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Quantitative determination of gastrointestinal bleeding utilizing CR-51 labeled red blood cells

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BY

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TABLE OF CONTENTS

Page

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I N T R O D U C T I O N

The occurence of occult gastrointestinal bleeding is a serious medical problem. The multiplicity of tests and techniques for detecting occult blood in feces suggests that none is entirely satisfactory. Blood which is swallowed or derived from a gastrointestinal lesion is evacuated in the feces, partly as acid hematin and partly as hematoporphyrin (Forshaw and Mason, 1954). The conventional techniques for qualitative determination of occult blood in the feces depend on the conversion of a substance with little or no color, such as Guaiac, Benzidine, or Orthotolidine, into a colored substance when oxidized by hydrogen peroxide in the presence of an oxygen carrier, such as hematin. Unfortunately these qualitative studies have their limitations. Hemoglobin or chlorophyll in the diet may give a positive result; e.g. Huntsman and Lidell (1961) obtained a large number of false positive results in normal subjects on meat diets. Also, small amounts of blood in the feces may be missed entirely. The effect of medicinal iron, which is not an oxygen carrier, on the occult blood tests remains controversial; some investigators (Hutchison and Hunter, 1949; Price 1950) report that medicinal iron gives false positive results with certain of

the occult blood tests, while other investigators (Todd et al, 1948; Needham and Simpson, 1952) state that medicinal iron does not give a positive result with the occult blood tests.

Therefore, it is obvious that a simple and precise quantitative and qualitative method for detection of blood in the stool would be highly desirable. Such a method, utilizing radioactive Cr-51 labeled erythrocytes, has been described and employed in the past (Owen et al, 1954; Cooper et al, 1955; Bannerman, 1957; Ebaugh et al, 1958; Izak et al, 1960). Briefly, the principle of this procedure involves: 1) the labeling of circulating red blood cells with radioactive Cr-51 (Cooper and Owen, 1956; Gray and Sterling, 1950; Ebaugh et al, 1953; Necheles et al, 1953), and 2) the detection of the radioactive Cr-51 in the stool as a measurement of fecal blood, thereby indicating bleeding into the alimentary canal.

O B J E C T I V E S

In this study, it has been our purpose to:

A. Measure accurately small amounts of blood (as Cr-51), if any, in patients who have no clinical evidence of gastrointestinal bleeding.

B. Make a comparative study between this method and that of a rapid, simple tablet test for occult blood in the stool, using Orthotolidine (Hematest), in patients

who have no clinical evidence of gastrointestinal bleeding.

C. Demonstrate that radioactive Cr-51 material, once in the gastrointestinal tract, is entirely excreted and not absorbed into the blood stream.

D. Quantitate the actual amount of fecal blood loss in patients with active bleeding.

E. Determine the point of cessation and/or the resumption of bleeding in patients with repetitive gastrointestinal hemorrhage.

MATERIALS AND METHODS

For this entire project, we used a total of sixty-three patients.

A. The following studies were performed on all patients:

1. Complete history and physical examination.
2. Laboratory evaluation.
   a. Complete blood count.
   b. Reticulocyte count.
   c. Platelet count.
   d. Serum iron and total iron binding capacity.
   e. Peripheral smear.
3. All patients were placed on a meat-free diet for four days; 24-hour stool specimens were collected and tested for occult blood during this period using Orthotolidine

(hematest). After four days, the patient was returned to his "normal" diet.

4. If clinical evidence warranted further examination, proctosigmoidoscopy, and upper and lower gastrointestinal surveys were performed. The patients used for control purposes were free of the following:
   a. Anemia, hypochromic.
   b. Gastrointestinal disease.
   c. Positive hematest for occult blood while on the meatless diet.
   d. Medications which may cause gastrointestinal bleeding (e.g., salicylates).
   e. Recent intake of radioactive material (RISA, iron, chromium, etc.).

The patients in the experimental group had some evidence of gastrointestinal blood loss (e.g., ulcers, gastritis, varices, drugs, etc.).

B. Red Blood Cell Labeling:

1. Twenty ml of blood was withdrawn from the patient into a heparinized syringe and aseptically added to a bottle containing ten ml of ACD solution.
2. Two-hundred uc of Cr-51 was then added to this mixture and incubated for twenty minutes.
3. One-hundred mgm of sodium ascorbate was then added.

