Pharmacokinetics of Salazosulfapyridine (Sulfasalazine, SASP) (III):

Metabolism and biliary excretion of SASP in the rat after a single intravenous or oral administration.

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Key words: Salazosulfapyridine, sulfasalazine, SASP, rat, metabolism, biliary excretion

Summary

The metabolites formed from [3H, 14C]salazosulfapyridine (sulfasalazine, SASP), were investigated in urine, faeces, bile and ten different organs from rats after a single oral or intravenous dose. The metabolites were fractionated by liquid chromatography and conclusively identified by mass spectrometry. The metabolites identified in urine were 5-amino salicylic acid (5ASA), 5-acetamidosalicylic acid (Ac5ASA), sulfapyridine (SP) and 5-hydroxysulfapyridine (SPOH) together with glucuronide, sulphate and/or acetyl conjugates of SPOH. The female rats formed no or very small amounts of hydroxylated and conjugated metabolites compared to male rats.

The collected organs and their major metabolite content were as follows: stomach (SASP), duodenum (SASP and SP), ileum (SASP and SP), caecum (SP, SPOH and AcSPOH), colon (SP), lung (SP and SASP), liver (SP, AcSP and SASP), kidney (SP, AcSP and SASP), thyroid (SASP) and testis (SP and AcSP).

The metabolites found in bile after an oral dose were SP, AcSP and glucuronide and sulphate conjugates of these as well as Ac5ASA and unchanged SASP. The bile from male rats contained higher concentrations of conjugated metabolites than bile from female rats. Only intact SASP was found after the i.v. dose.

The cumulative excretion of [14C, 3H]SASP was studied in bile collected during 24 hours after p.o. or i.v. administration. The recovery of total radioactivity in bile from female rats after i.v. administration was 101.6±1.8% (mean±SEM) and 103.5±1.4% of administered dose for 14C and 3H respectively. The corresponding figures for male rats were 88.0±4.1% for 14C and 88.1±4.2% for 3H. The excretion of SASP in bile after i.v. administration was 99.8% of the administered dose in female rats and 85.9% in the male rats. After an oral dose the biliary excretion of 14C radioactivity representing SASP and 5ASA metabolites was low, 3.86±0.79% for females and 5.71±0.95% for male rats. The 3H excretion including SASP but consisting most of SP metabolites was significantly higher in the male rats, 38.3±3.7% compared to 10.5±2.1% in female rats. The reason for this sex differences was the formation of hydroxylated SP and further conjugation in male rats. The biliary excretion of SASP after oral administration was similar in both sexes 2.74±0.37% for female rats and 2.52±0.45% for male rats. Since the biliary excretion of the unchanged drug was < 3% after p.o. administration enterohepatic cycling was not studied.

In conclusion, SASP underwent azoreduction to form 5ASA and SP. Both these metabolites were acetylated. SP was also hydroxylated particularly in the male rats. The SPOH was conjugated both with sulfate and glucuronic acid.
INTRODUCTION

The metabolism of SASP in the rat was studied 1972 by Peppercorn and Goldman and 1973 by Schröder and Gustavsson. They compared conventional rats with germ-free rats and could thus prove the importance of the intestinal microflora for the azo reduction of SASP to sulfapyridine and 5-aminosalicylic acid. Schröder and Gustavsson tentatively suggested 12 metabolites in rat urine according to thin layer chromatography with colour, UV, or Bratton-Marshall reagent for detection. In the present investigations we wanted to study the metabolites not only in urine but also in faeces, bile, and tissues. With aid of radioactivity and mass spectrometry we could make more conclusive identifications than have earlier been possible. Another aim with these studies was to investigate the quantitative importance of the biliary excretion which was observed already 1963 in mice by Svartz and Ullberg in autoradiographic studies. After i.v. administration the labelled compounds rapidly appeared in the bile duct and later on in the gastro-intestinal tract. Biliary excretion of about 2.5% of the administered dose has been reported in patients. In another study 9% of the dose was recovered in bile via a T-tube. In the latter study the collection of bile was not found to be complete. The urinary recovery in these patients was decreased from 55% in the healthy volunteers to 33% in the patients with the bile drain. In the same publication cats were found to excrete 20% of an absorbed dose into the bile and 50–60% of a dose infused directly into the portal vein. Similar results were obtained in rats. This paper presents the metabolism and biliary excretion of SASP in the rat.

