

The value of phenol red and chromic chloride as nonabsorbable gastric indicators

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SUMMARY A comparison is made of gastric loss of phenol red and chromic chloride other than by emptying. Chromic chloride appears to be lost by adsorption on to mucus to a considerably greater extent than does phenol red, suggesting that chromic chloride is less satisfactory as a non-absorbable gastric indicator.

Phenol red was introduced as a nonabsorbable indicator by Gorham (1923) and has since been used to study gastric emptying by a double sampling technique (George, 1968). Trivalent chromic salts were shown to be poorly absorbed in man (Donaldson and Barreras, 1966), and radioactive chromic chloride ($^{51}\text{CrCl}_3$) was recommended by Bloom, Jacobson, and Grossman (1967) and by Ivey and Schedl (1970) as a reliable gastric indicator; it has since been used in studies of gastric emptying by McKelvey (1970).

The reliability of $^{51}\text{CrCl}_3$ was questioned during the course of an experiment conducted in our laboratory as part of a clinical trial of gastric emptying. It was found that when a mixture of phenol red and $^{51}\text{CrCl}_3$ was used the $^{51}\text{CrCl}_3$ estimations gave consistently higher gastric volumes than did phenol red, and that when the gastric mucus residue was analysed separately it contained a much higher proportion of $^{51}\text{CrCl}_3$ than phenol red. These findings suggested that $^{51}\text{CrCl}_3$ might be adsorbed on to gastric mucus, and the present study was designed to make a quantitative assessment of the comparative losses of $^{51}\text{CrCl}_3$ and phenol red into the mucus fraction of gastric secretion.

Materials and Methods

TEST TECHNIQUE

Seven healthy student volunteers (A to G) and one postoperative hernia patient (H), aged between 18 and 23, took part in the study. All were starved overnight and a nasogastric tube was positioned under x-ray control just proximal to the antrum. The gastric contents were aspirated, and if the aspirate was not clear the stomach was washed out

with water. All the volunteers were given approximately 550 ml of a test meal containing a mixture of phenol red and $^{51}\text{CrCl}_3$, 20 ml of which had first been taken as a control. The test solution was drunk as rapidly as possible, the drinking time always being less than five minutes. Gastric samples of 20 ml were withdrawn five minutes after drinking and then every 15 or 20 minutes. Ten ml was first withdrawn and discarded to eliminate contamination from the tube dead space. This was continued until the stomach had emptied.

PREPARATION OF TEST MEAL

A solution of 550 ppm of phenol red is made up by dissolving 1 g of phenol red in 10 ml N NaOH in a 2 litre volumetric flask (as modified by Hunt, 1954). This is made up to nearly 2 litres with distilled water and the pH is adjusted between 3 and 4 with 5N HCl, before adding distilled water to the 2 litre mark.

The $^{51}\text{CrCl}_3$ initially contains 1 mCi in 1 ml; this is dissolved in 200 ml of the phenol red solution for ease of dispensing. Two hundred μCi of this solution (the actual volume has to take into account the 27.8 days half-life of ^{51}Cr) is then added to a 1 litre volumetric flask, and the volume made up to 1 litre with the phenol red solution to give a stock solution of 500 ppm phenol red and 200 μCi per litre $^{51}\text{CrCl}_3$. The acidification of phenol red solution was to prevent $^{51}\text{CrCl}_3$ being precipitated out as insoluble oxide, as suggested by Bloom and colleagues (1967). The test solutions for subjects (A, B, C, D, E, F, and H) were made up by adding 50 ml of the concentrated stock solution of phenol red and $^{51}\text{CrCl}_3$ to a 500 ml bottle of 10% dextrose. The 10% dextrose solution was chosen as this was used in the original studies on gastric emptying referred to above. As it is a hypertonic solution it

has a retarding effect on gastric emptying and more nearly reproduces the emptying conditions of a normal meal than does saline solution. In order to check that the 10% dextrose did not influence the adsorption of the indicators on to mucus the test meal for one subject (G) was made up in distilled water.

