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 [1]

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Since the work of Cruz (1) and Rhoads, Castle, Payne, and Lawson (2) iron deficiency has been attributed a pre-eminent role in the causation of the anemia associated with hookworm infection. At one time or another, however, this anemia has been blamed on the effect of hemolytic "toxins" from the hookworm, poor iron absorption, poor protein intake, and blood loss, or a combination of all or several of these factors. An exact evaluation of the role of each of these possible causes is not feasible until quantitative studies are carried out in the human being infected with the human hookworm.

In the present study, quantitative evaluation of one of these factors, namely blood loss due to the parasite, is attempted.

The radioactive isotope of chromium Cr^{51} has been used for marking red blood cells (3, 4), estimating their life span and measuring blood volume (4, 5). As a preliminary to our investigation on intestinal blood loss produced by hookworm, we wished to know: 1) How much Cr^{51} is absorbed from the intestinal tract when it is introduced into the stomach or duodenum of normal or hookworm infected patients, 2) whether Cr^{51} which is absorbed from the intestinal tract enters the circulating erythrocytes in significant quantities, 3) what proportion of Cr^{51} within the circulating red blood cells appears daily in the feces and urine of non-infected subjects.

In the present report, it is shown that most of the Cr^{51} from tagged circulating erythrocytes is excreted via the urine, that practically negligible amounts of Cr^{51} introduced into the gastrointestinal tract are absorbed, and that this absorbed Cr^{51} does not enter erythrocytes in measurable quantities. Furthermore, it appears from this study that blood loss, although less than the loss due to *A. caninum* in the dog (6, 7), is probably an important factor in the genesis of the anemia associated with human hookworm infection.[2]

METHODS

All subjects were hospitalized in the Medical or Surgical wards of the Hospital Vargas. Except for patients L.M. and J.F. (Table II), the non-infected subjects were patients who had been hospitalized for surgery, or for local skin conditions. In all subjects in this category, feces were free from hookworm ova in at least three examinations. Patient L.M. was a 30-year-old woman who had had an acute hemorrhage due to placenta previa, and was studied from day 13 to day 25 post partum. Patient J.F. had acute leukemia.

Tagging of circulating erythrocytes. Approximately 20 ml. of blood was taken from the antecubital vein and introduced into a sterile bottle containing 3 ml. of A.C.D. solution and approximately 80 microcuries of Cr^{51}.[3] The mixture was incubated at 37° C. and gently shaken every five minutes. The blood was then washed three times with 0.9 per cent saline solution in experiments in which intestinal absorption was studied, or reintroduced into the patient's circulation without further washing in the studies of Cr^{51} excretion from circulating erythrocytes.

Study of Cr^{51} absorption from the gastrointestinal tract. The washed erythrocytes were resuspended in 5 per cent dextrose. A Levine tube was placed in the patient's duodenum, under fluoroscopic control, and exactly 10 ml. of the suspension was introduced through the tube by gravity with a volumetric pipette, followed by six successive washings with 10 ml. of the diluting fluid. After thorough cleaning and decontamination of the pipette, a standard was prepared for each patient with the same

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[2] A preliminary note on this work has appeared (8). The discrepancies between the figures in the note and those in the present communication are due to the fact that at the time it was not realized that five of the cases were mixed *Ancylostoma-Necator* infections.

[3] From Abbott Laboratories, Oak Ridge, Tennessee.

pipette and in the same way used for intraduodenal administration and the washings were diluted up to 500 or 1,000 ml. with water. For the experiments in which sodium radio chromate in water was used, the dissolved radioactive substance was measured out volumetrically in a glass. The solution was then drunk by the patient, followed by six successive rinsings. Stools, 24-hour urine and venous blood were obtained daily, the radioactivity determined, and compared to radioactivity in the standard. The results were expressed in terms of per cent of the administered Cr^{51}, except for the activity of blood, in which results were expressed in terms of counts per minute per ml. of blood. Measurements were carried out until no more activity appeared in the feces.