MATERIALS AND METHODS

Materials

$^{[14C]}$SASP was synthesized from $^{14}$C labelled salicylic acid according to a synthetic route shown by Sjöquist et al. The specific activity of $^{[14C]}$SASP was 76MBq/mmol (2.05mCi/mmol) and the radiochemical purity was >97% judged by thin layer and liquid chromatography. $^{[3H]}$SASP was prepared according to a scheme also presented by Sjöquist et al. The specific activity was 1.09GBq/mmol (29.5mCi/mmol). The radiochemical purity was >97%. SASP was obtained from Pharmacia AB, Uppsala, Sweden. Sulfapyridine (SP), acetylsulfapyridine (AcSP), N-acetamidosalicylic acid (Ac5ASA), 5-hydroxysulfapyridine (SPOH) and N-acetyl-5-hydroxy sulfapyridine (AcSPOH) were synthesized in the department of Organic Chemistry, Pharmacia AB, Uppsala, Sweden. Other chemicals and solvents were of analytical grade and were obtained from various suppliers.

Animals and dosage

Male and female Sprague Dawley rats were purchased from Alab AB, Sollentuna, Sweden. The animals were 90–120 days old and weighed 215–330 g. The rats were housed in temperature and humidity controlled rooms (17–22°C and 31–60% humidity) with 12 hours light-dark cycle for at least one week before the experiment. The animals
were fed laboratory chow (EWOS R32 Södertälje, Sweden) but they were starved overnight before dosage. A day before and during the experiment the rats were housed individually in metabolism cages that allowed separate sampling of urine, faeces and expired air. Five animals were used in each group. The intravenous dose was 5mg/kg with 0.2MBq of $[^{14}\text{C}]$ and 0.4MBq of $[^{3}\text{H}]$SASP per kg body weight. The oral doses were 50, 200 and 500mg/kg body weight with 2 and 4MBq/kg body weight of $[^{14}\text{C}]$ and $[^{3}\text{H}]$ respectively. Only $[^{3}\text{H}]$ labelled SASP was used in the study where metabolites were investigated in organs.

**Bile duct cannulation**

The bile duct was cannulated with a 1.5cm silastic catheter with 3 holes (0.30mm ID $\times$ 0.64mm OD) glued to a 10cm silastic tube (Dow Corning gut catheter, 0.64mm ID $\times$ 1.19mm OD) equipped with glue cuffs 1.5cm from the end inserted into the intestine.

The animals were anaesthetized with Mebumal 30mg/kg body weight, i.p. The abdomen was shaved, washed with 70% ethanol and rinsed with saline. Laparatomy in linea alba was made.

The bile duct was uncovered and tied up against the intestine with silk, a small cut was done in the bile duct and the bile-catheter was inserted and secured with double sutures.

Half a cm down the bile duct entrance into the intestine a tobacco pouch was placed in the wall of the intestine. Then a small hole was made in the gut wall in the centre of the suture. The intestinal catheter was inserted through the hole. The suture was tied between the two cuffs.

A hole was done from the inside of the abdominal wall two cm beside linea alba on the abdomen. The bile fistula was guided through this hole and the base was stitched to the muscle layer of the abdomen. All catheters were guided through the hole in the bile fistula. The hole was closed with bone-wax and then with a thin layer of tissue glue from the inside. The spring on the bile fistula was thread on and the bile and the intestine catheters were connected to form a shunt. The abdominal wall and then the skin were sutured.

The rat was placed in a cage after operation until the beginning of the experiment. Intact bile circulation was checked every day.

**Sample collection**

After intravenous or oral administration bile was collected every 30 min during the first 6 hours and then during the period 6~16 hours and 16~24 hours.

Urine and faeces were collected after the following time periods: 0~4, 4~8, 8~24, 24~48, 48~72, 72~96, 96~120, 120~144 and 144~168 hours or until less than 0.1% of given radioactivity could be found in urine or faeces samples. The collection vials were protected from light during sampling of urine and faeces. Each faeces sample was homogenized in methanol, about 3 times the weight of the sample immediately after collection.

The animals were anaesthetized with ether and blood was removed by heart puncture. Following decapitation, the animals were perfused with saline. Liver, kidney, lung, thy-
roid, testis, stomach, duodenum + jejunum, ileum, caecum and colon were excised 40 min, 4 and 8 hours after oral administration of [3H]SASP (50mg/kg body weight). All samples were stored at -20°C until analysis.