As Bloom and colleagues (1967) suggested that a low pH was important for $^{51}\text{CrCl}_3$ recovery, the pH of the test solutions for G and H were altered and both solutions were adjusted to pH 1.4 with 5N HCl. To verify that the gastric samples from G and H remained acid, all samples were separately tested with narrow range low pH paper, and each pH was less than 2 apart from two samples from H which were 2.25 and 2.75.

The specific activity of the $^{51}\text{CrCl}_3$ was initially 167 mCi/mg as dispatched from the Radiochemicals Centre, Amersham, but allowing for decay was approximately 60mCi/mg at the time of these experiments. This is similar to the specific activity of $^{51}\text{CrCl}_3$ as used by Ivey and Schedl (1970).

ANALYSIS

All samples were centrifuged to give a clear supernatant solution. Four ml from these supernatant solutions and 4 ml from the control samples were pipetted into labelled 100 ml volumetric flasks for phenol red analysis, and 4 ml of the supernatant solutions and controls into labelled tubes for ^{51}Cr counting. Those samples with appreciable amounts of mucus were then selected, and the supernatant fluid was decanted off. If it was not possible to decant this fluid without disturbing the mucus, the mucus was washed gently once or twice with distilled water to remove as much as possible of the supernatant. The mucus samples were then made up to 10 ml with distilled water and were microhomogenized in a Potter-Elvehjem glass homogenizer. Four ml of the microhomogenized mucus was then pipetted into labelled 100 ml volumetric flasks for phenol red analysis and 4 ml into labelled tubes for ^{51}Cr counting.

To estimate phenol red a buffer solution was made by dissolving 27.5 g trisodium orthophosphate ($\text{Na}_3\text{PO}_4 \cdot 12\text{H}_2\text{O}$) in 1 litre of water. Twenty ml of buffer was added to the labelled volumetric flasks and the volume made up to 100 ml with distilled water. The purple colour is measured on a spectrophotometer at 560 m μ using a 1 cm cell. The phenol red result for each sample is expressed as spectrophotometer reading of sample over spectrophotometer reading of control.

To count ^{51}Cr an additional counting tube with 4 ml distilled water was used to estimate background radioactivity. The tubes were counted on a Nuclear

Chicago gamma counter using 5 cycled counts of 1,000 counts. Cycled counts were used to minimize the effects of any possible 'drift' in the counting conditions. The ^{51}Cr result for each sample is expressed as counts per minute of sample (less background) over counts per minute control (less background). For all supernatant and mucus samples a ratio of ^{51}Cr to phenol red is calculated.

Validity of Study

Any dilution of the indicator by gastric secretion or swallowed saliva and any loss of indicator by gastric emptying should apply equally to both indicators, and should not affect the final ratio of ^{51}Cr to phenol red for each sample, which should theoretically be 1. The only factors that can affect the ratio is differing absorption or adsorption of one indicator relative to the other. The exact amount of phenol red or $^{51}\text{CrCl}_3$ in the initial solution is immaterial, provided the concentrations are sufficient for accurate estimation, as all samples are compared against the initial control. The volumes of mucus and amount of distilled water for homogenization varied in each mucus specimen, but the effect on both the phenol red/control result and the ^{51}Cr /control result should be identical, so that this error will cancel itself out in the final ^{51}Cr /phenol red ratio, which should again theoretically be 1 if both indicators were equally adsorbed onto mucus.

Results

There were 34 samples of supernatant fluid, and 12 samples of mucus analysed for A, B, C, D, E, and F. There was at least one sample of mucus obtained from each subject. All but two of the ^{51}Cr /phenol red ratios were less than 1 for the supernatant fluid samples, average 0.8113 ($p < 0.0005$), indicating some loss of $^{51}\text{CrCl}_3$ relative to phenol red (Table I),

