JOURNAL OF NUCLEAR MEDICINE 7:612-619, 1966

Quantitation of Gastrointestinal Bleeding by Use of a Large Volume Scintillation Detector[2,3]

Earl T. Anderson, M.D.,[1] Mitchell Passovoy and Frank E. Trobaugh, Jr., M.D.[2]

Chicago, Illinois

INTRODUCTION

The estimation of gastrointestinal blood loss, by current widely accepted chemical techniques, is possibly the most poorly quantitated laboratory examination used in the practice of medicine. Since Van Deen first used tincture of guaiac in 1864 for the detection of occult blood in feces (1) many modifications of this basic technique have been reported. Although guaiac, benzidine and other chemical agents are regularly used in tests for occult blood, and great reliance placed on their results in formulating diagnoses and therapies, recent reviews have indicated that the sensitivity and accuracy of these tests are inconstant (2,3,4).

This paper describes a clinically practical method for detecting and quantitating blood in feces. With the availability of large volume scintillation detectors, previously described isotopic methods for quantitating gastrointestinal blood loss can be modified to add this valuable technique to the diagnostic armamentarium in many hospitals. The technique described herein utilizes a large volume scintillation detector and external agitation of an enclosed stool sample. The large volume scintillation detector ensures constant geometry and permits a

---

[1]Present address: The Mountainside Hospital, Montclair, New Jersey.

[2]From the Section of Hematology, Department of Medicine, Presbyterian-St. Luke's Hospital and the Department of Medicine, University of Illinois College of Medicine, Chicago, Illinois.

[3]These investigations were supported by General Research Support Grant FR 05477 from the National Institutes of Health, U.S. Public Health Service.

sensitive radioassay of the entire stool specimen. The techniques of sample collection and assay prevent specimen loss due to frequent handling and multiple transfers and reduce the esthetic objections to the handling of feces.

METHODS

Red Cell Tagging: The patient's red-blood cells are tagged with sodium chromate-51 according to a modification of Gray and Sterling's method (5). Sixteen milliliters of anticoagulated blood are incubated at room temperature for 30 min with 1.5 μC of ^{51}Cr[1] per kilogram of body weight, washed and resuspended in normal saline and reinjected into the patient.

Stool Collection and Assay: All stools in a 24 hour period are passed directly into a new one gallon tin container positioned under the seat of a commode, taking care to exclude contamination with urine. To eliminate offensive odors and to avoid specimen loss, the containers are kept in a portable refrigerator in the patient's room. At the end of each collection period, the containers are refrigerated in the laboratory until assayed. In general, the collection continues over a five day period.

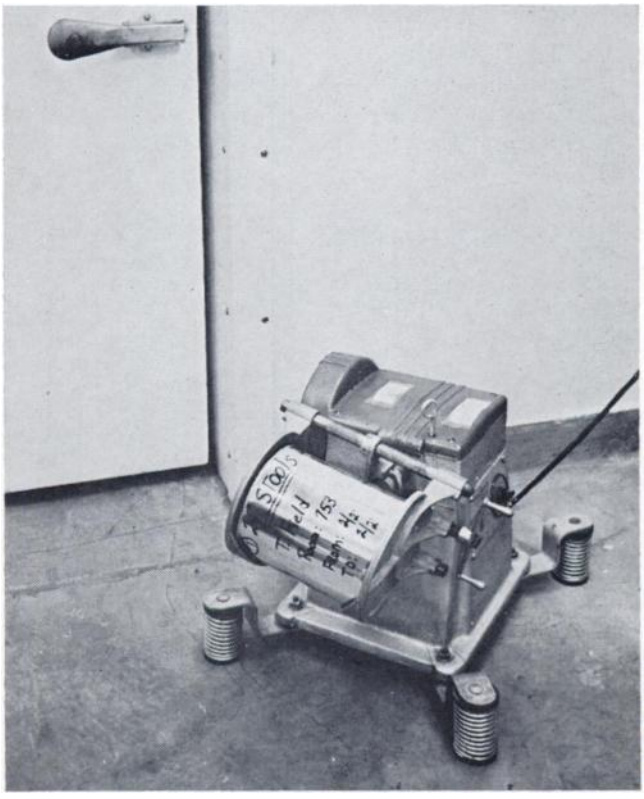

Fig. 1. Red Devil Paint Conditioner containing gallon tin container used for stool collection.

