+) result was taken as a deep blue staining on the test paper appearing within two minutes, otherwise a negative (—) result was recorded. The active ingredients of the "haematest" tablet are O-toluidine, strontium peroxide, calcium acetate, and tartaric acid.

Table V shows the results found in 10 consecutive stools passed by three patients who were taking a small dose of aspirin. The poor correlation shown in this small sample is typical of the whole series of 500 stools tested from 25 patients.

DISCUSSION

The sensitivity of the method described in this communication is shown by the recovery in a 24-hour stool of as little as $0.02\mu\text{C}$ of ingested ^{51}Cr contained in 1 ml. of whole blood. The average dried stool weighs about 40-50 g. and the whole can easily be counted but the stool is mixed so thoroughly by drying and powdering that it is unnecessary to count more than a 10 g. aliquot, representing 20%. By contrast the more usual method involves diluting the stool, homogenising and weighing it, and then measuring an aliquot which only represents about 2%, a malodorous and rather cumbersome process. By the use of this technique Bannerman (1957) was able to measure accurately only amounts of 3 ml. or more. The results of Ebaugh *et al.* (1958) and Hughes Jones (1958) suggest greater sensitivity, but their precise accuracy is difficult to determine because the dose of ^{51}Cr given and the volume of blood containing it was so large.

The recovery of 97.4% of relatively small orally administered doses of ^{51}Cr -labelled red cells suggests

TABLE V
CHEMICAL ESTIMATION OF OCCULT BLOOD COMPARED WITH RADIOACTIVITY MEASURED IN STOOLS OF
THREE PATIENTS ON SMALL DOSE OF ASPIRIN

| Case 1.—Male aged 56 | | | Case 2.—Male aged 59 | | | Case 3.—Male aged 75 | | |
|----------------------|-----------|---------------------------|----------------------|-----------|---------------------------|----------------------|-----------|---------------------------|
| Benzidine Test | Haematest | Measured Blood Loss (ml.) | Benzidine Test | Haematest | Measured Blood Loss (ml.) | Benzidine Test | Haematest | Measured Blood Loss (ml.) |
| ++ | + | 1.9 | +++ | — | 4.2 | + | + | 1.2 |
| — | + | 3.9 | +++ | — | 0.7 | ++ | + | 1.1 |
| — | + | 2.8 | +++ | + | 3.4 | + | — | 3.7 |
| ++ | + | 11.1 | ++ | + | 2.1 | + | + | 1.5 |
| ++ | + | 2.3 | +++ | + | 0.3 | + | — | 3.5 |
| + | — | 2.9 | +++ | + | 5.2 | + | — | 0.6 |
| + | — | 0.6 | +++ | + | 6.8 | + | — | 1.9 |
| + | — | 0.9 | +++ | + | 10.6 | + | — | 3.4 |
| +++ | — | 3.8 | +++ | — | 9.5 | + | + | 4.2 |
| +++ | — | 1.2 | ++ | + | 5.5 | ++ | + | 9.9 |

that apparently hardly any of the ^{51}Cr is absorbed from the gut, and thus agrees with the results of Owen *et al.* (1954), Roche *et al.* (1957), and Ebaugh *et al.* (1958). Hughes Jones (1958) gave oral doses of $21\ \mu\text{C}$ of ^{51}Cr and $8\ \mu\text{C}$ to two patients, and was only able to recover 66% and 87% of the administered dose. He concluded that it seemed reasonably certain that the discrepancy was due to reabsorption of some of the ^{51}Cr by the gut. This assumption seems unlikely because both Roche *et al.* (1957) and Ebaugh (1958) could recover less than 0.5% of the ^{51}Cr from the urine, and found less than 0.2% of the ingested ^{51}Cr to be present in the circulating blood.

