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Measurement of Gastrointestinal Loss of Plasma Albumin: A Clinical and Laboratory Evaluation of ^{51}Chromium Labeled Albumin[1,2]

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Hypoproteinemia is sometimes due to loss of plasma proteins, predominately albumin, into the gastrointestinal tract. This phenomenon, called exudative enteropathy by Gordon (1), has been demonstrated in many disorders by a number of less than ideal techniques. Over a decade ago, Gray and Sterling (2) introduced radioactive chromium-labeled albumin as a biologic tracer, and recently Waldmann (3) demonstrated its suitability for measuring gastrointestinal protein loss. We have evaluated the physical and biological characteristics of ^{51}chromium-labeled albumin, made estimates of its biologic hazards and obtained experience with it in measuring gastrointestinal protein loss in children and adults.

MATERIALS AND METHODS

$^{51}CrCl_3$ albumin (^{51}Cr-albumin)[1] was prepared according to a method devised by Waldmann using carrier free $^{51}CrCl_3$ of high specific activity, normal human serum albumin (Cohn fraction V) and Amberlite MB-1 resin (4). Radio-

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[1]We are indebted to Dr. Paul Numerof and E. R. Squibb and Sons, New York, N.Y. for the preparation and supply of ^{51}Cr-albumin.

dinated (^{131}I) human serum albumin (IHSA) was obtained from E. R. Squibb and Sons, New York, N.Y.; radiodinated (^{131}I) p-toluidine polyvinyl-pyrrolidone (PVP-^{131}I), 25.4 μc per mg, was obtained from Abbott Laboratories, Oak Ridge, Tenn.; $^{51}Cr\ Cl_3$, 17.1 μc per mg, was obtained from E. R. Squibb and Sons, New York, N.Y.; and normal human serum albumin (HSA), Cohn fraction V (salt poor, 25 gms per cent) was obtained from Cutter Laboratories, Berkeley, Calif. All materials were used shortly after receipt in our laboratory.

Electrophoretic, Dialysis and Protein Precipitable Characteristics. Three to 5μ liter of ^{51}Cr-albumin, IHSA and HSA were subjected to electrophoresis for two hours at low voltage using cellulose acetate supports (5). Subsequently they were stained with Ponceau S and scanned with a recording densitometer.[1] Duplicate 0.2 ml aliquots of ^{51}Cr-albumin were dialyzed against 500 ml of distilled water at 1 and 7 days after receipt. The distilled water was changed at 8 hours (0-8 hrs) and 24 hours (8-24 hrs), the temperature was kept at 4°C, and the specimens were counted in a well scintillation counter. To duplicate 0.25 ml aliquots of ^{51}Cr albumin 2.0 ml 95 per cent ethyl alcohol were added and the tubes agitated. Following centrifugation at 1000 X g for 1 hour, an aliquot of the supernatant was counted in a well scintillation counter. Using appropriate standards, the percentages of the dialyazable and nonprotein precipitated label were calculated.
Immunologic Characteristics. Immuno-precipitin studies, using the Ouchterlony technique with agar gel (6), were done with ^{51}Cr-albumin, IHSA and HSA as antigens and goat anti whole human serum, horse-anti whole serum and goat-anti human albumin as antibodies. Using the same antigens and antisera, immunoelectrophoretic studies were performed using cellulose acetate supports and Ponceau S stain (5,7).

Estimation of Biologic Inhomogeneity of Labeled Materials. Using a technique similar to that described by Dewey and Hunter for characterizing IHSA in the rat (8), the ratio of liver radioactivity to blood plasma radioactivity at 30 minutes after injection was measured with "screened" and "unscreened" ^{51}Cr albumin, IHSA, PVP-^{131}I and $^{51}CrCl_3$. Female albino Sprague-Dawley rats weighing 100-150 gm were used. The labeled materials were "screened" *in vivo* by administering 20-50 μc (0.3-0.5 ml) of the tracer per tail vein and allowing it to circulate for 1 hour. At this time, each rat was decapitated and the blood was collected in a heparinized tube. The plasma obtained from this blood contained the *in vivo*-"screened" materials. "Screened" and "unscreened" labeled materials (4-15 μc, 0.2-0.5 ml) were administered per tail vein to separate rat groups. At 30 minutes the rats were decapitated and their blood collected in separate heparinized tubes. The plasma and liver of each rat was placed, separately, in a 22 mm X 75 mm plastic vial with top; the mass of plasma or liver in each tube was determined by tare weighing. The radioactivity of each vial was measured in a well scintilla-

[1]Recording electrophoresis densitometer, Model 542; Electronic integrator, Model 49. The Photovolt Corporation, 1115 Broadway, New York, N.Y.

tion counter, and the liver accumulation was estimated by calculating net cpm per gm wt liver/net cpm per gm wt plasma. A ratio of greater than 0.1 indicated significant liver accumulation and thus biologic inhomogeneity. The "screened" material ratio should be less than that of "unscreened" material.