Time of Sample (min)	Subject					
	A	B	C	D	E	F
<i>Supernatant</i>						
5	0.752	0.851	0.969	0.858	0.834	0.837
25	0.614	0.897	0.704	0.873	0.801	0.713
45	0.561	0.865	0.799	0.890	0.745	0.724
65	0.962	0.842	0.860	0.778	0.785	0.674
85	0.717	0.736	0.754	1.114	0.776	1.052
105	—	0.769	—	0.818	0.965	0.770
<i>Mucus</i>						
45	—	—	—	—	11.210	3.250
65	6.520	—	—	—	—	2.700
85	6.180	—	9.320	13.980	—	16.730
105	—	15.000	—	5.360	9.330	11.500

Table I Ratio of ^{51}Cr to phenol red in supernatant and mucus in six subjects

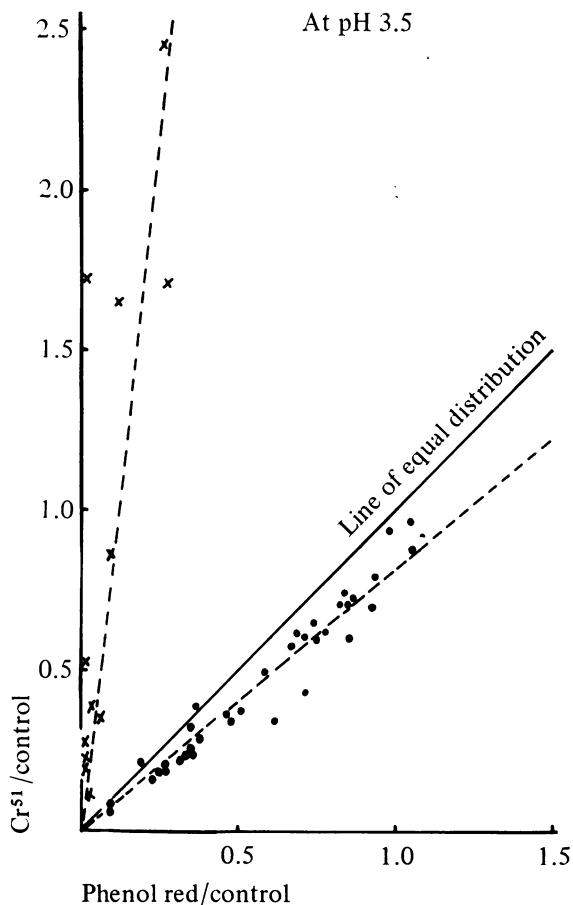


FIG. 1 Ratio of ⁵¹Cr to phenol red at pH 3.5 (six subjects) x mucus samples; ● supernatant samples.

and all the ⁵¹Cr/phenol red ratios for the mucus samples were greater than 1, average 8.461 ($P < 0.0005$) suggesting that ⁵¹CrCl₃ is adsorbed onto the gastric mucus (Table I). The actual results for each sample of phenol red and ⁵¹CrCl₃ relative to controls can be compared diagrammatically (see Fig. 1) where, assuming equal distribution of both substances, a perfect correlation should fall along a line drawn at 45° to both axes. As can be seen, all the supernatant fluid readings fall towards the phenol red axis and all the mucus samples fall towards the ⁵¹CrCl₃ axis.

All but one of the ⁵¹Cr/phenol red ratios for the 13 supernatant fluid samples in G and H were less than 1, average 0.9326 ($P = 0.01$) (Table II), and all the ⁵¹Cr/phenol red ratios for the seven mucus samples were greater than 1, average 3.940 ($P = 0.01$)

Time of Sample (min)	Subject	
	G	H
<i>Supernatant</i>		
5	0.972	0.979
20	0.882	1.008
35	0.946	0.967
50	0.876	0.958
65	0.654	0.850
80	0.649	0.684
95	—	0.924
<i>Mucus</i>		
50	2.197	31.040
65	15.320	2.250
80	10.310	4.600
90	—	17.230