The apparent average daily loss in normal people of 0.6 ml. of whole blood was found. A similar figure was obtained by Hughes Jones (1958), and Levin, Hart, and Bothwell (1959) but is rather less than the quantity found by Roche *et al.* (1957) and Ebaugh *et al.* (1958), namely 1.2 ml. It has been suggested that this difference may be due to the fact that these authors did not remove the unattached ^{51}Cr by washing their cells after labelling (Hughes Jones, 1958). It has been shown by Owen *et al.* (1954) in dogs, and by Roche *et al.* (1957) in man, that the greater proportion of chromium separated from the red cells and present in the plasma is excreted rapidly by the kidneys. Roche *et al.* (1957) found that after intravenous injection of labelled red cells 96.3% of the total daily recovery of radioactivity was in the urine. Mollison and Veall (1955) have shown that after the intravenous administration of labelled red cells, most of the chromium attached to senile cells or poorly bound to the cells is eluted within the first 24 hours. Hughes Jones and Mollison (1956) further estimated that thereafter the rate of elution is steady. If any significant amount of eluted chromium were to diffuse into the alimentary canal this should occur in the first 24 hours after injection. No evidence in support of this possibility has been found. There was no significant increase in faecal radioactivity in the first 48-hour stool samples as compared with later specimens. Previous workers in this field have given no figures of plasma radioactivity; such estimations were made and compared with the red cell radioactivity during the first 24-hour period and subsequently at intervals during the course of a four-week investigation and at no time was significant activity recorded. In the present series, in conformity with standard practice, the red cells were labelled with hexavalent $\text{Na}_2^{51}\text{CrO}_4$ which is thought not to cross the intestinal barrier, Owen *et al.* (1954) having shown that small quantities of the trivalent ion can pass through the gut wall. If, as has been shown (Roche *et al.*, 1957), most of the chromium eluted from the labelled red cells is excreted by the

kidneys, it is highly probable that very little ^{51}Cr can diffuse through the bowel wall.

Radioactivity in the saliva of patients labelled with ^{51}Cr was measured, but in no case was the background count exceeded. These results go some way to discount the suggestion that eluted ^{51}Cr might be stored and excreted into the intestine by an active glandular process.

It is therefore concluded that in patients labelled with well-washed hexavalent chromium-tagged red cells, the radioactivity in a 24-hour stool represents red cell loss into the intestine, and that contamination by eluted ^{51}Cr is insignificant. It is more likely that minor contamination by eluted chromium could occur from bile. Owen *et al.* (1954) have shown in dogs, but not in humans, that a small quantity of chromium is excreted in bile.

Great diversity of opinion has always existed about the usefulness of chemical occult blood tests as a quantitative measure of minimal faecal blood loss. Needham and Simpson (1952), using *in vitro* methods, have shown that a modification of the benzidine test would give a positive result with as little as 3 ml. of blood. Bannerman (1957), however, found little correlation between the benzidine test and isotope measurements when small quantities of blood were in question. The benzidine test has now been withdrawn because of carcinogenic risks incurred in its manufacture, and reliance has now been placed on O-toluidine as a chemical agent sensitive to occult blood. In the present experiments such variation was found between the results of the chemical tests in 500 stools containing less than 10 ml. of blood estimated radioactively that the only permissible conclusion which could be drawn was that these chemical tests possess some value as screening procedures but are useless as a quantitative measurement of alimentary bleeding. It is appreciated that the poor correlation between the radioactive measurement of occult blood and the chemical tests (particularly the "haematest" method) is partly due to sampling errors. False negative results can be obtained from incomplete mixing of blood in the stool which frequently occurs in patients who are bleeding intermittently. False positive results have always been the drawback of chemical tests which are too sensitive. Such false positive results are shown in Case 2 (Table V). It is thus extremely difficult to find a standard chemical test which is neither too sensitive nor too insensitive. It is therefore recommended that in order to minimize sampling errors the "haematest" method should be duplicated on each specimen.

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THE MARCH (1960) ISSUE

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