Organ Accumulation of ^{51}Cr Albumin in Young Dogs and Estimation of the Biologic Hazard. The distribution of microcurie quantities of intravenously administered ^{51}Cr albumin in dogs was measured. ^{51}Cr albumin in a dose of 18.4-158.4 μc (4.2-27.6 μc/kg) was administered intravenously to 22 young dogs; the larger dogs received the larger absolute doses, but the smaller dogs received the larger dose per unit weight. The dogs were sacrificed at 3, 14 and 30 days; the organs were removed and weighed individually; entire organs were weighed and all or weighed portions digested with concentrated H_2SO_4 in 22 mm $\times$ 75 mm plastic vials; the radioactivity in each specimen was measured in a well scintillation counter. Whole organ accumulations were calculated and compared with the original dose. In two dogs sacrificed at 3 days, 20 separate organs and tissues were examined, but almost all the radioactivity was found in the liver, spleen and kidney. Thus, in the remaining animals, only the liver, spleen, kidney and gonads were examined.

The dose of radioactivity within these organs was estimated by assuming that: 1) there was an essentially uniform distribution of radioactive material within the tissue, 2) the organs were spheres, 3) there was a volume of distribution much greater than the range of beta particles, 4) the ^{51}Cr remained for full physical decay and 5) the radiation emitted from electron K shell capture is equivalent to β radiation. The average absorbed doses from beta radiation (D_β) and from gamma radiation (D_γ) are given by (9):

$$D_\beta = 73.8 \quad \bar{E}_\beta \, Co \, T \text{ rad}$$

$$D_\gamma = 0.0346 \quad I_\gamma \, \bar{g} \, p \, Co \, T \text{ rad},$$

where 73.8 and 0.0346 are numerical constants comprising time and dose factors, $\bar{E}_\beta$ is the average K-capture energy per disintegration for ^{51}Cr in mev, l_γ is the γ-ray dose-rate constant in air in roentgens per hour (r/hr) at 1 cm from 1 mc, p is the density of tissue in grams per cubic centimeter (gm/cc)-essentially 1.0 $\bar{g}$ is the average geometrical factor in centimeters, C_o is the concentration of the ^{51}Cr in the tissue in microcuries per gram (μc/gm), and T is the effective half-life in days. The β dose equation is used for K capture radiation because of its low energy and β-like interaction with matter. These formulae give the radiation dose in rats.

Plasma Half-Times In Normal Human Adults. The effective plasma half-times of ^{51}Cr albumin and $^{51}CrCl_3$ were measured in the plasma of three normal adults separately over a 3-4 week period. ^{51}Cr albumin (25 μc in two and 200 μc in one and $^{51}CrCl_3$ (25 μc in three) were administered intravenously; 10 ml of venous blood was obtained, of which 4 ml of plasma (heparinized) was recovered and stored in 7 $\times$ 20 mm glass vials. At the end of 3-4 weeks the radioactivity in the accumulated samples was measured using an automatic sample changer and a

well scintillation counter. The percentage of the plasma radioactivity remaining in relation to the 10 minute sample was calculated and plotted on semi-logrithmic paper. The time course of radioactivity following equilibration between the body albumin pools and after initial loss via the urine or the rate of degradation was estimated. The samples were counted sufficiently to insure less than a three per cent statistical error in counting.

Recovery of Ingested ^{51}Cr Albumin in Stool and Urine. The radioactivity in four day stool collections and three day urine collections was measured following the oral administration of 10 μc ^{51}Cr albumin. Four convalescing male children without metabolic or gastrointestinal disorders and five normal adult male laboratory personnel were studied. The urines were collected in 24-hour aliquots, measured and 20 ml portions counted in a well scintillation counter. The stool collections for each subject were pooled in a two liter plastic bottle, the entire top cut off to facilitate collections; infant stool collections were facilitated by the use of paper diapers and cutting away the unsoiled portions. Sufficient water was added to make the final volumes equal. Standards equal to 1, 10, 90 and 100 per cent of the ingested doses were prepared in identical containers. The standards and the sealed complete stool specimens were counted using the recently developed opposed probe technique of Gibbs. (10) This technique, which minimizes geometry factors, permits the measurement of radioactivity in variable sized specimens.

Recovery of Intravenously Administered ^{51}Cr Albumin and $^{51}CrCl_3$ in Stool and Urine of Normal Individuals. Three normal adult male laboratory personnel were studied as part of the plasma half-time studies for each tracer. The ^{51}Cr albumin (25 μc for two, 200 μc for one) and $^{51}CrCl_3$ (25 μc for three) were administered intravenously by rapid injection; the dose was diluted with physiologic saline so that a minimum of 2 ml was administered. Urine and stools were collected separately and their radioactivity measured as noted before.