Study of excretion of Cr^{51} from tagged circulating erythrocytes. After waiting for a period of at least four days after tagging of the circulating erythrocytes, stool and urine collections were begun. Stools were obtained in four-day periods in wide mouthed jars lined with aluminum foil into which the patients defecated directly. The stools were weighed to the nearest gram and homogenized in a Waring blendor with measured quantities of water. An aliquot of the homogenate was introduced with a special applicator into the bottom of a test tube, so that the upper level of the sample reached approximately a 3-ml. calibrated mark on the tube. The fecal sample was weighed by difference to the nearest 0.1 mg. and the result corrected for the proportion of water introduced in the stool. The radioactivity of the fecal aliquot was compared to that of 3 ml. of blood obtained from the patient at the start of the collection period, four days previously. The results were expressed in terms of ml. of blood with a radioactivity equivalent to that found in the stools during the period of study. This value was obtained, for a four-day period, by the formula:

$$\frac{R_s \times Q}{R_b \times 4} = B \qquad (1)$$

in which,

R_s = Radioactivity in the four-day stool in counts per minute per gram of stool.
R_b = Radioactivity in the blood of the same patient obtained at the beginning of the four-day stool collection in counts per minute per ml. of blood.
Q = Quantity of stool in the four-day period, in grams.
B = Quantity of blood in ml. which contains the amount of radioactivity found in the stools in 24 hours.

The average of the studies from three such four-day periods is given for each subject. Patient J.F. (Table II) was studied, of necessity, in a somewhat different manner, his circulating erythrocytes having been tagged on day 0, and stool collections begun on day 4. As the patient was in poor condition and ate little, no feces were passed until day 10, shortly before death. At autopsy, performed 24 hours later, the contents of the small intestine and colon were collected and their radioactivity determined. It was reasoned that the total amount of radioactivity present in the feces, small intestine and colon represented the total amount of Cr^{51} which had been excreted into the gastrointestinal tract since stool collections had begun.

The use of the blood radioactivity at the start of a four-day period of stool collection as a standard for comparing with stool radioactivity during the subsequent four days was to some extent arbitrary. Ideally, the standard blood value to compare with fecal radioactivity should be the average blood radioactivity during the time in which Cr^{51} present in the four-day stools is being excreted into the intestinal tract. This would imply a knowledge of the intestinal transit time for each individual patient during the time of experiment and intestinal site at which Cr^{51} is being excreted. As it was not possible to obtain this value, the above compromise was used.

Urine radioactivity was measured in 3 ml. of undiluted urine, and the activity compared to that in 3 ml. of blood obtained on the same day. The results were expressed as for the stools. Urine was collected in three 24-hour samples and the results given are the average of three determinations.

Plasma volume was measured by the Evans blue method (9) and the blood volume calculated from the venous hematocrit multiplied by 0.9 (10), to correct for differences between venous and body hematocrit. The hematocrit was obtained by centrifuging the blood for 30 minutes at 3,000 revolutions. No correction for trapped plasma was used. Thus the value B, in formula (*1*) could be expressed in terms of per cent of the blood mass which contains the amount of radioactivity found in the stools in 24 hours.

Measurement of radioactivity. Radioactivity was determined in a well-type scintillation counter (Nuclear, model 3037-B). By mixing a water solution of radioactive sodium chromate of known radioactivity with stools of varying consistency, it was repeatedly shown that under the present experimental conditions there was no significant self-absorption. In Table I are shown the results of such an experiment; approximately 0.9 μc. of Cr^{51} was mixed with 1,000 ml. of water; a specimen of non-radioactive hard feces was chosen and mixed with chromated water in the proportions, by weight, shown in Table I. As may be seen, only when feces were mixed with water in proportion 2:1 was there any apparent reduction of the expected count, by 2.7 per

TABLE I

Effect on measured radioactivity of mixing Cr^{51} in water with hard feces

Dilution Cr^{51} in water/feces	Radioactivity Cr^{51} in water alone	Radioactivity Cr^{51} in feces	% Difference
	c.p.m./ml. of water	*c.p.m./ml. of water*	
1:2	7,058	6,866	−2.7
1:1	7,058	7,056	0.0
1:1	7,058	7,149	+0.1
2:1	7,058	7,177	+0.3

cent. In these experiments, a minimum of 20,000 counts was determined. In all other dilutions, and in experiments in which semi-soft, normal stools were used, there was no detectable self-absorption. Consequently, in our studies of fecal radioactivity, when a hard stool was obtained, a dilution of at least 1 : 1 was used.

For the sake of convenience, the quantity of blood in ml. which contains the amount of radioactivity found in the stools or in the urine in 24 hours is expressed hereafter as "ml. of blood per 24 hours" in the stools and in the urine, respectively. A minimum of 6400 counts was measured on each sample.