This reduces the remaining unbound chromate to chromic chloride (which does not tag red blood cells), but does not affect chromium bound within the red cells.

4. Fifteen ml of this blood tagged with Cr-51 was then aseptically injected intravenously into the patient. Approximately one hour later a two ml blood sample was drawn, placed into a test tube, labeled, dated, and set aside for later radioactive counts. Daily two ml blood samples were subsequently drawn for seven successive days. All blood samples were labeled, dated and set aside for subsequent radioactive counts.

C. Stool Collection:

1. Twenty-four-hour stool specimens were collected daily in individual containers and placed in a deep freeze.
2. The twenty-four-hour stool specimen was weighed and then mixed in a Waring Blendor. Smears of feces from at least four different portions of the mixed stool were tested for occult blood using Orthotolidine (hematest).
3. A known amount of water (stool was usually diluted to twice its original volume) was then added and the stool specimen was homogenized and re-weighed.
4. An aliquot of two ml of the homogenized stool specimen was then placed in a test tube, labeled, dated and

saved for counting.

D. Occult Blood Test (Orthotolidine - Hematest)

1. A smear of feces from at least four different portions of the twenty-four-hour stool specimen was made on filter paper before dilution of the stool.
2. Hematest tablets were placed in the center of each fecal smear.
3. One drop of water was placed on each tablet. After a five to ten second wait, a second drop of water was placed on each tablet in such a manner that it ran down the side of the tablet and onto the filter paper.
4. Readings were taken from the moistened areas on the filter paper around the tablets and recorded as follows:
   a. Strongly positive (+++) - an intense blue color appearing within sixty seconds.
   b. Positive (++) - a deep blue color appearing within 90 seconds.
   c. Weakly positive (+) - a blue color appearing within 120 seconds.
   d. Trace (tr) - a slight blue color appearing within 120 seconds.

e. Negative (-) - no color appearing within 120 seconds.

Any color changes on the tablet or color appearing on the filter paper after 120 seconds were disregarded.

E. Sample Counting:

1. The radioactivity count of the two ml sample of tagged venous blood and of the two ml aliquot of twenty-four-hour stool specimen was made in a well-type scintillation counter. Counts were performed daily and then repeated at the conclusion of the experiment on all daily samples.

2. All counts were corrected for background activity.

F. Measure of Radioactivity:

Blood radioactivity is measured and plotted on semi-logarithmic graph and calculated in counts per five minutes/two ml. (CP5M/ml) The radioactivity of an entire stool specimen was obtained from the aliquots of stool counted. This value was then compared with the radioactivity of the circulating blood obtained the day the stool was passed. Utilizing the following formula, it was possible to calculate the amount of blood (as Cr-51) in a twenty-four-hour fecal specimen.

FORMULA:

$$\frac{\text{Radioactivity cts/2 ml stool homogenate X total vol. stool homogenate}}{\text{Radioactivity counts/2 ml whole blood}} = \text{total volume (ml / 24 hours) of blood lost in stool.}$$

G. Ingestion of Cr-51 Labeled Red Blood Cells:

Twenty ml of the patient's own erythrocytes, tagged with two-hundred uc of Cr-51, were instilled directly into the stomach via a gastric tube. A two ml sample of the tagged blood was retained as the standard. Twenty-four-hour stool specimens were collected and processed in the same previously described manner. Two ml aliquots of the homogenated stool specimens were prepared daily over a seven-day period. Two ml blood samples were withdrawn daily from the patient for seven days to check for any blood absorption of radioactivity. The radioactivity of the homogenized stool specimens was compared with the radioactivity of the two ml sample of Cr-51 tagged blood standard. Utilizing the following formulae, it was possible to 1) calculate the amount of blood (as Cr-51) recovered in the stool, and 2) calculate the percentage of blood (as Cr-51) recovered in the stool.

1)

$$\frac{\text{Radioactive cts/2 ml stool homogenate X } \frac{\text{Total ml stool homogenate}}{2}}{\text{Radioactive counts/1 ml blood standard sample}}$$

= Total volume (ml / 24 hours) of blood in stool.