**Analyses of total radioactivity**

All samples of urine, faeces, bile and plasma were subjected to combustion in a TRICARB Model 306 Sample Oxidizer (Packard Instruments Inc., USA) in order to separate the two isotopes 3H and 14C. The radioactivity was then measured in a Model 2805 Liquid Scintillation Counter (Beckman Instruments Inc., USA).

**Quantification of SASP and its metabolites**

SASP was determined by liquid chromatography using a reversed phase C18 column with mixtures of methanol and phosphate buffer as mobile phase and with UV-detection.

The metabolites of SASP were determined by liquid chromatography after enzymatic hydrolysis of glucuronide and sulphate conjugates. A reversed phase C18 column was used with an aqueous metanolic eluent containing hexane sulphonic acid and triethylamine. A UV-detector and a fluorescence detector were coupled in series.

It was not possible to determine 5ASA due to interfering peaks from endogenous compounds in the chromatograms obtained with this system.

**Metabolite identification**

The urine samples containing the highest concentrations of radioactivity were diluted three times with NH4Ac buffer, 0.1M, pH=4.8, followed by fractionation of the metabolites by high performance liquid chromatography. The faeces samples were centrifuged and the supernatants were treated as urine samples.

Tissue samples were prepared as follows. After thawing the entire tissues, approx. 1 ~ 8 g, were homogenized. After dilution, with methanol, two times the tissue weight, the homogenates were centrifuged and the supernatants were evaporated to dryness. The samples were redissolved in about 0.4m1, 1M NH3 and pH were adjusted to pH 5 with 0.5m1, 1M HAc and diluted with 3.6m1, 0.025M tetrabutylammonium bromide.

A SepPac C18 cartridge (Waters Assoc., Inc.) was conditioned with 10m1, 0.02M tetrabutyl ammonium bromide and the extract was percolated through the cartridge followed by a wash with 4m1 of water. The metabolites were eluted with 2m1 of methanol/water (60 : 40), and the eluate was further concentrated about ten times prior to the following chromatography step.

A high performance liquid chromatography system equipped with a UV (Pharmacia LKB Biotechnology AB) and a radioisotope detector (Model 171, Beckman Instruments Inc.) and with a reversed phase column (Nucleosil C18, 5μm, 125 x 4 mm) was used with 0.1M NH4Ac/MeOH as mobile phase and with gradient elution. (MeOH(%) / time(min) : 0/0, 10/10, 10/14, 40/24, 100/26 and 100/30). Fractions containing radioactivity were collected for further analysis by mass spectrometry.

A triple stage quadrupole mass spectrometer (TSQ 70, Finnigan MAT, San José, USA) equipped with a thermospray interface was used for identification of metabolites in the
collected fractions. Aliqoutes of 50 $\mu l$ were injected directly via a sample injection loop into the mass spectrometer.

The flow rate of the mobile stream, MeOH/0.5M NH$_4$Ac (30:70), was set to 1.2ml/min. Argon (0.5 mtorr) was used as collision gas to obtain daughter ion mass spectra.

Vaporiser and block temperatures as well as repeller potential and collision energy was optimized for each substance.

RESULTS

**Urine and faeces metabolites**

The metabolites in urine and faeces samples were fractionated by liquid chromatogra-

Fig. 1 UV and radioactivity profiles of urine from a) female rat and b) male rat administered [14C, 3H]SASP 200mg/kg body weight p.o.

Fig. 2 UV and radioactivity profiles of faeces from a) female rat and b) male rat administered [14C, 3H] SASP 200mg/kg body weight p.o.
Table 1  Accumulated excretion of sulfasalazine, metabolites, $^{14}$C and $^{3}$H in urine and faeces from rats collected during 7 days after an oral administration of $[^{14}$C, $^{3}$H] SASP 200mg/kg bw, expressed as % of given dose.