Studies on hookworm-infected subjects. All subjects in this study were also hospitalized in the wards of the Hospital Vargas. Most of the patients with moderate or no anemia had been hospitalized for different unrelated complaints and were found incidentally to pass hookworm ova in their feces. Those with marked anemia came in with complaints related to the anemia.

Stool collections, marking of the circulating erythrocytes and determination of radioactivity were done as for non-infected patients. Hemoglobin was determined by the cyanomethemoglobin method (11). Ova counts were done by the method of Caldwell and Caldwell (12).

At the end of the initial 12-day stool study,[4] each patient was given 3 ml. of tetrachlorethylene by mouth, followed in two to three hours by a magnesium sulphate cathartic. During the ensuing days, stools were collected in 24-hour periods and washed repeatedly with tap water in a fine-meshed cloth bag until a thoroughly homogenized residue was obtained. The hookworms were then sought and picked out individually until no more worms could be found. The worms were placed in 70 per cent alcohol or 10 per cent formalin solution in a test tube. In some of the cases, the test tube was placed in the well-type scintillation detector and radioactivity determined. Stools were collected until they were found to be free from hookworms. Stool radioactivity was again studied during three successive four-day periods.

After gentle and thorough shaking of the hookworms in the test tube to insure uniform distribution, a representative sample of at least 100 worms was examined (or the whole sample when there were less than 100 worms). Each worm was cleared in creosote under a coverslip and identified under the microscope. Although it has been thought that *Ancylostoma* duodenale infection did not exist in Venezuela (13), five of the 20 cases examined were found to harbor this parasite, along with *Necator americanus* (14).

In some patients, in whom a good number of ova remained after the first vermifuge, tetrachlorethylene was administered a second time, and a third twelve-day study period was carried out.

The blood was "marked" again when radioactivity decreased below 100 counts per minute per ml. above background. The quantity of blood lost per hookworm per day was calculated from

$$H = \frac{A - B}{N} \qquad (2)$$

in which

A = Average amount of blood in the stool in ml. per day, determined from a 12-day study period before the administration of vermifuge.
B = Average amount of blood in the stool in ml. per day determined in the same way after the administration of tetrachlorethylene.
N = Number of hookworms recovered from the stools after the vermifuge given between periods A and B.

In order to attempt to establish a relationship between oviposition and blood-sucking activity of the worms, the blood loss per one hundred thousand ova per day was similarly calculated.

Since there were no pure *Ancylostoma* infections, a rough estimate of the blood loss occasioned by *Ancylostoma* was attempted by assuming that the *Necator* present were responsible for an intestinal blood loss calculated from the average found in pure *Necator* infections. Then,

$$B_a = \frac{100\,B_h - (3.11 \times 10^{-2}\,NH)}{AH}$$

in which

B_a = Blood loss per *Ancylostoma*, in ml. per day.
B_h = Total blood loss due to hookworms (*Necator* + *Ancylostoma*) as calculated by formula (*2*).
3.11×10^{-2} = Average blood lost per *Necator* (Table VI) in ml. per day.
N = Per cent *Necator* in the sample studied.
A = Per cent *Ancylostoma* in the sample studied.
H = Total number of hookworms (*Necator* + *Ancylostoma*) recovered.

An estimate of iron lost into the gastrointestinal tract was made by applying the formula:

$$Q_{Fe} = \frac{Hb \times B \times 3.40}{100}$$

in which

Q_{Fe} = Mg. of iron lost per day into the gastrointestinal tract.
Hb = Blood hemoglobin in Gm. per 100 ml.
B = Ml. of blood lost per day in the intestine.
3.40 = Mg. of iron per Gm. of hemoglobin.

Gastrointestinal studies, including X-ray visualization of esophagus, stomach, duodenum, small intestine and colon, and sigmoidoscopy were performed on patients 1 to 6, 11, 14, and 21. These revealed no source of bleeding. Patient 9 was autopsied and no source of bleeding other than hookworm infection was found.

RESULTS

The results are shown in Tables II to VII. In Tables III and IV, blood radioactivity is not tabulated, since in all cases except one it was not above background. In the case of M.P., Table IV, the

[4] Except on patient 1, who was studied for six days only because of his poor condition.