2)

$$\frac{\text{Radioactive cts/2 ml stool homogenate X } \frac{\text{Total ml stool homogenate}}{2}}{\text{Radioactive counts/2 ml blood standard sample X 10}}$$

X 100 = % of Blood recovered in stool.

R E S U L T S

Table 1 demonstrates the results of qualitative studies for occult blood in the feces of fifty-one patients placed on a meatless diet for four days. Thirty-eight patients had a trace to weakly positive reactions to Orthotolidine up to forty-eight hours after fasting from meat; thirteen patients had a trace to strongly positive reactions throughout the entire period. When placed on a "normal" meat diet for seven days, the occult blood tests were always positive for all fifty-one patients.

TABLE 1

OCCULT BLOOD TEST - ORTHOTOLIDINE

A. On Meatless Diet (4 days)

No. of Patients	Day 1	Day 2	Day 3	Day 4
38	tr to 1+	tr	neg.	neg.
13	tr to 3+	throughout		

B. On Meat Diet (7 days)

No. of Patients	Day 1	Day 2	Day 3	Day 4
51	Occult blood tests were always positive.			

From this study we were able to compare the results of this method with the Cr-51 labeled red blood cell technique for quantitative detection of fecal blood.

Table 2 demonstrates the results of the quantitative studies using Cr-51 for determination of blood in the stool on the same fifty-one patients. The clinical diagnoses of these patients are also summarized on this table. It was found that the thirty-eight patients who had a negative Orthotolidine test, after forty-eight hours on a meat-free diet, had less than four ml of blood in their stools. Conversely, more than four ml of blood was found in the stools of the thirteen patients whose stools had remained positive to Orthotolidine while on the meat-free diet. Moreover, all thirteen patients had some explanation or derangement to account for this excessive blood loss in the stool.

TABLE 2

QUANTITATIVE DETERMINATION OF GASTROINTESTINAL BLEEDING

USING CHROMIUM-51

Ml of Blood 24-hr Stool Specimen as Cr-51	Total Number of Cases	No G.I. Dis-order	Chronic Alcoholism No G.I. Disorder	Early Cirrhosis No.G.I. Disorder	Cirrhosis Gastritis Varices Etc.	G.I. Disorders Ulcer Hem. Gastritis Drugs, Etc.
1	20	10	4	3		3
2	8	2	3	2		1
3	4	1	1	1		1
4	6	3	1	2		
5	5	2	1	1		1
6	2	1			1	
7	4	2				2
12	1					1
17	1					1
TOTAL	51	21	10	9	1	10

DISTRIBUTION OF 51 CASES INTO DISEASE ENTITY CATEGORIES

Table 3 represents a summary of the results in the thirteen patients with more than four ml of fecal blood. Their clinical diagnoses plus other factors (see under comment) capable of contributing to excessive fecal blood are listed. Case No. 9 was discarded; this patient had previously received RISA, thereby invalidating his quantitative results.

TABLE 3

SUMMARY - 13 PATIENTS WITH POSITIVE
QUALITATIVE & QUANTITATIVE TESTS

Patient Case #	Clinical Diagnoses	Ortho-tolidine Test	Ml of Blood 24-hr Stool as Cr-51	Comment
#9*	No G.I. Disorder	tr	4.0 - 4.9	Had RISA
#23	Severe Chr Pulm Emphysema	1+	4.0 - 4.9	Teeth Extractions
#15	Chr Pulm Emphysema	1+	4.0 - 4.9	- - - -
#36	Duodenal Ulcer - Anemia	2+	4.0 - 4.9	Active Ulcer c Crater
#45	Cirrhosis, D.T.'s	3+	4.0 - 4.9	Gastritis
#37	Rheumatoid Spondylitis	1+	5.0 - 5.9	Salicylate Therapy
#34	Cirrhosis, Acute Alcoholism	2+	5.0 - 5.9	Gastritis
#47	Chr Pulm Emphysema	3+	6.0 - 6.9	Teeth Extractions
#5	Panniculitis - Anemia	2+	6.0 - 6.9	- - - -
#16	Severe Anemia, Etiol.?	2 to 3+	6.0 - 6.9	Site of Bleeding Unknown
#14	Pneumonitis	2+	6.0 - 6.9	Active Duodenal Ulcer
#46	Status Asthmaticus	tr	11.0 -11.9	ACTH Therapy
#35	Schizoid Personality	tr	16.0 -16.9	Hemorrhoids