<table>
<thead>
<tr>
<th>Animal</th>
<th>SASP (%)</th>
<th>Ac5ASA (%)</th>
<th>SP (%)</th>
<th>SPOH (%)</th>
<th>AcSP (%)</th>
<th>AcSPOH (%)</th>
<th>SASP+Ac5ASA (%)</th>
<th>$^{14}$C (%)</th>
<th>SASP+SP-metabolites (%)</th>
<th>$^{3}$H (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Faeces :</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>0.27</td>
<td>32.66</td>
<td>13.03</td>
<td>8.32</td>
<td>9.94</td>
<td>3.82</td>
<td>32.93</td>
<td>72.61</td>
<td>35.38</td>
<td>21.01</td>
</tr>
<tr>
<td>Males</td>
<td>0.12</td>
<td>32.23</td>
<td>3.46</td>
<td>17.92</td>
<td>0.14</td>
<td>61.49</td>
<td>32.35</td>
<td>59.18</td>
<td>83.13</td>
<td>41.06</td>
</tr>
<tr>
<td>Urine :</td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
<td></td>
</tr>
<tr>
<td>Females</td>
<td>0.02</td>
<td>15.59</td>
<td>8.19</td>
<td>4.37</td>
<td>23.48</td>
<td>1.13</td>
<td>15.61</td>
<td>16.89</td>
<td>37.19</td>
<td>45.13</td>
</tr>
<tr>
<td>Males</td>
<td>0.10</td>
<td>20.84</td>
<td>2.17</td>
<td>16.58</td>
<td>4.04</td>
<td>33.39</td>
<td>20.94</td>
<td>20.73</td>
<td>56.28</td>
<td>49.54</td>
</tr>
</tbody>
</table>

Mean, n=5.
phy. Only peaks that contained radioactivity were collected for further identification by mass spectrometry.

Similar UV and radioactivity profiles were obtained from male rats that received $^{14}$C, $^3$H]SASP 50, 200 or 500mg/kg body weight. However, the profiles from female rats were different from those of male rats.

The UV-chromatograms and radioactivity profiles of urine and faeces collected during the first 24 hours from one male and one female rat administered $^{14}$C,$^3$H]SASP 200mg/kg body weight are shown in Fig. 1 and 2.

The peaks that were collected and identified by mass spectrometry are indicated in the chromatograms. The metabolites, except glucuronide and sulphate conjugates, were identified by comparison of their daughter ion mass spectra with those of synthesized standards. Eleven metabolites were identified. The main metabolites after a oral dose in both faeces and urine from male rats were AcSPOH, Ac5ASA and SPOH (Table I). In female rats the hydroxylated metabolites were only found in small amounts, while SP and AcSP were found in higher amounts and Ac5ASA in equal amounts as in the male

![Fig. 3 Daughter ion mass spectrum of a) SP from rat urine and b) SP standard.](image)

![Fig. 4 Positive ion mass spectrum of a) SPgluc and of b) AcSPgluc.](image)
rats. There was a good correlation between the sum of all SP metabolites and \(^3\)H, and between Ac5ASA and \(^{14}\)C in urine. However in faeces the correlations were not as good due to difficulties with the quantitative analyses of metabolites in faeces by the chromatography method. Thus the data obtained from measurement of radioactivity is more reliable in this case.

Fig. 3 shows the comparison of the daughter ion mass spectrum of SP to that of the urine fraction containing this metabolite and illustrates the high selectivity obtained with the technique. Similar results were obtained from analyses of other non-conjugated metabolites.

The glucuronide and sulphate conjugated metabolites of SPOH and AcSPOH were
identified from their fragmentation patterns. Thus, the mass spectrum of SPglucuronide (Fig. 4 a) showed a relatively intense peak at m/z 442, [M+H]+, and the adduct ions with one or two sodium. An intense peak at m/z 266 was also seen corresponding to the protonated SPOH fragment ion that resulted from loss of the glucuronide moiety, 176 amu (atomic mass units).

A similar fragmentation pattern was obtained from AcSPglucuronide shown in Fig. 4 b. The characteristic loss of 176 amu from the protonated molecular ion, m/z = 484, of this glucuronide conjugate yielded a fragment ion at m/z 308, corresponding to the protonated AcSPOH. The peaks at m/z 506, [M+Na]+, and at m/z 266, [M–Ac–176]+, also belongs to the fragmentation pattern of AcSPglucuronide, while peaks at m/z 346, 368 and 390 comes from [M+H]+, [M+Na]+ and [M–H+2Na]+ of SPsulphate which were difficult to separate from AcSPglucuronide in this chromatography system.

