SALICYLAZOSULPHAPYRIDINE METABOLISM IN CLINICAL PRACTICE

A thesis presented in part fulfilment of the requirements for the admittance to the degree of Doctor of Philosophy of the University of Edinburgh.

by

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SUMMARY

This Thesis describes work on the metabolism of salicylazosulphapyridine in normal volunteers and in patients with ulcerative colitis or Crohn's disease. The effect of coincidental administration of iron and calcium in healthy volunteers on the absorption of sulphasalazine and its metabolites has been investigated. In patients with ulcerative colitis or Crohn's disease an attempt has been made to correlate the clinical state of the disease and the serum concentration of sulphasalazine or its metabolites. The relationship of the toxic effects due to sulphasalazine with the acetylator phenotype, dosage and serum concentration of the drug and its metabolites has also been explored. The feasibility of reintroducing the drug in patients with side effects has been studied. The metabolism of sulphasalazine in patients with ileostomy or colostomy was investigated.

NOMENCLATURE

The following abbreviations have been used.

Salicylazosulphapyridine, sulphasalazine	:	SASP
Sulphapyridine	:	SP
N^4-Acetylsulphapyridine	:	AcSP
Sulphapyridine-o-glucuronide	:	SP-Gluc
N^4-Acetylsulphapyridine-o-glucuronide	:	AcSP-Gluc
5-Aminosalicylic acid	:	5-ASA
Acetylated 5-Aminosalicylic acid	:	Ac5-ASA
Ulcerative Colitis	:	U.C.
Crohn's Disease	:	C.D.
Mean	:	M
Standard Deviation	:	S.D.
Standard Error of Mean	:	S.E.M.
Optical Density	:	O.D.
Hydrochloric acid	:	HCl
Sodium hydroxide	:	NaOH
Twice daily	:	B.D.
Three times a day	:	T.I.D.
Four times a day	:	Q.I.D.
Plain sulphasalazine tablet	:	P
Enteric coated tablet	:	E.C.
Statistically Not Significant	:	N.S.
Acetylator Phenotype	:	Ac.Ph.
Thin Layer Chromatography	:	T.L.C.

CHAPTER I

INTRODUCTION

INTRODUCTION

The clinical entity of "ulcerative colitis" was first described in the latter part of the nineteenth century (Wilks, 1859; Alchin, 1885; Hale-White, 1888), but it was not until 1932 that Crohn, Ginzburg and Oppenhiemer described fourteen cases of "regional ileitis" as a separate pathologic and clinical entity. This was probably due to the difficulty of distinguishing the latter disease from ileocaecal tuberculosis.

The management of these two diseases did not progress until the introduction of sulphonamide and later of corticosteroid therapy. Different sulphonamides, in absorbable and non-absorbable forms, were used initially with varying success (Svartz and Kallner, 1940(a); Gaspar, 1945; Streicher, 1945; Trier, 1948). During 1940 - 41 Nana Svartz tried to develop a compound combining sulphonamide and salicylic acid to treat the patients with "rheumatic polyarthritis". She had found that "sulfanilamide preparations are active in the septic form of arthritis but not in the common rheumatic form" and therefore she speculated on "whether medication with both salicyl and sulfanilamide preparations at the same time affects rheumatic polyarthritis. "These experiments yielded no tangible results". "In the next phase" salicylazo-sulphapyridine, a combination between 5-aminosalicylic acid and sulphapyridine, through an azo link, was prepared

in collaboration with AB Pharmacia Ltd., Upsalla.

The preparation "has been shown a definite effect in certain cases of polyarthritis". Simultaneously she started using this drug for patients with ulcerative colitis and in 1941 and 42 she reported the results of twelve successfully treated patients with ulcerative colitis. She later reported one hundred and twenty-four cases of ulcerative colitis treated successfully with this drug (1948). This encouraging initial result led to further investigative work, but it was not until the late fifties and early sixties that the value of salicylazo-sulphapyridine in active and quiescent colitis was formally established by controlled trials (Baron et al, 1962; Moertel and Bargen, 1959; Dick et al, 1964; Misiewicz et al 1965).

The role of sulphasalazine in the treatment of Crohn's disease remains unresolved (Lennard-Jones, 1971; Dyer, 1972). Favourable effects have been claimed by different workers (Bargen, 1957; Meeuwisse et al, 1970; Lennard-Jones, 1970; Goldstein & Murdock, 1971), but differing view exists (Cooke, 1972). No well-controlled trial has been reported to settle this issue, perhaps because of the difficulties in defining Crohn's disease and its response to treatment.

Though the drug has been extensively used in the treatment of inflammatory bowel diseases during the last thirty years very little was known about its metabolism in man. Recently Schröder and Campbell (1972) have

studied its metabolism in nine healthy volunteers after giving the drug at a dosage of 4G/day for ten days. In these normal persons in a 'steady state' (five days and after) the mean serum concentration of SASP, total SP (i.e. free sulphapyridine and its metabolites) and 5-ASA were found to be 8 ug, 33 ug, and < 2 ug/ml. of serum respectively. Twenty-four hour urinary excretion was largely (80% of the administered dose) in the form of sulphapyridine metabolites. These authors suggested that "about one third" of the administered dose of sulphasalazine is absorbed from small intestine and that the drug is split in the colon and then absorbed as sulphapyridine. It is not known whether there is any enterohepatic circulation of SASP and its metabolites. Sulphapyridine per se, if administered orally, is almost totally absorbed from the small intestine; it is partly excreted in the bile (Goodman and Gillman, 1970) and can be reabsorbed.

A scheme of the different metabolites of sulphasalazine is shown in Figure 1. Sulphapyridine constitutes about two thirds of the molecular weight of sulphasalazine. The main components of sulphapyridine found in the serum are free sulphapyridine and acetyl sulphapyridine in proportions dependent on the acetylator phenotype of the subject (Schröder and Evans, 1972(a)). Sulphapyridine is also subjected to hydroxylation followed by conjugation to glucuronic acid & N4 acetylation. Using TLC hydroxy SP could not be detected in the urine and it was suggested

that the hydroxylation is the rate limiting step in the formation of the glucuronide which is found in large amounts in the urine (Schröder and Campbell, 1972). The serum concentration of sulphapyridine glucuronides were found to be lower than SP or AcSP, yet more than 50% of the total recovery of sulphapyridine in the urine was in the form of the glucuronides, i.e. (SP-Gluc. and AcSP-Gluc.).

Acetylation is genetically determined and certain drugs, e.g. isoniazid (Evans et al, 1960) sulphadimidine and hydrallazine (Evans and White, 1964), and dapsone (Gelber et al, 1971) share the same acetylation polymorphism. However, sulphanilamide does not share it (Evans and White, 1964). Recently Schröder and Evans, (1972) have shown parallel acetylation of sulphapyridine with sulphadimidine. A knowledge of acetylation capacity is important as it has been shown that side effects are common in slow acetylators e.g. peripheral neuritis with isoniazid was predominently found in slow acetylators (Devadatta et al, 1960).

Müller-Wieland et al (1970) reported that when an increased dose of SASP methyl glucusamine salt, believed to be more readily absorbed than SASP, was introduced through a tube into the duodenum, the absorption of the drug did not appear to be increased. However, they did not indicate what percent of the drug is absorbed from the small intestine. Recently Harris et al (1972) have shown from "in vivo" and "in vitro" experiments that there is net secretion of Na and water from the colon in

patients with ulcerative colitis and Crohn's disease. They also reported that sulphasalazine reverses this abnormality.

There is no evidence of any significant change of gut flora after giving SASP (Gorbach et al, 1968; Cooke 1969). Golde (1968) suggested that the effect of the drug may be due to the action of aminosalicylic acid on mycobacteria whose role in the aetiology of ulcerative colitis or Crohn's disease is not clear. Schröder and Campbell (1972) have, however, found low serum levels of 5-ASA (0 - 2 ug/ml). Sulphonamides may also have an anti-inflammatory or antimetabolite action (Woods, 1962). Hanngren et al (1963) suggested that therapeutic success is related to the fixation of SASP in connective tissue in the colonic wall, but its significance in clinical practice is denied (Thayer, 1970).

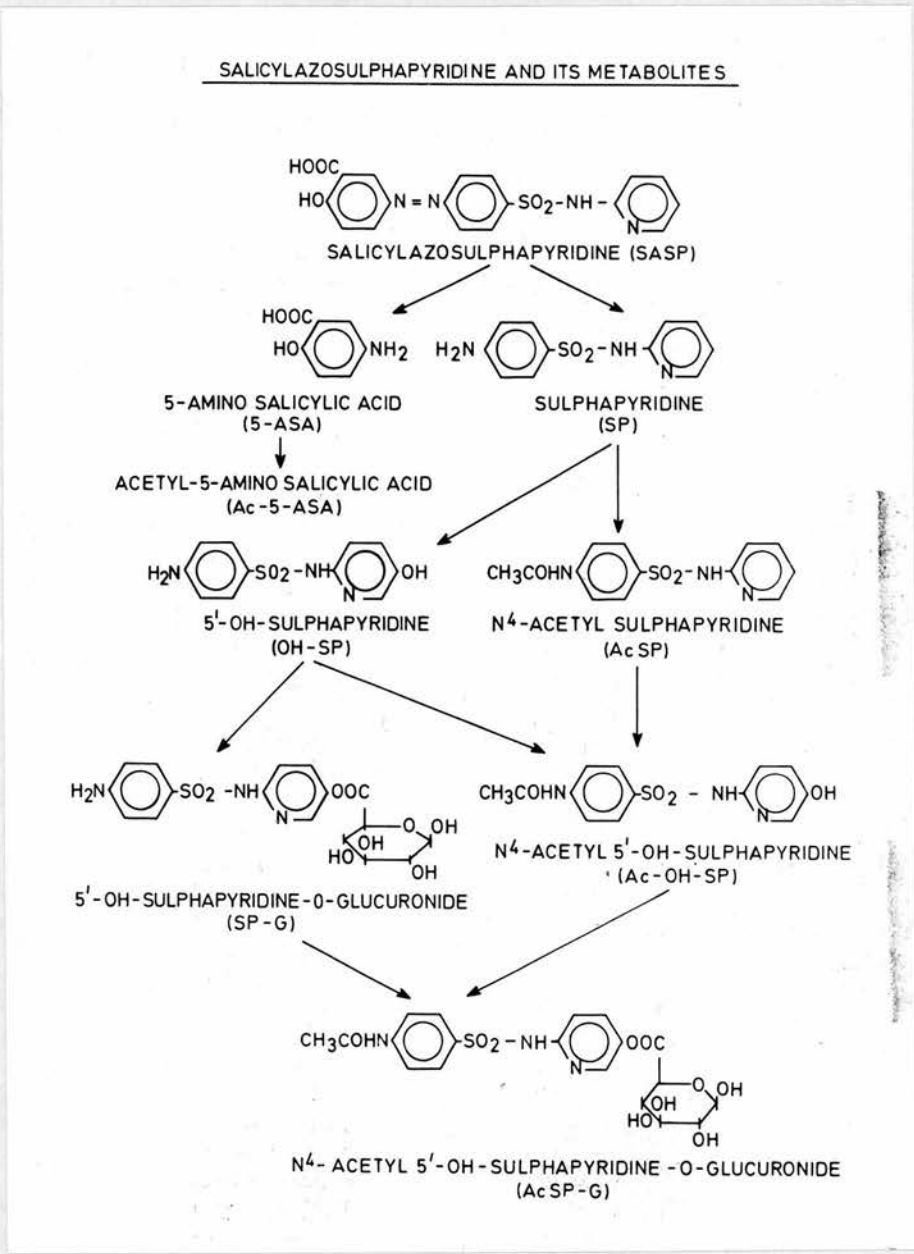
It has been reported in different papers (Svartz, 1942; Morrison, 1953; Moertel and Bargen, 1959; Lennard-Jones et al, 1960; Truelove and Watkinson, 1962; Dick et al, 1964; Misiewicz et al, 1965; Collins, 1968) that a variable percentage ("few" - 55%) of patients cannot tolerate the drug due to various side effects, e.g. nausea, headache, fever, rash and blood dyscrasias. Moertel and Bargen, (1959) and Truelove and Watkinson (1962) have suggested that by a reduction of the dose the side effects can be alleviated, but the dose needed to produce full therapeutic benefit without toxic effects is not clear. The recognition of such effects in long term therapy is particularly important

as the drug was found to have a special role in preventing relapses (Misiewicz et al, 1965), but such information during long term therapy is also lacking (Collins, 1968). The relationship of these toxic reactions to serum concentrations of SASP and its metabolites is also unknown.

This thesis describes a study of the metabolism of sulphasalazine in patients with ulcerative colitis and Crohn's disease. The relationship of the disease state to serum concentrations of sulphasalazine and its metabolites was studied during the initial attack and also in patients on long term therapy. The relationship between side effects and the serum concentration of the drug and its metabolites was also investigated. The role of acetylator phenotype was evaluated in relation to toxic symptoms and to therapeutic success. The feasability of reintroducing sulphasalazine to patients with unacceptable side effects has also been studied.

The effect of iron and calcium on the metabolism of SASP has been explored.

Figure 1



Salicylazosulphapyridine and its metabolites.

CHAPTER II

PATIENTS, MATERIALS AND METHODS.

SECTION I : PATIENTS

SECTION II : METHODOLOGY

SECTION I

DESCRIPTION OF PATIENTS STUDIED

The patients studied were all either admitted to the Gastro-intestinal Unit, Western General Hospital, Edinburgh or attended the Gastro-intestinal outpatient follow-up clinic. For the purpose of this study they were divided into two groups:-

1. Patients with ulcerative colitis and
2. Those with Crohn's disease of the small and/or large bowel.

Table 1 outlines all the patients studied. Ulcerative colitis was diagnosed on the basis of the clinical findings including sigmoidoscopic evidence of ulceration, friable and granular mucosa. Diagnosis was always confirmed by histological examination of the rectal biopsy showing ulceration, crypt abscess, excessive infiltration with polymorphs and mononuclear cells. Radiological examination of barium enema also helped the diagnosis. This in most of the cases of active colitis showed ulceration and granularity of mucosa, shortening especially of pelvic colon with increase in retro rectal space, absence of normal haustrations, disease starting distally and extending proximally in a continuous manner.

CROHN'S DISEASE OF THE SMALL AND/OR LARGE BOWEL

The diagnosis of Crohn's disease was based on analysis of structural abnormality as disclosed by

Table 1

TOTAL PATIENTS STUDIED (n = 133)

I. Ulcerative Colitis (n = 88)*

A. Inpatient Study (n = 23)*

(a) Introduction of SASP in new patients and follow-up = 13

(b) Patients established on SASP, during relapse = 3

(c) Patients with Ileostomy or Colostomy (n = 7)

Before and after ileostomy = 4

After ileostomy only (2), after Colostomy (1) = 3

B. Outpatient Study (n = 68 Ø)

II. Crohn's Disease (n = 45)

A. Inpatient Study (n = 14)

(a) New patients, introduction of SASP and follow-up = 2

(b) Established patients, SASP stopped for 1 year, reintroduction of SASP and follow-up = 2

(c) Patients with ileotransverse anastomosis, introduction of SASP and follow-up = 3

(d) Patients after ileostomy (2), after colostomy (1) = 3

(e) Patients included after having SASP toxicity = 4

B. Outpatient study (n = 31 Ø)

* - Three patients with ulcerative colitis were studied initially as outpatients. They were subsequently admitted and had colectomy. They were also included under ileostomy study.

Ø - Six outpatients (4 U.C., 2 C.D.) had no detectable SASP or metabolites in serum and were therefore assumed not to have taken the drug. None were included in any comparative study.

clinical examination, x-rays and biopsies. Small intestinal disease was clinically based on evidence of malabsorption or obstructive symptom. Evidence of clubbing, enterocutaneous fistula and perianal lesion were particularly noted. In some of the patients studied a mass was felt at the right iliac fossa.

Confirmation of the diagnosis from rectal biopsy, in some patients with rectal involvement, or in operative specimens, was based on the findings of non-specific inflammation with evidence of granuloma with giant and epithiloid cells, cracks and fissures deepening up to muscular coat.

Radiological findings were often helpful. Small bowel - in some patients ulceration, smooth narrowed segment (string sign) sometimes with normal intervening areas, fistulae, inflammatory mass. Disease affecting mostly distal small bowel especially terminal ileum; colon- ulceration, shortening especially of proximal colon, disease starting proximally and extending distally in patchy manner (skip lesions).

A. Inpatient study: (37 patients - U.C. = 23; C.D. = 14).

(i) This includes the patients admitted for the first time in the Unit without being treated with SASP before admission.

(ii) Those admitted with relapse after being established on SASP for some time.

(iii) Patients studied before and/or after ileostomy, colostomy and ileotransverse anastomosis.

B. Outpatient study: (99 patients, U.C. = 68; C.D. = 31).

Patients with established diagnosis of ulcerative colitis or Crohn's disease attending the follow-up clinic either in a stage of remission or with active disease, though less severe than inpatients.

In total one hundred and thirty-three patients with ulcerative colitis or Crohn's disease were studied. Three patients (A.D., C.S., W.M.) with ulcerative colitis are included under inpatient as well as outpatient study. These three patients were studied as outpatients and subsequently they were admitted for subtotal or total colectomy. They were also studied following the operation.

A. INPATIENT STUDY

Of the thirty-seven patients studied, twenty-three had ulcerative colitis (Group I) and fourteen had Crohn's disease (Group II).

Group I - ulcerative colitis

Inpatients: Thirteen patients (A.P., W.R., J.R., J.W., P.T., E.A., R.B., T.F., J.C., L.L., M.N., T.B., D.F.) with ulcerative colitis were admitted during their first attack (12) and during second attack (1). None of them had SASP during admission. In these patients, studies were carried out from day of admission to the day of

discharge and followed up to one year.

Three other patients (M.D., W.C., M.B.) with ulcerative colitis were admitted with relapse. They had been taking SASP for more than two months. From these three patients blood samples were collected on admission and subsequently two to three times a day on two different occasions, after improvement. Twenty-four hour urine samples were also collected.

One other patient (J.H.) with ulcerative colitis had transverse colostomy for left-sided colitis and diverticulitis of the pelvic colon. She was studied during introduction of the drug SASP six weeks following the operation.

Four other patients (A.D., C.S., W.M. and E.C.) were studied before and after they had undergone proctocolectomy or subtotal colectomy with ileostomy. Two other patients with ileostomy following subtotal colectomy (G.Mc., G.K.) were treated with SASP two to three weeks after surgery for rectal disease in situ. SASP metabolism was followed from the first day of therapy (i.e. fourteen to twenty-one days after surgery) and at variable intervals up to one year.

Group II - Patients suffering from Crohn's disease

Inpatients: Two new patients (J.W., R.M.) were admitted to the Unit and treated with SASP for the first time. They were studied from the day of admission to the day of discharge and then followed up at variable intervals up to one year.

Two other patients (M.M., A.B.) had been suffering from C.D. with protein losing enteropathy for two and five years respectively. They were previously treated with SASP but the drug had been discontinued for about one year because of haemolysis. These two patients were also reintroduced to the drug during their recent admission (in the course of the present study) and then followed up.

One other patient (M.C.) was treated with SASP for six months prior to the study. She was studied before and after (two weeks) she had an ileotransverse anastomosis. Two other patients (G.F., M.Y.) were given SASP only after (four to eight weeks) they had an ileotransverse anastomosis. They were also studied during initial stage of introduction of SASP and followed up to six months.

The two other patients (C.M., M.Mc.) were studied only after ileostomy which they had for more than six years. One patient (J.Cr.) was studied after having ileotransverse anastomosis (seven years ago) and also transverse colostomy (four years ago).

Four other patients (R.W., M.B., I.G., J.B.) were studied at a stage when they exhibited toxic symptoms related to SASP. Studies were continued until the toxic symptoms disappeared.

B. OUTPATIENT STUDY

This study consisted of patients with ulcerative colitis (n = 68) or Crohn's disease (n = 31) who were attending the Gastro-intestinal follow-up clinic and

who were established on SASP therapy. These ninety-nine patients were taken at random. A proforma which included details of age, sex, body weight, state of disease, the present dose of SASP, type of tablet (plain or enteric coated), approximate time that the most recent dose was taken by the patient, the time of blood collection, history of any bowel operation, other drugs used and presence of any toxic symptom, was completed by each attending physician. The clinical state of the disease was assessed by clinical and sigmoidoscopic findings and reported in the proforma. Blood samples were collected simultaneously for the estimate of SASP and its metabolites and also for the haematological and biochemical examinations.

DETAIL PROCEDURE FOR INPATIENT STUDY

The study was explained in detail to each patient prior to commencement. Patients who were admitted for the first time were classified into slow or fast acetylator, using sulphadimidine as described by Price Evans (1969) during the first twenty-four to thirty-six hours of admission. The patient was fasted overnight then in the morning sulphadimidine (500 - 750 mg.) was given orally on the basis of the body weight, i.e. less than 51 kg. the dose of sulphadimidine given was 500 mg. and between 51 - 83 kg. the dose of sulphadimidine was 750 mg. The patient did not eat for a further two hours and was then allowed normal breakfast and lunch. At the fifth hour patients were asked to evacuate the bladder and the samples discarded. Six hours after taking the sulphadimidine

a sample of blood was collected and all samples of urine passed during the fifth to sixth hour were also obtained. The total quantity of urine was measured and an aliquot was preserved for analysis. Sixteen patients with ulcerative colitis (thirteen new patients, one with transverse colostomy, two of the three (M.D., M.B.) patients admitted with relapse) were phenotyped. The last two patients were phenotyped with sulphadimidine at a later date after stopping SASP for five to six days. Five patients with Crohn's disease (J.W., M.M., A.B., G.F., M.Y.) were also phenotyped this way.

Following this, the conventional treatment was started in all the new patients with SASP 3 - 6 G/day (except in two patients who had smaller initial dose because of the history of rash) with or without corticosteroids and supportive therapy. Samples of blood were taken throughout at approximately 9 a.m., 12 mid-day and 5 p.m., or at 8 p.m., on day 1, 3, 5, 7 and 10 as far as possible. The blood specimens were allowed to coagulate at room temperature for one to two hours and the serum separated and stored at -20°C until analysis.

Twenty-four hour samples of urine were collected essentially parallel to the blood collections. However, blood samples, and twenty-four hour urine collections were avoided on those days when the patients were undergoing other more essential clinical investigations, e.g. collections were avoided on those days when the patient

had to fast and therefore did not take therapy at normal times. Urine samples were collected from 7 a.m. to 7 a.m. in one container without any preservative. After twenty-four hours the volume of urine collected was measured and an aliquot was stored at -20°C until analysed. Further samples, mostly serum and occasional samples of urine (twenty-four hours collection) were collected at intervals up to one year.

As it is difficult to ensure complete urine collection, e.g. even the creatinine test does not provide full proof (Henry, 1966), the validity of the twenty-four hour urine collection was checked by questioning the patients. In any patient, if there was the possibility of an incomplete urine collection, that collection was discarded and a subsequent twenty-four hour sample was taken.

In four patients with ileostomy (ulcerative colitis) twenty-four hour ileal effluent was collected, while the patients were on SASP therapy for one month or more. They were stored at -20°C until analysed.

Acetylation phenotyping with sulphadimidine was not done in seven patients with ulcerative colitis (i.e. six patients with ileostomy, one (W.C.) who was admitted with relapse) and nine patients with Crohn's disease. Of these nine patients with C.D., one patient (M.C.) was taking SASP for six months before admission to the hospital and so it was not practicable. In one of the two new patients (R.M.) it was not done because of the

demand of the clinical state, SASP had to be started immediately following admission. Two patients with C.D. having ileostomy, one with colostomy and four patients who were included only after exhibition of toxicity due to SASP did not have sulphadimidine test for detection of acetylation phenotype.

In all these patients samples of blood were taken for SASP and its metabolites at various times of the day from the time of admission (or inclusion to this study) and at intervals until the day of discharge and also subsequently up to about one year. Twenty-four hour urine samples were also collected during their stay in hospital.

PATIENTS WITH ULCERATIVE COLITIS

The patients with U.C. were always given plain SASP tablets (SASP 3 - 6G/day initially) except on three occasions (J.H., W.C. and D.F.) when plain tablet was changed to enteric coated tablet to avoid nausea. Corticosteroids were given as retention enema (Predsol Enema), orally (Prednisolone, 40 - 60 mg/day) or as parenteral preparation (Synacthen Depot = 0.25 to 0.5 mg. I.M. at intervals of two to four days). The initial dose of Prednisolone (40 - 60 mg/day) maintained for one to two weeks. After this the dose was progressively reduced over a period of four to eight weeks. Synthetic corticotrophin (Synacthen Depot) was also reduced over the course of four to eight weeks. Of the sixteen new patients and the patients admitted with relapse,

all had Predsol Enema except three (R.B., A.P. and W.C.) who did not have corticosteroids at all. These three patients were treated only with SASP and other supportive therapy as they had mild ulcerative colitis. Of the remaining thirteen patients, three had retention enema only, four had oral Prednisolone and retention enema and the rest had parenteral steroids with (4) or without (2) enema. Patients taking steroids were also prescribed oral potassium and Nystatin. Supportive therapy including iron, water, electrolytes and blood transfusion was given when indicated. No patient had any barbiturate, but ten had Nitrazepam (5 - 10 mg. nocte) and four had Diazepam (6 - 10 mg/day).

PATIENTS WITH CROHN'S DISEASE

They were also treated with SASP (Salazopyrin tablet, plain, 3 - 4 G per day). In one patient (R.M.) the initial dose was 6G/day and in two other patients (M.M., A.B.) who had past history (one year ago) of haemolyasis were given 2G/day. All the patients with C.D., except three (M.M., A.B. and G.F.) were treated with parenteral synthetic corticosteroid (Synacthen Depot 0.25 to 0.5 mg. at two to three days intervals) and one patient (J.W.) also had Predsol Enema as his rectum was involved. Other supportive therapy was the same as mentioned for the patients with U.C. In addition they had parenteral Vitamin B_{12} periodically as needed.