TABLE II

Fecal and urinary excretion of Cr^{51} tagged onto erythrocytes in non-infected patients

Subject Sex	Diagnosis	Feces		Urine		Blood hemoglobin
		ml. of blood/day	*% of blood mass/day*	*ml. of blood/day*	*% of blood mass/day*	*Gm./100 ml.*
J. S. M	Cutaneous leishmaniasis	0.71	1.22×10^{-2}	24.85	0.43	15.2
A. R. M	Chromoblastomycosis	1.05	2.03×10^{-2}	38.80	0.75	15.4
J. M. M	Cutaneous leishmaniasis	1.76	5.74×10^{-2}	49.09	1.40	14.4
J. R. M	Erythema induratum (Bazin)	1.04	2.01×10^{-2}	32.91	0.64	13.8
L. O. F	Bronchopneumonia (cured)	1.54	3.45×10^{-2}	28.33	0.59	12.5
M. B. F	Chronic cholecystitis	0.28		37.53		12.1
A. A. F	Erythema multiforme	2.14	6.72×10^{-2}	28.30	0.89	13.8
V. S. F	Cutaneous leishmaniasis	1.79	4.50×10^{-2}	26.82	0.67	14.0
L. M. F	Placenta previa-acute hemorrhage	0.42				6.1
J. F. M	Acute leukemia	1.98				4.2
Average		1.27	3.67×10^{-2}	33.33	0.77	
Range		0.28 2.14	1.22×10^{-2} 6.72×10^{-2}	24.85 49.09	0.43 1.40	

blood counted consistently 14 counts per min. per ml. above background throughout the period of study. In Table V, the patients are arranged according to the quantity of blood loss, which is roughly that of decreasing severity of infection, as judged from the total number of hookworms recovered and the ova remaining in the stools after vermifugation. In column 1 are shown the average of the first three ova counts in each patient. In column 2, the number of hookworms is the total number recovered after one or two vermifuges. In patients 9, 10, 12 and 21, recovery of the hookworms was not attempted. Column 3 shows the blood hemoglobin value upon admission to hospital and column 4 at the time of study. When values in columns 3 and 4 coincide, the patient was studied approximately during the first two weeks after admission, and the two similar values are one and the same determination. In column 5, the values represent the average of three successive four-day fecal samples. In patients 7, 10, 11, 13, 14, 15, 17 and 19, blood loss was not studied on admission, but only after partial recovery from anemia or, in case 8, after reduction of the blood hemoglobin, presumably because of bleeding due to hookworms. In Table VI, the number of hookworms is that recovered after the first vermifuge only, between periods A and B. In the column marked "patient" the numbers 1 and 2 in parentheses indicate two different studies in the same patient, before and after the first vermifuge, and before and after the second vermifuge, respectively. Thus there are fourteen studies in twelve patients.

DISCUSSION

Owen, Bollman and Grindlay (15) have shown in two dogs that most of the Cr^{51} tagged onto erythrocytes introduced into the stomach of these animals is recovered in the feces, and that most of the excreted radioactivity from Cr^{51} in circu-

lating erythrocytes appeared in the urine. In this respect, man seems to be similar to dog. On the other hand, in one dog who was given an aqueous solution of radiochromate by stomach tube, nearly half of the Cr^{51} was absorbed, whereas in our four subjects (Table IV) there seemed to be very little absorption of Cr^{51} under the same conditions. It has been suggested (15) that marking circulating erythrocytes with Cr^{51} and studying stool and blood radioactivity comparatively would be a means of determining the exact quantity of blood loss from gastrointestinal lesions. The same authors later reported two studies of intestinal bleeding in human patients (16), but they do not appear to have determined previously Cr^{51} excretion and absorption in the human. From our findings (Tables II, III and IV), it would seem that the method is a valid one in the human, since only negligible amounts of circulating Cr^{51} bound to erythrocytes (average 1.27 ml. of "blood" per day) are excreted in the feces (Table II) and since most of the radioactivity administered into the duodenum or stomach is recovered in the feces (Tables III and IV). Indeed, there seems to be little absorption of Cr^{51} even if this substance is given by mouth, either tagged on red cells or as sodium chromate in water (Table IV). It was felt that patient M.P. (Table IV) failed to collect feces on day 3, and that this accounted for the relatively low fecal recovery of 59.2 per cent, since the missing radioactivity was not recovered in the urine and since blood radioactivity was never above background in this patient.