Recovery of Intravenously Administered ^{51}Cr Albumin in Patients With Gastrointestinal Disorders. During an eighteen month period, ten infants and children and one adult with hypoproteinemia associated with gastrointestinal disorders were studied. The doses administered were 0.5-1.0 μc/kg with a maximum of 25 μc, and the stool collections were made free of urine. An indwelling Foley catheter and prophylactic oral sulfasoxazole were used with female patients. In most of these tests, the urine radioactivity was not counted and stool radioactivity was measured in a one quart paper cylinder with a scintillation probe at a distance of 1 meter. The specimens were counted sufficiently to limit the counting error to less than three per cent.

RESULTS

Electrophoretic, Dialysis and Protein Precipitable Characteristics. Electrophoresis: Almost all the ^{51}Cr albumin, IHSA and HSA, individually, migrated toward the cathode as a single homogenous moiety; but there was a trace band in the α-globulin area in all three preparations.

Dialysis: The percentage of activity recovered in the 24-hour duplicate ^{51}Cr albumin dialysates at one and seven days after preparation were 8.05 per cent

(6.01% for 0-8 hours and 2.04% for 8-24 hours) and 9.08 per cent (6.37% for 0-8 hours and 2.71% for 8-24 hours), respectively.

Protein Precipitation: The percentage of activity recovered in the duplicate ^{51}Cr albumin supernatants were 3.20 and 3.27 per cent.

Immunologic Characteristics. On all the studies, IHSA and HSA precipitin bands were continuous, smooth at their junctures and without "spurring". In contrast ^{51}Cr albumin produced "spurring" and migrated slightly different than IHSA and HSA on some of the immunoprecipitin studies. On immunoelectrophoresis, ^{51}Cr albumin, IHSA and HSA seemed to be identical. Moreover, at least two α-globulin precipitin crescents were present between these three agents and the antiseras. Some of the studies showing "spurring" are shown in Fig. 1.

Estimation of Biologic Inhomogeneity of Labeled Materials. There was a large and variable uptake by the rat liver of both "screened" and "unscreened" ^{51}Cr albumin, the "unscreened" being much greater. In contrast, there was mini-

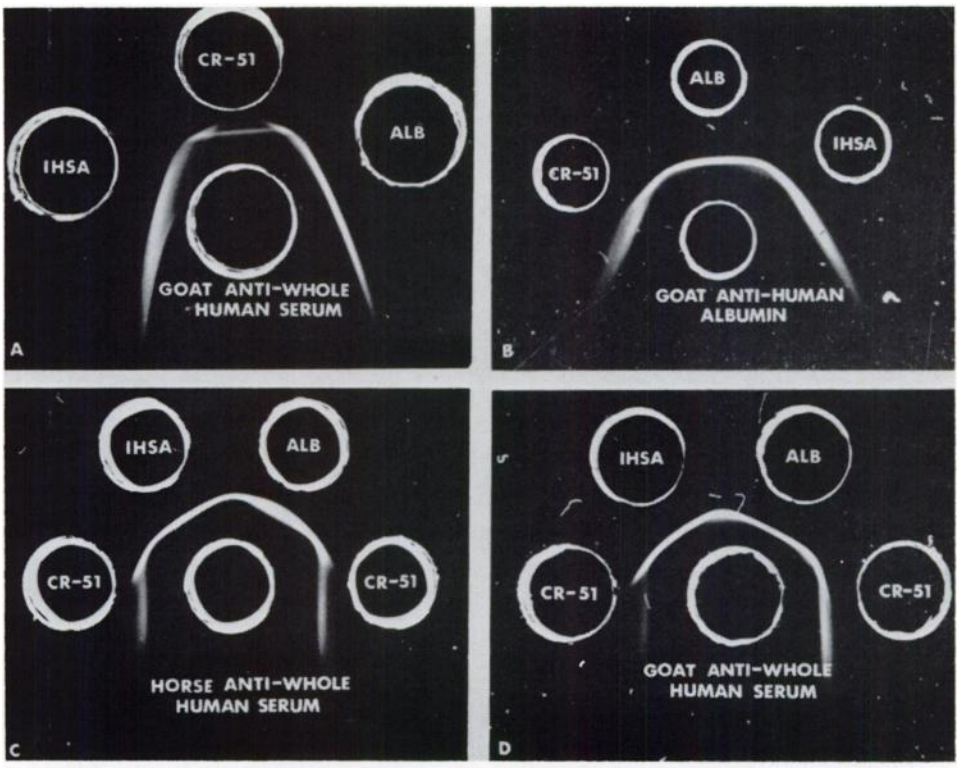

Fig. 1. Immunoprecipitin properties of radioiodinated albumin (IHSA), albumin (ALB) and ^{51}Cr labeled albumin (^{51}CR). Precipitin bands in agar by double diffusion. The IHSA and ALB were diluted 1:1,000-1,500 and ^{51}CR 1:50 prior to filling wells. The "spurring" in one direction at the precipitin band junctions between ^{51}CR:IHSA and ^{51}CR:ALB suggests the proteins are similar, but not identical.