The sulphate conjugates of SPOH and AcSPOH were identified from their negative ion thermospray mass spectra. Thus, the peaks at m/z 344 and 366 in the mass spectrum of SPsulphate in Fig. 5a correspond to the ions [M–H]– and [M–2H+Na]– respectively and m/z 324 to [M–SO3+Ac]–.

A similar fragmentation was obtained from AcSPsulphate, Fig. 5 b. Significant peaks in the molecular region of this spectrum were from m/z 306, 324, 342, 366, 386 and 404 corresponding to [M–H–SO3]–, [M+Ac–42–SO3], [M–SO3+C]–, [M–SO3+Ac]–, [M–H]– and [M+Ac–42] respectively. A minor peak was also seen at m/z 446 corresponding to [M+Ac]–. The identified metabolites are shown in Fig. 6.

Fig. 7 UV and radioactivity profiles of bile collected 16 hours after per oral administration of [14C, 3H]SASP 200mg/kg body weight, a) female rat and b) male rat.
Bile metabolites

The UV chromatogram and radioactivity profiles of the bile from rats that received $[^{14}C, {^3}H]$ SASP 200mg/kg body weight orally are shown in Fig. 7. The metabolites found in bile were also observed in urine and/or faeces. The radioactivity chromatogram of bile from female or male rat after intravenous administration contained only one peak corresponding to unchanged SASP, as illustrated for male rats in Fig. 8.

Tissue metabolites

The metabolites of SASP were analysed in ten different organs. The selection of organs was based on the quantitative determinations of radioactivity performed by liquid scintillation counting and also based on results from autoradiography studies.

Thus liver, kidney, lung, thyroid, testis, stomach, duodenum (+jejunum), ileum, caecum and colon were collected. The organs were homogenized and metabolite extracts were analysed by liquid chromatography using a UV and a radioactivity flow detector.

Fig. 9 and 10 show the UV traces and radioactivity profiles obtained from the tissue homogenates. The radioactivity profiles showed intense peaks corresponding to SP metabolites, due to the rather high specific activity of $^3H$ in the administered dose. No metabolites of the 5-aminosalicylic acid part of the molecular, could be detected since the administered SASP used in this experiment was only labelled with $^3H$ in the sulfophenyl ring.

The sample from stomach collected after 40 minutes contained unchanged SASP as expected, whereas a small $^3H$ peak corresponding to SP was seen in duodenum after 4
Fig. 9 UV and radioactivity profiles of tissue extracts after an oral administration of $^{[14]C, \ 3H}]$ SASP 50mg/kg body weight, a) stomach (40 min) b) duodenum (4 h) c) ileum (4 h) d) caecum (8 h) e) colon (8 h) f) lung (40min).

Fig. 10 UV and radioactivity profiles of tissue extracts after an oral administration of $^{[14]C, \ 3H}]$ SASP 50mg/kg body weight, a) liver (4 h) b) kidney (4 h) c) thyroid (8 h) d) testis (8 h).
Fig. 11 Accumulated excretion of $^{14}$C (a) and $^3$H (b) in bile from rats injected i.v. Mean ± SEM, n=5.

Female Male

- □ $^{14}$C
- ☐ $^3$H

Fig. 12 Accumulated excretion of $^{14}$C (a) and $^3$H (b) and SASP in bile from rats administered p.o. Mean ± SEM, n=5.

female Male

- ■
- ☐ $^{14}$C
- ○ $^3$H
- ● D
- ○ ○ SASP
Table II  Accumulated excretion of sulfasalazine, metabolites, $^{14}$C and $^3$H in bile from rats collected 24 hours after an oral administration of $[^{14}$C, $^3$H]SASP 200mg/kg bw, expressed as % of given dose.

<table>
<thead>
<tr>
<th>Animal</th>
<th>SASP (%)</th>
<th>Ac5ASA (%)</th>
<th>SP (%)</th>
<th>SPOH (%)</th>
<th>AcSP (%)</th>
<th>AcSPOH (%)</th>
<th>SASP+Ac5ASA (%)</th>
<th>$^{14}$C (%)</th>
<th>SASP+SP-metabolites (%)</th>
<th>$^3$H (%)</th>
</tr>
</thead>
<tbody>
<tr>
<td>Females</td>
<td>2.74±0.37</td>
<td>1.10±0.40</td>
<td>0.72±0.24</td>
<td>1.40±0.62</td>
<td>1.19±0.43</td>
<td>1.99±0.57</td>
<td>3.84±0.76</td>
<td>3.86±0.79</td>
<td>8.04±2.21</td>
<td>10.54±2.06</td>
</tr>
<tr>
<td>Males</td>
<td>2.52±0.45</td>
<td>2.54±0.65</td>
<td>1.28±0.16</td>
<td>3.76±0.64</td>
<td>0.87±0.53</td>
<td>35.20±4.19</td>
<td>5.06±1.10</td>
<td>5.71±0.95</td>
<td>43.63±5.97</td>
<td>38.30±3.72</td>
</tr>
</tbody>
</table>