[1]Specific activity—approximately 100 mC/mg Cr.

To assay a sample, approximately 500 ml of tap water are added to the specimen in the original container which is then resealed. The gallon tin is placed in a Red Devil paint conditioner (Fig. 1) and mixed thoroughly for five minutes, producing a fine liquid suspension of stool and ensuring uniform distribution of any chromium-51. This suspension is then transferred to a new one quart Mason jar made of low potassium glass. The original container is rinsed once with a small amount of water and the rinse water is also added to the jar. The specimen volume is then adjusted to exactly one quart and again mixed in the paint conditioner. The radioactivity of the entire specimen is then measured in a special large volume detector (Armac Scintillation Detector, Model 440, Packard Instrument Company) which is connected to a single channel autogamma spectrometer (Model 410A, Packard Instrument Company).

The counting chamber is an aluminum cylinder 4.25 inches in diameter and eight inches in length, enclosing a volume of 1,800 cc. It is surrounded by a scintillation fluid which contains five grams per liter of diphenyloxazole and 0.5 grams per liter of dimethyl POPOP[2] in a special high-flash solvent. Six three-inch magnetically shielded photomultiplier tubes are mounted at the rear of

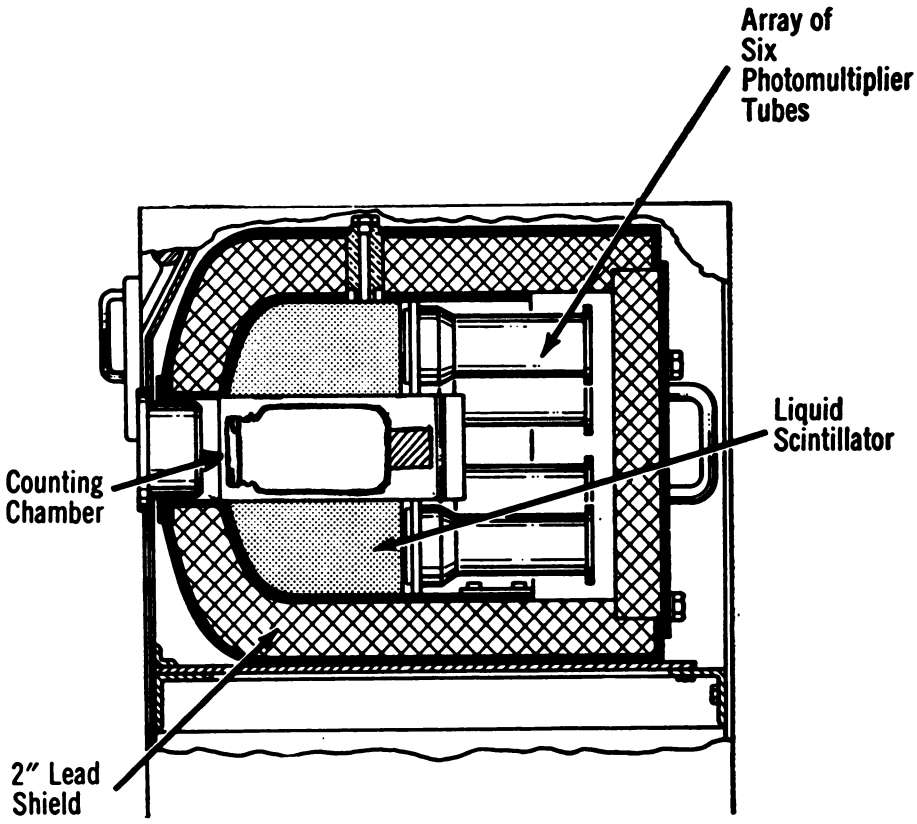

Fig. 2. Cross-sectional view of the Armac Scintillation Detector.