Patients with marked anemia did not seem to differ from patients without anemia either with respect to intestinal absorption of Cr^{51} (Table III, patients M.R., J.V., and A.G.) or with respect

TABLE III

Per cent recovery in feces and urine of red-cell-attached Cr^{51} introduced into the duodenum

Patient Sex Presence of hookworm infection	Blood hemoglobin	Excretum studied	Per cent recovery*										
			Day after administration									Total	Cumulative total
			1	2	3	4	5	6	7	8	9		
	Gm./100 ml.												
A. R. M no	15.4	Feces Urine	0 0.3	— 0	95.3 0	— 0	2.0 0	— 0	0			97.3 0.3	97.6
J. S. M no	15.2	Feces Urine	— 1.3	0.4 0	29.8 0	— 0	42.5 0	— 0	18.0 0	0.01 0.2		90.7 1.5	92.2
J. A. M yes	15.2	Feces Urine	4.7 0.1	50.8 0.1	24.3 0.1	11.2 0	0.1 0	0				91.1 0.4	91.5
P. B. M yes	12.4	Feces Urine	32.6 0.9	58.9 0.8	11.8 0.7	0						103.3 2.4	105.7
M. R. F yes	5.9	Feces Urine	67.5 4.1	25.4 0	12.1 0	— 0	0 1.2					94.5 5.3	99.8
J. V. M yes	6.2	Feces Urine	84.2 0	14.7 0	0 0							98.9 0	98.9
A. G. F yes	5.4	Feces Urine	0 1.3	83.5 0.3	19.0 0.2	1.0 0	0 0	0				103.5 1.8	105.3
J. J. M. M yes	11.5	Feces Urine	0	0	0.8	90.8 Not studied	—	2.1	—	0.5	0	94.2	
Average		Feces Urine										96.7 1.7	98.7

* 0 = no radioactivity present in sample; — = no feces obtained.

TABLE IV

Per cent recovery in feces and urine of Cr^{51} given by mouth

Patient Sex Presence of hookworm infection	Blood hemoglobin	Substance administered*	Excretum studied	Per cent recovery† Day 1	2	3	4	5	6	7 to 13	14	Total	Cumulative total
	Gm./100 ml.												
A. P. F no	10.1	W	Feces	68.4	13.3	3.6	0.1	0.8	0			86.2	95.2
			Urine	5.1	1.4	1.7	0.6	0.2	—			9.0	
J. M. M no	14.4	W	Feces	87.8	—	6.6	3.4	0.2	0			98.0	99.8
			Urine	1.3	0.3	0.2	—	—				1.8	
L. R. F no	14.3	W	Feces	0	48.0	—	—	—	47.7	— 0.1	0	95.8	103.2
			Urine	5.7	0.6	0.8	0.2	0.1	0	0 0		7.4	
M. P. F no	13.7	W	Feces	3.9	2.7	—	52.6	0				59.2	60.2
			Urine	0.6	0.3	0.1	0					1.0	
S. B. M yes	15.8	E	Feces	35.0	38.6	15.2	0					88.8	89.3
			Urine	0.5	0	0						0.5	
B. M. M yes	7.1	E	Feces	0	56.3	43.2	0.2	0				99.7	101.8
			Urine	2.1	0	0	0	—				2.1	

* W = Aqueous solution of sodium chromate; E = Chromium-tagged, washed erythrocytes.
† 0 = No radioactivity present in sample; — = No feces obtained.

to fecal excretion of Cr^{51} (Table II, patients L.M. and J. F.).

Most of the radioactivity which escapes into the urine when Cr^{51} is administered into the gastrointestinal tract usually appears during the first 24 hours after administration of the radioactive material. Whether this means that absorption from the upper gastrointestinal tract is greater