CLINICAL ASSESSMENT AND PROGRESS OF INPATIENTS

Following admission, the patients were clinically

assessed as described by Jalan et al (1970) and Lennard-Jones (1971) and the clinical data noted in a proforma. The details of investigation including radiology, bacteriology, haematology, biochemistry and histology were also recorded. The progress of the patients was closely observed regularly during the initial stage and then at variable intervals for up to one year. In patients with U.C. sigmoidoscopy was carried out at intervals of four to five days during initial stage.

During the initial stage of therapy any toxic symptom such as nausea, vomiting, epigastric discomfort, rashes, fever, giddiness, headache, depression, cyanosis were noted. Serial haematological examinations were also carried out and attention was given to detect evidence of haemolyasis, leucopaenia and agranulocytosis. If there was any evidence of haemolysis daily check of haemoglobin, reticulocyte count and index and the presence of any urobilinogen in the urine were carried out. Special investigations including Heinz bodies, fragments of R.B.C. were also searched for. Coombs test, haptoglobin and the presence of any methaemalbumin were also looked at. Any evidence of leucopaenia necessitated detailed investigations such as bone marrow and a daily check up of peripheral blood counts. In the event of cyanosis the presence of sulph and methaemoglobin were investigated. The estimations of sulph and methaemoglobin were carried out in the Department of Biochemistry,

Western General Hospital. The methods followed were described by Varley, (1967).

Patients in whom toxic symptoms were observed, the dose of SASP was reduced or temporarily stopped as needed and the patients were followed up until the toxic symptoms disappeared. During this period blood samples were collected to determine the serum concentration of SASP and its metabolites.

All the inpatients were examined clinically daily during the first week and the clinical progress was evaluated on the basis of the number of stools, presence or absence of blood and/or mucous, the general feeling of the patient, the increase in body weight, E.S.R., body temperature and also by sigmoidoscopy. Repeat radiological examination was carried out where indicated.

DESCRIPTION OF OUTPATIENT STUDY

A total of ninety-nine patients with U.C. (68) or C.D. (31) were studied while attending the follow-up clinic.

I. Ulcerative Colitis

They had been taking SASP for six months to fifteen years. In addition to SASP, six patients were being treated with Predsol Enema and eleven other patients were being treated with enema and systemic corticosteroids, i.e. Prednisolone (three patients, 10 - 40 mg/day, or Synacthen Depot, eight patients, 0.25 mg - 0.5 mg/bi-weekly). Five patients out of these eight patients who were taking Synacthen were also on Azathioprine

(1 - 2 mg/kg. of body weight). Those patients who were treated with corticosteroids were also given oral K and Nystatin. Other drugs used were iron, codeine phosphate, Diazepam and Nitrazepam. Blood samples were obtained from all these patients over one year period. Twenty patients had multiple collections (2 - 4) during active and/or remission state of the disease. Simultaneously, a sample of blood was sent for haemotological examination. Liver function tests were available in thirty patients during three months period close to collection.

II. Crohn's disease

The thirty-one patients studied had been taking SASP for six months to ten years. In addition to SASP two patients were also treated with systemic corticosteroids (one with Prednisolone 10 mg/day and the other with Synacthen Depot, 0.5 mg. twice a week). Other drugs used were mostly supplemental therapy, e.g. iron, Vitamin B_{12} and oral Potassium. Eleven of these patients had multiple collections (2 - 3) at different times. Simultaneously, samples of blood were also sent for haemoglobin, peripheral blood count, electrolytes and serum proteins.

SECTION II

METHODOLOGY

The instruments used were -

1. Pye Unicam spectrophotometer SP600
2. Pye Unicam spectrophotometer SP800
3. Vitatron Automatic Digital Colorimeter (Fisons)

The chemicals used are shown in Appendix I.

Measurement of salicylazosulphapyridine in serum and urine

Salicylazosulphapyridine (SASP) concentration in serum and urine was measured by the method of Sandberg and Hansson (1972). The reagents used were -

1. Hydrochloric acid, 0.5M, for urine analysis
2. Hydrochloric acid, 1M, for serum analysis
3. Amylacetate, Analar, washed twice with 2M sodium hydroxide and then once with 2M HCl and finally washed with distilled water to neutrality.
4. Sodium hydroxide solution, 0.5M.

METHOD

(a) In serum - 1 ml. of serum was pipetted in duplicate into centrifuge tubes, acidified with 1 ml. of 1M HCl. and then thoroughly mixed with 4 ml. of amylacetate. After centrifugation at 3,000 rpm for 15 minutes, 3 ml. of the organic phase were transferred to a second centrifuge tube. 4 ml. of 0.5M sodium hydroxide were added and the contents thoroughly mixed and then centrifuged. The organic phase was discarded and the optical density

(O.D.) of the aqueous phase was measured at 455 nm, in the SP600 or colorimeter. Serum blanks and standard were run in parallel with the experiments.

(b) In urine - the procedure was exactly the same as for serum, except that 0.2 ml. of urine sample was taken and diluted up to 1 ml. with distilled water. This was subsequently acidified with 1 ml. of 0.5M hydrochloric acid instead of 1M hydrechloric acid.

The wavelength of maximum absorption for the disodium salt of SASP was confirmed to be 455 nm with the recording spectrophotometer SP800 (Figure 7).

RESULTS

Efficiency of extraction of SASP by organic solvents

Different organic solvents were tried to determine the most efficient extraction of added SASP (up to 60 ug) from urine. The following Table 2 shows the results obtained with these different solvents.

Table 2

Solvents	Percentage extracted	O.D.of sample / O.D.of blank
Amylacetate	89%	3.00
IsoButylmethyl Ketone	87%	1.50
Chloroform	57%	7.50
Benzene	65%	21.9
Ether	83%	3.85

Benzene gave low urine blank but a poor extraction yield. Amylacetate gave the best extraction yield but a high urine blank. It was decided to use amylacetate

as the extracting solvent.

Recovery experiments

Recovery experiments were carried out by adding known amounts of SASP to sera and urine samples. The results are shown in Table 3. Recovery of SASP from serum was 95 ± 1% and from urine 90 ± 5%.

Calibration

A linear relationship existed between optical density and concentration of SASP up to 60 ug/ml. The means and 1 S.D. of 12 standard graphs are shown in Figure 2. The coefficient of variations within the assay and between the assays was within 5%.

The blank values were obtained using serum and urine from five healthy volunteers, five patients (not suffering from ulcerative colitis or Crohn's disease) and from ten patients prior to starting SASP therapy. The O.D. of the serum blank varied between 0.005 and 0.015, the urine blank varied in healthy persons from 0.05 - 0.10 and in patients from 0.015 - 0.020.

MEASUREMENT OF SULPHAPYRIDINE AND ITS METABOLITES, ACETYLSULPHAPYRIDINE, SULPHAPYRIDINE GLUCURONIDE AND ACETYLSULPHAPYRIDINEGLUCURONIDE

The method used for the measurement of sulphapyridine and its metabolites is that described by Hansson and Sandberg (1972) with some modifications.

Reagents used (vide Appendix 1)

1. β-Glucuronidase
2. Acetate buffer, pH 4.7

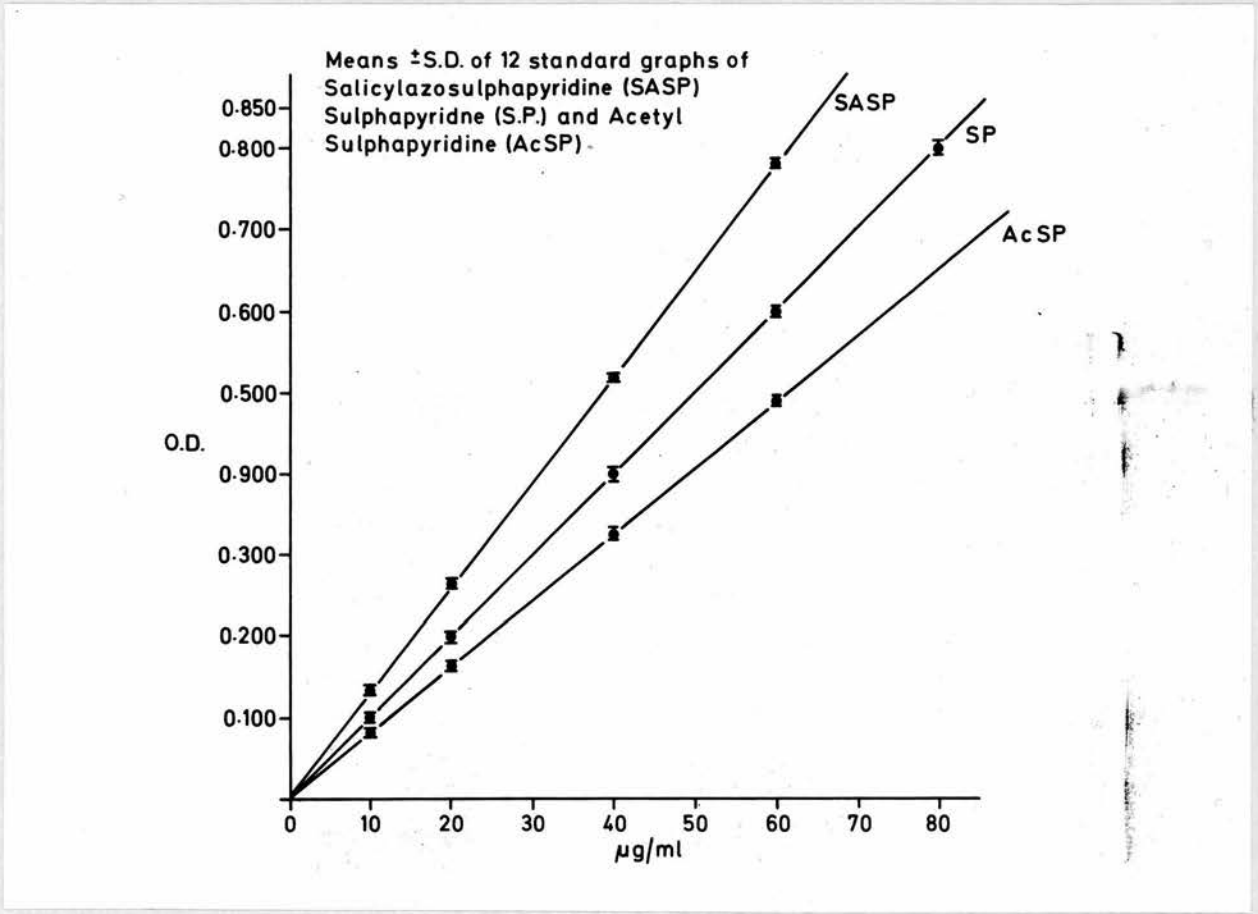
Table 3

RECOVERY EXPERIMENTS

Mean % Recovery (± SD) of Sulphasalazine, Sulphapyridine and Acetyl Sulphapyridine from serum and urine obtained from five healthy persons

Amount (ug/ml) added	SULPHASALAZINE		SULPHAPYRIDINE		ACETYLSULPHAPYRIDINE	
	Serum	Urine	Serum	Urine	Serum	Urine
10	96 ± 2.6	95 ± 3.6	95 ± 2.2	93 ± 2.7	96 ± 3.6	94 ± 4.6
20	95 ± 2.4	90 ± 3.0	95 ± 2.8	93 ± 3.1	94 ± 3.6	93 ± 4.8
40	95 ± 2.5	90 ± 3.2	94 ± 3.9	90 ± 4.0	95 ± 4. 0	90 ± 5.0
60	94 ± 3.2	89 ± 3.0	92 ± 4.0	88 ± 4.2	92 ± 3.9	90 ± 5.2

Figure 2



Standard graphs of salicylazosulphapyridine (SASP), sulphapyridine (SP) and acetylsulphspyridine (AcSP)

3. Sodium chloride
4. IsoButyl methyl ketone
5. Hydrochloric acid, 4M
6. Sodium nitrite, 0.12%, solution in distilled water
7. Ammonium sulphamate, 0.8% solution in distilled water
8. N-1-naphthylethylenediamine dihydrochloride - 0.8% solution in distilled water.

The last three solutions were freshly prepared before use as they have very limited stability.

Modifications

The original method recommended incubation at 38°C for three hours with β-glucuronidase to give maximum yield of glucuronides. In the experiments described in this thesis a longer incubation period (12 - 18 hours) was used to ensure maximum hydrolysis of glucuronides present. Table 4 shows the results of 5 serum and 2 urine samples.

It was found that 50 ul of β-glucuronidase (Limpet acetone powder suspension 1%; Sigma) was as effective as 20 ul of β-glucuronidase (Boehringer, Mannheim 15427EGAF) in hydrolysing glucuronides present in the urines from four ulcerative colitis patients treated with SASP. The results were 5.41 ± 4.0 and 5.13 ± 4.2 respectively. There was no significant difference in the release of SP from SP-glucuronides by 0.05 or 0.1 ml. of Limpet acetone powder suspension (1%). The mean values obtained from four serum samples were 0.224 ± 0.008 and 0.224 ± 0.007 respectively and from two urine

Table 4

Mean Optical Density

Time of incubation (hrs.)	Serum					Urine	
	1	2	3	4	5	1	2
1½	0.110	-	-	-	-	-	-
3	0.172	-	-	-	-	-	-
6	0.198	0.214	0.647	0.564	0.480	0.364	1.804
12	0.202	-	-	-	-	-	-
18	0.208	0.270	0.720	0.598	0.570	0.410	2.304

Serum 1 and 2 were from two normal subjects twelve and twenty-four hours after taking 4G of SASP.

Serum 3, 4 and 5 were from a patient (D.F.) with ulcerative colitis, treated with SASP.

Urine sample 1 was from a normal subject after taking 4G of SASP (0 - 24 hour collection).

Urine sample 2 was from a patient (D.F.) with ulcerative colitis, twenty-four collection, SASP 3G/day.

samples were 1.015 and 1.025 ± 0.015 respectively. Sigma β -glucuronidase (Limpet acetone powder suspension 1%) 0.05 ml. was, therefore, used.

Activity of each new batch of Limpet acetone powder purchased was confirmed by using the phenophthalein glucuronide assay method (Sigma Technical Bulletin No. 105, 1958).

METHOD

(a) For serum - duplicate aliquots of serum (1 ml.) were pipetted into two groups of centrifuge tubes A and B and 1 ml. of acetate buffer added. 50 ul. of β -glucuronidase suspension was then added to the tubes in Group B which were incubated at 38°C overnight. Sodium chloride (0.5G) was added to both sets of tubes and shaken. 5 ml. of isobutylmethyl ketone were pipetted into each tube, mixed on the Whirlimixer (Fisons) for thirty seconds and then centrifuged. After centrifugation, 3ml. of the organic phase were transferred into another two sets of Tubes A and B containing 3 ml. of 4M HCl. These tubes were kept in ice water for five minutes before the organic phase was added. The contents were then thoroughly mixed for fifteen seconds and centrifuged. Immediately after centrifugation the tubes were replaced in ice water and the organic phase was discarded. 1 ml. of the acidic aqueous phase was pipetted into two sets of tubes corresponding to each Group A and B, i.e. A1, A2 and B1, B2. The tubes, A1 and B1 were immediately placed in ice water,

whereas the tubes A2 and B2 were stoppered and placed in a boiling water bath for ten minutes. After ten minutes the tubes A2 and B2 were allowed to cool to room temperature. The next step for all the four sets of tubes was development of Bratton-Marshall reaction. This was carried out as follows:-

To all four sets of tubes (A1, A2, B1, B2) sodium nitrite solution (1 ml.) was added, the tubes shaken and allowed to stand for three minutes. This was followed by the addition of 1 ml. of ammonium sulphamate solution, the tubes shaken and allowed to stand for another three minutes. Subsequently, 1 ml. of N-1-napthylethylene-diamine dihydrochloride was added. Colour immediately developed in the presence of sulphapyridine. Ten minutes following the development of colour the O.D. of the contents of the tubes was measured in the colorimeter at 544 nm with distilled water as reference. The wavelength of maximum absorption for sulphapyridine was confirmed to be 544 nm with SP800.

The readings were completed within one hour as the colour tends to fade. If the O.D. of the sample was outwith the range of the calibration graph, the solution was diluted. A maximum dilution of four times its value was allowed with distilled water. If the absorbence exceeded 0.8, even after four times dilution, the analysis

was repeated with a diluted sample. Standards were run parallel with each experiment. A reagent blank was taken through the procedure as well as a serum or urine blank as appropriate.

(b) Urine - the same procedure was used with urine, except that the urine sample (0.2 ml.) was made up to 1 ml. with distilled water. Dilution is corrected accordingly.

A flow diagram of the extraction procedure is shown in Figure 3.

CALCULATION

The concentration of the different metabolites were calculated as follows -

O.D. of A1 = sample not treated with enzyme and not hydrolyzed.

O.D. of A2 = not treated with enzyme but hydrolyzed.

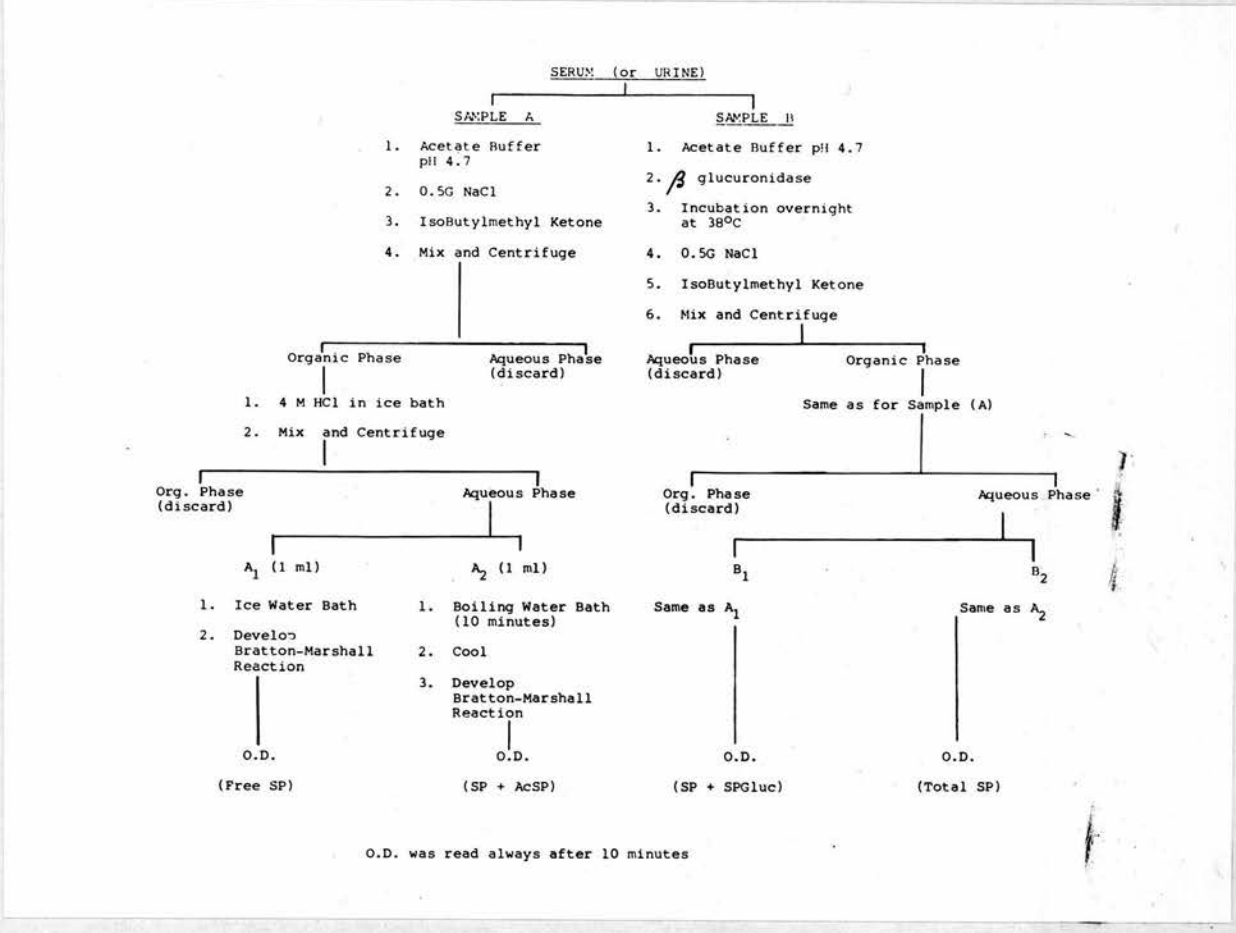
O.D. of B1 = sample treated with enzyme but not hydrolyzed.

O.D. of B2 = sample treated with the enzyme and hydrolyzed.

Therefore, the concentration of the four compounds are -

1. Amount of free sulphapyridine, ug/ml. of sample = K1 (A1 - A_{B1}) where K1 is the constant, A1 is the O.D. of the sample and A_{B1} is O.D. of the blank.
2. Concentration of acetyl sulphapyridine, ug/ml. of the sample = K2 (A2 - A1), where K_2 is the constant.
3. Concentration of sulphapyridine glucuronide, ug/ml. of sample = K1 (B1 - A1).
4. Concentration of acetyl sulphapyridine glucuronide, ug/ml. of sample = K2 (B2 - B1 - A2 + A1).

Figure 3



Flow diagram for the measurement of free sulphapyridine (SP) acetyl sulphapyridine (AcSP), sulphapyridine-glucuronide (SP-Gluc) and acetylsulphapyridine-glucuronide (AcSP-Gluc) in serum and urine.

The constants K1 and K2 were obtained from standards which were run parallel with the experiments. For serum, the O.D. of the blank values varied from 0.015 to 0.025 and for urine, the blank values were higher and varied from 0.020 to 0.030. The serum and urine blank values were obtained from patients before treatment with SASP. In those patients where no control samples of serum or urine was available, O.D. was corrected with a mean blank value of serum (0.020) or urine (0.025) of five healthy persons and five patients without any bowel disease.

1. Calibration - The Figure 2 (Page 27) shows the standard graphs of sulphapyridine and acetyl sulphapyridine obtained from the mean values (with standard deviations) of twelve graphs. A linear graph was obtained up to 60 ug/ml.

2. Recovery Experiments - Table 3 (Page 26) also shows the recovery of sulphapyridine and acetyl sulphapyridine from serum and urine.

3. Coefficient of variation within the assay and in between different assays -

The mean coefficient of variation within the assays was $4.0 \pm 1.8\%$ and in between the assays was 6.6 ± 2.8.

The percentage acetylation of sulphapyridine (SP) (i.e. $\frac{\text{AcSP + ASPG}}{\text{Total SP}} \times 100$) in serum and urine of each patient collected on different occasions was found to be fairly constant (Page 71). The mean coefficient of variations for all the patients with ulcerative colitis was $< 10\%$ (Page 74). These results also indicate a low interassay analytical error.

Total amount of sulphapyridine was measured by adding sulphapyridine and its metabolites (i.e. SP + AcSP + SP-Gluc. + AcSP-Gluc).

EFFECT OF STORAGE ON SPECIMENS

1. Serum. Five serum samples from five different patients treated with SASP were estimated for SASP and SP metabolites before storage and one year after storage. The mean O.D. ($\pm$ S.D.) of these samples for SASP, SP and AcSP were 0.275 ± 0.06; 0.231 ± 0.69, 0.104 ± 0.03 before storage and 0.269 ± 0.07, 0.234 ± 0.68 and 0.100 ± 0.04 after storage respectively.

2. Urine. The urine samples of some patients (especially patients with Crohn's disease) contained considerable amount of sediments after thawing. This was at times yellow in colour. The precipitates from four such urine samples were centrifuged and the sediments were washed with N.Saline. The sediment obtained from one of the samples, when examined under the microscope, showed some crystals (). This sediment when estimated for SASP, SP and AcSP was found to contain moderate amounts of SP and AcSP. Some other sediments also contained SASP in addition to SP and AcSP. To evaluate this situation four fresh samples of urine of four different patients (two ulcerative colitis and two Crohn's disease) were estimated for SASP, SP and its metabolites. Subsequently six months after storage they were thawed when sediments were noted. The samples were vigorously shaken and an aliquot of 1 ml. and also 0.2 ml. (as described

in the method) were taken immediately after shaking. Extraction procedure was followed as usual except that excess (20 ml.) of organic solvents (either Amylacetate for SASP or Isobutylmethyl ketone for SP) were used for 1 ml. sample of urine. For the yield of SP glucuronides 0.2 ml. of β-glucuronidase was used instead of 0.05 ml in cases where 1 ml. sample of urine was taken. The O.D.(mean $\pm$ S.D.) for SASP, SP and its metabolites in the equivalent amount of urine (0.2 ml.) are shown in Table 5.

The results were found to be quite consistent when properly thawed and vigorously shaken.

EFFECT OF OTHER DRUGS COMMONLY ADMINISTERED WITH SASP

As various substances are commonly administered in the disease state with SASP, their influence on the measurement of SASP and its metabolites were investigated.. Substances considered were -

(a) Prednisolone
(b) Nitrazepam.
(c) Diazepam
(d) Iron
(e) Calcium

(a) Effect of Prednisolone, Nitrazepam and Diazepam

Two serum samples were collected from different patients taking Prednisolone 30 mg., Nitrazepam 10 mg., and Diazepam 10 mg. in twenty-four hours, but not SASP. The samples were estimated for SASP and SP and AcSP along with a blank serum from a healthy person. O.D's. of these samples are shown in Table 6 .

Table 5

The effect of storage and thawing (B) and volume of aliquot (C) for analysis on the O.D. of SASP, SP and its metabolites in four urine samples from different patients.