*Case Discarded

Table 4 demonstrates the results for the recovery of ingested Cr-51 labeled red blood cells. The results in three of our patients showed a 97% average recovery of the ingested blood in the stools. The results in three other patients revealed only partial recovery of the ingested blood in the stools. This partial recovery was attributed to loss of one or more of the patients' stools. Daily blood samples from all six patients were negative for radioactivity.

TABLE 4

RECOVERY OF INGESTED CR-51 LABELED BLOOD

Patient	Blood Ingested (ml)	Recovered Blood in Stool (ml)	Recovered Blood in Stool (%)	Radioactivity in Blood
1	20 ml	19.5	97.5	None
2	20 ml	20.0	100.0	None
3	20 ml	18.9	93.7	None
4*	20 ml	17.5	87.6	None
5*	20 ml	16.6	83.0	None
6*	20 ml	16.3	81.5	None

*Stool lost on one or more days.

CASE STUDY #1

This study was done in an attempt to quantitate the actual fecal blood loss in patients with active bleeding and to determine the point of cessation and/or resumption of bleeding in those with repetitive gastrointestinal hemorrhage.

Case #54 is a representative case of six cases with active gastrointestinal bleeding.

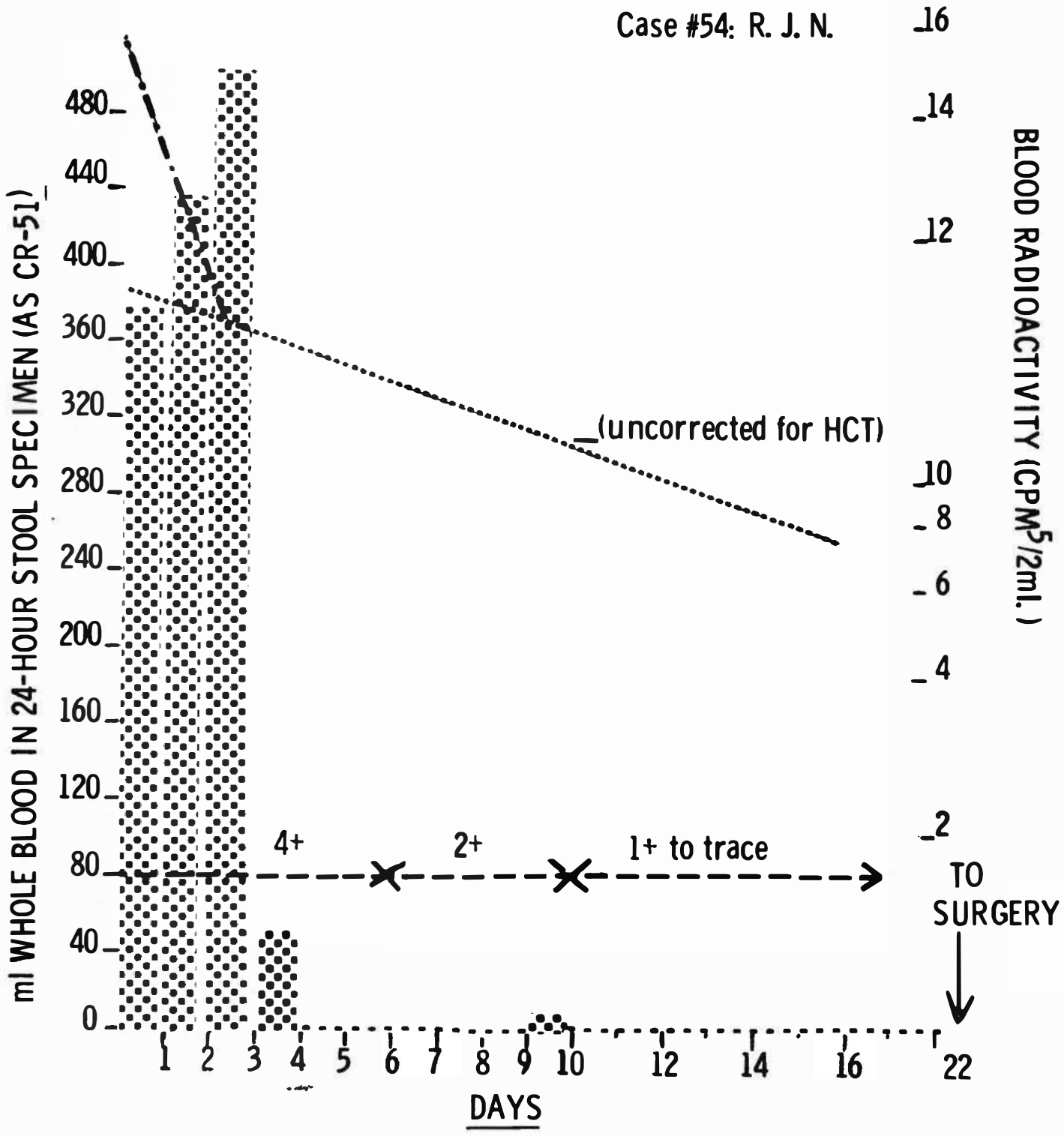
Case #54: R.J.N. U8913

1. 36-year old white single male
2. Chief Complaint: Hematemesis and Melena
3. Past History: Duodenal Ulcer for past 12 years confirmed by x-ray. Hematemesis and melena 5 times. Numerous transfusions.
4. Hospital Course:
   a) Repetitive hemorrhage
   b) Numerous transfusions
   c) Surgery: Bilateral Vagectomy Gastro-jejunostomy
5. Diagnosis:
   a) Duodenal Ulcer with Hemorrhage
   b) Multiple Pseudo-diverticula

Blood volume studies were done on the initial day of the study and the day prior to surgery. Red blood cell labeling with Cr-51, stool collections, and sample counting were done as previously described.