Mean ± SEM, n=5.
hours beside the bigger peak from SASP. A similar profile was seen in ileum after 4 hours.

The sample from caecum collected after 8 hours showed an almost complete conversion of SASP to SP, SPOH and AcSPOH metabolites. The two latter most likely resulting from biliary excretion in non-conjugated forms or as sulphate or glucuronide conjugates of SPOH and AcSPOH followed by intestinal enzymatic hydrolysis.

Samples of colon collected after 8 hours contained mainly SP and some minor metabolites. Except from samples of the gastro-intestinal tract, we collected samples of lung, liver, kidney, thyroid and testis. Thus, the lung contained SP and SASP whereas the major peaks in the liver were AcSP together with SP and SASP as well as some minor non-identified peaks with shorter retention times. The metabolic profiles of the kidney showed similarities to the liver but contained relatively higher concentrations of SPOH and AcSPOH. The peak corresponding to SASP in thyroid in Fig. 10c is probably due to contamination from larynx during dissection.

The summary of identified metabolites in organs were SP, SPOH. AcSP, AcSPOH and unmetabolized SASP.

**Bile excretion**

The cumulative excretion of radioactivity after intravenous administration is shown in Fig. 11. The recovery of both $^{14}$C and $^3$H radioactivity from the female rats was quantitative, 101.6±1.8% and 103.5±1.4%, respectively. For the male rats the recovery was lower 88.0±4.1% and 88.1±4.2% for $^{14}$C and $^3$H, respectively. The sex difference was highly significant ($p < 0.001$) for both isotopes. The excretion of SASP in bile during 24 hours after intravenous administration was 99.8% of the administered dose in the female rats and 85.9% in the male rats. The cumulative excretion of radioactivity and SASP after oral administration is illustrated in Fig. 12. The biliary excretion of $^{14}$C was low and did not differ significantly between the sexes, 3.86±0.79% for female and 5.71±0.95% for male rats ($p < 0.02$). The $^3$H excretion was significantly higher in the male rats bile 38.3±3.7% compared to 10.5±2.1% in the female rats ($p < 0.001$). The sum of the cumulative excretion of SASP and SP and SP metabolites were 8.04% which is slightly below 10.5% found for $^3$H. In the male rats the corresponding figures were 43.6% and 38.3% respectively (Table II). The excretion of SASP in bile during 24 hours after oral administration was 2.74±0.37% of the administered dose for the female rats and 2.52±0.45% in the male rats (Table II and Fig. 12). Thus there was no sex difference in the excretion of SASP. The sum of the cumulative excretion of SASP and Ac5ASA (2.74+1.10=) 3.84% of given dose was the same as the excreted $^{14}$C radioactivity 3.86%. Adding together SASP and Ac5ASA from male rats (2.52+2.54=) 5.06%, gave a figure close to the amount of $^{14}$C in bile from male rats 5.71% (Table II).

**DISCUSSION**

The metabolism of SASP is extensive in the rat. The main site for azo reduction has been claimed to be in caecum. However, after in vitro incubation of various human tissue homogenates and bacteria it has been shown that all bacteria tested whether colonic
or noncolonic, aerobic or anaerobic, show roughly the same level of activity. In human tissues, the liver showed pronounced activity, while the difference between other tissues was not marked\(^5\). However the red-ox potential and the residence time in different tissues is critical and thus colon becomes the major site of reduction.

The metabolites in rat urine were tentatively proposed by Schröder and Gustavsson, 1973\(^2\).

The data from identification of isolated metabolites reported in the present study were obtained by tandem mass spectrometry, which is an unbiased technique that facilitated conclusive identification of isolated SASP metabolites. The results we obtained are in agreement with those obtained by other groups using other techniques. However, in addition to the metabolites that have been identified or tentatively identified previously in rat urine we found other metabolites as indicated in the radioactivity profiles.