	SASP and SP metabolites	Before Storage (A) Fresh Specimen	After Storage (B) Thawed, vigorously shaken	After Storage (C) Thawed vigorously shaken
O.D. (M ± S.D.) of four urine samples from different patients	SASP	0.283 ± 0.4	0.279 ± 0.5	0.276 ± 0.5
	A1	1.20 ± 0.8	1.18 ± 0.7	1.20 ± 0.8
	A2	2.06 ± 1.2	2.05 ± 1.1	2.06 ± 1.1
	B1	2.13 ± 1.2	2.11 ± 1.2	2.12 ± 1.1
	B2	3.29 ± 2.1	3.40 ± 1.8	3.34 ± 1.8

The extraction procedures for SASP and the SP metabolites are described in Section II, Pages 23 & 25. Volume of urine sample was 0.2 ul (A and B) and 1 ml. (C). Volume of β-glucuronidase added was 0.05 ul. (A and B) and 0.2 ul. (C). The volume of amylacetate and isoButylmethyl ketone was 20 ul. (C) compared with 4& 5 ml. respectively. (A & B) The O.D. values are corrected to 0.2 ul. urine.

Table 6

Effect of Prednisolone, Nitrazepam and Diazepam on the methods for estimation of SASP, SP and ASP

	Prednisolone			Nitrazepam			Diazepam		
	SASP	SP	ACSP	SASP	SP	ACSP	SASP	SP	ACSP
Sample 1	0.012	0.022	0.023	0.010	0.015	0.020	0.011	0.011	0.022
Sample 2	0.010	0.021	0.023	0.009	0.016	0.019	0.010	0.020	0.023
Blank Serum	0.010	0.018	0.020	0.010	0.018	0.020	0.010	0.018	0.020

The contents after the extraction procedures were run in SP800 to confirm further whether there is any deflection at 455 (SASP) or 544 (SP and AcSP). No deflection was found.

Interaction of Iron and Calcium were studied in some detail in five volunteers. These are discussed at length in the next Chapter (III).

ESTIMATION OF SULPHASALAZINE IN FAECES

This method has been described by Hansson and Sandberg (1972).

The reagents used (vide Appendix I)

1. Titanium trichloride, $TiCl_3$, 15% solution in distilled water.
2. Sodium hydroxide, 6M
3. Acetate buffer solution, 1M, pH 4.6
4. IscButylmethyl ketone
5. Hydrochloric acid, 1M
6. Sodium nitrite 0.12% solution in distilled water
7. Ammonium sulphamate, 0.8% solution in distilled water
8. N-1-naphthylethylenediamine dihydrochloride, 0.8% solution in distilled water.

The last three solutions were always freshly prepared.

PROCEDURE

Duplicate aliquots (approximately 0.2 - 0.4G) of well homogenised faeces were transferred into two separate stoppered centrifuge tubes A and B. In tube A titanium trichloride solution (50 ul) was added and the contents

well mixed for approximately fifteen seconds. Sodium hydroxide (50 ul) was added to neutralise the excess of $TiCl_3$. Acetate buffer (1 ml.) was added in both the tubes A and B. The extraction procedure from this stage onward is the same for both A and B. IsoButylmethyl ketone (5 ml.) was added, the tubes were shaken vigorously for fifteen seconds and then centrifuged and an aliquot of 3 ml. of the organic phase was re-extracted with 3 ml. of 1M Hcl and the mixture centrifuged. The organic phase following centrifugation was discarded and 1 ml. of the aqueous phase was pipetted off, and well mixed with 1 ml. of sodium nitrite. The mixture was allowed to stand for three minutes. Ammonium sulphamate solution (1 ml.) was then added and the mixture allowed to stand for another three minutes. Finally, N-1-naphthylethylenediamine dihydrochloride solution (1 ml.) was added and the colour was allowed to develop completely for ten minutes before the O.D. of the contents of the tubes were read in the colorimeter at 544 nm. The colorimeter readings were then corrected by the reagent blank which had been taken through the experiment.

If the O.D. exceeded 0.8 the solution was diluted up to four times its volume with distilled water. Should the O.D. still exceed 0.8 after this dilution, the sample was re-estimated after further dilution before analysis.

As sulphapyridine is a metabolite of SASP correction has to be made for SP which may be present in the faeces. For this reason a sample blank, i.e. tube B, was taken

through the procedure and the free SP present already in the faeces was measured. This value was then subtracted from the amount present after the action of $TiCl_3$. The difference represents the amount of unchanged SASP present in the stool.

The amount of sulphapyridine was measured from the calibration graph (Figure 2) which was found to be the same.

RESULTS

SASP is rapidly reduced by $TiCl_3$. The SP thus released is estimated.

The recovery of SP was 80 ± 5% when extracted from the faeces. The blank value for the faeces was found to be low and it ranged from 0.010 to 0.015.

ESTIMATION OF FREE AND ACETYLATED 5-AMINOSALICYLIC ACID IN SERUM AND URINE AFTER ADMINISTRATION OF SALICYLAZOSULPHAPYRIDINE

This method was described in detail by Hannson (1972).

Reagents used (vide Appendix I)

1. Hydrochloric acid, 1M
2. Acetic anhydride
3. Isobutylmethyl ketone
4. Phosphate buffer, 0.5 M, pH 6.0.

PROCEDURE

(a) Estimation in serum - Duplicate aliquot of serum (1 ml.) were transferred to two centrifuge tubes. To one of these tubes acetic anhydride (20 ul) was added. Hydrochloric

acid (1 ml.) was then added to each of the two tubes and the mixture was then extracted with 5 ml. of isoButyl methyl ketone. The contents were mixed and centrifuged. An aliquot (4 ml.) of the organic phase was then removed and re-extracted with 3 ml.of phosphate buffer. The fluorescence of buffer solution was then measured in a spectrofluorometer. The excitation wave length was 310 nm and the fluorescence wave length was 430 nm.

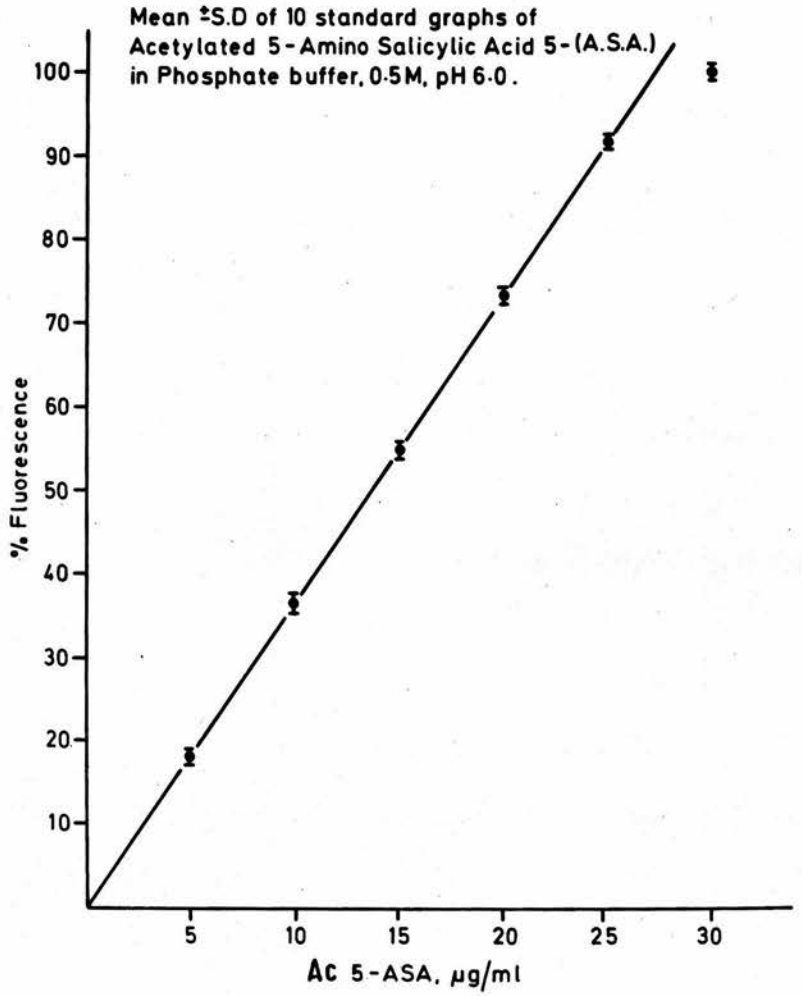
(b) Estimation of urine - A sample (0.1 - 0.2 ml.) of urine was taken and diluted to 1 ml. with distilled water. The extraction procedure was followed as described for serum.

If the concentration of the sample exceeded 25 ug/ml. the original sample was diluted further before analysis. Standards and appropriate serum or urine blanks were always run in parallel with each experiment.

RESULTS

The sample in which acetic anhydride was not added represents the amount of acetylated 5-ASA and the difference between the two samples represents the amount of free 5-ASA which was acetylated by the addition of acetic anhydride. The mean and standard deviation of ten calibration graphs of acetylated 5-ASA for different estimations in phosphate buffer are shown in Figure 4. This was linear up to the concentration of 25 ug/ml. Approximately 90% of acetyl 5-aminosalicylic acid was recovered by this extraction procedure from the serum and urine (Table 7). The blank values of serum and urine obtained from five healthy

Figure 4



Standard graph of acetylated 5-aminosalicylic acid (Ac5-ASA

Table 7

Recovery of added acetylated 5-aminosalicylic acid (Ac5-ASA) to serum and urine samples

Amount of Ac5-ASA added - ug/ml.	Mean % Recovery from serum (n = 2)	Mean % Recovery from urine (n - 2)
5	90	95
10	90	95
15	88	92
20	87	90
25	86	90

volunteers were found to be quite low. For serum approximately 0.1 to 0.2 ug/ml. and for urine it varied from 0.5 to 1 ug/ml.

DISCUSSION

The methods used for the estimation of SASP and its

different metabolites are those described by Hansson and Sandberg (1972). These methods were made available to me by Pharmacia of Great Britain Ltd., who provided the grant for this research.

In the original method amylacetate was used for the extraction of SASP. I investigated the possibility of using another organic solvent but found that when efficiency of recovery and intensity of the blank were taken into account, amylacetate was the most suitable solvent (Table 2).

Recovery experiments of SASP, SP and AcSP from serum and urine were also carried out and were found to be satisfactory (Table 3). According to Hansson and Sandberg, the optimum incubation time for the hydrolysis of sulphapyridine glucuronides was three hours but it was found that longer incubation (twelve to eighteen hours) gave better results and hence the incubation was carried out overnight (Table 4). This was also more convenient.

In the studies to be carried out it was envisaged that storage of serum and urine samples would occur. A sediment had, in fact, been observed when some of the stored urine samples were thawed. This did not occur in serum. It was, therefore, necessary to determine whether storage at -20°C followed by thawing had any effect on the concentration of SASP and its metabolites in serum and urine. The sediments from urine samples were found to contain variable amounts of SASP, SP and AcSP. The concentration of these compounds in the

sediment was higher in urine samples containing high concentrations of SASP and SP metabolites. The practice was therefore to mix the thawed samples thoroughly before aliquots were taken for analysis. From Table 5 it can be seen that after thawing and thorough mixing, the optical densities did not vary significantly compared with the fresh specimens..

Many of the patients treated with SASP were also coincidentally treated with other drugs, e.g. Prednisolone, Nitrazepam, Diazepam and iron. It was therefore important to determine whether the administration of these drugs interfered with the methods of analysis for SASP and its metabolites. It was found that Prednisolone, Nitrazepam and Diazepam did not interfere with the analytical methods (Table 6). The effects of coincidental administration of iron and also calcium were investigated in detail. (Chapter III).

As SASP is split into sulphapyridine and 5-aminosalicylic acid the estimation of 5-aminosalicylic acid in the serum and urine was carried out. The method used was described by Hansson (1972). Recovery experiments for 5-ASA showed that about 86 - 90% of the added amount (up to 25 ug/ml.) of acetyl 5-ASA in the serum and 90 - 95% in the urine could be recovered.

From these results it appears that the methods described by Hansson and Sandberg (1972) are quite satisfactory. Schröder and Campbell (1972) also used the same methods in their experiments with healthy volunteers and they also claimed satisfactory results with these methods.

CHAPTER III.

ACUTE EXPERIMENTS IN HEALTHY VOLUNTEERS AND ROLE OF IRON AND CALCIUM ON SULPHASALAZINE METABOLISM

ACUTE EXPERIMENTS IN HEALTHY VOLUNTEERS

Acute experiments after administering a single dose of SASP (50 mg/kg. of body weight) were performed in five healthy volunteers. Their age ranged from 30 - 40 years, body weight ranged from 60 - 83 kg. The drug was given one hour after breakfast and subsequently samples of blood were collected at intervals of 1½ hours, 3 hours, 5 - 6 hours, 12 hours, 24 hours and 48 hours, and in one person up to 120 hours. Twenty-four hour urine samples were collected for two to seven days. These procedures were repeated after an interval of three to four weeks when the same dose of SASP was given with calcium (2 Sandocal tablets, i.e. 40 m.Eq. Ca^{++}) and then with iron (Ferrous sulphate, 400 mg. equivalent to 80 mg.Fe). In two volunteers, the first experiments were with iron and SASP followed by SASP alone, and in one volunteer calcium and SASP were given before SASP only.

These experiments were done in order to -

(a) Determine the serum concentrations of SASP and SP metabolites in the serum, the amount and type of metabolites excreted in the urine over the first and subsequent days after a single dose of SASP.

(b) Determine the effect of iron on the absorption of SASP and its metabolites as it is commonly administered

in patients with ulcerative colitis or Crohn's disease. The effect of simultaneous administration of calcium on the metabolism of SASP was also studied.

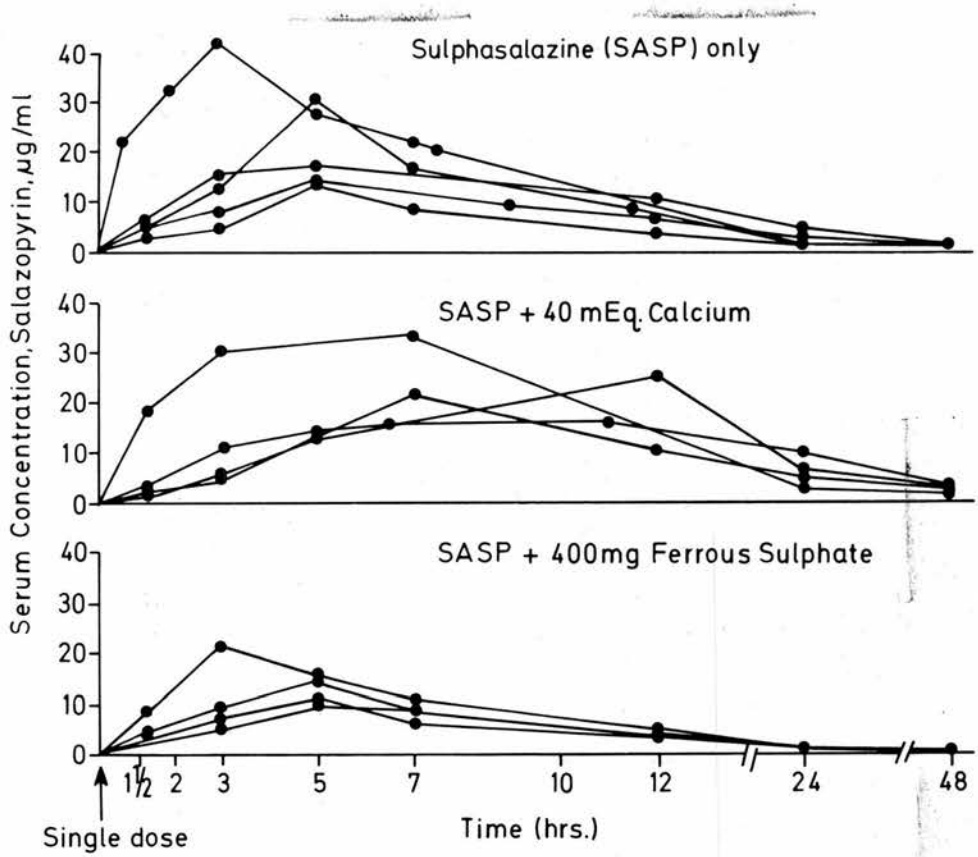
In addition to the acute experiment acetylation phenotype was detected in four of the five volunteers giving a single dose of sulphadimidine (Evans, 1969). The same four volunteers were also phenotyped on the basis of the amount of free and acetylated SP detected after administering the same dose of SP and the procedure was adopted in the same way as for sulphadimidine.

RESULTS

Figures 5 and 6 and Table 8 show the results of these acute experiments with SASP alone and SASP with calcium and SASP with iron. The peak serum concentration of SASP occurred at three to five hours and became negligible by twenty-four hours. Approximately 1 - 3% of the administered dose was recovered in the urine as SASP during the first twenty-four hours. Sulphapyridine appeared in the serum at five to six hours, reaching a maximum concentration at about twenty-four hours, after which it gradually declined. The maximum excretion of total sulphapyridine in the urine occurred during the twenty-four to forty-eight hours in contrast to SASP (Table 9). In one volunteer sulphapyridine was found in the urine six days after the administration of the single dose of SASP (Table 10).

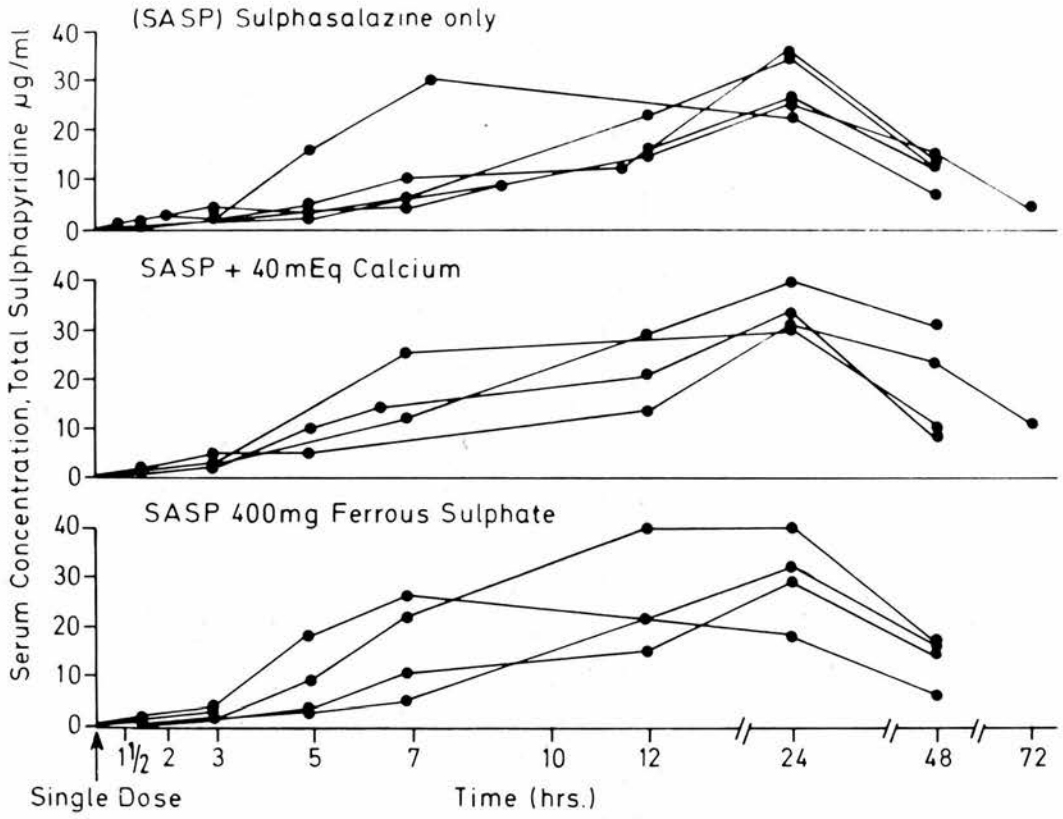
The different metabolites of sulphapyridine measured

Figure 5



Concentrations of salicylazosulphapyridine (SASP) at time intervals after an oral dose of SASP alone and with calcium and ferrous sulphate.

Figure 6



Concentrations of total sulphapyridine at time intervals after an oral dose of salicylazosulphapyridine (SASP) alone and with calcium and ferrous sulphate.

Table 8

Serum Concentration of Salicylazosulphapyridine (SASP) and total sulphapyridine (SP) in the three groups.

	Drug Taken	1½ hrs.	3 hrs.	5 hrs.	7 hrs.	12 hrs.	24 hrs.	48 hrs.
SASP ug/ml Mean ± S.D.	SASP only	8.9 ± 10	16.3 ± 15	20.3 ± 7.8[1]	14.1 ± 5.2[2]	7.6 ± 2.8[3]	2.8 ± 1.8	0.5 ± 0.4
	SASP + Fe	4.9 ± 1.8	10.3 ± 7.3	12.2 ± 2.4[1]	8.7 ± 2[2]	3.6 ± 0.5[3]	1.4 ± 0.3	0.5 ± 0.1
	SASP + Ca	12.7 ± 12.2	19.8 ± 11.4	19.5 ± 3.5	16.9 ± 7.9	5.5 ± 4.2	1 ± 0.1	
Total SP ug/ml Mean ± S.D.	SASP only	2.1 ± 0.8	1.9 ± 0.7	6.1 ± 5.9	13.8 ±10.9	15.8 ± 4.6	28.9 ± 6.8	12.7 ± 3.6
	SASP + Fe	0.8 ± 1	2 ± 1.1	7.9 ± 7	15.9 ± 9.8	25.5 ± 12.9	29.3 ± 9.4	17.5 ± 12
	SASP + Ca	0.9 ± 0.9	3.5 ± 0.9	11.8 ± 3.9	16.9 ± 7.1	24.7 ± 7.1	32.9 ± 3.7	20.9 ± 11.1

1, 2 and 3 indicate a significant difference ($p < 0.025$) between results with the same superscript

Table 9

Mean Urinary Excretion of SASP and Total Sulphapyridine in twenty-four hours.

Day	SASP (% of dose)			Total Sulphapyridine (% of dose)		
	SASP only	SASP & Iron	SASP & Calcium	SASP only	SASP & Iron	SASP & Calcium
1	2.0	1.1	2.2	13.5	13.2	14.3
2	0.3	0.1	0.3	29	26.5	28.7

Table 10

Serum Concentration and Urinary Excretion of SASP and its SP metabolites after a single dose (3G) in one volunteer

Serum, ug/ml						
	SASP	SP	AcSP	SP-Gluc	AcSP Gluc	Total SP
1½ hrs.	4.8	0.8	0	0	0	0.8
3 hrs.	6.2	1.0	0	0	0	1.0
5 hrs.	12.5	2.0	0.2	0	0	2.2
12 hrs.	9.3	12.1	1.2	0	1.1	14.4
24 hrs.	3.5	19.2	4.1	1.7	0.3	25.3
48 hrs.	1.4	12.2	3.0	0	0.4	15.6
72 hrs.	0	4.8	1.3	0	0	6.1
120 hrs.	0	0.9	0	0	0	0.9

Urine, mg/24 hours						
Time (Hours)	SASP	SP	AcSP	SPGl	AcSP Gluc.	Total SP (% of dose)
0 - 24	80.9 (2.7%)	103.9	51.7	47.7	12.2	11.6%
up to 48	12.3 (0.4%)	153	82.3	66.1	38.1	18.3%
up to 72	5.3 (0.1%)	100.3	91.0	57.3	48	16%
up to 96	-	30	31.5	0.8	31.6	5%
up to 120	-	13	11.4	0	25.4	2.7%
up to 144	-	7.2	2.6	0	7.9	0.9%

Bracketed figures indicate the % of the administered dose excreted

in the serum and urine were free sulphapyridine, acetyl sulphapyridine, sulphapyridine glucuronide and acetyl sulphapyridine glucuronide. Table 10 shows the concentration of the different metabolites at different times in the serum and urine in one of the healthy volunteers.

When iron was given with SASP the time required to reach a maximum concentration of SASP and total sulphapyridine did not differ from that obtained with SASP given alone. However, the serum concentration of SASP was significantly low (Figure 5 and Table 8) with $p < 0.025$. However, there was no significant difference in the concentration of total sulphapyridine. When the experiments were repeated with calcium there was a significant delay in the absorption of SASP, the peak concentration of SASP in the serum extended over five to twelve hours in contrast to three to five hours found when SASP was given alone (Figure 5 and Table 8). However, there was no significant difference in the time required to reach the maximum serum total sulphapyridine concentration. After forty-eight hours the total sulphapyridine concentration was still considerably higher compared with the concentration obtained after administration of SASP only (Table 8).

Table 9 shows the urinary excretion of SASP and total sulphapyridine (Mean $\pm$ S.D.) during the first forty-eight hours. SASP excretion was considerably lower when it was given with iron. This was not seen with calcium. The excretion of total sulphapyridine was, however,

more or less the same during these two days.

One of the volunteers had a peak serum SASP concentration at three hours and also sulphapyridine appeared in the serum quicker, within 3 hours, with the peak concentration at twelve hours. This person was subsequently found to have a jejunal diverticulum which was removed and following operation eighteen months later, a similar experiment was carried out with the same dose of SASP. The concentration of SASP and the serum total sulphapyridine before and after operation, are shown in Table 11. The results after operation are more parallel with the other volunteers.

Experiments for acetylation phenotyping in four out of the five volunteers with sulphadimidine and sulphapyridine showed parallel results (Table 12). All of them were slow acetylators.

IN VITRO EXPERIMENTS

SASP (20 ug) was dissolved in 3 ml. of 0.5 M sodium hydroxide and the optical density measured in colorimeter at 455 nm. Increasing amounts of ferrous sulphate (1 - 3 mg., 1 mg. ferrous sulphate is equivalent to 0.2 mg of iron) were added. A precipitate developed in each tube and after centrifugation the optical density of the supernatant was measured. Similar amounts of calcium (1 - 3 mg) as calcium gluconate were added to separate aliquots of SASP solution and in this case no precipitation was formed. The optical density of the solution was measured. The results are shown in Table 13.