mal rat liver uptake of "screened" or "unscreened" IHSA, PVP-^{131}I and $^{51}CrCl_3$ These findings show that ^{51}Cr albumin is rapidly concentrated in the liver and, presumably, the reticuloendothelial system of the rat; the liver accumulation of the tracers are summarized in Table I.

Organ Accumulation in Young Dogs. The concentration of radioactivity in twenty organs and tissues of two dogs sacrificed at three days following intravenous administration of ^{51}Cr albumin (158.4 and 54.2 μc) showed only negligible amounts outside the liver, spleen and kidney. The isotope accumulations in these three organs and the gonads, along with the estimated radiation absorbed doses (rads), are shown in Table II for the 22 dogs studied. Though the dose administered to each dog varied, the percentage accumulated in the organs studied varied independently of the dose and the calculated rads per organ varied greatly, the accumulated amounts of radioactivity were not excessive. Also, with time the amount of activity in the liver, spleen and gonads diminished, while the amounts in the kidney remained the same or increased slightly. The kidney, undoubtedly, is the final pathway in the removal of parenterally administered ^{51}Cr albumin. So, with time there is a biological elimination of ^{51}Cr; it does not remain in the organ until full physical decay.

TABLE I

LIVER: PLASMA RATIOS OF EARLY ISOTOPE DISTRIBUTION IN YOUNG RATS

Tracer	*No. Rats Studied*	*Liver: Plasma Ratio*
^{51}Cr Albumin:		
Unscreened	10	0.716 *(0.486–1.107)**
Screened	7	0.127 (0.097–0.157)
IHSA:		
Unscreened	4	0.089 (0.085–0.098)
Screened	4	0.085 (0.079–0.091)
PVP-^{131}I:		
Unscreened	5	0.059 (0.054–0.063)
Screened	5	0.065 (0.058–0.070)
$^{51}CrCl_3$:		
Unscreened	4	0.078 (0.071–0.081)
Screened	3	0.068 (0.063–0.073)

*mean
**range

Plasma Half-Times in Normal Human Adults. The biological half-time in plasma of ^{51}Cr albumin was 8-9 days in the three individuals studied. The biological half-time in plasma of $^{51}CrCl_3$ was 7-8 days in the three individuals studied. These data are shown in Figs. 2 and 3, respectively. There was a greater initial drop in plasma radioactivity with ^{51}Cr albumin, presumably due to its uptake by the reticuloendothelial system. With both ^{51}Cr albumin and $^{51}CrCl_3$, the initial drops in plasma activity were greater than usually seen with IHSA.

Recovery of Ingested ^{51}Cr Albumin in Stool and Urine. Almost all (92.9-100.0%) of the ingested radioactivity was recovered in the stool and, essentially,

TABLE II

ORGAN ACCUMULATION OF ^{51}CHROMIUM ALBUMIN

Organ, (Day Sacrificed)	*No. Dogs Studied*	*Range of Per Cent Dose Conc in Organ*	*Range of rads**
Liver:			
3 Day	11	17.4–64.1	0.42–5.58
14 Day	4	20.9–49.1	0.69–2.40
30 Day	7	22.3–46.6	0.77–1.30
			5.0**
Spleen:			
3 Day	11	1.1–7.1	0.40–3.62
14 Day	4	0.9–3.9	0.23–3.82
30 Day	7	0.9–2.7	0.23–1.94
			5.0**
Kidney:			
3 Day	11	0.7–1.4	0.11–0.93
14 Day	4	0.5–1.8	0.04–0.48
30 Day	7	0.7–4.2	0.11–0.87
			5.0**
Gonads:			
3 Day	11	.001–.057	<.001–1.28
14 Day	4	<.001–.003	<.001–0.05
30 Day	7	<.001–.003	<.001–0.08
			1.25**

*Estimated value in rads assumes maximum concentration in organ occurred on designated day.