Table II Ratio of ⁵¹Cr and phenol red in supernatant and mucus in two subjects

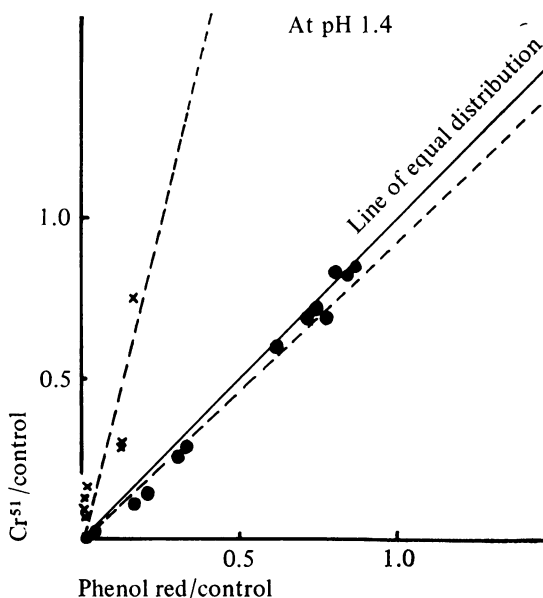


FIG. 2. Ratio of ⁵¹Cr to phenol red at pH 1.4 (two subjects) x mucus samples; ● supernatant samples.

(Table II). These results can also be compared diagrammatically (Fig. 2).

Discussion

Donaldson and Barreras (1966) concluded that ⁵¹CrCl₃ was a good nonabsorbable indicator for studies of intestinal absorption and excretion, on the basis that virtually all the ingested ⁵¹CrCl₃ could be recovered in the faeces. Bloom and colleagues (1967) compared various substances for use as gastric dilution indicators, including ⁵¹CrCl₃ and

phenol red. They found that there was a 5-8% loss of phenol red from the Heidenhain pouch in the dog (less if the stomach was actively secreting), but found that $^{51}\text{CrCl}_3$ could be recovered almost 100% when the stomach was highly acidic. Recovery was not satisfactory at neutral solutions possibly due to precipitation of chromic oxide. Schanker, Shore, Brodie, and Hogben (1957) found, however, a 98% recovery rate of phenol red from the rat stomach.

Ivey and Schedl (1970), in a search for gastric nonabsorbable indicators for study in man, compared the absorption of $^{51}\text{CrCl}_3$, phenol red, and polyethylene glycol by the human stomach at pH 1. They stated in their summary that there was minimal absorption from the stomach of all three indicators, and that all were equally valid. They did state, however, that for the ^{51}Cr /phenol red ratios, charted as in Fig. 1, slightly more points lie towards the phenol red axis, and in their discussion they state that there was less complete recovery of an indicator on first instillation into the stomach, possibly due to adsorption to gastric surface or contents, and this was found for all indicators. Since then $^{51}\text{CrCl}_3$ has been used as a means of studying gastric emptying by McKelvey (1970) using a balanced meal containing protein, fat, and carbohydrate at pH 6.3. This study does not measure absolute gastric absorption rates for phenol red and $^{51}\text{CrCl}_3$ as this can only be done by total indicator recovery studies in animals with gastric pouches. However, indicator recovery from animals with gastric pouches may well not reflect what happens in the intact stomach of man.

From this present study it is felt that $^{51}\text{CrCl}_3$ appears to be bound in some way to gastric mucus, although the actual amount may not be very great. This tendency was lessened but not preventable by a low pH (as Bloom *et al* suggest) as both subjects G and H had a high ^{51}Cr /phenol red ratio in the

mucus. There was little difference between the results in G and H and so it seems unlikely that 10% dextrose has any effect on the distribution of these indicators in the stomach. These results agree with other observations (R. J. Clarke, unpublished data) from counting mucus in gastric emptying studies in duodenal ulcer patients when a ^{51}Cr /phenol red ratio in the mucus of about 9 : 1 has been found. Even if a pH of 1 helps to prevent $^{51}\text{CrCl}_3$ adsorption, there are practical difficulties keeping such a low pH in emptying studies, and both subjects G and H complained bitterly of the unpleasant vinegar taste after drinking the solutions at pH 1.4. It is therefore suggested that $^{51}\text{CrCl}_3$ is not an entirely suitable indicator for gastric emptying studies.

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Please send requests for reprints to J. A. W.

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