THE ASHING TECHNIQUE AS A MORE PRECISE METHOD FOR THE RADIOASSAY OF ^{51}Cr IN FECES

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A well-accepted and effective method of measuring the occult loss of blood into the gastrointestinal tract uses the tagging of erythrocytes with ^{51}Cr-sodium chomate (*1–3*). These tagged red cells are subsequently re-injected into the donor, and timed fecal collections are made. Many investigators (*1–5*) currently use a "wet technique" for the stool radioassay in which a 2.0–5.0-gm sample of the stool homogenate, representing 3–4% of a total 24-hr stool collection, is counted in a well scintillation counter. This method requires long counting periods because of a low sample-to-background ratio.

To circumvent the inherent error associated with the radioactive counting of trace levels of ^{51}Cr, Cameron (*6*) suggested that the total fecal collection be dehydrated to reduce the stool volume for counting purposes. In this communication we wish to present data derived from a modification of Cameron's method for the fecal radioassay of trace levels of occult blood. These data support the hypothesis that the ashing technique is superior to the wet technique; therefore we would like to recommend the use of the ashing technique as the best method of radioassay of stool for trace levels of isotope.

TABLE 1. RECOVERY OF ^{51}Cr COUNTS FROM STOOL

			Ash technique results		Wet technique results	
Stool No.	Weight (gm)	Vol. blood added	Counts recovered (%)	Mean cpm	Counts recovered (%)	Mean cpm
1	345.5	0.4g	95	127	0	0
2	395.9	1.5	97	364	134	8
3	398.0	2.0	92	245	125	13
4	338.4	2.0	98	2003	97	67
5	338.2	2.5	93	2458	92	88
6	336.3	3.0	98	2719	101	102
7	324.7	0.6	98	425	110	12
8	320.2	0.8	99	571	103	9
9	275.2	1.0	102	827	113	25
10	330.7	3.5	99	3611	111	87
11	305.1	4.0	95	2699	103	97
12	306.7	4.5	93	3319	103	109
			M = 97		M = 99	
			s.d. ± 3		s.d. ± 32	

METHODS

Twelve 24-hr fecal samples were obtained from patients who had not received radionuclides.

Stool weights ranged from 275.0 to 398.0 gm. Fresh whole blood was obtained from laboratory personnel and was placed into 6.0 ml of ACD solution (2.5% sodium citrate, 0.8% citric acid and 1.2% dextrose). One microcurie of ^{51}Cr-sodium chromate was added to the 20.0 ml of the whole blood and was incubated in a water bath at 37°C for 120 min with gentle mixing to minimize trauma to the red cell membrane. A weighed quantity of the tagged whole blood was added to each stool (Table 1). The weight of the blood added ranged from 0.4 to 4.5 gm. After weighing, each stool was brought to constant volume by the addition of 1 part water to 3 parts of stool and was then homogenized for approximately 5 min using the Waring blender.

The stools were treated in two ways:

1. **Wet method:** Approximately 2.0 gm of the stool-blood homogenate were placed into preweighed counting tubes. This was done in triplicate, and the samples were counted for three 10-min periods concurrently with the blood standard. Calculations were based upon the corrected mean of the three 10-min counts.
2. **Ashing method:** The stool-blood homogenate minus the three samples removed from the wet assay was poured into preweighed, 100-gm capacity porcelain evaporating dishes. The filled dishes were again weighed and placed on a hotplate at the low setting for 12 hr. Stools were dehydrated to avoid splatter in the muffle furnace. The dishes were then transferred into a muffle furnace, and the temperature was gradually raised to 500°C where it remained for 12 hr. At the completion of the ashing, the dishes were allowed to cool and the ash was transferred with a spatula into a

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counting tube. Each dish was rinsed with 1.0–2.0 ml of 6 *N* HCl, and the contents were added to the counting tube. The addition of the acid further reduced the volume of the ash by an exothermic reaction. Each tube was then counted for three 10-min periods along with the blood standard.

A Packard Autogamma spectrometer (Model #410-A) was used for all counting. The window settings corresponded to the peak of the spectrum scan for ^{51}Cr which were 270 MeV and 370 MeV for the lower and upper windows, respectively. The geometry of all samples was identical.

RESULTS

The mean recovery, expressed as the percent of the counts added, is summarized in Table 1. Using the wet method, the mean percent recovery ranged from 0 to 134% with a mean of 99% ± 32 (1 s.d.). If sample number 1 is omitted from the calculations, the mean percent recovery is 108% ± 12 (1 s.d.). However, we have no explanation for the data in the sample; therefore, we do not feel justified in omitting it from our table.

When the ashing technique was used, the percent recovery ranged from 92 to 102% with the mean of 97% ± 3 (1 s.d.). The mean counting rates for the two techniques are shown in Table 1. These data clearly support the advantages of the ashing technique whereby trace levels of blood may be detected in feces with better precision in shorter counting time because of less error in counting.