**Maximal permissible 13 week dose for workers subject to chronic radiation exposure.

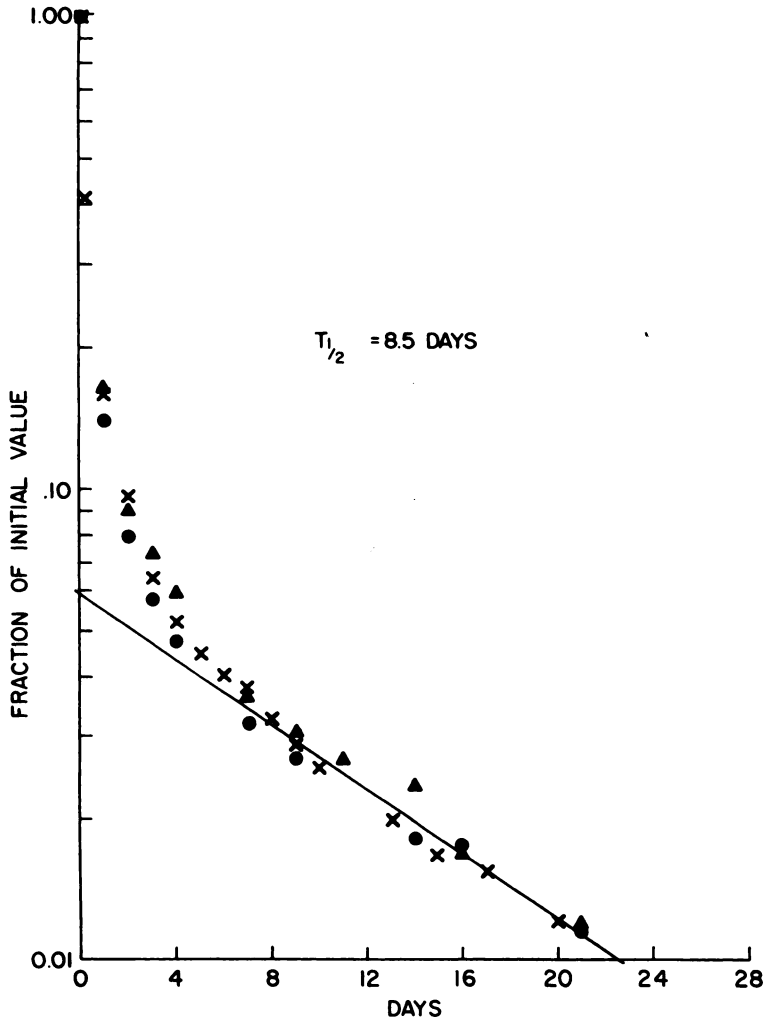

Fig. 2. The plasma disappearance of ^{51}Cr albumin in three normal adults. The composite estimate of biologic half-life is 8.5 days. The major factors determining the initial disappearance of ^{51}Cr albumin are distribution into extravascular spaces, avid uptake by the reticuloendothelial system and urine excretion.

Circle, C. M.; Triangles, E. K.; Crosses, H. K. Twenty-eight day sample for H. K. was 0.0084 of initial value.

none appeared in the urine. These data are recorded in Table III. These findings confirm the observations that essentially no chromium is absorbed by the intestine.

Recovery of Intravenously Administered ^{51}Cr Albumin and $^{51}CrCl_3$ in Stool and Urine in Normal Individuals. In all studies, less than 1.0 per cent of the intra-

