Effect of five salicylate-containing compounds upon loss of $^{51}$chromium-labelled erythrocytes from the gastrointestinal tract of normal man

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The adverse effects of salicylates upon the gastrointestinal tract of both man and experimental animals have been documented in numerous reports. These studies indicate that salicylate ingestion frequently causes various upper gastrointestinal symptoms (Caravati and Cosgrove, 1946; Freemont-Smith, 1955; Muir and Cossar, 1955;waterson, 1955; Lange, 1957b; Batterman, 1958; Muir and Cossar, 1959), gross changes in the gastric mucosa consisting of oedema, erythema, ulceration, and bleeding (Dodd, Minot, and Arena, 1937; Barbour and Dickerson, 1938;Douthwaite and Lintott, 1938; Hurst and Lintott, 1939; Stutzman, Orth, and Mellish, 1941; Seeberg, Hansen and Whitney, 1951; Muir and Cossar, 1955; Jones, 1956; Lange, 1957b; Muir and Cossar, 1961; Weiss, Pitman, and Graham, 1961; Roth, Valdes-Dapena, Pieses, and Buchman, 1963; Vickers and Stanley, 1963; Fishler, 1964), and microscopical abnormalities of congestion and loss of superficial epithelium (Roth and Valdes-Dapena, 1963; Hurley and Crandall, 1964). Biochemical studies (Croft, 1963; Menguy and Masters, 1964, 1965) indicate that marked alterations in gastric mucus are caused by salicylates, and bleeding of the gastric mucosa resulting from these drugs has been experimentally related to events resulting from an increase in the permeability of the epithelial membrane (Davenport, 1964, 1965, 1966, and 1967). Retrospective clinical studies suggest that salicylate ingestion precipitates gastrointestinal bleeding (Brown and Mitchell, 1956; Kelly, 1956; Allibone and Flint, 1958; Alvarez and Summerskill, 1958; Muir and Cossar, 1959; Levrat and Lambert, 1960), and receive support from qualitative (Lange, 1957a; Stubbé, 1958; Porter, Lewis, and Dixon, 1959; Stubbé, 1962; Stubbé, Pietersen, and Van Heuken, 1962; Bannerman, Beveridge, and Witts, 1964; Lane, Holmes and Moyer, 1964) and quantitative (Matsumoto and Grossman, 1959; Cameron, 1960; Holt, 1960; Watson and Pierson, 1960; Grossman, Matsumoto, and Lichter, 1961; Pierson, Holt, Watson, and Keating, 1961; Scott, Porter, Lewis, and Dixon, 1961; Izak, Galewsky-Stein, Menczel, and Groen, 1962; Wood, Harvey-Smith, and Dixon, 1962; Leonards, 1963) assay of faecal blood loss during the consumption of these compounds. Most quantitative evaluations have been performed in groups consisting largely of patients with various disease states and there have been few systematic quantitative comparisons of gastrointestinal blood loss induced in normal subjects by common proprietary salicylates available in the U.S.A. Watson and Pierson (1960) compared faecal blood loss induced by regular aspirin, enteric-coated aspirin, calcium salicylate complex, and choline salicylates in normal volunteers and report this in abstract form, but later communications from these authors (Pierson et al, 1961) do not clearly separate results in normal volunteers from patients in hospital. Scott and his colleagues (1961) evaluated different salicylate preparations in a large group of subjects, most of whom had rheumatoid arthritis or other diseases. Grossman, Matsumoto, and Lichter (1961) also evaluated various salicylate compounds, but again their studies were carried out largely in a hospital population rather than in normal subjects. This study was undertaken to assess in a controlled fashion the effect of five popular salicylate compounds upon the magnitude of erythrocyte and equivalent whole blood losses from the gastrointestinal tract of normal adults.

METHODS

SALICYLATES TESTED The composition of the five salicylates evaluated and their doses were:

1. Acetylsalicylic acid 0.325 g . . . 2 tabs T.I.D.
2. Acetylsalicylic acid 0.325 g
   Aluminium glycinate 0.049 g
   Magnesium carbonate 0.098 g
   2 tabs T.I.D.
Acetophenetidin was not present in some of the tablets of compound 3, but these were distributed so that all subjects in this group received equal proportions of each type. The drugs were taken randomly throughout the day in no specific relationship to meals. Placebo in the appropriate form of tablets or powder were taken on the same schedule as the salicylates. Ten subjects were studied with each drug.