Table 11

Concentration of SASP and sulphapyridine before and after removal of jejunal diverticulum.

Time of collection of blood after taking this drug	Serum SASP, ug/ml		Serum Total Sulphapyridine, ug/ml	
	Before Operation	After Operation	Before Operation	After Operation
1½ hrs.	27.5	15.0	1.5	1.5
3 hrs.	42.5	17.6	1.5	1.7
5 hrs.	27.5	25.8	16.5	13.7
7 hrs.	20.0	25.0	30.0	21.0
12 hrs.	4.0	10.1	35.4	28.7
24 hrs.	1.5	2.6	22.5	32.0

Table 12

Comparison of results for Acetylator Phenotyping

Dose Used	Sulphadimidine (750 mg)		Sulphapyridine (750 mg)	
	% Acetylation		% Acetylation	
	Serum	Urine	Serum	Urine
Volunteer 1	25	63	16	29
Volunteer 2	43	59	9	42
Volunteer 3	19	65	13	35
Volunteer 4	33	59	9	26

Table 13

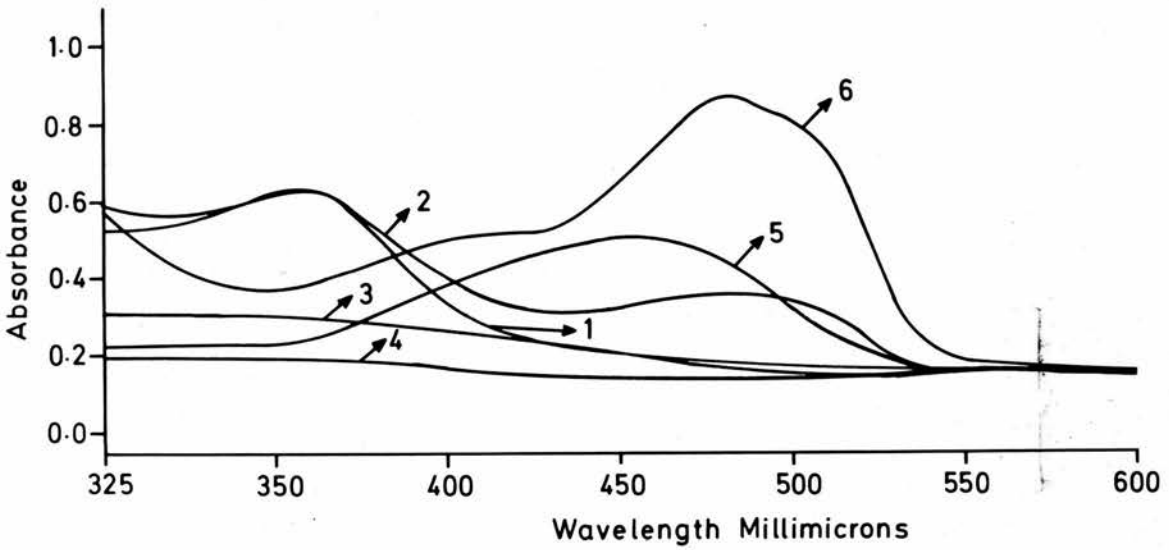
The Effect of Iron and Calcium on SASP solution measured by optical density.

Amount of $FeSO_4$ added	20 ug of SASP solution in 3 ml. of 0.5 NNaOH			
	0 mg	1 mg (= 0.2 mg Fe)	2 mg	3 mg
O.D.	0.468	0.273	0.021	0
Amount of Ca added	0 mg	1 mg	2 mg	3 mg
O.D.	0.466	0.460	0.459	0.458

In a separate experiment 1 mg of standard SASP solution was dissolved in 50 ml. of redistilled water (i.e. 20 ug/ml). The pH of the solution was 4.8 which was then made up to pH 6.7 by adding a few drops of dilute sodium hydroxide solution (0.1M) with constant stirring and recording with a pH meter.

1 ml. of ferrous sulphate solution (1%) was added to one of the above solutions and the pH changed to 5.8. The pH was again gradually raised by adding a few drops of dilute sodium hydroxide and at pH 6.2 turbidity developed. The solution was then filtered off and the supernatant retained. 5 ml. of the same ferrous sulphate solution was added to another SASP solution (pH 6.7). The pH after the addition of ferrous sulphate came down to 5.6. After a short time turbidity was noted and this was filtered and the supernatant was retained. Similarly 1 ml. of calcium gluconate (containing 0.8 m.eq.Ca^{++}) was added and the pH was kept at 6.5. There was no precipitate. Figure 7 shows the tracings of all the solutions in spectrophotometer - SP 800 (Pye Unicam). Figure 7 shows two spectra for SASP in alkaline (extracted with 0.1 M NaOH solution, i.e. sodium salt of SASP with maximum absorbence at 455 nm (No. 5) and also SASP in water (pH 6.7) with maximum absorbence at 360 nm (No. 1). The deflection at 506 nm with calcium and SASP (No. 2) was confirmed to be due to calcium gluconate solution No. 6). Tracings (3) and (4) show the effect of addition of iron.

Figure 7



The absorbence spectra (run in SP-800) of different solutions.

(1) Salicylazosulphapyridine (SASP) solution in 500 ml. of water, 20 ug/ml., pH - 6.7.

(2) Same as (1) + calcium gluconate (0.8 m.equiv. Ca^{++}), pH - 6.9

(3) Same as (1) + ferrous sulphate (5 mg Fe^{++}), pH - f.6, filtered.

(4) Same as (1) + ferrous sulphate (1 mg Fe^{++}), pH - 5.8; pH was brought to 6.2, precipitate, filtered.

(5) Same as (1), extracted with 0.1 M NaOH

(6) Calcium gluconate solution in water

SUMMARY

SASP alone (in a single dose of 50 mg/kg) and with 400 mg ferrous sulphate or 40 m.eq. calcium was given to five volunteers, and serum and urine concentrations of SASP and its SP metabolites measured.

The peak serum concentration of SASP occurred within three to five hours whereas that of total SP was found at twelve to twenty-four hours. Iron caused a significant decrease in serum SASP but no change in serum total SP whereas calcium delayed SASP absorption without reducing its eventual amount or the total serum SP.

The presence of a jejunal diverticulum in one subject accelerated the appearance of SP in the serum.

CHAPTER IV

ACETYLATION POLYMORPHISM OF SULPHAPYRIDINE IN PATIENTS WITH ULCERATIVE COLITIS AND CROHN'S DISEASE

ACETYLATION POLYMORPHISM OF SULPHAPYRIDINE

A total of one hundred and twenty-two patients out of one hundred and thirty-three (Table 1) with ulcerative colitis or Crohn's disease were included for the study of acetylation polymorphism. Four outpatients with ulcerative colitis and two outpatients with Crohn's disease did not take the drug at all during collection of samples. Therefore they were excluded from this study. Four other patients (G.Mc, G.K., C.Mc, M.Mc) who were studied only after ileostomy and J.Cr. with ileotransverse anastomosis and transverse colostomy were also not included in the acetylation polymorphism study.

The one hundred and twenty-two patients are divided into four groups (Table 14).

Group (A) Inpatients - acetylation phenotyping test with sulphadimidine (Evans, 1969) was available.

Group (B) Inpatients - multiple collections of serum and urine samples were available while they were treated with SASP but they did not have the acetylation test with sulphadimidine.

Group (C) Outpatients - multiple collections of serum on different occasions whilst they were being treated with SASP.

Group (D) Outpatients - one collection of serum only while they were taking SASP.

Table 14.

Groups of patients studied for acetylation polymorphism.

		Group A	Group B	Group C	Group D	Total
No. of Patients		21	11	31	59	122
Disease	U.C.	16	5	20	41	82
	C.D.	5	6	11	18	40
Age (yrs.) range	U.C.	20 - 80	17 - 50	15 - 80	17 - 74	
	C.D.	17 - 41	23 - 46	17 - 72	21 - 62	
U.C.	Male	6	3	8	22	39
	Female	10	2	12	19	43
C.D.	Male	2	2	6	8	18
	Female	3	4	5	10	22

Group A. Thirteen new untreated patients with ulcerative colitis, one patient with ulcerative colitis and transverse colostomy and two other patients with ulcerative colitis who were admitted to the Unit with relapse, are included in this group. The last two patients (M.D. and M.B.) were phenotyped subsequently with sulphadimidine after stopping SASP for five to six days at a stage when they were in remission. Of the five patients with Crohn's disease who had sulphadimidine test, one was a new patient (J.W.) and the other four (A.B., M.M., G.F. and M.Y.) belonged to Group A (b) and (c) Table 1, Page 10). The extent and severity of the disease state of all these patients on admission are indicated in Table 15.

During the first twenty-four hours of admission new patients and the other two patients subsequently were identified as slow or fast acetylators using the simplified method with sulphadimidine. After this the patients were treated with SASP steroids and other standard therapy as discussed in Chapter II. Blood samples were collected two to three times each day during the acute phase (days 1, 3, 5, 7 and 10) and at irregular intervals for a period of up to one year (Page 16). Parallel to these, twenty-four hour urine collections were obtained during the acute phase.

Group B. The severity and extent of the disease in these patients (five ulcerative colitis; six Crohn's disease) are shown in Table 15. These patients had been admitted to the ward with a relapse, or who were already established on SASP for some time. Therefore, preliminary sulphadimi-

Table 15

Extent of Disease of Patients in Group A and B

Extent of Disease		Group A	Group B
Ulcerative colitis	:- entire colon	3	2
	L.colon	13	3
Crohn's disease	: small intestine	2	2
	S.I. with colonic involvement	2	4
	colonic only	1	-
Assessment of severity			
	mild	6	-
Ulcerative colitis	moderate	7	2
	severe	3	3

S.I. = Small intestine

dine phenotyping was not possible. Samples of blood and urine were collected after admission at variable intervals until the day of discharge and followed-up to eight months.

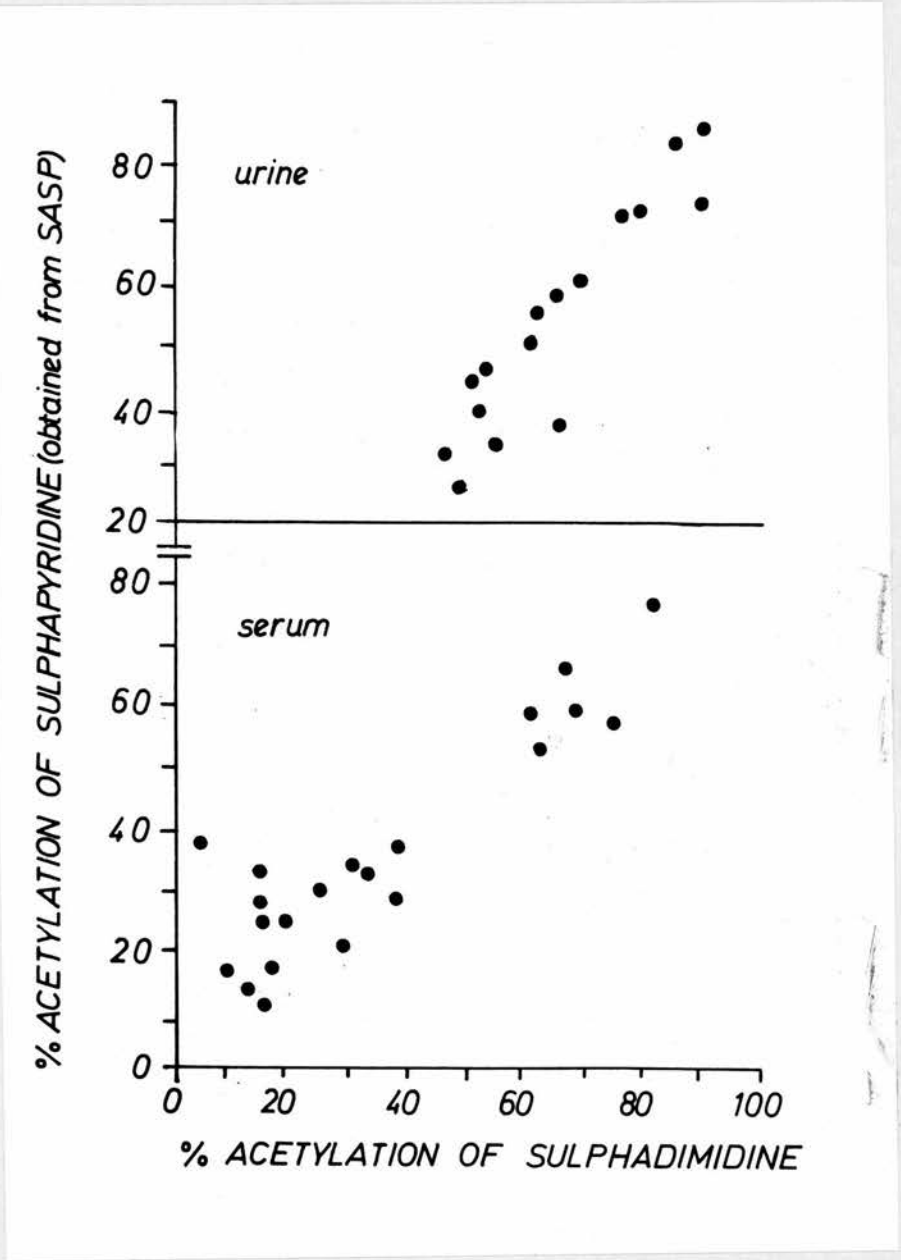
Group C. These patients were attending the follow-up clinic and were either in remission or with mild colitis only. There were twenty patients with ulcerative colitis and eleven patients with Crohn's disease. SASP therapy had been used for between six months to fifteen years. Several blood samples were obtained at irregular intervals during one year in this group.

Group D. This group consists of fifty-nine patients (41 ulcerative colitis; 18 Crohn's disease) who had been taking SASP for more than six months . Only one sample of blood was available from these patients to estimate the acetylator phenotype from the serum concentrations of SP and its metabolites. This group (with ulcerative colitis or Crohn's disease) enabled me to screen this population for acetylation polymorphism on the basis of SP and its metabolites present in the serum.

RESULTS

Figure 8 shows that sulphapyridine shares the same acetylation polymorphism as sulphadimidine. It is shown by the results in both serum and urine. All patients (Group A) who had been phenotyped as slow or fast acetylators by sulphadimidine method belonged to the same acetylation groups with sulphapyridine.

Figure 8



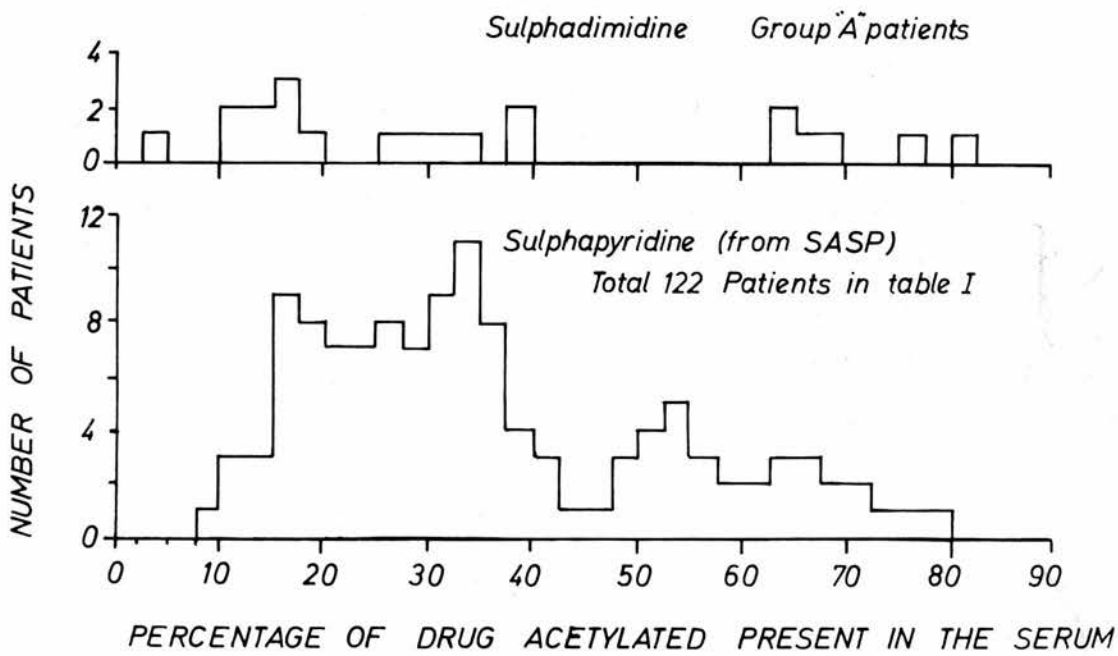
The relationship between %acetylation of sulphadimidine and %acetylation of sulphapyridine in serum and urine.

Figure 9 shows the frequency distribution histogram of the percentage acetylation of sulphadimidine (twenty-one patients) and sulphapyridine (one hundred and twenty-two patients) in the serum. This shows bimodal distribution of acetylation polymorphism.

Table 16 shows the mean ± S.D. of the percentage acetylation of sulphapyridine (obtained from SASP) in serum and urine for both slow and fast acetylators amongst patients with ulcerative colitis or Crohn's disease. Where there were more than two values, the mean value was taken. This further confirms the two distinct population groups in terms of acetylator phenotype and this can be done quite efficiently from both serum and urine results.

Table 17 shows the mean coefficient of variations (± S.D.) of the acetylation percentage in serum and urine collected at variable intervals during the period of one year from all the patients in Groups A, B and C. Variations of the acetylation capability of each individual was found to be quite small. This is more significant when more than three or four different samples are tested though they were collected at variable intervals. In patients with collection of two samples though the difference of acetylation percentage was within the range of 10 - 15%, compared with the previous estimate, the coefficient of variation obviously will vary, depending on the mean value which has a wide difference in slow and fast acetylators (Table 16).

Figure 9



The frequency distribution histogram of the percentage acetylation of sulphadimidine (Group A) and sulphapyridine (122 patients) in serum.

Table 16

Comparison of mean values for slow and fast acetylators

	Slow Acetylators		Fast Acetylators	
	% Acetylation (Serum)	% Acetylation (Urine)	% Acetylation (Serum)	% Acetylation (Urine)
Mean of all means with S.D.'s	26.0±8.5	40.6±9.7	63.3±9.2	75.7±8.2
U.C. (means with S.D.'s)	26.3±9.2	41.2±9.2	62.4±7.6	75.7±8.2
C.D. (means with S.D.'s)	25.9±7.3	40.3±13.2	56.3±9.5	76% 1 patient only.

Table 17

Variation of multiple observations of sulphapyridine acetylation in the same individual.

No. of Estimations, on different occasions.	No. of Patients U.C./C.D.		Mean of coefficient of variation ± SD.			
			U.C.		C.D.	
			Serum	Urine	Serum	Urine
2	18	13	9.5 ± 7.9	7.9 ± 3.1	24.2 ± 13.4	10.5 ± 8.9
3 - 8	15	5	7.7 ± 3.2	7.9 ± 4.4	23.2 ± 11.5	8.4 ± 6.6
>8	10	1	8.6 ± 5.5	-	17.7	-

U.C. - Ulcerative Colitis;

C.D. - Crohn's Disease.

Throughout the period of study of one year, this acetylation capacity remained fairly constant. The range was relatively wider in patients with Crohn's disease (Table 18). However, at every stage the percentage acetylation was well within the range of respective slow or fast acetylator groups.

The results of three patients in whom the dose of SASP was reduced and two patients in whom the dose was increased are shown in Table 18. Constancy of acetylation can be seen irrespective of dosage at the steady state at the total SP concentration range of >10 - 80 ug/ml.

Table 19 shows the age and sex distribution of all the one hundred and twenty-two patients studied, among slow and fast acetylators. There is a significant increase of fast acetylators in females (or decrease in males). Number of fast acetylators after the age of 60 years was also comparatively low, though the sex distribution at this age group was more or less the same.

Liver function tests including bilirubin, transaminases, B.S.P. were all within normal limits in all the patients with ulcerative colitis or Crohn's disease. However, serum total protein and albumin were low in three patients with ulcerative colitis during the initial stage and in seven patients with Crohn's disease.

Table 18

Constancy of acetylation in five subjects on different dose of SASP.

Patient	Dose of SASP G/day	Day of therapy	% Acetylation in serum	Serum total SP ug/ml	% Acetylation in urine
1. W.R.	4	(3	20.0	32	25
		(5	18.0	38.3	35
		-(20	18.0	50.5	32.5
	from day 21,2	(27	18.5	22.6	-
		(36	21.0	30.2	-
		(89	20.0	27.9	-
2. J.C.	2	(3	64.0	13.3	82.6
		(10	66.0	10.7	81.4
	from day 11,3	15	68.0	12.2	-
	from day 16,4	18	70.0	11.1	80.8
		25	62.0	13.5	82.0
		32	62.0	13.5	-
	from day 190,6	233	67.0	42.4	-
3. P.T.	8	10	20.5	70.1	32
	from day 16,4	23	25.0	44.1	-
	from day 27,1	30	26.5	18.2	39
	from day 32,2	38	30.0	20.2	41
4. A.P.	4	(5	15.3	78.1	34
		(10	17.0	80.0	36.5
	from day 20,2	(30	17	27.8	-
		(43	18.0	41.7	-
		(142	14.0	29.2	-
5. L.L.	from day 10, 1.5	(11	37.0	20.9	-
		(16	36.0	29.9	-
	from day 17,2	(40	32.0	33.7	-
		(100	38.0	27.7	-

Table 19

Age and Sex distribution in slow and fast acetylators.

	Acetylator Phenotype					
	Slow (n = 87)			Fast (n = 35)		
	U.C.	C.D.	Total No. of Patients	U.C.	C.D.	Total No.of Patients
< 20 years	2	2	4	0	0	0
20-40 years	30	16	46	11	5	16
41-60 years	16	10	26	11	4	15
61-80 years	9	2	11	3	1	4
Sex						
Male (n = 57)	31	16	47 (82.5%)	8	2	10 (17.5%)
Female (n = 65)	26	14	40 (61.5%)	17	8	25 (38.5%)

SUMMARY

Sulphapyridine shared the same acetylation polymorphism as sulphadimidine in patients with ulcerative colitis or Crohn's disease. The acetylation capability of each patient was constant in serum and urine irrespective of activity of the disease and dosage (2 - 8G/day). A single estimation of serum or urine for SP and AcSP can determine the acetylator phenotype in patients on sulphasalazine therapy.

CHAPTER V

SALICYLAZOSULPHAPYRIDINE METABOLISM IN ULCERATIVE COLITIS

SECTION I : INPATIENT STUDY

SECTION II : OUTPATIENT STUDY

SALICYLAZOSULPHAPYRIDINE METABOLISM IN ULCERATIVE COLITIS

SECTION I

INPATIENT STUDY

This section describes thirteen new, untreated patients with active colitis and three patients who were already on SASP treatment but were admitted with relapse.

RESULTS

Table 20 shows the age (range), sex, body weight, severity and extent of the disease in these sixteen patients.

Clinical outcome

Of the thirteen previously untreated patients, eleven responded within ten days to the treatment as described in Chapter II, Section I (Page 18). The mean period of stay in the hospital of these eleven patients was thirteen days. Prior to discharge sigmoidoscopic examination revealed a normal rectum in five, marked improvement with only minimal friability in two and minimal granularity only in four.

Two patients continued to have active disease despite adequate treatment as outlined before (Chapter II Section I). Three patients who were admitted with relapse after previously being established on SASP and with a maintenance does of 2 - 3G/day were treated with 2 - 4G of SASP/day and corticosteroids. They

Table 20

Clinical data of the sixteen patients studied.

Clinical data		Number of patients
Previously untreated		13
Previously treated		3
Ages :	<20	1
	20 - 39	10
	40 - 59	3
	60 - 79	3
Sex :	Male	7
	Female	9
Body weight	<50 kg.	4
	50 - 70 kg.	10
	71 - 90 kg.	2
Severity of Disease		
Mild		6
Moderate		7
Severe		3
Extent of Involvement		
Entire colon		3
Distal		13

went into remission within one week.

Figures 10 and 11 show the serum concentrations of SASP and total SP during the study. Eleven patients who responded to therapy are represented together (Mean ± S.E.M.). The other two who did not respond within ten days are shown individually.