TABLE V

Intestinal blood and iron loss in patients with hookworm infection

Column number			1	2	3	4	5	6
Patient	Age	Sex	Hookworm ova	Total no. of hookworms recovered	Blood hemoglobin: On admission	Blood hemoglobin: At time of study	Fecal blood loss	Calculated iron loss
			1,000/day		*(Gm./100 ml.)*		*ml./day*	*mg./day*
1*	24	M	8,714	3,534	2.0	2.0	251.5	17.1
2	18	F	2,983	3,043	3.7	6.6	99.3	22.3
3	14	M	6,748	1,641	7.8	9.3	92.0	29.1
4	60	M	1,639	1,121	8.8	8.8	65.0	19.5
5	36	M	1,708	1,684	3.9	3.9	57.2	7.6
6	35	M	3,281	840	3.6	8.9	56.1	6.7
7*	24	F	1,824	258	3.1	11.4	49.8	19.3
8*	56	M	3,511	502	10.2	7.1	46.3	11.2
9	50	M	583		4.9	4.9	39.6	6.6
10	38	M	2,154		2.0	6.9	29.2	6.9
11*	14	M		700	4.6	11.5	25.2	9.4
12	70	M	447		6.2	6.2	24.1	5.1
13	35	M	440	417	7.2	11.4	29.4	11.4
14*	20	F	1,662	72	5.9	9.0	18.9	5.8
15	14	M		609	5.2	12.0	15.4	6.3
16	21	M	482	189	11.6	11.6	7.8	3.1
17	36	M	1,060	509	12.4	15.0	7.3	3.7
18	17	M	421	109	13.4	13.4	4.4	2.0
19	48	M	160	80	16.1	13.9	3.5	1.7
20	15	F	38	67	14.4	14.4	2.8	1.4
21	16	M	56		18.1	18.1	2.0	1.2

* Mixed *Ancylostoma duodenale-Necator americanus* infection.

TABLE VI

Blood loss per hookworm and relationship between blood loss and number of ova in pure Necator americanus infection

Patient	Fecal blood loss period* A	Fecal blood loss period* B	Number of *Necator* recovered	*Necator* ova period A	*Necator* ova period B	Blood loss	Blood loss
	ml./day			*thousand/day*		*ml.* $\times 10^{-2}$*/ Necator/day*	*ml./*10^4 *ova/ day*
2 (1)	43.17	7.36	2,586	2,983	379	1.38	1.38
2 (2)	7.36	0.96	457	379	75	1.40	2.11
3	96.94	3.47	1,641	6,748	138	5.69	1.41
4 (1)	65.00	8.13	1,023	1,639	147	6.35	3.81
4 (2)	8.13	5.73	98	147	47	2.44	2.40
5	29.02	3.33	1,684	1,708	27	1.53	1.53
6	51.30	8.75	840	3,281	240	5.07	1.40
13	17.87	1.45	417	440	0	3.94	3.73
15	15.40	0.73	609			2.41	
16	7.74	1.84	189	482	0	3.12	1.22
17	7.26	1.23	509	1,060	0.9	1.18	0.57
18	4.41	0.53	109	421	1	3.55	0.92
19	3.49	0.17	80	160	0	4.15	2.08
20	2.74	1.81	67	38	13	1.39	3.72
Average						3.11	2.02
Range						1.18–6.35	0.57–3.73
Standard deviation						±1.73	±1.12

* A = Study shortly before vermifuge; B = Study after vermifuge.

than from the lower bowel cannot be decided but appears quite possible. It is improbable that this radioactivity should represent Cr^{51} which remained suspended outside the injected erythrocytes, since the cells were washed three times before being administered by mouth or stomach tube, and since absorption of radiochromate, when given in water, although apparently significant is also small (Table IV).

Except in one case, there was no detectable radioactivity in the erythrocytes of patients given Cr^{51} into their intestinal tract. It would seem, then, that what little Cr^{51} is absorbed does not tag the circulating erythrocytes.

From these studies it appears that, within certain limits, measurement of fecal radioactivity and its comparison with blood radioactivity are valid ways of estimating the amount of blood lost into the gastrointestinal tract through a bleeding lesion, in patients whose erythrocytes have been marked with Cr^{51}. On the other hand, while studies with radioactive chromium tell us fairly accurately the amount of blood which is lost into the gastrointestinal tract, they give us no information regarding possible reabsorption of the various blood elements from the intestinal lumen.

The total loss of blood and iron per patient

The total blood loss per day may be considerable (Table V), and is roughly proportional to the intensity of infection. To obtain a more exact value in the column indicating fecal blood loss (Table V), one would have to subtract as a "blank" the small quantity of Cr^{51} which would

TABLE VII

Estimate of blood loss in Ancylostoma duodenale infection

Patient	Blood loss*	No. of *Necator*	Probable blood loss due to *Necator*	Probable blood loss due to *Ancylostoma*	No. of *Ancylostoma*	Probable blood loss per *Ancylostoma*
	ml./day		*ml./day*	*ml./day*		*ml.* $\times 10^{-2}$*/day*
1	64.18	2,987	92.90	?	124	?
7	40.07	76	2.36	37.71	182	20.72
8	29.70	453	14.09	15.61	49	31.86
11	16.72	327	10.17	6.55	32	20.47
14	2.09	53	1.65	0.44	9	4.89

* Calculated from formula (*1*).

be excreted by the individual under study if he were not infected by hookworm. Such a "blank," however, appears to be quite variable from patient to patient (Table II) and using the average fecal Cr^{51} found for the 10 non-infected subjects as "blank" does not appear justified.

