Use of $^{51}\text{CrCl}_3$ in the diagnosis of protein-losing enteropathy

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EDITORIAL COMMENT This appears to be a simple and effective technique for measuring enteric protein loss.

A number of isotopically labelled compounds, including ^{131}I albumin, ^{131}I polyvinylpyrrolidone (^{131}I PVP), *in vitro* labelled ^{51}Cr albumin, and *in vivo* labelled plasma proteins with $^{51}\text{Cr Cl}_3$, have been used to detect protein loss into the gastrointestinal tract. ^{131}I albumin (Veall and Vetter, 1958) is most suitable for determining albumin turnover rate, but is of little value in the assessment of enteric loss. ^{131}I PVP (Gordon, 1958, 1959), a synthetic polymer with a short half life (Rubini and Sheehy, 1961), is not suitable for estimating albumin degradation and its value in enteric protein loss is purely qualitative.

In vitro ^{51}Cr albumin (Waldmann, 1961, 1964) has the advantage of being a natural compound, the chromium released after digestion is not reabsorbed, and the stool activity is a measure of enteric protein loss. However, the short half life makes this method unsuitable for albumin turnover studies.

The labelling of plasma proteins *in vivo* would theoretically be the most physiological means of assessing enteric protein loss and possibly albumin turnover. However, only two studies using this method (Rubini and Sheehy, 1961; Guillen and Peterson, 1964) have so far been published. The present report evaluates the use of $^{51}\text{Cr Cl}_3$ *in vivo* as a method for determining enteric loss and daily turnover of plasma proteins.

Thirty-one patients were given $^{51}\text{Cr Cl}_3$ intravenously, and the distribution of the isotope in the blood, the daily loss in the urine and faeces, and the half life of the isotope were measured.

Our studies show that the intravenous injection

of $^{51}\text{Cr Cl}_3$ results in an instantaneous labelling of plasma proteins. It is a simple and effective method for the detection of abnormal enteric protein loss, but would appear to be an unsuitable method for parallel studies of daily albumin turnover.

METHODS

Chromic chloride (^{51}Cr) suspended in saline was given intravenously in a dose of 100 to 200/ μC . to 31 patients. In 24 patients blood was taken at five minutes and then every second day for 10 days. The stools were collected, homogenized, and made up to a final volume of 500 ml., and counted for 300 seconds using a sodium iodide activated crystal (2 in. diameter) in a specially constructed stool counter. The total radioactivity in the five-day collection was expressed as a percentage of the injected dose. The five-day urinary radioactivity was also expressed as a percentage of the injected radioactivity.

The six specimens of blood from each patient were centrifuged and the activity in 4 ml. plasma and 4 ml. red blood cells (washed twice) was measured. The uptake of radioactivity by the plasma was expressed as a percentage of the total radioactivity in the blood, correction being made for the haematocrit. The percentage of bound and free radioactivity in 1 ml. plasma after dialysis at 0°C. for 24 hours was also determined in 14 patients. In 10 patients the total plasma proteins were precipitated with saturated ammonium sulphate (4.1 ml. added to 1.9 ml. plasma with final concentration 68% saturated) at 25°C. and pH 6.5. The radioactivity in the protein fraction was expressed as a percentage of the total activity.

The radioactive half life was assessed by plotting the radioactivity of each plasma sample on a semilogarithmic scale, the five-minute sample being taken as 100%. The half life of the injected material was assessed by extrapolation of the slow component of the curve.

The 31 patients studied with $^{51}\text{Cr Cl}_3$ were divided into four groups: group I, nine control patients, in whom

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excessive enteric protein loss was not expected (Table I); group II, six patients with ulcerative disease of the bowel in whom enteric protein loss was anticipated (Table II); group III, nine patients with the malabsorption syndrome (six patients with subtotal villous atrophy and three with partial villous atrophy) (Table II); and group IV, seven patients with miscellaneous diseases (Table II).

RESULTS

STOOL LOSS Nine control patients (Table I) with a normal serum albumin concentration had a total faecal radioactivity in a five-day stool collection of 1% or less of the injected dose. In five of the six patients with ulcerative disease of the colon (Table II) the total faecal radioactivity was greater than 2%. In the remaining patient in whom the ulcerative disease was confined to the recto-sigmoid region the faecal radioactivity was 1.0%.

Eight of the nine patients with the malabsorption syndrome had faecal radioactivity greater than 1% (Table II). This included five patients with subtotal villous atrophy (4.0%, 5.7%, 7.4%, 2.3%, 1.1%)

TABLE I

RESULTS IN GROUP I

Patient	Age	Sex	Diagnosis	Stool Radioactivity (%)
L.R.	40	F	Normal	0.9
J.T.	66	F	Myocardial infarct	0.5
H.S.	50	M	Myocardial infarct	0.2
L.O'N.	43	F	Small gastric ulcer	0.3
J.M.	72	M	Small gastric ulcer	0.3
M.B.	24	F	Duodenal ulcer	1.0
K.McA.	53	F	Small bowel resection	0.7
D.T.	48	F	Malnutrition	0.6
J.C.	36	F	Thyrotoxicosis	0.1

Patients used as controls (normal serum albumin $>3.8\text{g. \%}$).