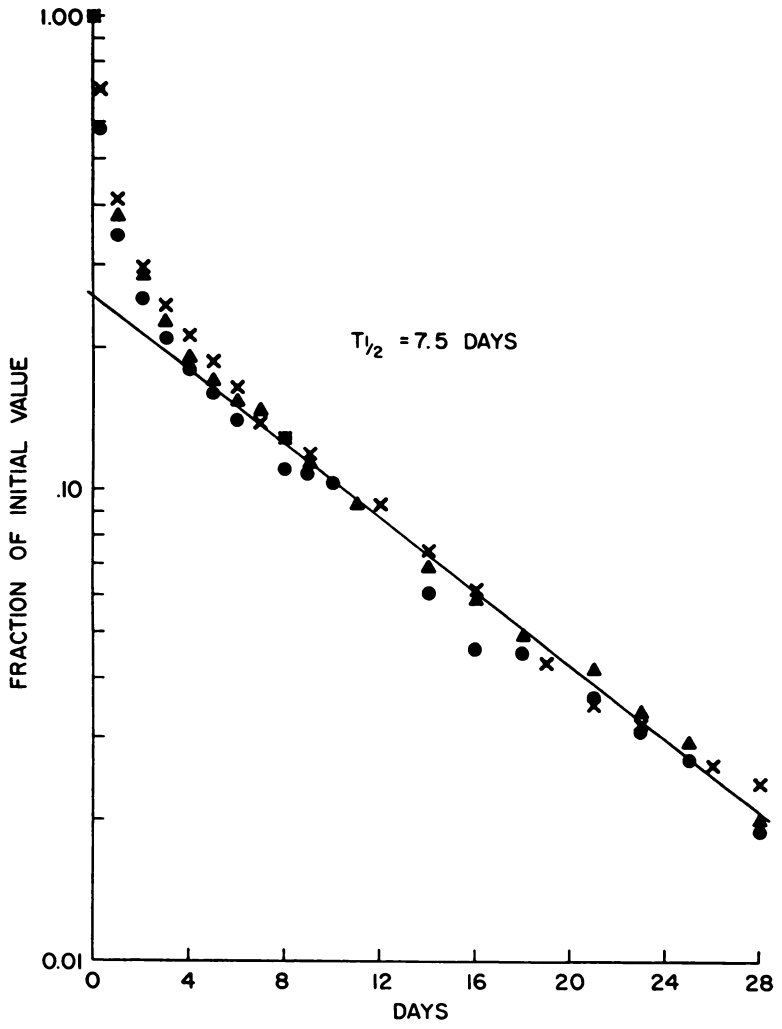

Fig. 3. The plasma disappearance of $^{51}CrCl_3$ in three normal adults. The composite estimate of biologic half-life is 7.5 days. The major factors determining the initial disappearance of $^{51}CrCl_3$ are distribution into extravascular spaces and urine excretion.

Circles, G. E.; Triangles, Z. T.; Crosses, W. R.

TABLE III

RECOVERY OF ^{51}Cr IN STOOL AND URINE AFTER INGESTION OF ^{51}Cr ALBUMIN

Patient	*Per Cent Recovered in Stool (0-96 hours)*	*Per Cent Recovered in Urine (0-72 hours)*
Adult:		
CM	97.4	0.02
EK	100.0	0.17
UB	97.4	0.02
LC	100.0	0.07
PM	98.7	0.20
Children:		
DP	92.9	0.00
ER	98.6	0.02
EC	100.0	0.02
GS	96.5	0.20
Mean	97.9	0.03

TABLE IV

RECOVERY OF INTRAVENOUSLY ADMINISTERED ^{51}Cr ALBUMIN AND $^{51}CrCl_3$ IN URINE AND STOOL OF NORMAL INDIVIDUALS

	^{51}Cr *Albumin* % DOSE RECOVERED			$^{51}CrCl_3$ % DOSE RECOVERED		
	CM	*EK*	*HK*	*ZT*	*WR*	*GE*
URINE, DAY 1	10.9	9.6	7.3	38.8	40.1	43.3
URINE, DAY 2	2.2	2.1	1.9	11.6	9.5	17.0
URINE, DAY 3	1.3	1.5	1.2	4.6	5.2	4.6
TOTAL (3 DAY)	14.4	13.2	10.4	55.0	54.8	64.9
STOOL (4 DAY)	0.0	0.3	0.3	1.0	1.0	0.8

TABLE V

RECOVERY OF ^{51}Cr IN 3 DAY STOOL AFTER IV ADMINISTRATION OF ^{51}Cr ALBUMIN

Patient	*Age (yrs)*	*Serum Albumin (GM %)*	*Clinical Condition*	*Per Cent of Dose Recovered*
I. NORMAL SERUM ALBUMIN, NO EDEMA:				
RH	3 4/12	5.1	Gluten coeliac disease	0.3
CC	5/12	4.8	Deprivation malnutrition	0.3
NC	57	4.2	Chronic pancreatitis	0.7
DT	1 7/12	4.6	Cystic fibrosis	0.5
CM	34	5.1	Normal adult	0.0*
EK	25	5.1	Normal adult	0.3*
HK	27	4.9	Normal adult	0.3*
II. HYPOPROTEINEMIA, NO EDEMA:				
HC	3 8/12	2.2	Gluten coeliac disease; deprivation malnutrition	1.6
III. RECOVERING FROM HYPOPROTEINEMIA AND EDEMA:				
CH	1 4/12	1.9→2.8**	Transient disorder associated with iron deficiency anemia	0.8
KH	3/12	1.6→2.8**	Transient disorder associated with iron deficiency anemia	1.5
DT	2/12	1.9→2.4**	Cystic fibrosis, soybean diet, associated with hemorrhagic diathesis	5.4
IV. HYPOPROTEINEMIA AND EDEMA:				
JB	6 6/12	2.9	Acute leukemia in remission, receiving aminopterin; chronic diarrhea	12.5
CB	10/12	2.3	Transient disorder associated with iron deficiency anemia	7.9
BC	3 8/12	1.9	Deprivation malnutrition associated with chronic diarrhea	12.5
HC	4 9/12	1.7	Gluten coeliac disease; deprivation malnutrition	0.9

*4 day stool collection

**Serum albumin concentrations just prior and during 3 day stool collection period

venously administered dose was recovered in the four day stool collection. Recovery of the ^{51}Cr albumin in the 3-day urine collections was much less (11.1-14.4%) than with $CrCl_3$ (54.8-64.9%). These data (3 adults each) are shown in Table IV. These findings suggest that when more than 1 per cent of the dose is detected in the stool, there is an abnormal loss of plasma proteins into the gastrointestinal tract.