[2]One, 4-bis-2-(5-phenyloxazolyl)-benzene.

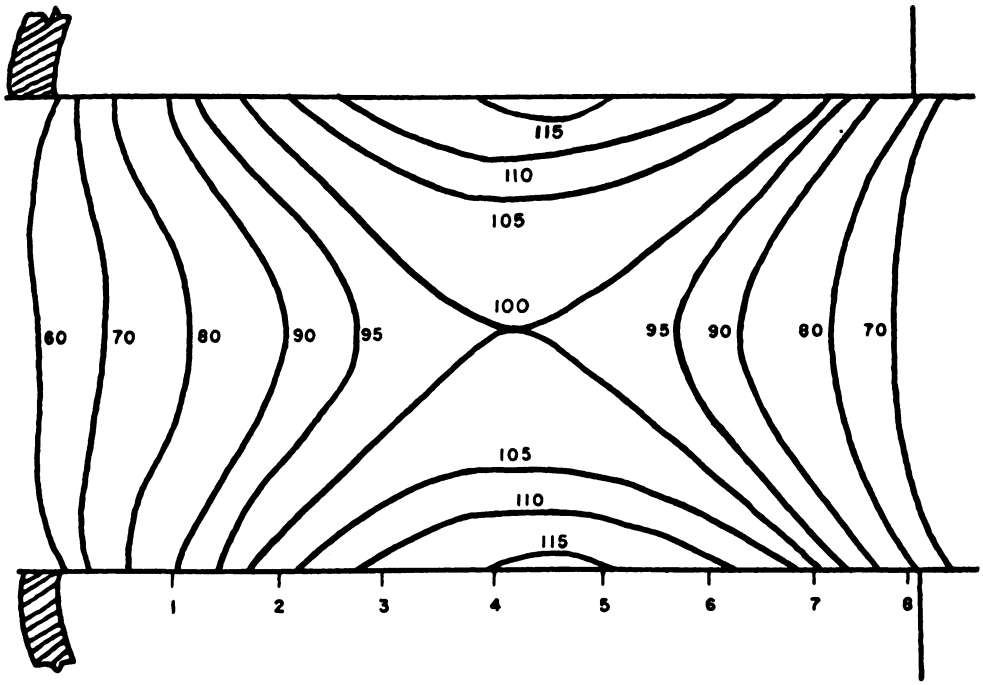

Fig. 3. Isoresponse curves of the Armac detector. These curves were obtained from a series of 0.3 minute counts of a 2.8 ml ^{51}Cr source positioned at vertical and horizontal intervals of 0.5" in a vertical plane through the axis of the chamber. The counts are expressed as a per cent of the count obtained in the center of the chamber.

the scintillator tank, and the entire detector is surrounded by two inches of lead shielding (Fig. 2). Because of the isoresponse curves of the detector (Fig. 3), sample position is critical and each must be placed in the center of the chamber to ensure good counting reproducibility. This is readily achieved by inserting each specimen jar against a backstop installed at the rear of the counting chamber.

The radioactivity of each sample is determined by counting for five minutes at a window energy width of 800 keV. The background activity of each jar filled with clean tap water is determined prior to the sample preparation.

A sample of venous blood is drawn daily and its radioactivity determined as follows. Two ml of blood, drawn on the same day the stool was collected, are added to a quart jar filled with tap water and counted for five minutes under conditions identical to those used for counting the stool specimen. The amount of blood in each stool specimen is then calculated from the following formula:

$$\text{ml of blood in specimen} = \frac{\text{corrected counts per min in stool specimen}}{\text{corrected counts per min per ml of venous blood}}$$

RESULTS

To determine the accuracy, sensitivity and the validity of this technique the following studies were made.