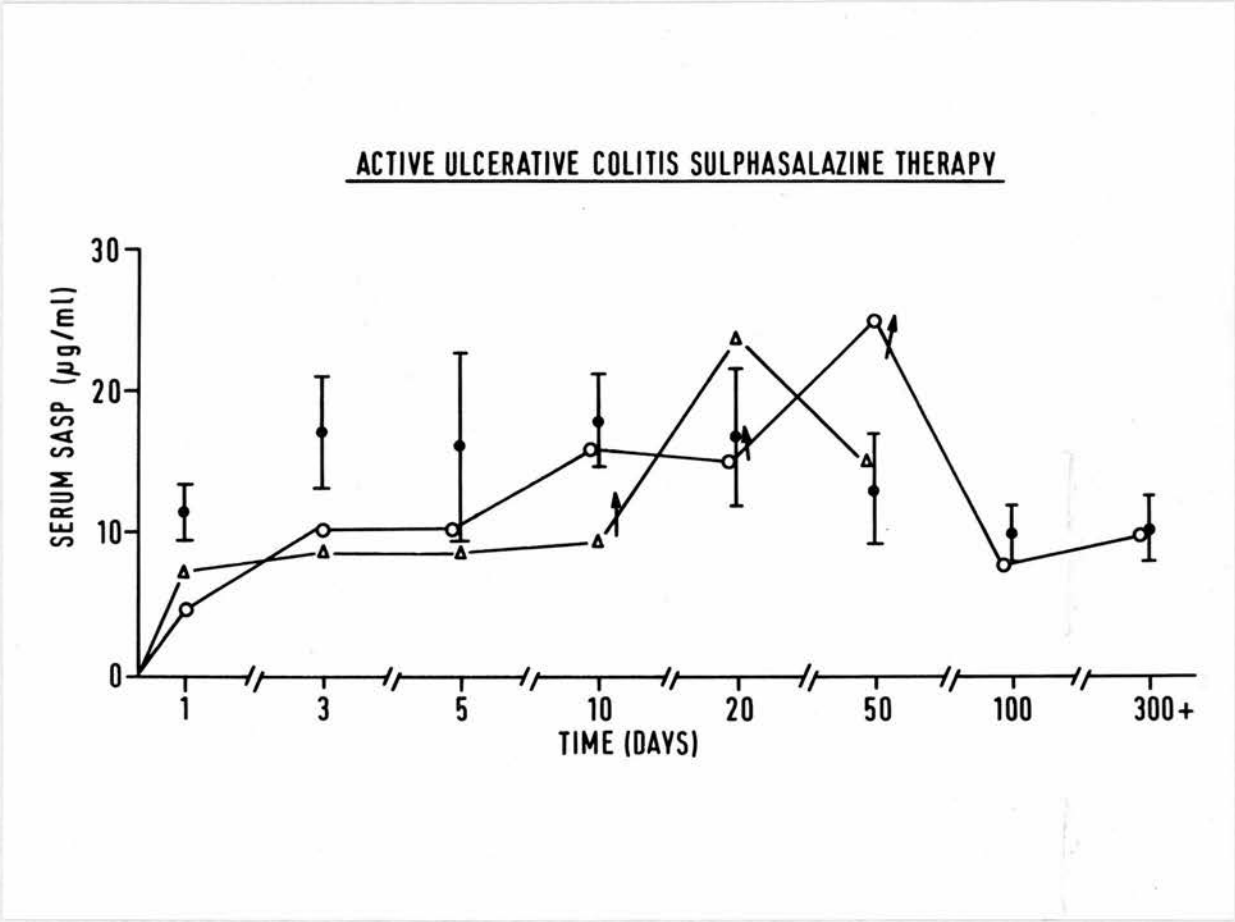
Serum SASP concentration reached a steady state within three days at a mean level of 10 - 15 ug/ml. (Figure 10). There was no significant difference in serum SASP concentration between the patients who responded within ten days of therapy and those who did not. The serum concentration of SASP decreased after three weeks of treatment. This was probably due to reduction of the dose (2 - 3G per day) in most of the patients during or shortly following discharge from hospital.

In the eleven patients who responded to the treatment the steady state of the serum total SP (SP + AcSP + SP-Gluc. + AcSP-Gluc.) was achieved within five days with a mean level of 43 ug/ml. (Figure 11).

The two patients who did not improve during the first ten days had total SP concentrations > 10 ug/ml. On increasing the dosage of SASP in these two patients improvement in the clinical state coincided with an increase in the serum concentration of total SP to within the range observed in those patients who had shown an early clinical response.

SP metabolites appeared in the serum of all thirteen patients four to eight hours after the first dose, although

Figure 10

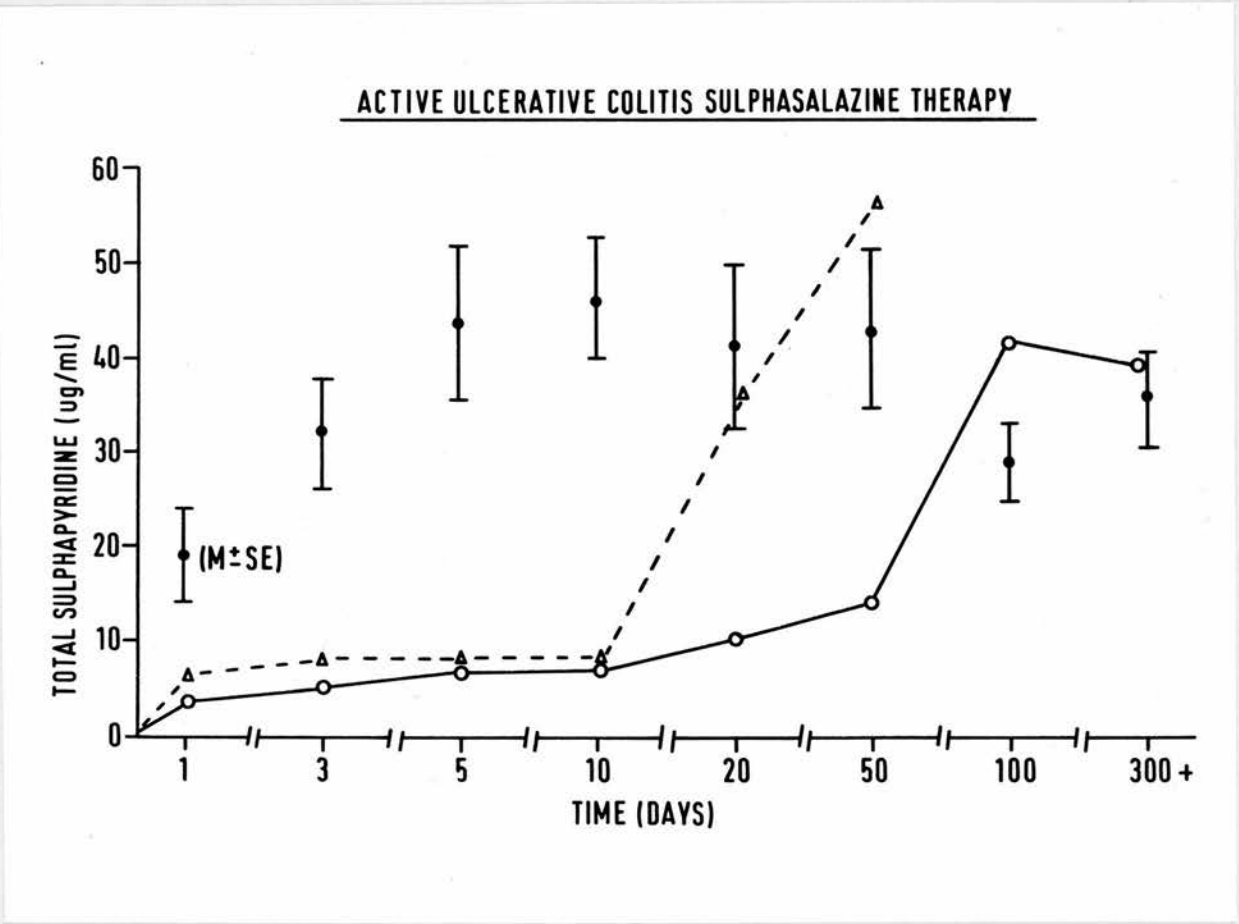


Serum sulphasalazine concentration related to duration of treatment.

I represents mean ± S.E.M. of eleven patients, who responded to treatment.

O,Δ represent two patients who did not initially respond to treatment.

Figure 11



Serum total sulphapyridine concentration related to duration of treatment.

I● represents mean ± S.E.M. of eleven patients who responded to treatment.

o,Δ represent two patients who did not initially respond to treatment.

SASP reached its peak concentration within three to five hours of the first dose. Serum concentration of total SP did not reach a peak level until twelve to twenty-four hours after the first administration of SASP. However, a steady state of total SP was achieved within day five (Figure 11).

The results of the three patients who were admitted in relapse are shown in Table 21. The serum total SP concentration in these three patients on admission, was less than 20 ug/ml. and the total SP, but not SASP and individual SP metabolites, was significantly lower than that observed when clinical improvement was attained ($p < 0.05$) (Table 21).

The influence of acetylator phenotype

Six of the sixteen patients studied were fast acetylators and ten were slow acetylators on the basis of the sulphadimidine test and the concentration of free and acetylated SP. In slow acetylators most of the total SP was free SP whereas in fast acetylators it was mainly AcSP. SP-Gluc. and AcSP-Gluc. were present only in small concentrations in the serum in both groups (Table 22).

There did not appear to be a diurnal variation in the total SP levels in the steady state, although there is a tendency to gradual increase in the serum concentration of total SP over a prolonged period of time among slow acetylators (Figure 12). Serum SASP concentration, however, had a wider diurnal variation depending on time of collection of blood in relation to the last dose taken

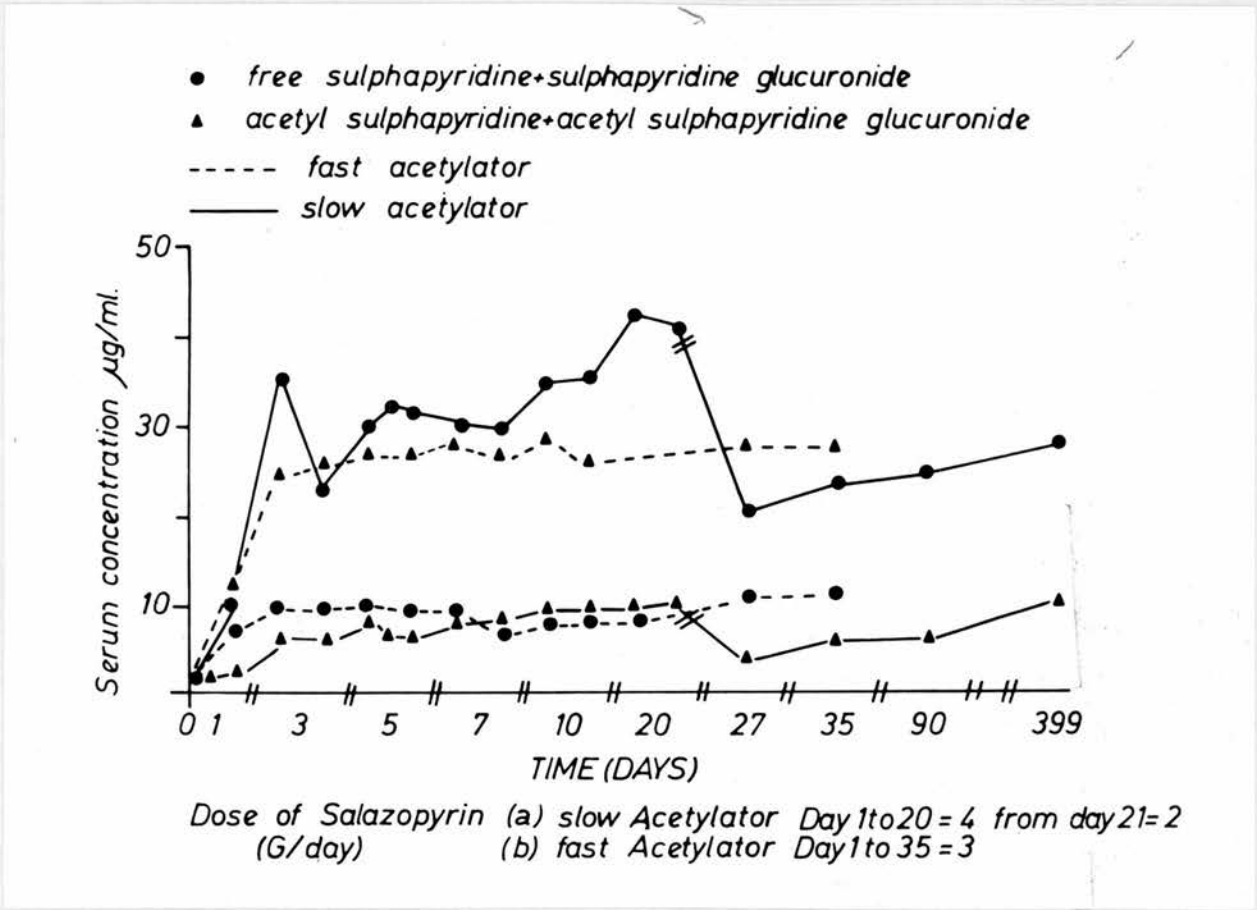
Table 21

Serum Concentrations of sulphasalazine and its Metabolites in three patients admitted with relapse.

Serum Concentrations ug/ml. (Mean ± S.D.)	On Admission	After Improvement	't' test
SASP	11.4 ± 8.3	15.3 ± 8.6	N.S.
Free SP	9.5 ± 1.5	19.5 ± 12.0	N.S.
AcSP	6.2 ± 4.7	10.3 ± 6.0	N.S.
SP-Gluc.	0.4 ± 0.5	3.2 ± 4.3	N.S.
AcSP-Gluc.	2.6 ± 1.9	3.6 ± 1.0	N.S.
Total SP	18.7 ± 1.9	36.7 ± 13.4	$P<0.05$

N.S. Not Significant

Figure 12



The effect of time on the serum concentration of sulphapyridine and its metabolites in one slow acetylator and one fast acetylator.

by the patients.

Table 22 shows the serum concentration of SASP and its metabolites in the slow and fast acetylators, when a steady state had been achieved (8 ± 2 days). There was no significant difference in SASP concentration between the slow and fast acetylators. There was, however, a significant difference in free SP, AcSP and also total SP concentrations between the two phenotypes.

Urinary Excretion

The twenty-four hour urinary excretion of SASP and SP metabolites at different times of starting the therapy from day 1 are shown in Table 23. Daily SASP excretion varied from 1 - 13% (mean 4.6 ± 3.1). Individually there was no significant difference in the excretion of SASP on different days although interindividual variations occurred. The total sulphapyridine metabolites recovered from twenty-four hour urine samples of the responding patients ranged from 9 - 23% of administered dose on day 1, 31 - 58% on day 3, 38 - 60% on day 5 and 42 - 63% on day 10. The mean values (± S.D.) are shown in Table 23. Individual metabolites of SP did not show much variation once the steady state was achieved.

The mean recovery of total SP and SASP in twenty-four hours urine in the two patients who did not respond to the treatment was 14.4% ± 11.3 and 4.9% ± 4.8 of the dose respectively during the stage of activity. Following improvement, the total SP excretion was 49.8% and SASP was 4.7% ± 2.8 of the administered dose.

Table 22

Serum concentrations of sulphasalazine and its sulphapyridine metabolites during steady state (8 ± 2 days) in slow and fast acetylators.

M. ± S.D.	Slow Acetylators (n = 10)	Fast Acetylators (n = 6)	't' test
Percent Acetylation of SP	25.2 ± 9.6	62.9 ± 8.5	P<0.0005
SASP ug/ml	18.7 ± 12.8	17.6 ± 7.1	NS
Free SP ug/ml	42.2 ± 24.4	8.5 ± 2.8	P<0.0005
AcSP ug/ml	7.6 ± 3.1	15 ± 5.5	P<0.005
SP-Gluc ug/ml	2.5 ± 2.5	2.2 ± 2.2	NS
AcSP-Gluc ug/ml	4.4 ± 3.4	4.4 ± 3.3	NS
Total SP ug/ml	53.7 ± 23.1	31.7 ± 9.0	P<0.01

NS = Not significant

Table 23

Twenty-four hour excretion of sulphasalazine and its SP metabolites in patients with Ulcerative Colitis

Time of Urine Collection	Sulphasalazine % of dose (M ± S.D.) recovered in 24 hrs.	Total Sulphapyridine % of dose (M ± S.D.) recovered in 24 hrs.
In new patients who responded to therapy (n = 11)		
Day 1 of SASP therapy	3.2 ± 2.8	16.3 ± 6.0*
Day 3 of SASP therapy	4.5 ± 4.0	41.7 ± 9.0
Day 5 of SASP therapy	3.5 ± 1.8	45.9 ± 9.4* **
Day 10 of SASP therapy	4.6 ± 3.1	52.0 ± 9.6** Ø
2 weeks - 20 weeks (5 samples)	4.3 ± 2.3	49.2 ± 7.0 Ø
In Chronic Patients admitted with relapse (n = 3)		
After improvement (8 ± 2 days)	5.0 ± 2.3	74.3 ± 15.8

* 't' test (between day 1 & 5) = $P < 0.001$

** 't' test (between day 5 & 10) = Not Significant

Ø 't' test (between day 5 &>2 weeks)= Not Significant

The proportions of the different metabolites excreted in twenty-four hours during the steady state (8 ± 2 days) by the slow and fast acetylators (patients responded to the treatment) are shown in Table 24. There was no significant difference in the excretion of SASP and total SP metabolites, but slow acetylators excreted the drug mostly as free SP and SP-Gluc. whereas fast acetylators excreted it as AcSP and AcSP-Gluc. The differences were statistically significant. There was no significant difference in the total amount of excretion of the sulphapyridine as glucuronides (i.e. SP-Gluc. + AcSP-Gluc.) in twenty-four hours between fast and slow acetylators (Table 24).

The mean percentage of hydroxylated (i.e. glucuronides) sulphapyridine calculcated from the total excretion of SP in twenty-four hours urine for the slow and fast acetylators was 38.4 ± 7.8 and 46.2 ± 14.9 respectively. This did not differ significantly in the two groups. Unlike the percentage acetylation (Chapter IV), the percentage hydroxylation varied widely in the same individual on different days. The mean co-efficient of variation of the percentage hydroxylation (i.e. percentage of the amount of SP glucuronides out of twenty-four hour urinary total SP excretion) in all these patients was 25.2 ± 15.4.

Serum concentration of 5-aminosalicylis acid and its urinary excretion

The concentration of 5-ASA measured in forty-eight representative serum samples from the sixteen patients

Table 24

Twenty-four hour Urinary Excretion of Sulphasalazine and its metabolites in 16 patients with ulcerative colitis in the steady state (8 ± 2 days)

		Slow Acetylators (n = 10)	Fast Acetylators (n = 6)	't' Test
Urinary Excretion (Mean ± S.D.)	SASP[1]	4.3 ± 3.8	4.7 ± 2.5	NS
	Total SP[1]	57.7 ± 11.3	57.6 ± 15.9	NS
	Free SP[2]	36.1 ± 12.7	13.1 ± 0.9	$P < 0.0005$
	ACSP[2]	23.6 ± 5.3	39.9 ± 17.5	$P < 0.01$
	SP-Gluc[2]	23.4 ± 7.7	12.1 ± 8.2	$P < 0.01$
	ACSP-Gluc[2]	16.0 ± 6.8	35.0 ± 17.6	$P < 0.0005$
	Proportion of Acetylated SP[2]	38.5 ± 10.4	72.8 ± 8.8	$P < 0.0005$
	Proportion of Glucuronidised SP[2]	38.4 ± 7.8	46.2 ± 14.9	NS

1 = Calculated as % of the administered dose
2 = % of the total recovery of sulphapyridine
NS - Not Significant

ranged from 0 - 4 ug/ml. (mean 1 $\pm$ 0.9) and was mainly (> 90%) in the form of free 5-ASA.

The urinary 5-ASA was recovered mostly as acetylated 5-ASA (> 80%) irrespective of slow or fast acetylators. The twenty-four hour urinary excretion ranged from 1 - 10% on day 1, 16 - 39.3% (24.8 $\pm$ 9.6) on day 3, 16 - 32% (21.2 $\pm$ 7) on day 5 and 5 - 37% (22.3 $\pm$ 13.7) on day 10 (Table 25). Of the two patients who did not improve within the first ten days of treatment, one had very low excretion in twenty-four hours of total 5-ASA (0.8 to 1%) but the second patient excreted 9 to 19% of the administered dose, during the first ten days.

Follow-up results of the patients studied

Figures 10 and 11 show the mean ($\pm$ S.E.M.) of the serum concentration of SASP and total sulphapyridine during follow-up study up to one year. The maintenance dose was found to be adequate both clinically and in terms of serum concentration at 3G SASP/day in fast acetylators and 2G/day in slow acetylators. One fast acetylator received 6G SASP/day for at least one year and yet his serum total SP concentration was less than 50 ug/ml. One patient with radiological evidence of total involvement of the colon and a fast acetylator who improved initially with the described combined regime, did not have a complete remission despite adequate serum concentration. Mild persistent activity continued over a year, and eventually she had a total colectomy. Two other patients stopped treatment themselves (after four months and one

TABLE 25

Urinary excretion of 5-ASA component of SASP (> 80% as acetylated 5-ASA) in twenty-four hours on different days.

Day of therapy	Range (percentage of dose administered in twenty-four hours)	Mean ± S.D.
1	1 - 10	6.4 ± 3.2
3	16 - 39.3	24.8 ± 9.6
5	16 - 32	21.2 ± 7.0
10	15 - 73	22.3 ± 6.7

and a half years of introduction of therapy). Both of them had relapses. One patient (P.T.) was readmitted and treated successfully with SASP and corticosteroids. Serum SASP and total SP showed similar results as before. The second patient was treated as outpatient.

Side effects

Side effects related to SASP were observed in nine out of the sixteen patients. Seven of these nine patients had high serum total SP > 50 ug/ml. at the time of toxicity. The side effects are described in more detail in Chapter VII.

SUMMARY

The mean serum concentrations (at steady state 8 ± 2 days in responding patients) of SASP, total SP and 5-ASA were approximately 15, 43, and 1 ug/ml. of serum respectively. Twenty-four hour urinary excretion of SASP, total SP and 5-ASA were approximately 3.5%, 57% and 22% of the administered dose respectively.

Serum total SP concentration of 20 - 50 ug/ml. appeared to be consistent with clinical improvement in the absence of any side effects related to SASP. No such relationship could be shown with serum SASP, individual SP metabolites, or 5-ASA.

SECTION II

OUTPATIENT STUDY

This section describes sixty-eight patients with ulcerative colitis who were studied while they were attending the Gastro-intestinal follow-up clinic. Four out of these sixty-eight patients were eventually excluded from the study as they were not taking the drug at all(Table I) Of the remaining sixty-four patients, forty-one patients were in remission, twenty-one had mild to moderate activity and two had severe activity. No division was made between mild to moderate cases as it was found difficult to separate this on outpatient basis. Table 26 summarises the patients.

RESULTS

Table 27 shows the serum concentration of SASP and its metabolites in patients during activity of the disease and in remission. There was a significant difference in the serum concentration of both free SP ($p < 0.001$) and total SP ($p < 0.001$). AcSP difference was not statistically significant. The serum glucuronides (i.e. SP-Gluc. and AcSP-Gluc.) were low in the active and the remission state of the disease, and there was no significant difference in the mean serum concentrations of AcSP-Gluc. and SP-Gluc.in two stages of the disease.

The 5-ASA component of SASP was found to be very low irrespective of the disease state.

Table 26

Ulcerative colitis, outpatient study

Clinical data

Male : 31 Female : 33 Age Range : 17 - 80 yrs.

Dose range of SASP : 1.5 - 5 G/day

Duration of SASP therapy : 6 months - 15 years

Disease state : Remission - 41 patients

Active - Mild - 21 patients

Severe - 2 patients.

Table 27

Serum Concentration (M ± S.D.) of Sulphasalazine and its metabolites in active and remission state of the disease

Serum Concentration ug/ml (Mean ± S.D.)	Patients with activity (n = 23)	Patients in Remission (n = 41)	Statical Significance, P Value
SASP	15.6 ± 15	12.1 ± 11.6	NS
Free SP	9.8 ± 6.1	16.9 ± 8.7	P < 0.001
ACSP	6.8 ± 4.8	8.6 ± 6.6	NS
SP-Gluc	1.2 ± 1	1.8 ± 1.6	NS
ACSP-Gluc	1.6 ± 1.9	3.0 ± 2.2	NS
Total SP	18.1 ± 10.5	30.8 ± 9.7	P < 0.001
5 - ASA*	0.6 ± 0.4	0.9 ± 0.7	NS

* Estimated in 25 patients - 10 active and 15 in remission

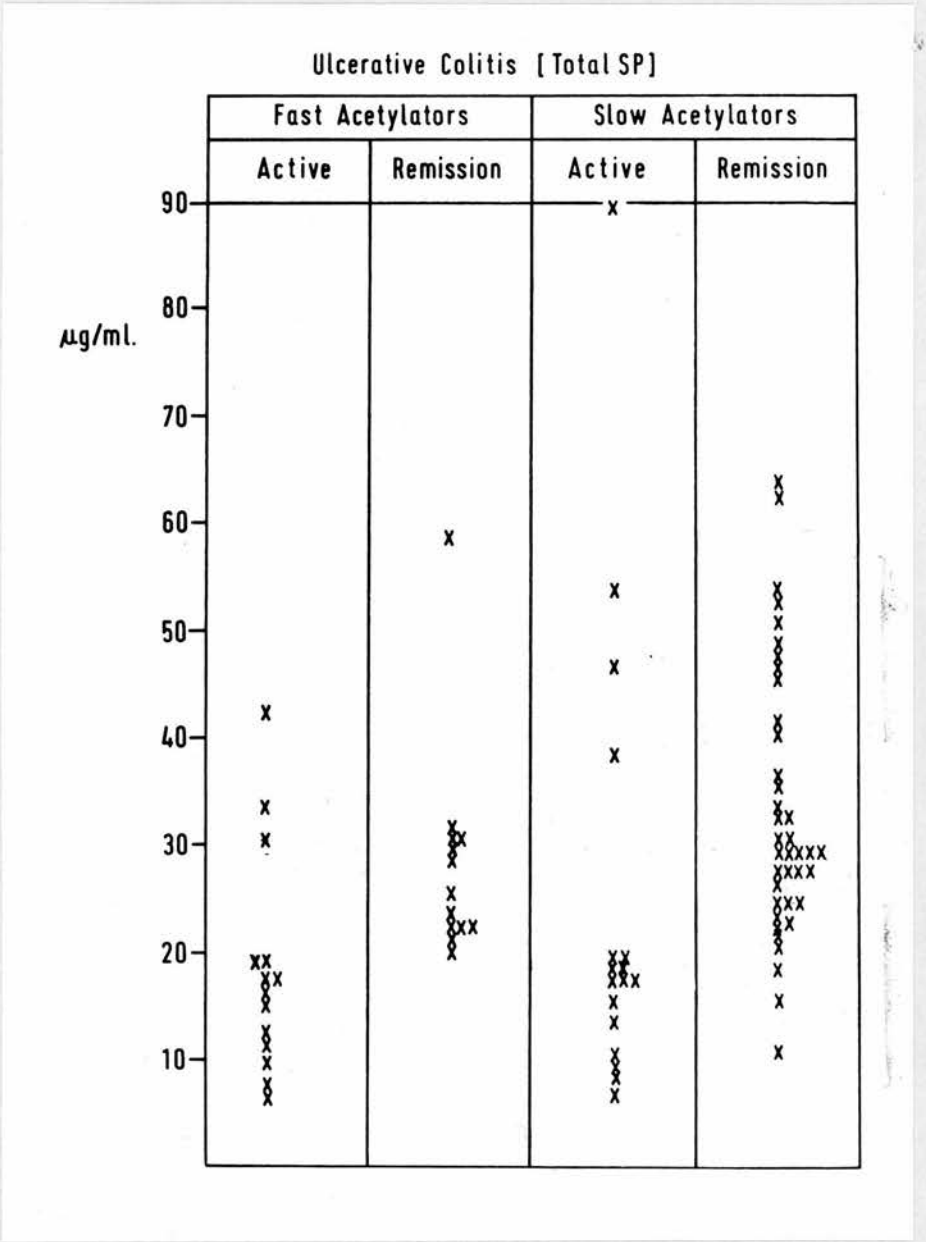
NS - not significant

Of the sixty-four patients studied, forty-five patients were slow acetylators and nineteen patients were fast acetylators.