Recovery of Intravenously Administered ^{51}Cr Albumin in Patients With Gastrointestinal Disorders. The data shown in Table V summarize our clinical experience with ^{51}Cr albumin. The patients selected for study were those who were highly or remotely suspected of having exudative enteropathy and in whom circumstances made the study easy to accomplish. The results in group I were as expected. H.C. in group II and group IV is the same patient studied on separate occasions. One could question whether the 1.6 per cent recovery on one occasion was more significant than the 0.9 per cent recovery on another occasion when the child was more debilitated. The fact that negligible to moderate per cent of dose recoveries were obtained in Group III is probably explained by the fact these children were recovering rapidly at the time of the study, and, thus, exudative enteropathy had or was ceasing. In group IV the percentages of dose recovered in the stool cannot be transposed to a precise estimate of the magnitude of exudative enteropathy, but the severity of the disorder, except for patient H.C., did parallel the excessive amounts of radioactivity recovered.

DISCUSSION

Development of techniques for studying exudative enteropathy. A quarter century ago excessive loss of body protein via the gastrointestinal tract was demonstrated by Welch, Adams and Wakefield (11) in cases of ulcerative colitis using the nitrogen balance method, and they first proposed the concept of excessive loss of plasma proteins via the gastrointestinal tract. Further documentation of the concept was not possible with the techniques of that day. In 1957 Citrin, Sterling and Halsted (12), using radioiodinated albumin, demonstrated a rapid turnover of serum albumin and excessive loss into the stomach in a patient with hypoalbuminemia and giant hypertrophy of the gastric mucosa. Their application of radioactive tracers to this problem revived the concept of exudative enteropathy, but quantitative recovery of labeled albumin from gastrointestinal contents is not possible with IHSA; the protein is digested and the iodine label absorbed. To circumvent this aspect of the problem, Gordon (1) introduced a synthetic albumin-like polymer, PVP-^{131}I. Because PVP-^{131}I is biologically inert, all that is lost into the lumen of the bowel can be recovered in stool. He found all nine of his patients with idiopathic hypercatabolic hypoproteinemia to have excessive stool excretion of PVP-^{131}I. PVP, however, has not proven to be a good tracer because it has been impossible to manufacture uniform-sized molecules. Clinical standardization is required for each batch, and then too, it is not albumin and not a tracer in the usual sense.

A large amount of recent work has shown that the gastrointestinal tract is normally a site for albumin catabolism which suggests that the mere demonstration of albumin in gastrointestinal excretions would not document exudative enteropathy. Moreover, Barandum and associates (13), using immunodiffusion techniques, have shown that stool protein in cases of protein-losing enteropathy, could not be differentiated, qualitatively or quantitatively, from the normal state. Next Jeejeebhoy and Coghill (14), in an effort to quantitate plasma protein loss into the gut, introduced the technique of feeding an anion-binding resin which binds the digestion products of IHSA and prevents their reabsorption during the radioiodinated albumin turnover study. This technique was initially thought to quantitate the magnitude of the plasma protein leakage into the bowel since it utilized a good biological tracer. However, recent study by Høedt-Rassmussen and Kemp (15) demonstrated that this technique does not give a precise measure of protein loss from the alimentary tract. Using IHSA in conjunction with an orally-administered resin may take up to two weeks for completion of a study in a single patient.

In vivo labeling of plasma proteins with $^{51}Cr\ Cl_3$ and subsequent measurement of stool radioactivity has been used with some degree of success (16,17). Our studies, however, indicate it is less suitable than *in vitro* chromium-labeled albumin because the former has a slightly shorter plasma half time and because large amounts are excreted in the urine shortly after its intravenous administration.

The above problems led Waldmann (3) to begin using ^{51}Cr albumin, largely because chromium, for practical purposes, is not absorbed. Thus, any radioactive chromium recovered in stool following its intravenous injection is a measure of exudation into the gastrointestinal contents. Our studies show that chromium is not absorbed and that chromated albumin, with certain qualifications, can be used to estimate the magnitude of plasma protein loss into the gut.