Figure 1 demonstrates dramatically the "explosive" nature of gastrointestinal hemorrhage during the first four days of his hospital stay. On the fifth day, there was an apparent cessation of bleeding and he had only minimal radioactivity (7 to 20 ml) in his stools during the subsequent forty-five-day period. The un-hatched horizontal line on the graph (Figure 1) represents the Orthotolidine Test results during this period. Initially, black tarry stools were collected but as bleeding subsided the stool color returned to normal. The curve in the upper half of the graph (Figure 1) shows the decline in the blood radioactivity (as Cr-51) during this period (whole blood--uncorrected for hematocrit).

Figure 1



CALCULATED LOSS OF BLOOD IN 24-HOUR STOOL SPECIMEN (AS CR-51)
BLOOD RADIOACTIVITY -- I. V. CR-51 LABELLED ERYTHROCYTES

Table 5 demonstrates an attempt to quantitate the actual blood loss over a fifty-day period in this same patient.

TABLE 5

Case #54: R.J.N.

Calculated Loss of Blood - 50 Day Period

Blood Volume (12-14-61)	3858 ml	
Transfusions (12-14-61 to 2-1-62)	1750 ml	
Total		5608 ml
Blood Recovered (12-15-61 to 2-1-62) in Stool	1664 ml	
Other Blood Loss	370 ml	
		2034 ml
		3574 ml
Blood Volume (2-2-62)		3333 ml
Whole Blood Unaccounted for		241 ml

The whole blood volume on day one was 3858 ml. Blood transfusions over a fifty-day period amounted to 1750 ml. If no bleeding had occurred during this period, the whole blood volume would have been 5608 ml. However, blood loss in the stool and via other exits totaled 2034 ml. Subtracting the blood loss (2034 ml) from the blood "gain" (5608 ml), an apparent blood volume of 3574 ml was calculated in this study. The actual blood volume done on the day prior to surgery (day 50) was reported as 3333 ml. Therefore, 241 ml of blood was not accounted for in this study. Since this represents less than five ml of blood per day, the numerous laboratory

procedures done over a fifty-day period can adequately explain for this "unaccounted" blood loss.