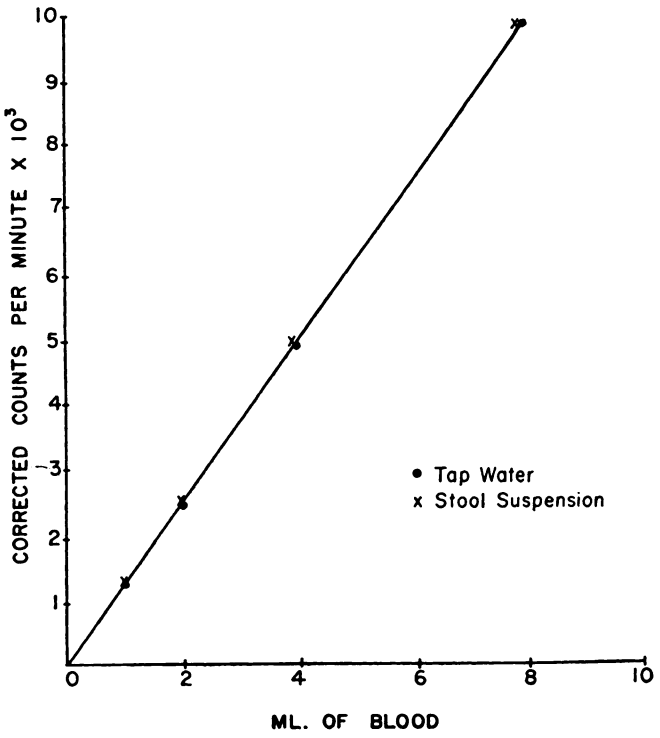

Fig. 4. Relationship between counts obtained and volume of ^{51}Cr labeled blood in tap water and in stool suspension.

The Counting System: The standard critical studies of the counting apparatus were performed. The efficiency of the detector for ^{51}Cr determined at a window energy width of 800 keV, ranged from 26.9% to 31.9% for a point source in the center of the chamber, while with the quart jars it was only slightly less, ranging from 24.0% to 27.4%. The background activity has been quantitated and minimized. The mean five minute background count of the empty chamber was 5,251 (1,050 counts per minute) with a standard deviation of 43.78. Each jar contributes to the background according to its potassium content. This component of the background has been minimized and kept contant by using jars made from the same batch of low potassium glass[3]. The mean five minute background count obtained from empty glass jars was 5,611 (1,122 cts/min) with a standard deviation of 78.09. The addition of tap water to the jars does not add any counts to the background. The maximum counting error (coefficient of variation) of the system, in counting one ml of tagged blood for five minutes, was two per cent.

Accuracy of Measurement: The relationship between the quantity of blood present and the counts obtained was determined as follows. Using volumetric pipettes, blood from a subject whose red-blood cells had been tagged with ^{51}Cr in the amount of 1.5 μC/kg on the preceding day was delivered in amounts

[3]Supplied by Kerr Glass Manufacturing Corporation, Sand Springs, Oklahoma.

of 1 ml, 2 ml, 4 ml and 8 ml, either to jars containing tap water, or to jars containing pooled stool suspension. The stool suspension was prepared by pooling stools from patients known to have received no radioactive substance. The stool was weighed, water added and the sample agitated in the paint conditioner. An amount of suspension equal to about 150 g of undiluted stool was added to each jar, the volume adjusted to one quart, and the whole sample agitated again. When corrected counts per minute are plotted against the amount of blood added both to plain water and to stool suspension, straight lines with identical slopes are obtained as illustrated in Figure 4. This indicates that there is no demonstrable absorption of gamma-rays by the stool.

Chromium-51 Excretion in Control Subjects: Thirty-two stool specimens were studied from seven patients with no evidence of gastrointestinal disease and whose red-blood cells had been tagged with chromium-51. There was an average apparent daily blood loss of 0.489 ml with a range of 0.02 to 1.44 ml (Table I). Whether this indeed represents small amounts of blood loss in *normal* individuals or whether it represents excretion of eluted ^{51}Cr has not been determined.

Clinical Studies: To date we have performed studies on approximately one-hundred patients suspected of gastrointestinal hemorrhage. A later paper will report details on these patients. A preliminary analysis of these investigations would suggest that this technique provides a powerful and reliable diagnostic tool, especially in the study of patients with hypochromic microcytic anemia, and that the various chemical tests used for the detection of occult blood are unreliable and difficult to interpret.