Tracer Characteristics The loose binding of chromium to albumin that we demonstrated is undoubtedly offset by some spontaneous *in vivo* binding, the degree of which was shown following the intravenous administration of $^{51}Cr\ Cl_3$. Though firm binding was in the order of only ninety per cent with our preparation, similarly prepared material by others has been shown to have firmer binding. (18) Ideally, a radiolabeled tracer should retain most of its natural biologic properties; this is perhaps the greatest drawback of ^{51}Cr albumin. Our results show that the *in vitro* labeling process has indeed altered the albumin molecule. Immunologically it is different from its starting material, Cohn fraction V. The changes shown in our immunodiffusion and immunoelectrophoretic studies suggest that these changes are moderate and that the material still is similar to natural albumin. The avid uptake of ^{51}Cr albumin by the rat liver was demonstrated even after it was "biologically screened" for one hour. These findings parallel our findings with patients of whom we have frequently been able to obtain a "liver scan" following the intravenous administration of ^{51}Cr albumin. Also, ^{51}Cr albumin was concentrated almost entirely in the liver and spleens of our dogs. These findings suggest that the labeling process creates a macromolecule when compared

to normal abumin. Ideally prepared IHSA and biosynthetically ^{14}C labeled albumin have plasma half times between three and four weeks; our half times of slightly over one week are further evidence that ^{51}Cr albumin is biologically different from normal plasma albumin. Moreover, because of its relatively rapid removal from the circulation, it cannot be used to measure plasma volume or distributions. Thus, as a tracer for the study of exudative enteropathy, its values are practical. Our studies corroberate Waldmann's (3) in that almost all orally administered chromium can be readily recovered in stool. Also, much less radioactivity appears in the urine during the first few days after its administration when compared with PVP; this makes it a better test for infants and children. Most importantly, in the few cases where exudative enteropathy was suspected as the primary cause of the hypoproteinemia, losses in excess of five per cent of the administered dose were demonstrated. In the instances where the dose excreted was between one and five per cent, we had evidence that the patient either was recovering rapidly or that gastrointestinal loss of plasma proteins was only a contributing cause of their hypoproteinemia. Thus, though chromium labeled albumin is not an ideal biological tracer, and probably does not lend itself to precise measurement of plasma protein loss into the bowel, it is the best agent for clinical uses now available for the documentation and assessment of exudative enteropathy.

Radiologic Hazards It is appreciated that any increase in radiation dosage above background radiation is not desirable, especially in children. This, however, should not deter the judicious use of isotopes when their use is indispensable for accurate diagnosis. Because there is some hesitation in using radioisotopes for diagnosis, especially in infants and children, a more realistic estimation of the hazard is obtained when radioisotope use is compared to background radiation or radiation obtained during roentgen ray studies. We have estimated, using the average whole body exposures rates presented by Seltzer, Kereiakas and Saenger (19), that a single ^{51}Cr albumin study utilizing our standard dose (0.5 μc/kg with maximum of 25 μc) would be equivalent to background radiation in our locale for three to ten weeks, or only one fiftieth to one two hundredth the radiation acquired during a five-film exposure intravenous pyelogram. More specifically, ^{51}chromium is less hazardous than ^{131}I agents, since it decays without beta emmission. In our studies on organ concentration of ^{51}chromium albumin we employed doses eight to sixty-four times our suggested dose for patients. Even with these relatively large doses there was no indication of radiation hazard from organ concentration of the isotope. This is further supported by the fact that all assumptions made in our calculation would result in higher rather than lower dose estimate.

Practical Considerations We now appreciate that the gastrointestinal tract is normally a major site for albumin catabolism, and the difference between normal and excessive plasma protein exudation is one of degree. Accurate assessment of this phenomena has not been possible clinically, because all methods used to date have been either lacking in specificity, only semiquantative, or not practical

for regular clinical use. ^{51}Cr albumin also is not a perfect tracer, mostly because the labeled molecule is altered from the natural.

There are, however, definite advantages in using ^{51}Cr albumin to establish and estimate the passage of plasma proteins into the gut: 1) It can be prepared with no unusual difficulty and it has a longer "shelf-life" than radioiodinated products. 2) ^{51}Chromium emits a monoenergetic gamma ray (0.32 mev) that makes it satisfactory for scintillation counting. 3) Absence of beta emission decreases the radiation dosage. 4) A usual clinical study requires no preparation and only three days to complete. When radioiodinated albumin is used in conjunction with resin feedings, up to one or two weeks may be required to complete the study. 5) Only moderate amounts of radioactivity appear in the urine during the first few days following intravenous injection. This is in contrast to PVP-^{131}I and $^{51}CrCl_3$ which appear in the urine in large amount during this period. Thus, with ^{51}Cr albumin, trace contamination of stool with urine does not invalidate the study; this is of especial importance when caring for infants and children.

Thus, until a better plasma protein tracer is designed, chromium-labeled albumin as now prepared is the best agent available for clinical documentation and assessment of exudative enteropathy.

SUMMARY

^{51}Chromium albumin, prepared by a standardized method in a commercial radiopharmaceutical laboratory, has been characterized and found to be altered from the parent material and, to a degree, biologically inhomogeneous. It has, however, been shown to be a suitable agent for the clinical evaluation of plasma protein loss into the gastrointestinal tract and to have advantages over other methods and radioisotopes tracers for this purpose. In tracer doses, it does not constitute a radiologic health hazard.

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