DISCUSSION

The radionuclide ^{51}Cr has been widely used in the study of gastrointestinal physiology both in health and in disease. The trivalent cationic form is used as a tag for serum albumin in quantitating gastrointestinal protein loss (*7*). In the hexavalent anionic state, chromate is permeable to the red cell membrane and attaches to the hemoglobin molecule (*8*). This property has been used by other investigators (*5,9,10*) to measure blood loss into the gastrointestinal tract. The number of milliliters of blood lost is calculated using the formula:

$$\frac{\text{corrected cpm in 24-hr stool collection}}{\text{corrected cpm in 1.0-ml whole blood standard}}.$$

When activities of 100 μCi of ^{51}Cr are used in the average 70.0-kg adult, there is an activity of about 0.02 μCi/ml of whole blood. If we assume, for purposes of discussion, a blood loss of 5.0 ml into a 24-hr stool collection, an activity of 0.10 μCi is present in the 24-hr stool. Assuming that the activity is equally distributed throughout the stool (weight of 250.0 gm), there is a net activity of 4×10^{-4}–2×10^{-3} μCi in each tube. It can readily be appreciated from this model that these activities do indeed result in exceedingly low counts over background as is shown in our experiments and the work of others (*7*). Faced with this problem, several avenues are open to the investigator to improve his counting reliability:

1. Increase the counting time.
2. Use shielding devices to reduce the background counts.
3. Increase the activity of the administered radionuclide.
4. Use a large volume counter (*11,12*) so that most of the activity may be the object of counting and thus increase the absolute detection efficiency.

One can also use the ashing technique to improve the precision in the radioassay of trace levels of blood. Several investigators have used all of the above or various combinations of them. The use of shields may be cumbersome and ineffective. The administration of greater activities of radionuclides increases the radiation exposure and, therefore, should be avoided. The large-volume counter is not readily

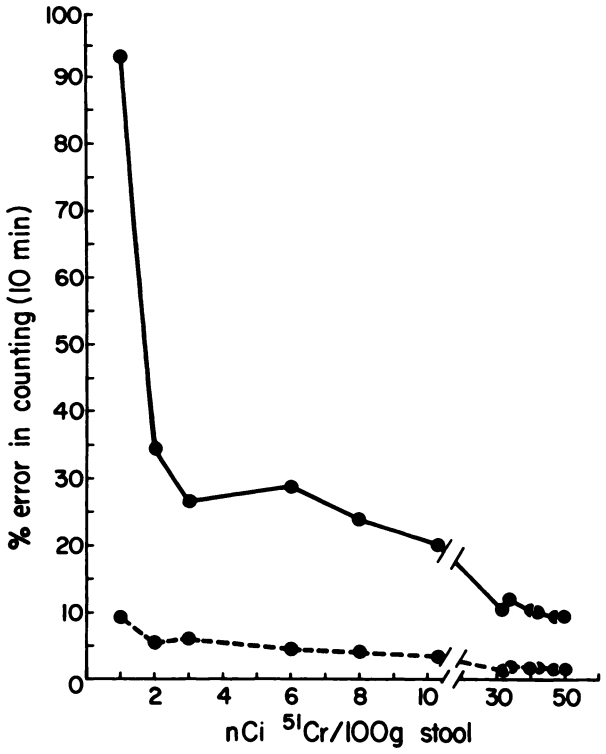

FIG. 1. Counting error is plotted against activity of ^{51}Cr/100 gm stool homogenate. Each point represent mean of three stool samples for a given activity. Solid line is for wet technique; broken line is for ash technique.

available and the scintillation well counter is found in most hospitals. The main advantage of the ashing technique is clear (Fig. 1) when the technique is compared to the wet technique and other methods: i.e., better precision in the counting statistics and therefore better recovery of the trace amounts of radiochromium. With the ashing technique, it may be possible to use less initial activity and yet obtain very precise counting statistics. Of course, the radiation to the patient will also be less.

The ashing technique might well be expanded to detect other isotopically labeled trace elements in stool. We have not had occasion to test the method with other isotopes. It is important to determine the vaporization temperature of the element prior to ashing because certain elements such as ^{131}I may be vaporized in the ashing process and thus give falsely low results. This information is readily available in chemistry handbooks.

SUMMARY

Two methods for the radioassay of stool for blood loss using ^{51}Cr tagged erythrocytes were compared. One method assayed a sample of the 24-hr stool homogenate, while the other method assayed the ash of the entire 24-hr fecal collection. The ashing technique revealed better precision in recovery as shown by a s.d. of 3.0 as compared to a s.d. of 32.0 using the wet technique. The ashing method concentrates a large volume of stool, thereby yielding higher counts per sample and thus reducing the error in counting.

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