CASE STUDY #2

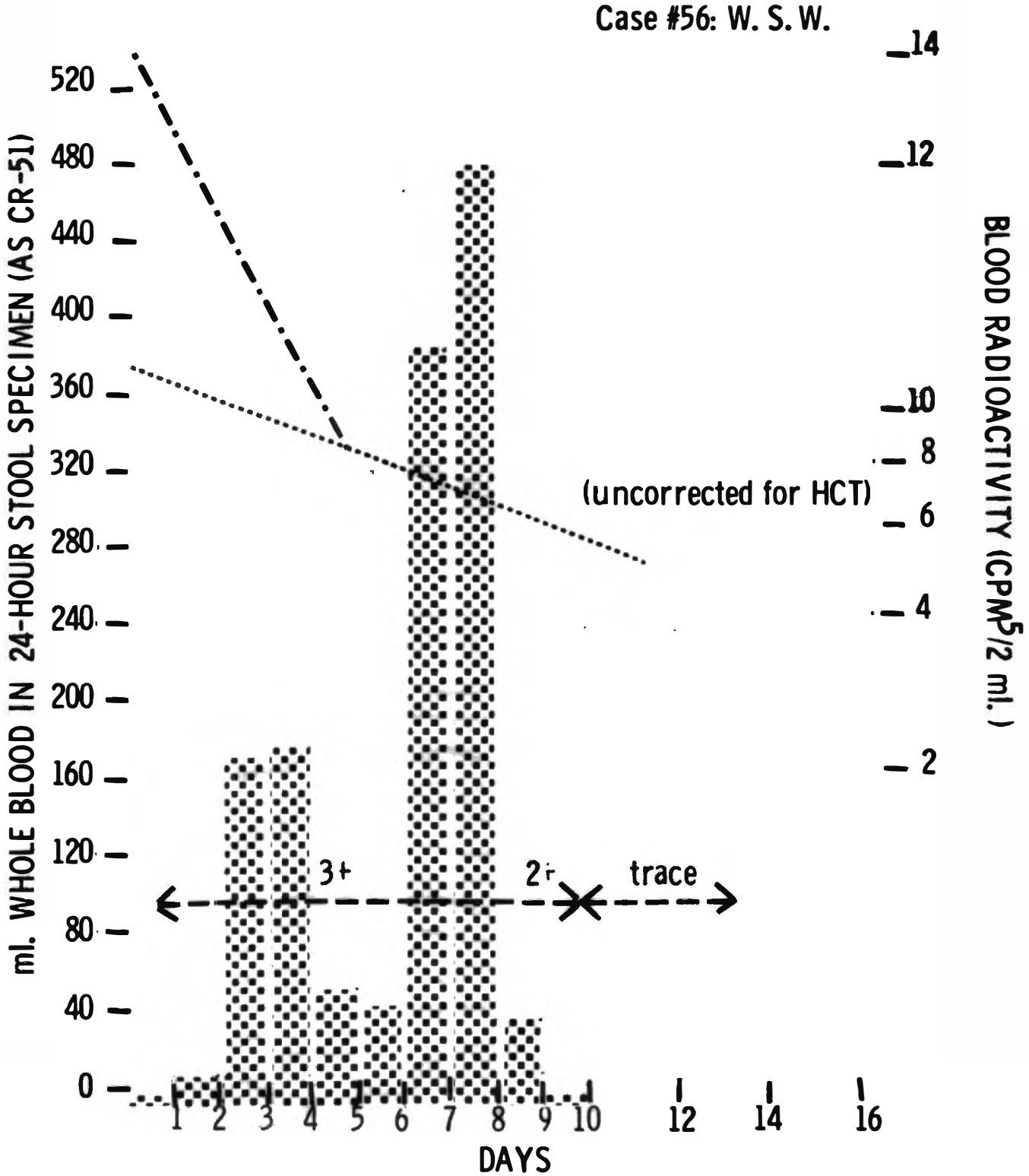
Case #56 is a representative case in our studies of a patient demonstrating cessation and resumption of gastrointestinal bleeding.