DISCUSSION

In 1950, Sterling and Gray (5) demonstrated that hexavalent ^{51}Cr combines with the hemoglobin molecule, thereby providing an erythrocyte "tag" that can be followed in studies of erythrokinetics. In 1954, Owen *et al* (6) first described the use of radiochromium-labeled erythrocytes for the detection of gastrointestinal hemorrhage in dogs. In 1957, Roche *et al* (7) applied this technique to the study of intestinal blood loss in humans suffering from hookworm infestation. Since that time several reports (8-14) have appeared in the literature dealing with the detection and quantitation of gastrointestinal blood loss with chromium-51. There is general agreement that this method is the most reliable test for either detecting or quantitating gastrointestinal bleeding. However, none of these methods has enjoyed clinical acceptance. Most of the previously described methods have depended upon measuring the radioactivity of small weighed aliquots of stool homogenates in well-type scintillation counters. Elaborate manipulations and the troublesome and disagreeable tasks of handling stool specimens have made these techniques cumbersome, malodorous, and therefore unacceptable as standard diagnostic techniques. Other techniques utilized crystal scintillation detectors with unfavorable geometry which resulted in very low counting efficiency and/or poor counting reproducibility. This not only necessitated the use of complex calculations in correcting for geometric factors, but severely limited the sensitivity of the methods.

Warner and Oliver (15) have proposed using a plastic phosphor well-counter for measuring ^{51}Cr in feces. Their instrument, however, could accommodate samples only up to 400 ml in volume; the efficiency of the system for counting ^{51}Cr was only 0.4 per cent. Cook and Valberg (16) have also described a well-counter which utilizes a plastic phosphor. Their counter was capable of accommodating a 1,000 ml sample. Its efficiency was considerably better than the one described by Warner and Oliver. However, a correction for sample volume had to be made routinely. Although they proposed that the instrument could be used for measuring fecal blood loss, no details were reported.

The method described herein circumvents most of the objections raised to previously described techniques. The procedure for the collection and homogenization of the stool specimens, first described by Gordon (17) and later by Jover and Gordon (18) is convenient for the patient, inoffensive in the laboratory, relatively inexpensive and uses disposable containers. By radioassaying the entire specimen in a closed system, the unpleasant task of taking aliquots or performing elaborate manipulations on stool specimens is avoided. The validity of the method is confirmed by the straight line relationship between the amount of blood in the stool specimen and the counts obtained, and the fact that there is no demonstrable absorption of gamma-rays by stool. Finally, in the absence of gastrointestinal bleeding, the amount of ^{51}Cr in a 24 hour stool sample is equivalent to approximately 1 ml, an amount in accord with that reported by others (7, 8, 11).

SUMMARY

An essentially closed-system of stool collection and radioessay which involves only one specimen transfer has been coupled with sodium chromate-51 labeling of red-blood cells to provide a sensitive and accurate estimate of blood loss in feces.

TABLE I

Subject \ Day	1	2	3	4	5	6	7	Mean
J.S.	0.64	0.63	1.44					0.903
D.A.	0.30	0.56	0.74	0.23	0.57	0.16	0.32	0.411
H.N.	0.36	0.33	0.13	0.20	0.37			0.278
R.V.	0.50	0.50	1.14	0.12				0.565
L.D.	0.59	0.29	0.50	0.37				0.437
K.J.	0.72	0.23	0.23	0.10				0.320
H.W.	0.02	0.82	0.92	0.87	0.76			0.842

The mean for the 32 specimens is 0.4893.

Table I. Apparent daily fecal blood loss (ml) in patients thought not to be bleeding from the gastrointestinal tract.

After a patient's red-blood cells are labeled with ^{51}Cr, his stools are collected into one gallon tins, mixed by external agitation, transferred to low potassium glass jars, and counted in a large volume scintillation counter.

Amounts of stool blood as small as 1 ml can be detected. This should provide a practical means of estimating the amount of blood lost in stools and provide a useful clinical diagnostic procedure.