TABLE II

RESULTS IN GROUPS II AND III

Patient	Age	Sex	Serum Albumin (g. %)	Stool Radioactivity (%)
<i>Group II: Ulcerative Lesions</i>				
1 G.B.	45	M	2.8	3.7
2 R.H.	36	M	3.8	2.8
3 B.H.	13	M	3.9	6.4
4 L.S.	57	M	2.9	1.0
5 D.W.	37	F	2.5	4.8
6 G.B.	65	F	1.9	16.5
<i>Group III: Adult Coeliac Disease</i>				
7 E.P.	33	F	3.5	5.7
8 F.E.	59	M	3.4	0.7
9 K.P.	70	M	3.5	1.1
10 P.A.	28	F	2.2	4.0
11 R.D.	41	M	2.4	7.4
12 J.C.	44	F	3.5	2.3
13 G.L.	35	F	2.1	3.0
14 E.N.	56	F	1.2	1.3
15 I.M.	63	F	3.1	1.2

Cases 1-4 had ulcerative colitis, case 5 Crohn's colitis, case 6 radiation enteritis.

Cases 7-12 had subtotal villous atrophy, cases 13-15 partial villous atrophy.

and three patients with partial villous atrophy (3.0%, 1.2%, 1.3%). In the group of patients with miscellaneous diseases (Table III) increased faecal radioactivity was detected in five cases (1.8%, 4.5%, 2%, 1.9%, 17.1%).

TABLE III

RESULTS IN GROUP IV

Patient	Age	Sex	Diagnosis	Serum Albumin (g. %)	Stool Radioactivity (%)
M.C.	49	F	Lymphangiectasia	2.8	2.0
A.W.	65	M	Idiopathic hypogammaglobulinaemia	2.5	1.9
L.S.	40	M	Small bowel melanomatosis	1.5	17.1
C.C.	51	F	Dermatomyositis	1.9	4.5
G.J.	37	F	Alcoholism	2.7	1.8
J.L.	14	M	Acute constrictive pericarditis	2.6	0.5
S.L.	36	M	Nephrotic syndrome	2.8	0.7

There was a significant quantitative relationship between the degree of hypoalbuminaemia and the stool radioactivity. The correlation coefficient was -0.52 giving a probability of less than 0.01.

URINE LOSS In the control group the average total five-day urinary radioactivity was 30% of the injected dose (range 18% to 61%). In 11 patients with faecal radioactivity above 1%, the average five-day urinary radioactivity excreted was 29.3% (range 13.4% to 50%). In all patients in whom urine radioactivity was measured, the major portion of the activity appeared in the first 24 hours, being 41% to 92% of the total five-day urinary radioactivity.

HALF LIFE STUDIES The half life of the injection radio-chromium was determined in 13 patients. The average half life of five control patients with a faecal radioactivity of 1.0% or less was 9.2 days (range 5-13.7 days). In the eight patients in whom faecal radioactivity was greater than 1.0% the average half life of the injected radioactivity was calculated to be 5.6 days (range 2.8-9.4 days).

DISTRIBUTION IN BLOOD In 24 patients studied, an average of 95% of the total blood radioactivity was present in the plasma at five minutes and an average of 85% at four days.

Plasma samples at five minutes and four days were subjected to dialysis and the radioactivity remaining in the bag was expressed as a percentage of the total plasma radioactivity. In 14 patients, the average radioactivity remaining in the dialysis sac was 83% (range 67%-91%) in the five-minute plasma samples and 92% (range 82%-100%) in the four-day samples.

When saturated ammonium sulphate was added to precipitate total protein, 85% (69–100%) of total radioactivity was found in the precipitate in the five-minute samples, and 92% (67–99%) in the four-day samples. When a final concentration of 50% saturated ammonium sulphate was used to precipitate differentially the globulin fraction, an average of only 29% (11%–64%) of the total radioactivity appeared in the precipitate, the remainder in the supernatant. The total recoverable activity in all precipitation studies was greater than 90%.

DISCUSSION

Intravenously administered chromic⁵¹ chloride would appear from our studies to be an adequate label for plasma proteins. Binding to these proteins is instantaneous, most of the radioactivity at five minutes appearing in the plasma, only a small amount being detected in red cells. The binding is tight, most of the activity not being dialysable. This radioactivity can be shown by protein precipitation to be distributed both to the albumin and globulin fractions, and may be of some practical importance, as enteric protein loss is not confined to serum albumin but involves other plasma proteins.

As there was a striking difference between the faecal radioactivity in the control group (1% or less of the injected dose) compared with the cases where protein loss was expected (1.0%–16.5%), the use of labelling of plasma proteins with chromic chloride *in vivo* would appear to be a suitable method for demonstrating enteric protein loss. The variable half life of the isotope in the control group, however, makes it unsuitable for estimating daily albumin turnover. The large and unpredictable urinary loss of radioactivity following the injection of the isotope possibly accounts for the wide range in the half-life of the labelled plasma proteins. None of the patients had significant proteinuria and the urinary loss could be due either to free radioactivity in the plasma or to leak of the isotope from the proteins during circulation. However, we have shown that binding to plasma proteins was virtually instant-

aneous and was sufficiently tight to resist protein precipitation.

SUMMARY

Thirty-one patients were given an intravenous injection of ⁵¹Cr Cl₃ and the subsequent labelling of plasma proteins and enteric stool loss was studied in both controls and subjects with suspected enteric protein loss.

Following intravenous injection of ⁵¹Cr Cl₃ the plasma proteins were selectively and instantaneously labelled. The binding was tight and undialysable and the activity found in both albumin and globulin fractions.

The labelling *in vivo* of plasma proteins has been shown to be a simple and effective method of demonstrating enteric protein loss. In addition there is some quantitative relationship between the degree of hypoalbuminaemia and the degree of enteric loss as measured by the recoverable stool radioactivity. The results of our studies, however, indicate that this method will not permit concurrent estimation of the daily turnover of albumin.

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