**SUBJECTS STUDIED** Normal volunteers were selected from groups of students, hospital personnel, and physicians. Ages ranged from 22 to 63 years, with a mean age of 30 years. None had a history of anaemia, gastrointestinal disease or bleeding, and each had a normal baseline microhaematocrit and submitted faecal specimens for determination of occult blood by the benzidine dihydrochloride method. One woman was included in group 1 and 4, and two were in group 5. Females were not studied during menstruation. A written protocol was distributed to each subject before the study to minimize errors in procedure.

**ERYTHROCYTE AND EQUIVALENT WHOLE BLOOD LOSS**

$^{51}$Cr-erythrocyte clearance studies were conducted as previously described (Beeken, 1967). Red cells were labeled with $^{51}$Cr by the method of Ebaugh, Clements, Rodnan, and Petersen (1958). Twelve to thirteen ml of the subject’s blood was added to 2-5 ml of acid-citratedextrose solution (Squibb) and mixed, and 50-100 μc of Na$_3$$^{51}$CrO$_4$ was added, the solution incubated at room temperature for one hour and injected intravenously. The sequence of the study is illustrated in Figure 1. After administration of the labelled erythrocytes three or more days elapsed to permit urinary clearance of free circulating isotope and the subjects began a seven-day course of the appropriate form of placebo. Complete four-day stool collections were made during the last four days of the placebo period and blood samples were drawn for microhaematocrit determinations and radioactivity monitoring at the beginning and end of the stool collection. The seven-day course of salicylate ingestion was then started, and after three days a second four-day stool collection was begun. Blood was again drawn for radioactivity and microhaematocrit determinations at the beginning and end of this collection period.

Complete four-day stool specimens were collected in paint tins containing alcohol and fitted to portable commode chairs to aid collection. The specimens were diluted with tap water to facilitate pipetting, homogenized, and weighed aliquots were then removed for radioactivity monitoring. Blood and stool radioactivity was measured in a scintillation well counter.

$^{51}$Cr-erythrocyte and equivalent whole blood losses were calculated from stool and whole blood radioactivity according to the following equations:

- **Erythrocytes (ml) lost/day** = \(\frac{\text{radioactivity in stool/day}}{\text{mean radioactivity/ml whole blood}} \times \text{haematocrit}\)
- **Whole blood (ml) lost/day** = \(\frac{\text{radioactivity in stool/day}}{\text{mean radioactivity/ml whole blood}}\)

Means, standard deviations, and significance of differences of means at 95% confidence levels were calculated on a General Electric model 235 digital computer.

**RESULTS**

$^{51}$Cr-erythrocyte and equivalent whole blood losses are presented in Table I as mean values for each group during the periods of ingesting placebo and salicylates. Mean drug loss-placebo loss (D/P) ratios are also indicated. Note that erythrocyte and equivalent whole blood losses were significantly greater during salicylate ingestion than during placebo periods in each of the five groups. Forty-eight of 50 subjects showed increased losses during drug administration. Furthermore, mean losses during drug ingestion were all in excess of 2-5 times mean losses during placebo periods; the mean D/P ratios are conservative in that they do not include D/P of infinity occurring in subjects having no detectable stool radioactivity in the placebo period. The greatest erythrocyte loss during salicylate ingestion was 3-80 ml per day, equivalent of 8-48 ml of whole blood. One subject in group 1 had erythrocyte and equivalent whole blood losses of 3-56 and 7-92 ml,
TABLE I

GROUP MEANS FOR 51CHROMIUM ERYTHROCYTE STUDY

<table>
<thead>
<tr>
<th>Group</th>
<th>Erythrocyte Loss (ml/day)</th>
<th>Equivalent Whole Blood Loss (ml/day)</th>
</tr>
</thead>
<tbody>
<tr>
<td></td>
<td>Placebo</td>
<td>Drug</td>
</tr>
<tr>
<td>1(\text{a}) M</td>
<td>0.33</td>
<td>0.96</td>
</tr>
<tr>
<td>SD</td>
<td>0.18</td>
<td>0.51</td>
</tr>
<tr>
<td>2 M</td>
<td>0.69</td>
<td>1.97</td>
</tr>
<tr>
<td>SD</td>
<td>0.47</td>
<td>1.03</td>
</tr>
<tr>
<td>3(\text{a}) M</td>
<td>0.52</td>
<td>1.27</td>
</tr>
<tr>
<td>SD</td>
<td>0.45</td>
<td>0.58</td>
</tr>
<tr>
<td>4 M</td>
<td>0.43</td>
<td>0.86</td>
</tr>
<tr>
<td>SD</td>
<td>0.28</td>
<td>0.44</td>
</tr>
<tr>
<td>5 M</td>
<td>0.07</td>
<td>0.61</td>
</tr>
<tr>
<td>SD</td>
<td>0.08</td>
<td>0.28</td>
</tr>
</tbody>
</table>