ACKNOWLEDGEMENT

The authors gratefully acknowledge the technical assistance of Miss Mary-Ann Cardello and Mr. Roger Benson.

REFERENCES

PERANIO, A., AND BRUGER, M.: The detection of occult blood in feces including observations on the ingestion of iron and whole blood. *J. Lab. and Clin. Med.* **38**:433, 1951.

2. DERMAN, H., AND PAUKER, S.: Detection of occult blood in feces and urine. p. 70. In F. W. SUNDERMAN AND F. W. SUNDERMAN, JR. (ed.) Hemoglobin—Its precursors and metabolites. Lippincott, Philadelphia, 1964.

3. CULVER, P. J.: Examination of the stool. p. 374. In L. B. PAGE AND P. J. CULVER (ed.). A syllabus of laboratory examinations in clinical diagnosis. Harvard University Press, Cambridge, 1960.

4. MENDELOFF, A. I.: Selection of a screening procedure for detecting occult blood in feces. *J.A.M.A.* **152**:798, 1953.

5. GRAY, S. J., AND STERLING, K.: Tagging of red cells and plasma proteins with radioactive chromium. *J. Clin. Invest.* **29**:1604, 1950.

6. OWEN, C. A. JR., BOLLMAN, J. L., AND GRINDLAY, J. H.: Radio-chromium-labeled erythrocytes for the detection of gastrointestinal hemorrhage. *J. Lab. and Clin. Med.* **44**:238, 1954.

7. ROCHE, M., PEREZ-GIMENEZ, M. E., LAYRISSE, M., AND DIPRISCO, E.: Study of urinary and fecal excretion of radioactive chromium Cr^{51} in man. Its use in the measurement of intestinal blood loss associated with hookworm infection. *J. Clin. Invest.* **36**:1183, 1957.

8. EBAUGH, F. G. JR., CLEMENS, T. JR., RODNAN, G., AND PETERSON, R. E.: Quantitative measurement of gastrointestinal blood loss. I. The use of radioactive Cr^{51} in patients with gastrointestinal hemorrhage. *Amer. J. Med.* **25**:169, 1958.

9. BANNERMAN, R. M.: Measurement of gastro-intestinal bleeding using radioactive chromium. *Brit. Med. J.* **5052**:1032, 1957.

10. LEVIN, N., HART, D., AND BOTHWELL, T. H.: The measurement of gastrointestinal blood loss using radioactive chromium. *South African J. Lab. and Clin. Med.* **5**:93, 1959.

11. CAMERON, A. D.: Gastrointestinal blood loss measured by radioactive chromium. *Brit. Med. J.* **1**:177, 1960.

12. JONES, N. C. H.: Measurement of red-cell loss from gastrointestinal tract using radioactive chromium. *Brit. Med. J.* **1**:493, 1958.

13. BOOTH, C. C., AND MOLLIN, D. L.: Plasma, tissue and urinary radioactivity after oral administration of ^{58}CO-labelled vitamin B_{12}. *Brit. J. Haemat* **2**:223, 1956.

14. LEWIS, S. M., AND PORTER, I. H.: Erythrocyte survival in rheumatoid arthritis. *Ann. Rheumatic Diseases.* **19**:54, 1960.

15. WARNER, G. T., AND OLIVER, R.: A plastic phosphor well counter for sample volumes up to 400 ml. *Brit. J. Rad.* **35**:349, 1962.

16. COOK, J. D., AND VALBERG, L. S.: Evaluation and use of a large plastic scintillator for gamma counting. *J. Lab. and Clin. Med.* **61**:317, 1963.

17. GORDON, R. S. JR.: Exudative enteropathy. Abnormal permeability of the gastrointestinal tract demonstrable with labeled polyvinyl-pyrrolidone. *Lancet.* **1**:325, 1959.

18. JOVER, A., AND GORDON, R. S. JR.: Procedure for quantitative analysis of feces with special reference to fecal fatty acids. *J. Lab. and Clin. Med.* **59**:878, 1962.