Case #56: W.S.W. U913

1. 47-year old white male
2. Chief Complaint: Abdominal pain
   Frank melena
   Syncopal attacks
3. Past History: Epigastric distress and abdominal pain, several years.
4. Diagnosis: Marked anemia, 2° to Chr Blood Loss
   Pre-pyloric Ulcer of Greater Curvature of stomach (x-ray)
5. Hospital Course: Numerous transfusions
   Ulcer regimen
   To be transferred to surgery for partial gastrectomy

Figure 2 demonstrates the cessation and resumption of bleeding in a patient with repetitive gastrointestinal hemorrhage. On the seventh day there was a 480 ml blood loss in the stool. On the eighth day blood loss in the stool had diminished to 38 ml. By the ninth day his bleeding had apparently ceased since subsequent counts for radioactivity in his stools were negative. Orthotolidine test results during this period are represented by the un-hatched horizontal line on the graph (Figure 2). The curve in the upper half of the graph (Figure 2) shows the decline in blood radioactivity (as Cr-51) during this period (whole blood -- uncorrected for hematocrit).

Figure 2



CALCULATED LOSS OF BLOOD IN 24-HOUR STOOL SPECIMEN (AS CR-51)
BLOOD RADIOACTIVITY -- I. V. CR-51 LABELLED ERYTHROCYTES

D I S C U S S I O N A N D C O N C L U S I O N S

These studies demonstrate and confirm the fact that the Cr-51 labeled red blood cell technique is a simple and precise method for quantitative measurement of blood in the feces. The amount of blood (as Cr-51) found in thirty-eight patients ranged from 0.03 ml to 3.99 ml of blood (as Cr-51) in a twenty-four-hour stool specimen. Thirteen patients had 4.0 ml or more of blood in their feces and all thirteen patients had some explanation or derangement to account for this excessive blood loss.

Our investigations show that the commonly used Orthotolidine (hematest) tablet test for occult blood is of some value only in those patients who were on an absolute meat-free diet for a minimum of forty-eight hours. On a regular diet containing meat, these tests were always positive. An adequate mixture of the twenty-four-hour stool specimen and samples taken from various areas of the stool are also required before proper qualitative analyses can be made. In addition, our findings confirm previous studies in showing that four to five ml of blood in the stool is necessary before a positive reaction is obtained with this test.

We found, in our studies, that Cr-51 tagged red blood cells, instilled directly into the stomach, were nearly completely recovered in the stools and that absorption of radioactivity into the blood stream was negative during this period.

The use of intravenously injected Cr-51 tagged red blood cells was found to be a reliable method for quantitating actual blood loss in the stool in those who have frank melena. This test correlated well with the clinical findings and other laboratory studies. It was also a good indicator of the cessation and/or the resumption of active bleeding in those with repetitive gastrointestinal hemorrhage.

SUMMARY

Twenty-four hour stool specimens were collected and tested for occult blood with Orthotolidine (hematest) on fifty-one patients after four days of meatless diet. Autogenous red cells were tagged with two-hundred uc of Cr-51 and reinjected into the patient. Stools were collected for seven days. Comparison of the radioactivity of the two ml aliquot of homogenized twenty-four hour stool specimen with the radioactivity of a two ml sample of circulating blood obtained the day the stool was passed, allowed the calculation of the amount of blood in twenty-four hour stool specimens.

In fifty-one patients, the Orthotolidine test was always positive on a well mixed twenty-four hour stool specimen up to forty-eight hours after fasting from meat. After forty-eight hours, thirty-eight of these patients had a negative test. The thirteen patients who remained positive had more than four ml of blood in the stool as determined by the Cr-51 red blood cell method.

Twenty ml of Cr-51 tagged red blood cells were instilled into the stomachs of six patients. Stools collected for seven days revealed nearly complete recovery of the administered Cr-51 red blood cells and daily blood samples were negative for radioactivity.

The calculated fecal blood loss using Cr-51 in patients with active hemorrhage correlated well with clinical and other laboratory findings.

A C K N O W L E D G E M E N T S

It is a pleasant obligation to express by indebtedness to those who have been most helpful to me in this research endeavor. I am especially grateful to Dr. R. E. Ogborn, Chief, Radioisotope Service, Veterans Administration Hospital, for: 1) the opportunity and privilege of working on a research project in this extraordinary department, and 2) his invaluable assistance, recommendations, and criticisms of this work. To Dr. E. A. Novak, I am most appreciative for his supervision, advice, and extensive help on all phases of this project.

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