One of these was identified as sulfanilamide which is known to have antibacterial effects. Sulfanilamide was only found in male rat urine and in relatively low concentration. This metabolite was not found in a similar study performed on dogs\(^7\).

The faeces samples contained relatively higher concentrations of \(^{14}\)C metabolites compared to urine which was in accordance with the data of excretion of the total activities of \(^{14}\)C and \(^3\)H in these matrices. In addition to 5ASA and Ac5ASA a \(^{14}\)C metabolite was also excreted at retention time 28 minutes as concluded from the \(^3\)H/\(^{14}\)C ratio of the radioactivity profiles of faeces samples. It was not possible to make any conclusive mass spectrometric identification of this peak due to its contents of other substances like unmetabolized SASP. Further work is however in progress to identify this unknown metabolite.

A sex difference in terms of metabolite hydroxylation and conjugation was observed. The urine from female rats contained no or relatively very low concentrations of SPOH and AcSPOH compared to urine from male rats that received the same dose. Furthermore, the concentrations of conjugates were also low as a consequence of the low concentrations of hydroxylated metabolites. A similar sex difference was also observed in the metabolite pattern in bile. Sex related differences in drug metabolism are more often observed in the rat than in other species\(^8\text{-}10\). The sex difference in the formation of hydroxylated and conjugated metabolites of SASP was not observed in the metabolism study performed on dogs\(^7\). In man no metabolic sex differences have been reported.

A dose of \([\text{H}]\text{SASP} 50\text{mg/kg}\) was used in the study of metabolic profiles of tissues. Thus peaks corresponding to metabolites were small in the UV chromatograms due to the relatively low concentrations and to interfering noise from endogenous compounds. Consequently, it was only possible to identify the major peaks corresponding to SP metabolites, in the radioactivity profiles by mass spectrometry. All identified metabolites in tissues were also identified in urine and/or faeces samples. Conjugates make the compounds hydrophilic and facilitate excretion. For example the high concentrations of sulphate and glucuronide conjugated metabolites observed in rat urine were not reflected in the metabolite profile of the kidney sample. As expected no sulphate or glucuronide metabolites were found in the tissues.

The finding that SP appeared after 4 hours both in duodenum and ileum indicates that the administered SASP reacts by cleavage of the azo bridge to a minor extent already
in duodenum. The small amounts of SP found in duodenum and ileum could of course have arisen from biliary excretion. However, if that was the case, other hydroxylated or acetylated metabolites of SP would also have been expected to occur in these samples which was not the case.

After intravenous administration of $^{14}$C, $^{3}$H-SASP the excretion of $^{14}$C and $^{3}$H radioactivity as well as of SASP in the bile of females rats was quantitative. From the male rats 88% of both $^{14}$C and $^{3}$H and 86% of SASP were recovered in the bile. The biliary excretion was rapid and almost completed after 2 hours. All radioactivity could be accounted for as the intact drug. In accordance no metabolites were found in bile after intravenous administration. This is in agreement with the findings in the bile excretion studies in dogs$^{7}$.

After oral administration the biliary excretion of SASP had almost ceased after 24 hours and less than 3% of the dose was found as SASP in bile from both sexes and the recovery of $^{14}$C radioactivity was also low (4% from female rats and 6% from male rats). The $^{14}$C radioactivity excreted was closely related to the sum of intact SASP and Ac5ASA. However, the biliary excretion of $^{3}$H was 38% in the male rats compared to 11% from the female rats. These data indicate that the biliary excretion of sulfapyridine and its metabolites are higher in males than in female rats. This was also shown by quantitative analysis of sulfapyridine and its hydroxylated and acetylated metabolites. The major metabolite in bile was acetylated and hydroxylated sulfapyridine (AcSPOH). In male rats this metabolite accounted for more than 80% of the sum of metabolites.

To conclude, the rat showed an extensive metabolism of SASP. Primarily the azo bridge was reduced and hydroxylation of sulfapyridine occurred. Further reactions as acetylation and conjugation with sulfate or glucuronic acid took place. The biliary excretion of the intact drug was prominent after i.v. administration but low after oral administration, thus the enterohepatic cycling was not studied.

References
7) Sjöquist, B., Ahnfelt, N.-O., Andersson, S., d’Argy, R., Fjellner, G.,