Figure 13 relates the serum concentration of total SP in between the slow and fast acetylators during active or remission state of the disease. All but three of the fifty-one samples of serum taken from patients in remission had total SP concentration > 20ug/ml. whereas twenty-four of the thirty-one patients examined during an active phase of the colitis had serum total SP concentration < 20 ug/ml. Seven others (four slow acetylators and three fast acetylators) were in an active phase despite total SP concentration > 20 ug/ml. Of these seven patients, two had recent relapses following which the dose of SASP was increased and four other patients were also on azathioprine and steroids. The concentration of SASP and each of its metabolites had no such clear distinction.

The major component of total sulphapyridine in the serum of slow acetylators was free SP, whereas in fast acetylators this was AcSP. SP-Glucuronides were present in low concentrations both in fast and slow acetylators in active and remission state (Table 28). When the individual metabolites were analysed for slow and fast acetylators during active and remission state of the disease it was found that only total SP was significantly different between active and remission state irrespective of slow or fast acetylation. Free SP, however, showed no significant difference. In fast acetylators free SP

Figure 13



Serum concentration of total sulphapyridine (i.e. SP + AcSP + SP-Gluc + AcSP-Gluc) in slow and fast acetylators during active and remission states of ulcerative colitis.

Table 28

Serum Concentrations of Sulphasalazine and its Sulphapyridine metabolites in slow and fast acetylators during active and remission states of the disease

M ± S.D. of SASP & its SP metabolites ug/ml	Fast Acetylator		Slow Acetylator	
	Active	Remission	Active	Remission
SASP	21.6 ± 18.7	12.6 ± 14.5	11.6 ± 10.3	11.1 ± 8.6
SP	6.3 ± 2.8	9.4 ± 2.3	11.4 ± 3.9	20.1 ± 8.7
ACSP	8.4 ± 4.2	10.5 ± 3.4	4.4 ± 2.5	5.8 ± 3.0
SP-Gluc	1.4 ± 1.1	1.5 ± 0.5	1.0 ± 1.0	1.8 ± 1.7
ACSP-Gluc	2.6 ± 2.3	3.9 ± 2.3	0.7 ± 0.5	2.5 ± 1.8
Total SP	18.8 ± 10.1	26.3 ± 4	19.5 ± 10.8	32.1 ± 10.5

level (9.4 ± 2.3 ug/ml) in the remission state was less than that found for the slow acetylator in the active state (11.4 ± 3.9 ug/ml)(Table 28).

In Table 29 the serum concentration of SASP and total SP in the two acetylator groups is compared with the different dosage schedules. There was no significant difference in the SASP concentration in the serum of either group at any dosage (between 2-4 G/day). However, the serum concentration of total SP was progressively higher with increasing dose and this was more marked in slow acetylators compared with the fast acetylators at the same dosage.

Though the serum concentration of total SP could be correlated with the dosage of SASP in terms of G/day no correlation was found in relation to body weight.

In Figure 14 the serum total SP concentration is related to different dosages of SASP in patients in activity and in remission. 55% of the patients taking 2G or less of SASP per day had active colitis and their serum total SP was < 20ug/ml. All but four of the forty-seven patients who were taking 3G or more SASP per day when the serum was analysed were in remission and serum total SP concentrations were > 20 ug/ml. Of the four whose serum total SP were < 20 ug/ml., one was in remission. In a total of seven instances (16%) the disease was active despite a serum total SP > 20 ug/ml. Of the eight patients who had side effects six patients were taking 4G or more and only two patients had 3G of SASP/day.

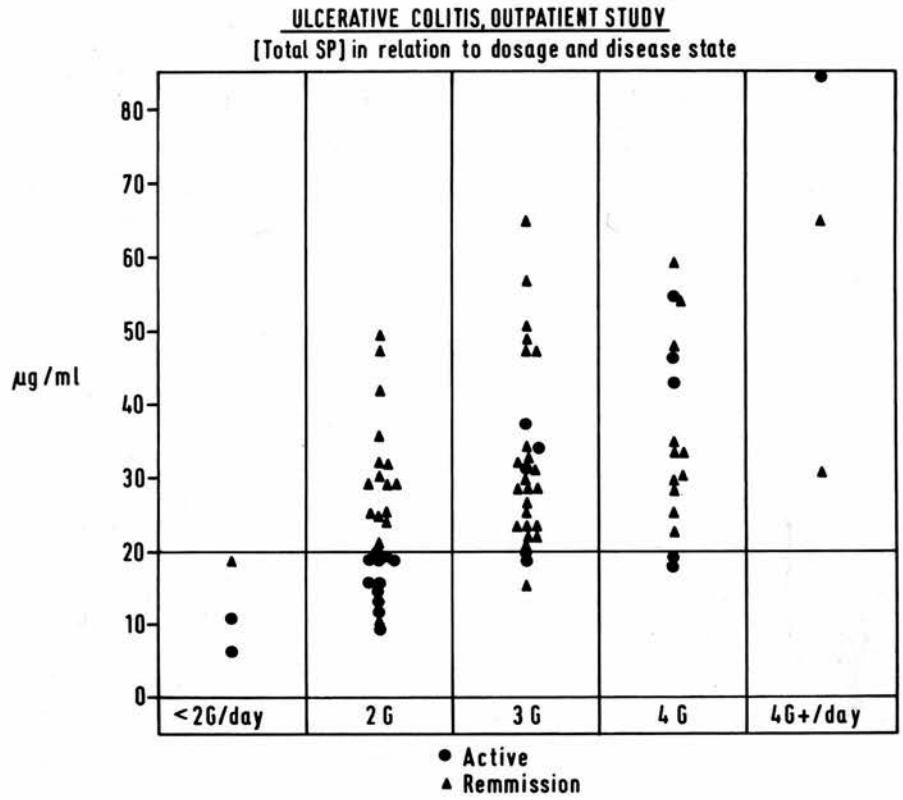
Table 29

Serum concentration of Sulphasalazine and total Sulphapyridine in sixty-four outpatients in relation to dosage of SASP.

Dose of sulphasalazine G/day	No. of patients at first examination	Mean (± S.D.) Serum concentration ug/ml	
		SASP	Total SP
<2	3	9.4 ± 1.1	9.8 ± 2.9
2	27	14.1 ± 14.6	22.3 ± 10.5
3	19	11.2 ± 11	30.4 ± 10.8
4	12	14.8 ± 10.7	37.1 ± 12.7
>4	3	23.1 ± 23.5	69.7 ± 28.5

Out of the sixty-four patients studied twenty-one were taking enteric coated tablets and the other forty-three plain tablets. Of these twenty-one, seven were in an active stage of the disease as were approximately one third of the patients taking plain tablets. The serum concentration of SASP was slightly lower in patients taking enteric coated tablets (11 ± 11.5) as opposed to that in patients who were on plain tablets (16.5 ± 13.9). The concentration of metabolites of SP and total SP

Figure 14



Serum concentration of total sulphapyridine in relation to dosage of sulphasalazine and the clinical states of the disease.

did not differ significantly between patients taking plain and enteric coated tablets at the same dosage.

SUMMARY

About 90% of the patients in remission had serum total SP concentration above 20 ug/ml. Seven patients, however, had active disease despite the serum total SP concentration > 20 ug/ml. SASP and the individual SP metabolites did not show any correlation with the disease state. 3G per day seems to be the most effective dose. Plain and enteric coated tablets gave similar results. Side effects due to SASP were frequent in patients who had a total SP concentration > 50ug/ml of serum.

CHAPTER VI

SALICYLAZOSULPHAPYRIDINE METABOLISM IN PATIENTS WITH CROHN'S DISEASE

SALICYLAZOSULPHAPYRIDINE METABOLISM IN CROHN'S DISEASE

This chapter describes SASP metabolism in patients with Crohn's disease who were studied during admission to the Gastro-intestinal Unit (seven patients) and also patients attending the outpatient follow-up clinic (Twenty-nine patients).

For other patients who were studied only whilst exhibiting side effects are included with toxicity study (Chapter VII, page 127). Two other patients with C.D. each with an ileostomy and one other patient with transverse colostomy, are described under the study of SASP metabolism in patients with ileostomy and colostomy (Chapter VIII, page 161).

INPATIENT STUDY

Table 30 shows the clinical data of the seven in-patients studied. Of the seven patients, three had had an ileotransverse anastomosis between one and three months before the study. One of these three patients (M.C.) was also studied just prior to surgery when she had been taking SASP for six months.

The sex distribution, age range, body weight, and site of lesion are shown in Table 30. The extent of the disease was judged on the basis of radiological (in all) and operative findings wherever available.

Total serum protein on admission was $< 6G$ in three of the seven patients but serum albumin was low ($< 50\%$ of the total protein) in all. Schilling test was $< 10\%$ recovery in all except one (J.W.) who had Crohn's colitis

Table 30

Clinical Data of the "Inpatient" with Crohn's disease (n = 7)

	No. of Patients	Site of Lesion
A. Patients with no history of bowel surgery -		
(a) New and previously untreated patients -	2	1. L.I. only 1. S.I. & L.I.
(b) Chronic patients with protein losing enteropathy, SASP stopped one year ago for haemolysis. Reintroduction of SASP	2	S.I.only
B. Patients with bowel surgery -		
Ileotransverse anastomosis	3	1. S.I. 2. S.I. & L.I.

3 male, 4 female, Age (range) years = 20 - 46 years.
Weight (Kg) = 40 - 66.

S.I. = Small intestine

L.I. = Large intestine.

with 11.5% recovery.

All seven patients also had < 12G Hb on admission. After leaving the hospital the serum albumin continued to be low in four patients (M.M., A.B., M.C., M.Y.) and Hb was < 12G in two patients (M.M., M.Y.).

Clinical outcome

Group A. (a) New patients -(1), J.W. had segmental proctocolitis, without radiological evidence of small intestine. He was treated with 3G SASP/day, oral Prednisolone 30 mgm/day and Predsol Enema. The dosage of corticosteroids was gradually reduced over eight weeks period. With this treatment he responded within ten days and remained well for at least four hundred and ten days follow-up with SASP as his only therapy.
(2), R.M. had acute Crohn's disease and was severely ill on admission. He was found to have lesions both in the ileum and colon. He was treated with SASP 6G/day parenteral steroids, i.e. hydrocortisone injection for forty-eight hours followed by Synacthen Depot 0.5 mg at two to three days intervals and replacement of iron, fluid, electrolytes and blood. Synacthen was discontinued after twelve weeks. He gradually improved in the course of four weeks and allowed home after five weeks. He had evidence of cyanosis and haemolysis after two weeks of therapy in the ward, these symptoms disappeared after stopping the SASP (Page 148). SASP was reintroduced after one week at a dosage of 2G/day which he has been tolerating for at least one year with coincidental

clinical remission.

(b) Two patients (M.M., A.B.) had been suffering from Crohn's disease with protein loosing enteropathy for two and five years respectively. They had been treated with SASP in the past and this was discontinued about one year prior to the study for suspected haemolysis. SASP was reintroduced to both of them with 2G/day without any corticosteroids. The dose was well tolerated for up to six months and one year follow-up, but the clinical state was still equivocal as there was continued protein loss from the intestine leading to hypoproteinaemia. A.B. had also been treated with azathioprine three months after reintroduction of SASP.

Group B Patients having had ileotransverse anastomosis -

(1) M.C. had been suffering from Crohn's disease for seven years. She was treated with Synacthen Depot, SASP (4G/Day) for six months prior to the study but she required an ileotransverse anastomosis because of an entero-vesical fistula. Two weeks after this surgery she was treated with azathioprine in addition to SASP. Synacthen Depot was continued for twelve weeks after operation. SASP metabolism was studied before and after operation. The dose of SASP after operation was 2G/day as she had had 'cyanosis' on 4G before operation (Page 133). During eight months follow-up she was in remission with SASP (2G/day) and azathioprine.

(2) G.F. had been suffering from Crohn's disease for three years prior to the study. He had an ileotransverse

anastomosis fashioned two months prior to introduction of SASP (4G/day). He was not given any corticosteroids or azathioprine. He has been keeping well for over seven months.

(3) M.Y. had an ileotransverse anastomosis following right hemicolectomy two months prior to this study. She was treated with SASP (3G/day) and synthetic steroids (Synacthen Depot). She did not improve and was readmitted with a recurrence after eight months.

SASP/METABOLITES IN THE SEVEN PATIENTS

Table 31 shows the serum concentration and twenty-four hour urinary excretion (Mean ± S.D.) of SASP and total SP in the four unoperated patients (Table 30, Group A) on different days of SASP therapy.

Serum SASP concentration was steady from day 1 at a level of 4 - 7 ug/ml and did not differ in patients with active disease or in remission. The serum concentration of total SP (Mean ± S.D.) reached a steady concentration (approximately 20 - 30 ug/ml) from day 5 onwards. These concentrations of both SASP and total SP in the steady state (8 ± 2 days) were lower than those in ulcerative colitis (SASP < 10 ug/ml, total SP < 40 ug/ml) (Pages 83 and 84)

All seven patients were slow acetylators and free SP was the main component of the serum total SP. The mean SP glucuronides concentration (SP-Gluc.+ AcSP-Gluc.) were < 2 ug/ml.

Twenty-four hour urinary excretion of SASP (M ± S.D.) was steady from day 1 at about 3% of the administered

Table 31

Serum Concentration and 24 hour Urinary Excretion (Mean ± S.D.) of SASP and Total Sulphapyridine in 4 patients with Crohn's Disease (unoperated) on different days

	Serum ug/ml		Urine % of dose administered in 24 hours	
Day of Therapy	SASP	Total SP	SASP	Total SP
1	4.2 ± 1.8	14.7 ± 6.7	3.4 ± 0.7	27 ± 8.5 [3]
5	4.6 ± 2.4	22.2 ± 12.6	3.0 ± 1	37.1 ± 19.9 [2]
10	7 ± 3.9	21.2 ± 6.7 [1]	3.5 ± 1.9	48.9 ± 19.4 [2 3]
6 - 8 weeks	6.3 ± 2.6	27.5 ± 11.3		
6 - 8 months	3.8 ± 3.0	32.9 ± 13.4 [1]		

't' test between 1 - 1 and 2 - 2 was not significant between 3 - 3, $P < 0.05$

dose. Total SP was comparatively higher on day five and ten compared with day 1 (Table 31) with significant difference between day 1 and 10 ($p < 0.05$) but not between day 1 and 5 or between 5 and 10.

The urinary SP metabolites were mostly in the form SP and SP-Gluc. which was also found in slow acetylators with ulcerative colitis (Page 92).

Table 32 shows the serum concentration and urinary excretion ($M \pm S.D.$) of SASP and total SP in the three patients with ileotransverse anastomosis on the tenth day of SASP therapy. The serum concentrations of SASP and total SP, the twenty-four hour urinary excretion of both SASP and total SP did not differ significantly from similar measurements of patients with no such operative interventions. One of the new patients (J.W.) who had segmental colitis without small intestinal involvement was treated with SASP 3G/day had total SP concentration in the range of 32 - 45 ug/ml of serum from day 4 - 10 and in urine 41 - 91% of the administered dose from day 5 - 22. Serum SASP ranged from 5 - 10 ug/ml with recovery of 3 - 6% of the dose in twenty-four hour urine. Conversely a patient (M.Y.) with right hemi colectomy and ileotransverse anastomosis had lower concentration of both SASP (2 - 6 ug/ml of serum) and total SP (10 - 25 ug/ml of serum) and in urine (SASP 0.2 - 0.9% dose, total SP 10 - 27% of the dose) although she was also taking 3G SASP/day.

This individual difference was not significant

TABLE 32

Serum Concentration and 24 hour Urinary Excretion (Mean ± S.D.) of SASP and Total SP in 3 patients with Crohn's Disease who had Ileotransverse Anastomosis

Serum ug/ml		Urine % of dose administered in 24 hours.	
SASP	Total SP	SASP	Total SP
9.4 ± 5.6	18.6 ± 3.8	2.1 ± 2.9	30.2 ± 7.5

when the two groups (patients with and without bowel surgery) were compared (Table 31 and 32) because of the less number of patients and different dosage of SASP.

SIDE EFFECTS RELATED TO SASP

Of these seven patients, two patients (R.M. and M.C.) who did not have ileotransverse anastomosis had cyanosis and R.M. also had haemolysis.

Of the four other patients who were studied only during the stage of toxicity two (R.M., J.B.) had haemolysis, one (I.G.) cyanosis and one (M.B.) had nausea and vomiting from the beginning of therapy which disappeared after replacing enteric coated tablet for plain tablet. They are included in the toxicity study (Chapter VII, Page 127).

OUTPATIENT STUDY

Table 33 shows the clinical data of the twenty-nine patients studied. The dose of SASP ranged from 2 - 4G/day and these patients had been taking the drug for six months to ten years. Of the twenty-nine patients, fifteen patients had received an ileotransverse anastomosis; ten patients had active disease and nineteen patients were in remission. The site of the lesion (obtained from radiological findings and operative notes) were confined to the small intestine in nineteen patients and in ten both small and large intestine were involved.

Of the twenty-nine patients studied eleven were fast acetylators and eighteen were slow acetylators on

Table 33

Clinical Data of the "Outpatients" with Crohn's disease (n = 29)

Age (range) years = 17 - 72 years

Male - 14, Female - 15.

Dose of SASP G/day - 2-4

Duration of SASP therapy - 6 months to 10 years.

Patients without bowel surgery = 14

Patients with bowel surgery = 15

Disease state, Active = 10 patients

Inactive = 19 patients

Site of lesion - small intestine = 19 patients

(judged from both small and

the x-ray or large intestine = 10 patients

operative report)

the basis of serum SP and AcSP concentrations. Figure 15 shows the serum concentration of SASP in these two groups in active and remission states. This shows a wide range.

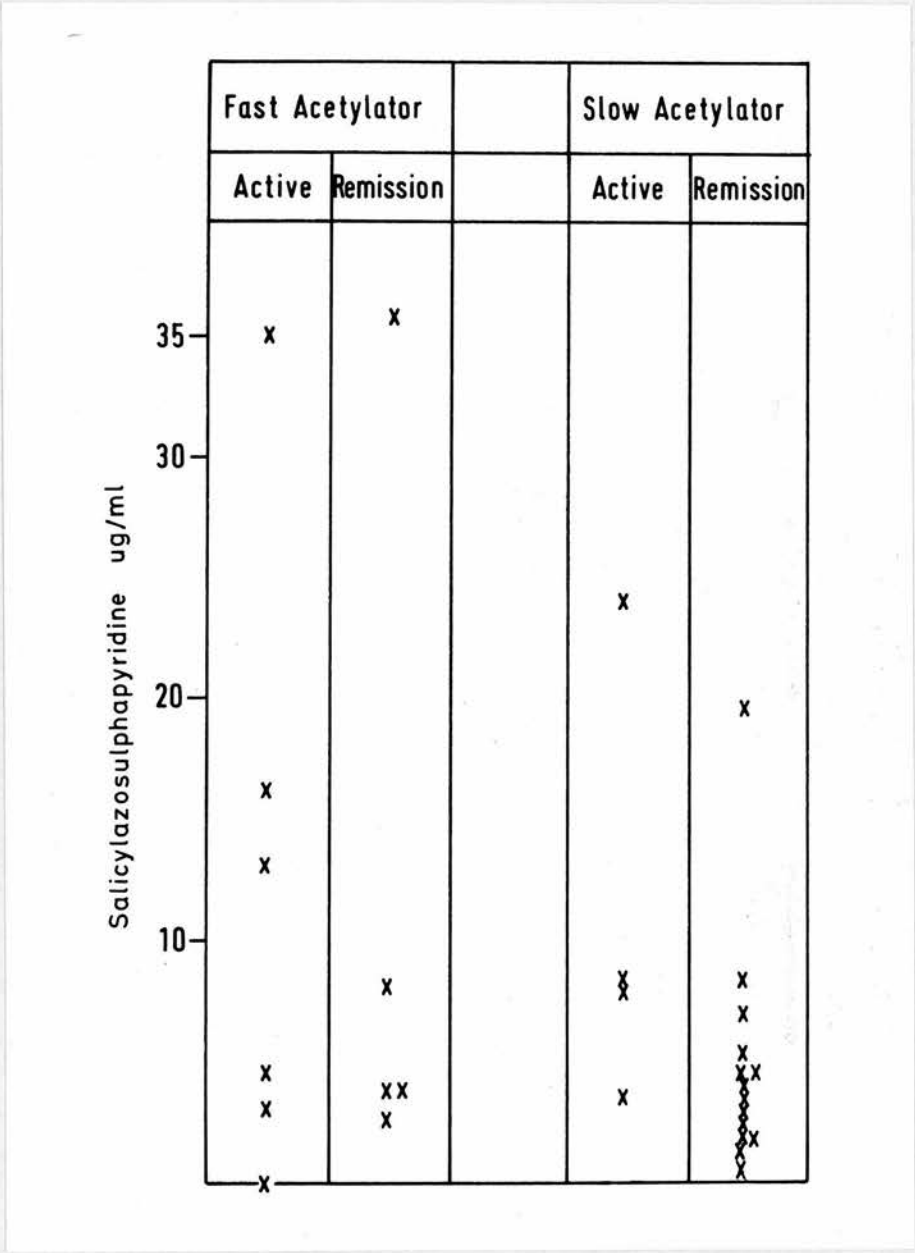
Figure 16 shows the serum total SP concentration in the two groups in active and remission states. Six of the ten patients in active state of the disease had serum total SP < 20 ug/ml whereas five of the nineteen patients in remission had serum total SP $<$ 20ug/ml.

Table 34 shows the serum concentration of SASP and the different SP metabolites (Mean $\pm$ S.D.) in patients with and without bowel surgery. The serum SASP and total SP were higher in patients without bowel surgery but this failed to reach any statistical significane. The SP-Glucuronides in patients with ileotransverse anastomosis were significantly higher ($p < 0.025$).

Table 35 shows the serum concentration of SASP and total SP (Mean $\pm$ S.D.) in the patients with involvement of only small intestine and patients with involvement of both small and large intestine. The latter group had significantly higher concentrations ($p < 0.005$) of total SP.

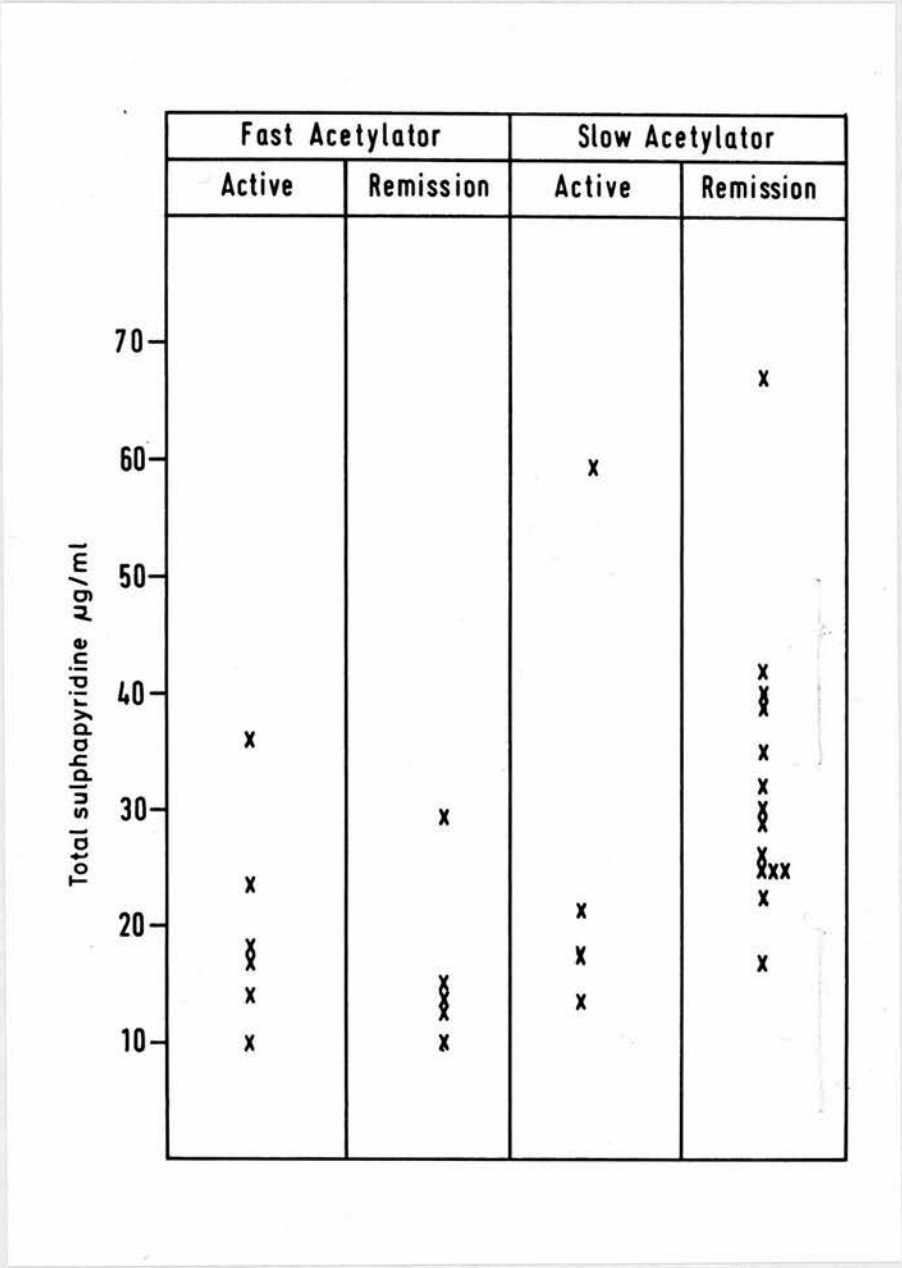
The relation of serum SASP and total SP (Mean $\pm$ S.D.) with different dosage of SASP are shown in Table 36. SASP concentration did not change significantly but total SP was significantly higher ($p < 0.05$) in patients taking 3 and 4G of SASP/day compared with the patients who were being treated with 2G/day. The difference

Figure 15



Serum concentration of sulphasalazine in fast and slow acetylators during active and remission states of Crohn's disease.