In patients 1, 2, 3, 4, and 6 who entered the hospital in poor condition, with a low blood hemoglobin, blood loss soon after admission (Table V, column 5) was distinctly greater than after they had been treated with iron and diet, or transfusions. These patients will be reported elsewhere in more detail (17). Conversely, some patients with a rather intense infection bled relatively little. Thus, patient 17, who harbored at least 509 worms, bled 7.3 ml. per day and patient 15, with at least 609 worms, 15.4 ml. per day. Patient 15 entered the hospital with a hemoglobin of 5.2 Gm. per 100 ml., but he was not studied at this time. At the time of study, he had recovered from the anemia and was in excellent state of nutrition. Patient 17, who had no significant anemia on admission, had a hemoglobin of 15.0 Gm. per 100 ml. at the time of study. Contrary to most of the patients in this study, his dietary history was good. Being both a fisherman and a farmer, he ate fish and fresh vegetable twice daily, and drank milk daily.

The data on iron loss in Table V are an approximation only since iron may be in part reabsorbed after it arrives in the intestinal tract. In Venezuela, the average daily iron intake has been estimated to be around 12 mg. per day (18). If it is assumed that of those 12 mg. a maximum of 3 to 4 mg. is absorbed, it is plain that the patients with severe infection are in negative iron balance, with the possible exception of patient 17. It is interesting that only those six patients (from 16 to 21) who had calculated iron losses of less than 4 mg. per day were free from significant anemia on admission.

The loss of blood per hookworm per day

The work of Wells (6) and of Nishi (7) showed clearly that the dog hookworm, *Ancylostoma caninum,* consumed considerable quantities of blood. Wells arrived at an estimate of this quantity by sucking into a calibrated pipette the blood being ejected from the anus of the worms fixed to the intestine of the anesthetized dog, diluting it in known amounts of counting fluid and comparing the red blood count thus obtained with the dog's red blood count. He concluded that, if the same rate of suction which prevailed during the period of observation (approximately 20 minutes) continued during the 24 hours in the intact dog, each hookworm could consume as much as 0.84 ml. of blood per day. Nishi (7) with similar methods, concluded that the blood loss per *Ancylostoma* per 24 hours was on the average "0.144 ml.," and could be as high as "0.392 ml." if the hookworm continued sucking with a maximum activity throughout the 24 hours. Apart from the hypothesis of the constant rate of suction, two objections may be made to this interesting work: 1) it is known that the red blood counting method has a large intrinsic error, and 2) these results would apply only to the dog hookworm, a larger and probably more voracious animal than the human parasite, specially the *Necator.* If we assumed 0.84 ml. per worm as an average daily loss for the human hookworm, some of the patients in the present series would be losing more than two liters of blood per day into their intestinal tract, an improbable figure. Gerritsen, Heinz, and Stafford (19), using radioiron and methods similar to the ones described here, studied three patients with hookworm infection. They did not mention the exact species involved, but they found respectively 0.026, 0.22 and 0.053 ml. per day per hookworm, values much closer to the present ones. It is possible that the higher value of 0.22 may have been due to the presence of *Ancylostoma duodenale.* Since an unknown quantity of the lost iron is possibly reabsorbed, Gerritsen's values are presumably closer to the actual amount of iron lost by the patient, while the present values should give a more exact indication of the amount of blood actually lost into the intestine through the action of the worms.

The amount of blood loss is variable, ranging in the various patients with pure *Necator* infections from extremes of 1.18×10^{-2} and 6.35×10^{-2} ml. per worm per day (Table VI). As for *Ancylostoma duodenale* (Table VII), it would appear from the limited number of patients herein presented that blood loss due to these worms is distinctly larger than that due to *Necator.* The data expressing the probable loss per *Ancylostoma* are of course unreliable, since it is assumed, for

the sake of calculation, that the *Necator* present in mixed infections are consuming a quantity of blood equal to the average in pure *Necator* infection, this parameter being subject to considerable variations (Table VI), and since the percentage of *Ancylostoma* is generally low. In patient 7 (Table VII), the figure is more reliable, since 70 per cent of the hookworms were *Ancylostoma*. It is plausible that *Ancylostoma,* a larger worm, will consume more blood than *Necator,* and 0.2 ml. per day per worm appears to be a fair estimate of its capacity to produce blood loss from the host.