\(\text{a}\) Difference of drug means significantly higher than placebo means (P = 0.05).

respectively, and one in group 3 lost 2.51 ml of erythrocytes, equivalent to 5.97 ml of blood during the placebo period. These values were greater than two standard deviations from the means of their groups and hence the data from these subjects are not included in the tabulation. Microhaematocrit values and stool occult blood tests were normal in both of these men. Four subjects ingested drugs other than the prescribed compounds at some time during the study. These included primidine, propoxyphene (Darvon), and emmipin in various dosages. The results for these subjects did not differ from the remainder of their respective groups and omitting their data did not reduce the significance of the mean differences. Another subject omitted salicylate for several days without altering significantly the group mean value. Two subjects had positive baseline tests for faecal occult blood, and quantitative blood loss in each during the placebo period was normal. There was no apparent decrease in mean serial haematocrit determinations during the study.

**DISCUSSION**

The use of 51Cr-erythrocyte to quantify gastrointestinal blood loss is a well-established technique. It has been shown that the gut is impervious to circulating hexavalent 51Cr not bound to erythrocytes (Owen, Bollman, and Grindlay, 1954), and when 51Cr-erythrocytes enter the lumen the isotope is excreted quantitatively in the faeces (Owen et al., 1954; Roche, Perez-Gimenez, Layrisse, and di Prisco, 1957; Ebaugh et al., 1958, Leonards, 1963). Reported values (Ebaugh et al., 1958; Ebaugh and Beeken, 1959; Cameron, 1960; Holt, 1960; Watson and Pierson, 1960; Grossman et al., 1961; Pierson et al., 1961; Leonards, 1963) of faecal blood loss in normal subjects agree quite well with the losses during the placebo period in this study. Other investigators have found whole blood losses in varied populations during salicylate ingestion to range from 2.3 to over 5 ml per day, results again consistent with our values.

The mechanism of injury due to salicylates responsible for the increased bleeding is not yet fully understood. Decreases in carbohydrate moieties (Menguy and Masters, 1964, 1965) of gastric mucus resulting in a less effective protective barrier probably play an important role. In addition, increases in DNA concentrations in gastric aspirates after salicylates is thought to reflect more rapid mucosal exfoliation induced by these drugs (Croft, 1963). Recent investigations by Davenport (1964, 1965, 1966, and 1967) and others (Johnson and Overholt, 1967) indicate that ingesting salicylates initiates the following sequence. Increased permeability of the epithelial membrane permits the influx of luminal hydrogen ion; an increased intracellular hydrogen ion concentration triggers histamine release, as evidenced by the increased content in gastric venous blood; and capillary vasodilatation and rupture with subsequent bleeding occur. The back diffusion of hydrogen ion also provides an explanation for the conflicting data regarding the influences of salicylates upon gastric acid secretion.

Should blood losses of the magnitude induced by salicylates in this study be perpetuated in menstruating females with marginal iron reserves, iron-deficiency anaemia would probably result. However, normal males and non-menstruating females with adequate iron stores could be expected to compensate adequately for losses of this order. It is noteworthy that salicylates increased blood loss in 48 of the 50 normal subjects, thus suggesting that it is not necessary to implicate any predisposing gastrointestinal lesion in this degree of bleeding. The documented increase in faecal blood loss is clearly abnormal and represents further evidence that salicylates exert an untoward effect upon the gastrointestinal tract of normal man.
SUMMARY

Gastrointestinal losses of $^{51}$Cr-erythrocytes and whole blood were quantitated in separate studies of five groups of 10 normal subjects during the ingestion of placebo preparations and five commonly used salicylate compounds. Group mean losses were significantly greater during the ingestion of each salicylate than during placebo control periods. Mean erythrocyte losses increased by factors ranging from 2.92 to 12.39 during drug ingestion and equivalent whole blood losses increased from 2.89 to 10.97 times during the same period. It is concluded that salicylates produce abnormalities in normal man resulting in increased gastrointestinal erythrocyte and equivalent whole blood losses. Menstruating females with marginal iron stores may be expected to develop iron-deficiency anaemia if losses of this magnitude are continued, but otherwise such losses could be compensated for by other normal subjects.

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REFERENCES


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