Figure 16



Serum concentration of total sulphapyridine in fast and slow acetylators during active and remission states of Crohn's disease.

Table 34

Serum Concentration (M ± S.D.) of SASP and its Sulphapyridine Metabolites in Crohn's Disease (outpatients) with or without bowel surgery

Serum ug/ml Mean ± S.D.	Patients without Bowel Surgery (n = 14)	Patients with Bowel Surgery (n = 15)
SASP	8.5 ± 10.5	4.2 ± 3.5
SP	15.2 ± 7.0	15.9 ± 12.4
ACSP	7.8 ± 4.8	7.0 ± 4.0
SP-Glucs	2.7 ± 1.5	5.3 ± 3.2 *
Total SP	27.2 ± 7	23.5 ± 11 NS

't' test * $P < 0.025$

NS Not Significant

TABLE 35

Serum Concentration (Mean ± S.D.) of SASP and Total Sulphapyridine in Patients with Crohn's Disease in relation to extent of the disease

Serum ug/ml	"Regional Ileitis" n = 19	Ileitis and Segmental Colitis n = 10
SASP	5.1 ± 6.9	15.4 ± 16
Total SP	17.3 ± 9.0	28.4 ± 11.3*

* 't' test; $P < 0.005$

Table 36

Serum Concentration (Mean ± S.D.) of SASP and Total Sulphapyridine in patients with Crohn's Disease treated with different dosage of SASP

Dose G/day	No. of Patients	SASP ug/ml	Total SP ug/ml
2	11	4.3 ± 3.5	20.4 ± 8.0* Ø
3	9	6.8 ± 7.8	28.5 ± 10.6* ⌀
4	9	8.1 ± 11.3	33.5 ± 13.4⌀ Ø

't' test * $P < 0.05$

Ø $P < 0.07$

⌀ Not Significant

Table 37

Serum concentration (M ± S.D.) of SASP and Total Sulphapyridine in Crohn's disease (outpatients) treated with Plain or Enteric Coated (E.C.) Tablets.

Type of Tablet	SASP ug/ml	Total SP ug/ml
Plain	11.7 ± 14.1	24.4 ± 12.1
E.C.	3.7 ± 2.8	20.1 ± 8.4

between 3 and 4G/day was however not significant.

The effect of plain and enteric coated (E.C.) tablet on serum SASP and total SP (Mean ± S.D.) concentration are shown in Table 37. Serum SASP was lower in patients taking E.C. tablets compared with plain tablet but total SP did not show any difference. These findings are parallel to those of ulcerative colitis (page 103).

SUMMARY

The study of SASP metabolism in the limited number of patients with Crohn's disease was further complicated by the variation of anatomical site of lesion, and also through consequences of surgical intervention. A steady state in serum was obtained for SASP from day 1 and for total SP from day 5 at a level of 4 ug/ml and 22 ug/ml respectively. Serum concentration and twenty-four hour urinary excretion of SASP and the SP metabolites were lower than those in ulcerative colitis. The relationship between the clinical state and total SP was not as marked as in ulcerative colitis. About 70% of the patients in remission and 40% of the patients with activity had serum total SP > 20 ug/ml.

CHAPTER VII

SIDE EFFECTS RELATED TO SULPHASALAZINE THERAPY

SECTION I : Side effects and their relation to acetylator phenotype and serum concentration of SASP and its metabolites

SECTION II : Procedures adopted toovercome toxic effects

SIDE EFFECTS RELATED TO SULPHASALAZINE THERAPY

This chapter deals with the side effects which developed during SASP therapy. Section I describes the patients who exhibited the toxicity and their correlation with dosage, acetylator phenotype and serum concentrations of SASP and its metabolites.

In Section II the procedures adopted to overcome these adverse reactions are described along with the follow-up of drug metabolites level in the blood during the stage when toxicity disappeared.

SECTION I

PATIENTS WITH TOXICITY AND THEIR ACETYLATOR PHENOTYPE, SERUM CONCENTRATIONS OF SASP/METABOLITES

Side effects for the inpatients - a total of sixteen out of thirty-four patients who were treated as inpatients showed various side effects. These sixteen patients include nine of the sixteen patients with ulcerative colitis (Page 95), one other U.C.patient (J.H.) with colostomy (Page161) two (M.C. and R.M.) of the seven patients with C.D. (Page 109) and four other patients with C.D. who were included during the stage of exhibiting side effects (Page107).

Side effects for the outpatients - in twelve out of ninety-nine patients studied, side effects were observed at the stage when the patients had been taking the drug for six months to fifteen years.

RESULTS

Table 38 shows the twenty-eight patients with different toxic manifestations. The overal incidence of side effects was 21.5%. Of the twenty-eight patients, nineteen patients had ulcerative colitis and nine

Table 38

Different Side Effects in a total of 28 patients with ulcerative colitis or Crohn's Disease during SASP therapy and their Acetylator Phenotype

Side Effects*	Disease		Acetylator Phenotype	
	U.C. (n = 19)	C.D. (n = 9)	Slow (24)	Fast (4)
Sickness of early onset	1	1	0	2
Sickness of late onset	4	0	4	0
Rashes	2	0	1	1
Drug Fever	1	0	1	0
'Cyanosis'	5	5	9	1
Frank Haemolysis	2	3	5	0
Transient Reticulocytosis	6	0	6	0
Leucopaenia	1	0	0	1
Agranulocytosis	1	0	1	0
Headache	3	0	2	0
Dizziness	2	0	2	0

* 9 patients had multiple side effects

patients had Crohn's disease. Nine patients had multiple side effects, e.g. sickness, headache, dizziness and drug fever. Patients with gastro-intestinal side effects or headache and dizziness did not have haemolytic anaemia or cyanosis. Two patients who were on 6 and 8G of SASP/day respectively showed both cyanosis followed by evidence of frank haemolysis with Heinz bodies present (Figure 17).

Of these twenty-eight patients, twelve patients were outpatients who had been taking SASP for six months to fifteen years. Among these twelve patients, six had "cyanosis", two patients complained of headache and four developed reticulocytosis (three with maintained haemoglobin) and one (E.C.) with a drop of haemoglobin and presence of fragmented R.B.C. in peripheral blood. Two patients with cyanosis and all four with reticulocytosis had their SASP dosage increased in the recent past (two weeks to sixteen weeks).

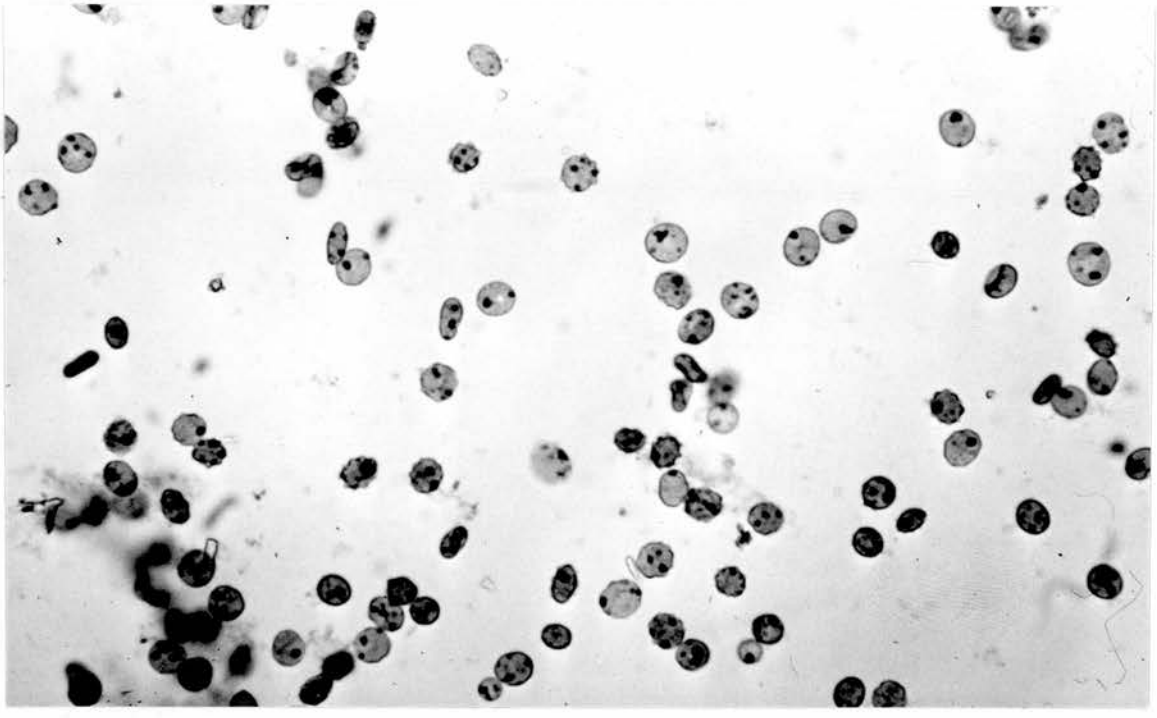
Of the sixteen inpatients fourteen showed side effects while they were in the ward (first four weeks) or subsequently during the next four weeks. The remaining two patients (M.C. and I.G.) had "cyanosis" during their readmission.

Of these twenty-eight patients, twenty-four patients were slow acetylators and four patients were fast acetylators, judged on the basis of sulphadimidine test or from the concentration of free and acetyl sulphapyridine in the serum or urine (Chapter IV)

NAUSEA AND/OR VOMITING

Table 39 shows four patients all with U.C. with persistent nausea and sickness which developed within a few days of starting treatment. Two other patients

Figure 17



Presence of Heinz bodies in red blood corpuscle.

Table 39

Nausea/Sickness of late onset during SASP therapy

Name	Age	Disease	Ac. Ph	Dose of SASP/day	Duration of Therapy before symptom developed	SASP ug/ml Serum	Total SP ug/ml Serum
A.P.	34	U.C.	Slow	4G(P) Cr I	10 days	10.4	87.1
				3G Cr II	28 days	3.4	50.0
W.R.	22	U.C.	Slow	4G(P)	16 days	60	50.1
D.F.	35	U.C.	Slow	3G(P and E.C.)	72 hours	13.6	72.0
J.H.	80	U.C.	Slow	3G(P and E.C.)	96 hours	2.4*	40.7*

* This sample of blood was collected 30 hours after stoppage of SASP

Cr I = 1st Course of SASP therapy

Cr II = 2nd Course of SASP therapy

who felt sick with the first dose of SASP therapy are excluded from the correlation with the serum concentration of SASP and total sulphapyridine.

All four patients were slow acetylators. Serum SASP concentration had a wide range (2.4 - 60 ug/ml.). However, three had considerably increased concentration of total sulphapyridine at the time of symptom (> 1 S.D. from mean of all other patients). In one patient (J.H.) blood was collected thirty hours after the stoppage of therapy when the sickness had started improving. The serum total SP in her was comparatively low (40.7 ug/ml.). Symptoms disappeared in other patients when the drug was stopped (Section II).

BLUISH DISCOLOURATION OF THE SKIN AND MUCOUS MEMBRANE, "CYANOSIS"

Table 40 shows the patients with "cyanosis". The disease, acetylator phenotype, dosage of SASP, duration before side effect developed, serum concentration of SASP and total sulphapyridine and the absence of sulphaemoglobin and methaemoglobin are also given. In all these ten patients SASP levels were within the expected range (Chapter V) but the total sulphapyridine level was significantly high (i.e.>1 S.D. from the overall mean) derived from patients who did not have side effects (Table 43). "Cyanosis" did not disappear immediately after stopping the drug but took five to seven days. Two patients also had "cyanosis" and haemolyasis with Heinz bodies. Sulph and Methaemoglobin could not be

TABLE 40

'Cyanosis' during SASP therapy

Name	Age	Disease	Ac. Ph	Dosage G/day	Duration	SASP ug/ml Serum	Total SP ug/ml Serum	Sulph. & Met. Hb.
P.T.	20	U.C.	Slow	8	2 weeks	11.3	71.8	N.P.
R.M.	24	C.D.	Slow	6	2 weeks	8.2	62.8	N.P.
M.C.	23	C.D.	Slow	4	6 months	4.8	54.2	N.P.
A.D.	51	C.D.	Slow	4	1 year	5.0	67.4	N.P.
C.B.	49	U.C.	Slow	3	1 year	15.0	63.5	N.P.
D.F.	74	U.C.	Fast	4	1 year	19.7	58.6	N.P.
T.B.	55	U.C.	Slow	4	1 year	10.0	70.0	N.P.
W.C.	61	U.C.	Slow	6	3G - 15 yrs. 4G - 1 month	29.5	54.5	N.P.
W.D.	49	C.D.	slow	8	4G - 1 year 8G - 2 weeks	6.7	94.5	N.P.
I.G.	51	C.D.	Slow	4	3G - 2 years 4G - 3 weeks	8.1	54.8	N.P.

N.P. - Not Present

detected in the blood of any of these patients.

"Cyanosis" developed both during the initial stage of therapy (two weeks) and during prolonged treatment (two years). All but one of the patients who were taking SASP for several years developed cyanosis following a recent increase in their dose. The dose with which the "cyanosis" developed was 4G or more except in one patient who was taking 3G of SASP per day for one year, (slow acetylator).

HAEMOLYSIS

Table 41 shows the patients who had evidence of haemolytic anaemia due to SASP therapy. The dose of SASP varied from 3 - 8 G/day and the toxic effect was noted from ten days onward up to six weeks. Four out of these five patients were inpatients and SASP was given to them for the first time. The one outpatient had recently increased the dose of SASP to 4.5 G/day following a relapse six weeks before. Two patients, as mentioned earlier, out of five had Heinz bodies (Figure 17). The fall of haemoglobin (G/100 ml.) and the highest retics index which developed are shown in Table 41. There was no evidence of blood loss from the bowel at this stage of improvement. The reticulocytosis and the fall in haemoglobin were noted suddenly about two to three weeks after starting treatment. Urobilingen was present in excess only in three patients and the Coombs' test was negative in all of them. Haptoglobin was within the normal range, methaemalbumin was absent. However,

Table 41

Frank Haemolysis and Transient Reticulocytosis during SASP therapy

Name		Age	Dosage G/per day	Duration	Fall of Hb, g%	Highest Retics Index	H.B.	F.E.	Coombs Test	Haptogl. & Metalb	SASP ug/ml Serum	Total SP ug/ml Serum
Frank Haemolysis												
P.T.	U.C.	20	8	10 days	1.7	10.4	Pr.	Pr.	Neg.	N.; N.D.	20	70
R.M.	C.D.	24	6	2 weeks	3.7	7.6	Pr.	Pr.	Neg.	N.; N.D.	8.2	62.8
E.C.	U.C.	28	5	6 weeks	3.0	8.4	Neg.	Pr.	-	-	57.7	91.0
R.W.	C.D.	20	3	2½ weeks	4	23.9	Neg.	Pr.	Neg.	N.; N.D.	7.4	47.8
			2	8 weeks	3	9.5	Neg.	Neg.	Neg.	N.; N.D.	6.1	42.5
J.B.	C.D.	17	4	2 weeks	2.4	6.7	Neg.	Pr.	Neg.	N.; N.D.	2.6	59.4
Transient Reticulocytosis												
J.R.	U.C.	25	4	22 weeks	0.4	8.2	Neg.	Neg.	Neg.	-	14.0	89.7
W.R.	U.C.	22	4	3 weeks	0.3	4.0	-	Neg.	-	-	47.4	50.9
A.P.	U.C.	34	4	12 days	0.8	3.7	Neg.	Neg.	Neg.	-	20.8	87.1
R.T.	U.C.	26	2 for 1 yr. then 3	4 weeks	0.0	3.8	-	Neg.	-	-	2.5	49.2
E.M.	U.C.	60	2 for 1 yr. then 4	4 months	0.0	5.2	-	-	-	-	30.0	37.8
M.L.	U.C.	72	2 for 1 yr. then 4.5	4 months	1.4	5.6	Neg.	-	-	-	18.3	63.5

Pr. - Present; Neg. - Negative; N.D. - Not Detected; - not known; F.E. Fragments of Erythrocytes; H.B. - Heinz Bodies; Haptogl. - Haptoglobin; Metalb - Methaemalbumin; N. - Normal

fragments of R.B.C. were noted in the peripheral blood in all of them.

Serum SASP concentration varied widely (2.6 - 57.7 ug/ml.) whereas the serum total sulphapyridine concentration was greater than 48 ug/ml. in all these patients.

TRANSIENT RETICULOCYTOSIS

Table 41 shows six patients with ulcerative colitis who had transient reticulocytosis without any evidence of rectal bleeding. There was no fall of haemoglobin and no other evidence of haemolysis. This was noted during the initial four weeks of treatment with 4G of SASP per day or four to sixteen weeks following a recent increase of dosage. Serum SASP concentration had a wide range (2.5 - 47.4 ug/ml.) but the total sulphapyridine concentration was high (i.e. > 1 S.D. higher than the mean of the patients without side effects)(Table 43).

LEUCOPAENIA AND AGRANULOCYTOSIS

Table 42 shows one patient with leucopaenia (W.B.C. < 4,000/cum.m.) and one with agranulocytosis. Toxicity developed six to eight weeks after starting the treatment. Both the patients were studied during the initial attack (Chapter V). The serum concentration of SASP was approximately 30 ug/ml. (Leucopaenia) and 19 mg/ml. (Agranulocytosis) whereas in both the cases the total sulphapyridine concentration was approximately 50 ug/ml. at the stage when toxicity developed. The detail case report of the patient with agranulocytosis is given below. Figure 18a and b show his bone marrow picture which shows

Table 42

Leucopaenia, Agranulocytosis, Drug Fever and Skin Rashes during SASP therapy

Name	Age	Disease	Ac. Ph	Dose of SASP	Duration of therapy before toxicity developed	SASP ug/ml Serum	Total SP ug/ml serum
Leucopaenia							
J.W.	72	U.C.	Fast	1G T.I.D.	6 weeks	2 samples 33.3) 28.0)	41.3) 50.0)
Agranulocytosis							
T.F.	47	U.C.	Slow	1G T.I.D.	7½ weeks	19	50
Drug Fever and Exanthema							
J.R.	25	U.C.	Slow	1G Q.I.D.	8th day	17.2	100.2
Rashes							
L.L.	21	U.C.	Slow	0.5G B.D.	24 hours	10	10
J.C.	28	U.C.	Fast	0.5G B.D.	10 - 24 hours	4.5	4.3

Figure 18a

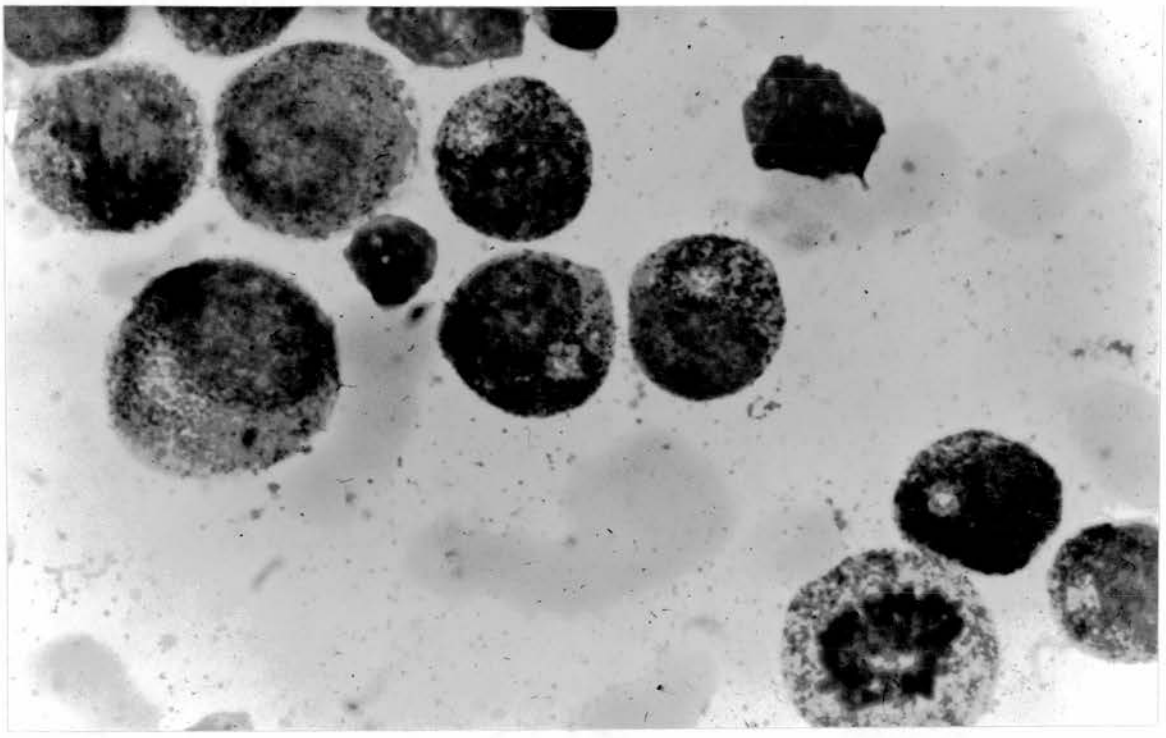
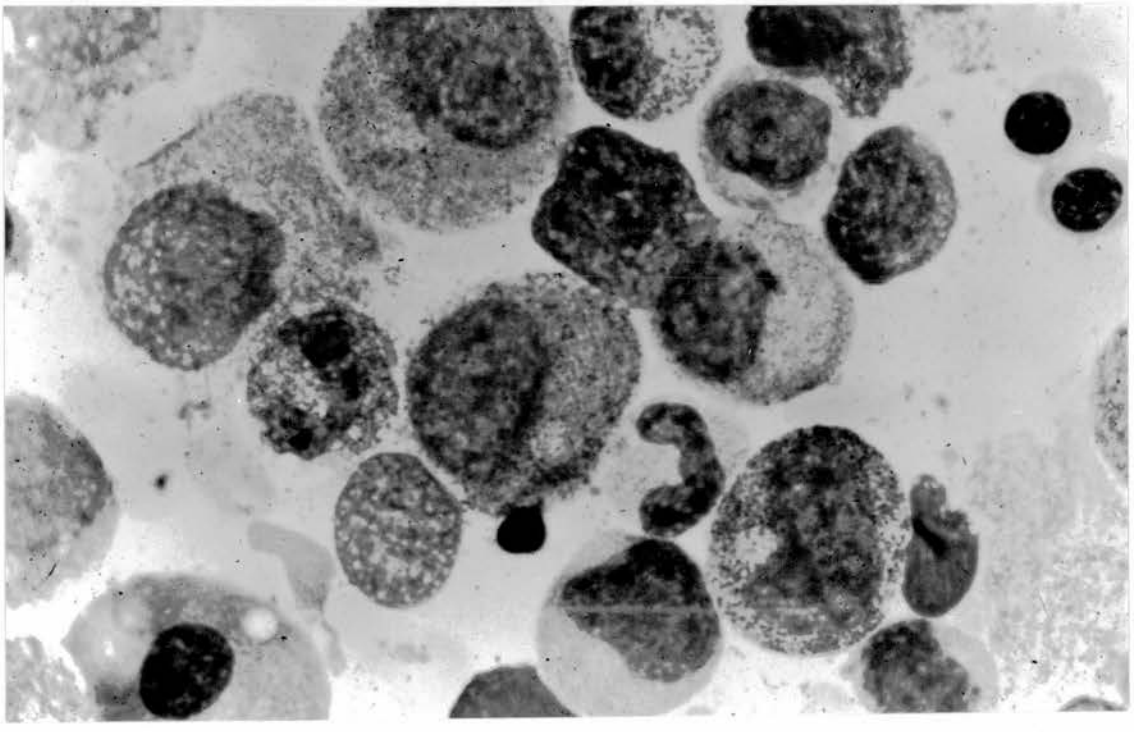


Figure 18b



Bone marrow picture showing maturation arrest of W.B.C. at myelocytic stage.

maturation arrest of granulocytes at the mylocyte stage. Following the stoppage of drug and other supportive measures the patient with agranulocytosis improved within seven to ten days. Figure 19 depicts his serum SASP and total SP concentrations from the beginning of therapy up to five days after stopping the drug.