In cases in which the recovered hookworms were placed in a test tube and their radioactivity determined, it was a surprise to find that in none of the tubes was radioactivity above background. This finding would imply that the hookworm contains little or no blood, possibly because they eject it under the action of the vermifuge, or lose it during the washing process. According to Wells (6), a number of observers have seen that specimens of *Ancylostoma duodenale* removed from the host at autopsy eject blood both from the mouth and the anus.

The relationship between number of ova in the stools and blood loss

The amount of blood lost per day per million ova is on the average 2.02 ml. ± 1.12 for pure *Necator* infections (Table VI). Thus, there is a rough correlation between blood loss and egg laying, which is to be expected, since both are expressions of the biology of the worm, and hence should depend upon the number of worms present.

The above method of calculating blood loss, while interesting from a biological point of view, is impractical for the clinician who wishes to gain an idea of the amount of blood his patient is losing from the number of ova which he finds in the stools. Since it will usually be difficult for the clinician to collect feces in four-day periods in a quantitative way, the result of the first three ova counts performed on each patient was expressed also in terms of ova per Gm. of feces, and it was attempted to correlate this value with the amount of blood lost per day during the same period of time. By averaging the three ova counts, it was found that the patient lost on the average 2.74 ml. per thousand ova per gram of stool, with range of 0.82 to 7.14 ml. and standard deviation of ± 1.50. Thus the range is rather wide, depending in part on the presence of *Ancylostoma* in some of the cases, but the method has been found useful in roughly predicting the blood loss, and in judging whether or not an anemia associated with hookworm infection is due chiefly to that infection.

Contribution of blood loss to the anemia of hookworm infection

Whether bleeding due to the hookworm is sufficient to account entirely for the anemia cannot be said from the present data alone. Certainly there appears to be a rough inverse correlation between the amount of bleeding and iron loss and the hemoglobin values. Ultimately, whether a patient with hookworm infection develops anemia or not probably depends on a balance between what goes into the red blood cell and what comes out of the body of the patient. This does not necessarily involve iron intake and blood output alone; factors of protein ingestion, as well as of iron absorption, red cell destruction and possibly bone marrow function may enter the picture. Until these factors are quantitatively studied, no definite conclusion can be reached, although it appears probable that bleeding does play an important, if not the most important, role in the genesis of hookworm anemia.

SUMMARY

1. Two non-infected and six hookworm-infected subjects were given their own chromium-tagged erythrocytes via a duodenal tube. Fecal recovery was on the average 96.7 per cent of administered radioactivity, and urinary recovery 1.7 per cent.

2. In ten non-infected subjects, two of them with marked anemia, whose circulating erythrocytes had been tagged with Cr^{51}, there was little fecal excretion of radioactivity (average 1.27 ml. of "blood" per day). Most of the daily excreted radioactivity appeared in the urine (96.3 per cent of the total activity recovered daily, on the average) in the eight non-anemic subjects.

3. A comparison of the radioactivity in blood and feces of patients with Cr^{51}-marked erythrocytes appears to be a valid way of estimating intestinal blood loss.

4. The circulating erythrocytes of 21 patients with varying severity of hookworm infection were

marked with Cr^{51}, and intestinal blood loss due to the hookworm was measured by comparing stool and blood radioactivity.

5. Blood loss per day in the stool was found to range from 2.0 to 251.5 ml., in rough proportion to the severity of infection.

6. In twelve patients with pure *Necator americanus* infection, the blood loss per day per hookworm was on the average $3.11 \times 10^{-2} \pm 1.73 \times 10^{-2}$ per ml.

7. Five patients were found to harbor mixed *Necator-Ancylostoma* worms. Blood loss per *Ancylostoma duodenale* was estimated to lie in the neighborhood of 0.2 ml. per hookworm per day.

8. Calculated iron loss ranged from 1.2 to 29.1 mg. per day.

9. There was a rough correlation between number of ova in the stools and amount of blood lost. On the average, patients lost 2.74 ml. per day per 1000 ova per gram of stool, with standard deviation of $\pm$ 1.50 and range of 0.82 to 7.14 ml.

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