The patient with leucopaenia improved following the stoppage of SASP therapy, however, the drug was reintroduced subsequently in a smaller dose (2G/day).

SKIN RASHES (MACULOPAPULER)

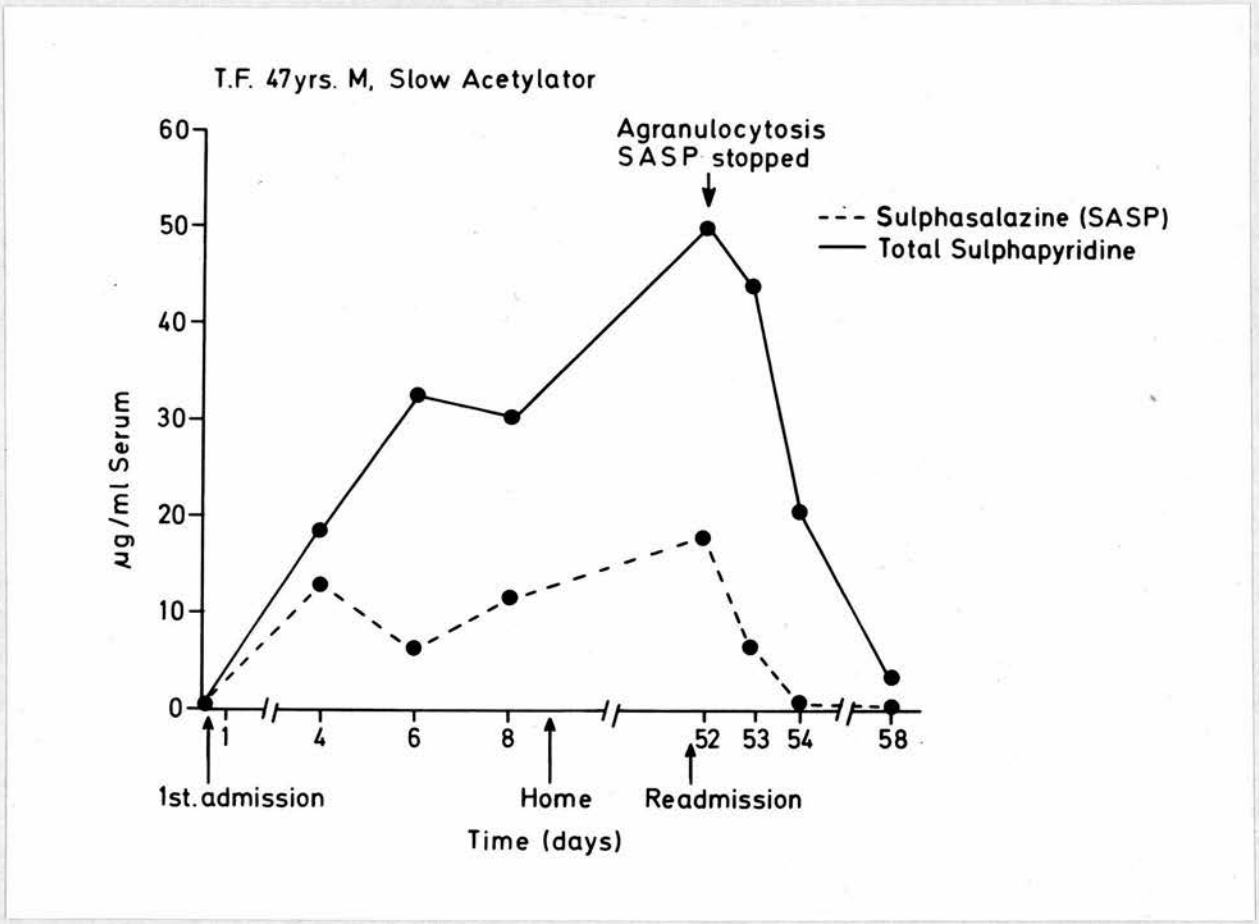
Table 42 shows two patients with drug rash and also one patient with drug fever and exanthema. Both the patients with drug rash had previous history of such a reaction with SASP after a short exposure (two to five days). A small dose of SASP (1G/day) was started yet they developed a rash within twelve to twenty-four hours despite low concentration of SASP and the serum total sulphapyridine.

The patient with fever and exanthema (J.R.) developed the side effect on the eighth day of treatment and she had a very high concentration of serum total sulphapyridine (i.e. 100 ug/ml.) at that stage. SASP concentration, however, was not high (17.2 ug/ml.). The symptoms cleared up within four to five days following stoppage of treatment. At this stage serum SASP was not detectable and total SP concentration was 3 ug/ml.

HEADACHE AND DIZZINESS

One patient with headache (A.P.) and two patients

Figure 19



Serum concentration of sulphasalazine and total sulphapyridine in the patient with agranulocytosis.

with dizziness (J.H., W.R.) had these complaints along with nausea and sickness (Table 39). One other patient (outpatient) complained of headache after an increase of dosage to 3G of SASP/day. Data on this patient was available after the dosage was reduced to 1.5G/day when he was symptom free (SASP 3.5 ug/ml., Total SP 20.2 ug/ml.).

Table 43 shows the serum concentration (M $\pm$ S.D.) of SASP, free sulphapyridine, acetyl sulphapyridine, sulphapyridine glucuronides, total sulphapyridine and 5-ASA in patients with and without side effects. In the patients with side effects there was a significant difference in the serum total sulphapyridine concentration ($p < 0.001$) and free SP ($p < 0.005$), compared to the patients without toxicity. The mean serum concentration of 5-aminosalicylic acid was < 1 ug. in fifteen out of the twenty-eight patients with side effects. The 5-aminosalicylic acid concentration in the serum was not measured in the remaining thirteen patients.

The patients without side effects include the inpatients and outpatients in remission and the twenty-eight patients described here prior to development of toxic symptom.

ACETYLATOR PHENOTYPE AND THE SIDE EFFECTS

About two thirds of the total patients studied were slow acetylators whereas 86% of the patients with various side effects were slow acetylators. Of the four fast acetylators with side effects, one had skin rash,

Table 43

Serum Concentration (M ± S.D.) of SASP and its Metabolites in patients with and without side effects during SASP therapy

Serum, ug/ml M ± S.D.	(A) Patients with toxicity	(B) Patients without toxicity		"t" test between (A) & (B)
		Inpatients in remission	Outpatients in remission	
SASP	9.9 ± 9.5	18.4 ± 11.8	12.1 ± 11.6	NS
Free SP	46.5 ± 15.6	28.6 ± 20.3	16.9 ± 8.7	P < 0.005
AcSP	10.5 ± 4.8	9.1 ± 5.2	8.6 ± 6.6	NS
SP-Glucuronides (SP - Gluc + AcSP Gluc)	6.4 ± 5.3	5.9 ± 3.2	4.2 ± 2.0	NS
Total SP	63.6 ± 16.6	43.9 ± 19.5	30.8 ±	P < 0.001
5-ASA	< 1.0	1.0 ± 0.9	< 1.0	NS

one patient had "cyanosis" and the third patient had leucopaenia. The fourth patient and the same patient with leucopaenia had nausea from the beginning of the therapy (i.e. immediately after first dose). All the patients with nausea and sickness of late onset, haemolysis, transient reticulocytotis, and nine out of ten patients with cyanosis were, therefore, slow acetylators.

SUMMARY

Most of the toxic symptoms observed with SASP could be related with a high concentration of total SP ($\geq$ 50 ug/ml). No such correlation was observed with SASP, AcSP, SP-Gluc., AcSP-Gluc and 5-ASA. The majority of the patients with side effects were taking 4G or more of SASP per day.

SECTION II

PROCEDURES ADOPTED TO OVERCOME SASP TOXICITY AND FOLLOW-UP RESULTS

This section describes the procedures adopted to overcome the toxic symptoms due to SASP therapy developed in twenty-eight patients in the course of this study. In two of the twenty-eight patients the drug had to be stopped altogether; these included one patient with agranulocytosis, one with haemolysis.

Four additional patients in whom side effects (haemolysis -2, skin rash - 1, persistent vomiting - 1) had led to discontinuation of SASP therapy in the past (1 - 10 years) are also included.

The basic principles adopted were as follows -

(a) Patients with nausea/sickness - stoppage of drug for five to seven days and then restarted in a smaller dose, usually 2G/day.

(b) Patients with "cyanosis" - reduction of dosage.

(c) Patients with haemolysis and transient reticulocytosis - stoppage of the drug for five to seven days or reduction of dosage by about 50%.

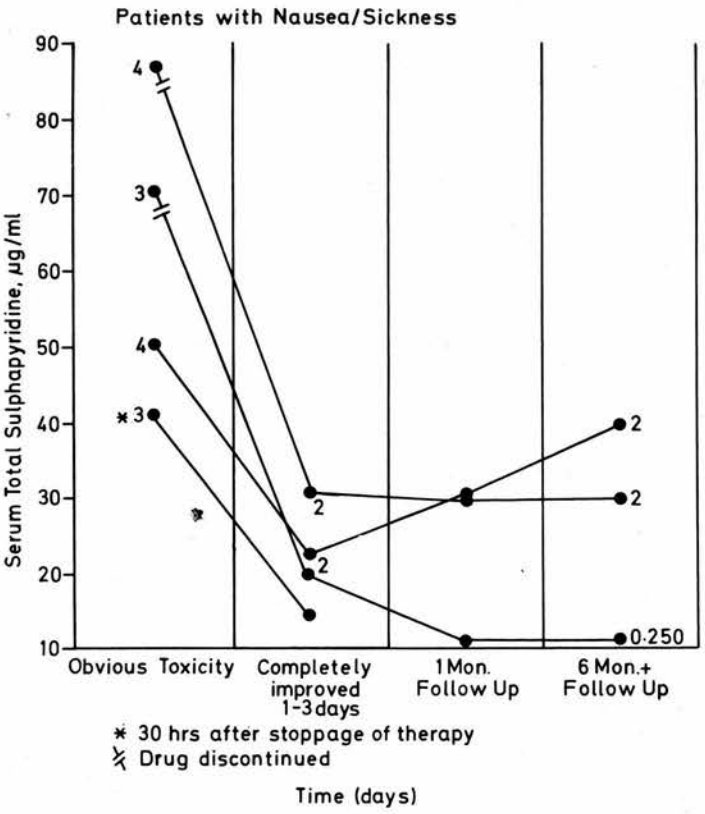
(d) Patients with history of rash - initially 1G/day then increased it to 2G/day after a week. Antihistamine was also added.

RESULTS

PATIENTS WITH NAUSEA/SICKNESS OF LATE ONSET

Figure 20 shows the serum concentration of total sulphapyridine in the four patients with nausea and

Figure 20



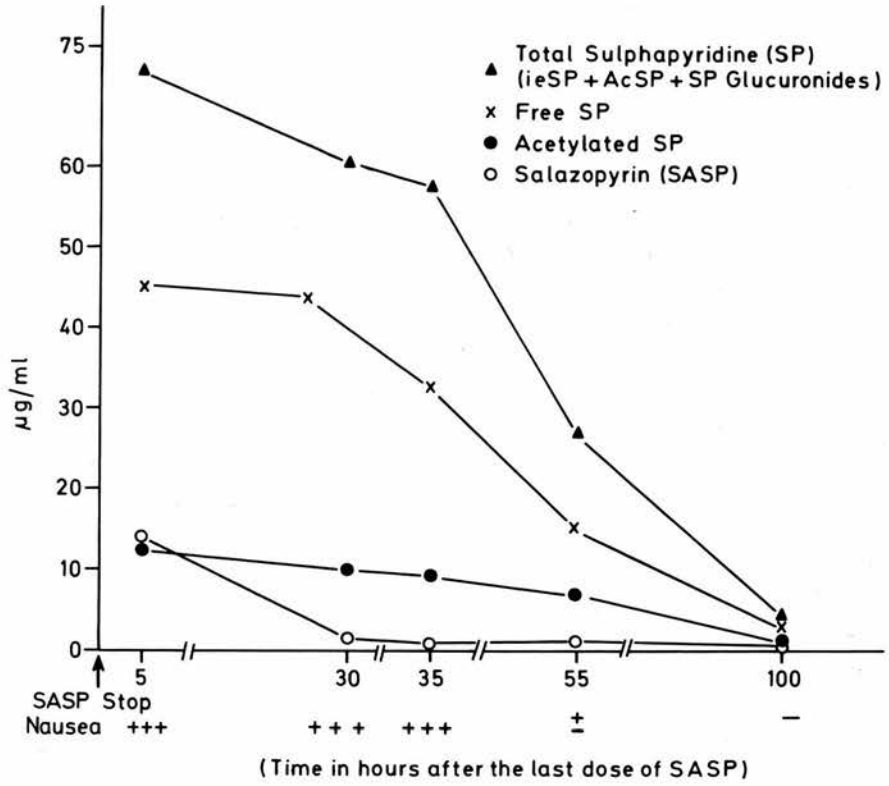
The effect of stoppage and reintroduction of a smaller dose of sulphasalazine (SASP) on the serum concentration of total SP in four patients with nausea and sickness.

Figures (0.250, 2, 3, and 4) indicate the dose of SASP, G/day

sickness of late onset during symptoms and following improvement. In three out of the four patients the drug had to be discontinued. In two of them plain tablets were changed into enteric coated tablets but sickness continued and serum total sulphapyridine concentration also did not change with enteric coated tablets so long as the dose was the same. Symptoms only improved after stoppage of the drug. Figure 21 shows the regression of symptom in one patient (D.F.) and the serum concentrations of SASP, total SP, free SP and AcSP. The serum concentrations were followed until the patient improved completely during the next three to four days. In one patient only who had slight nausea reduction of the dose from 4G to 2G/day improved the situation.

In one other patient the drug was reintroduced in a very small dose 1G/day but the patient complained of some abdominal discomfort after ten days (blood level not available). The dose was reduced to 0.25G/day and symptoms disappeared. The dosage was continued for more than six months and his serum total sulphapyridine level at six months follow-up was 10.5 ug/ml. He was in remission at that stage. One patient developed sickness seventy-two hours after taking 3G of SASP/day. Her serum total SP level was 41 ug/ml. thirty hours after the stoppage of the drug by which time her sickness started improving. Symptom completely improved after a further twenty-four hours when serum total SP level was 15 ug/ml. She was subsequently given SASP 2G/day

Figure 21



The relationship between the symptom and the serum concentration of SASP, SP and its metabolites after stopping SASP therapy.

and has been tolerating this for six months.

PATIENTS WITH NAUSEA OF EARLY ONSET

Two other patients complained of nausea from the beginning of the therapy. Enteric coated tablet improved the situation in one and in the other patient the drug could be continued after reassurance and some perseverence without further symptom.

PATIENTS WITH "CYANOSIS"

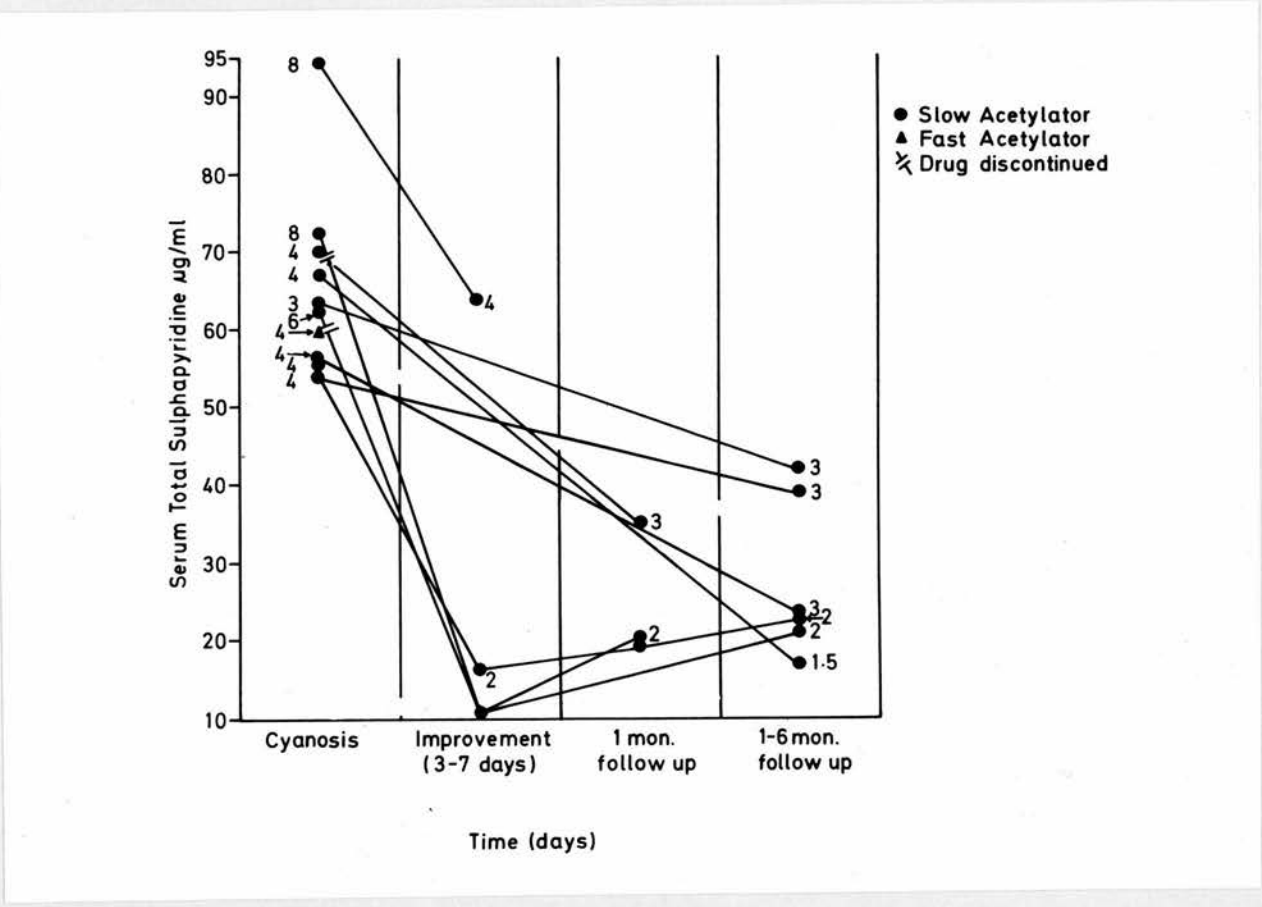
Figure 22 shows the serum concentration of total sulphapyridine in the twelve patients with "cyanosis" initially and after improvement following stoppage or reduction of the dose.

PATIENTS WITH FRANK HAEMOLYSIS AND TRANSIENT RETICULOCYTOSIS

Figures 23 and 24 show the serum total SP concentration of the patients with obvious haemolysis and patients with reticulocytosis without any other evidence of haemolysis during the stage of toxicity and following improvement. Reduction of the dose stopped haemolysis in two patients whereas in three patients stoppage of the drug was necessary (Figure 23). In these three patients SASP was subsequently reintroduced in a smaller dose 1 - 2 G/day. Peripheral blood picture was closely observed frequently. The patients tolerated it well up to six months follow-up but one patient continued to have evidence of haemolysis as soon as the dose was increased to 2G/day and the drug was stopped altogether for him.

All the patients except one with reticulocytosis improved, after reduction of the dose (Figure 24) and one

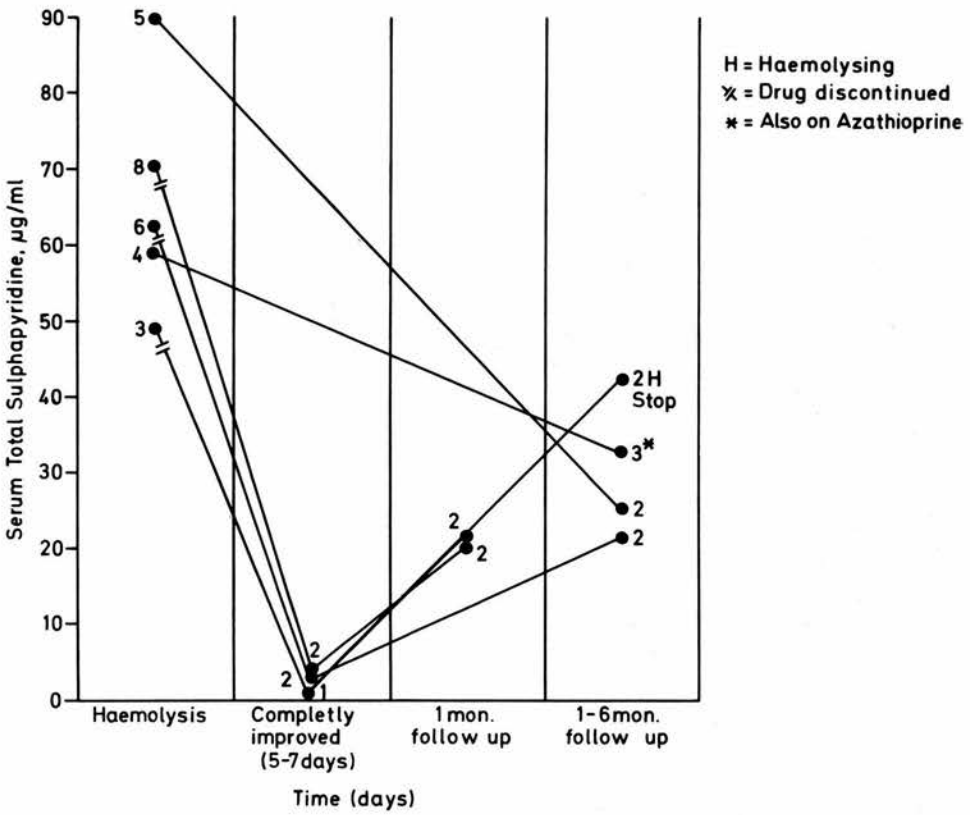
Figure 22



The effect of stoppage or reduction of sulphasalazine on the serum concentration of total sulphapyridine in patients with "cyanosis".

Figures (1.5, 2, 3, 4, 6, 8) indicate the dosage of SASP, G/day.

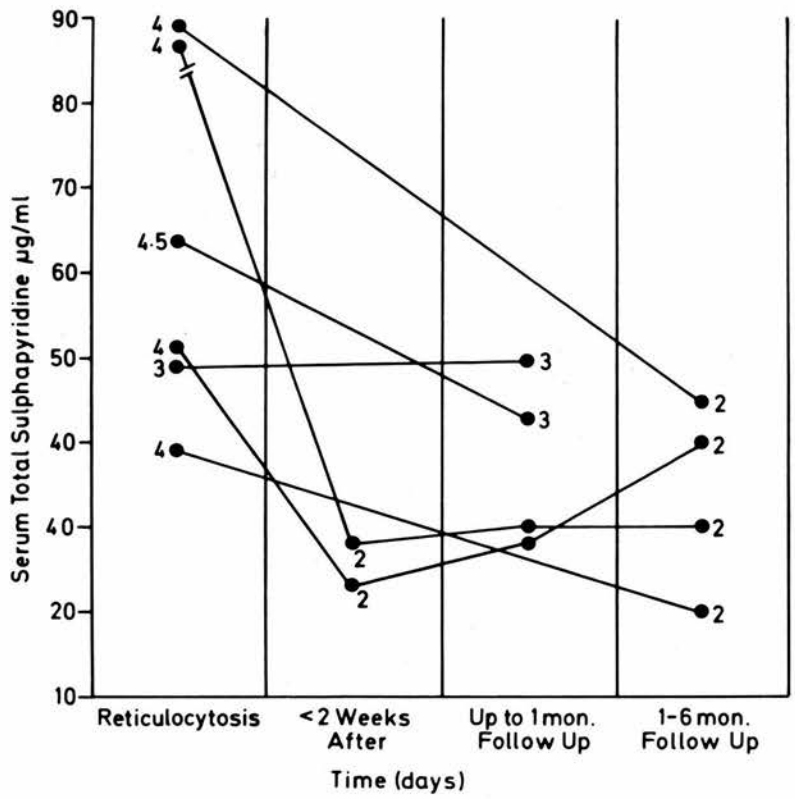
Figure 23



The effect of stoppage and reduction of sulphasalazine (SASP)therapy on the serum concentration of total sulphapyridine in patients with haemolysis.

Figures (1, 2, 3, 4, 5, 6, 8) indicate the dosage of SASP, G/day.

Figure 24



The effect of stoppage and reduction of sulphasalazine (SASP) therapy on the serum concentration of total sulphapyridine in patients with reticulocytosis.

Figures (2, 3, 4, 4.5) indicate the dosage of SASP, G/day.

patient improved without change of the dose (3G). The patient in whom the drug was discontinued temporarily also had sickness in addition to reticulocytosis.

PATIENTS WITH SKIN RASH

One patient (U.C. fast acetylator) had fever and rash five days following SASP therapy about eight months ago. This initial attack was then treated with Prednisolone only in some other hospital. Prednisolone was stopped after eight weeks. Figure 25 shows the attempt to reintroduce the drug when he was admitted to this Unit with a relapse. SASP was started at the dose of 1G/day (without any steroids) then gradually built up over a long period. Within twelve to twenty-four hours he developed rash over the exposed areas of the body. This was associated with flushing of the skin and itching. The drug was continued at the same dose, 0.5G twice daily and Phenergan was added at the dose of 10 mg. three times daily. By the seventh day rashes disappeared completely and hence SASP was increased to 0.5G three times daily and continued until the fourteenth day when it was increased to four times daily. Injection of Synacthen was added to the regime at the end of the third week as the patient was not improving from colitis. SASP was subsequently increased to 3G followed by 4G/day because he did not have the blood level of serum total sulphapyridine in the "therapeutic range". Synacthen was discontinued after five weeks as it did not change the patient's clinical state. He continued to have mild symptoms of colitis

Figure 25

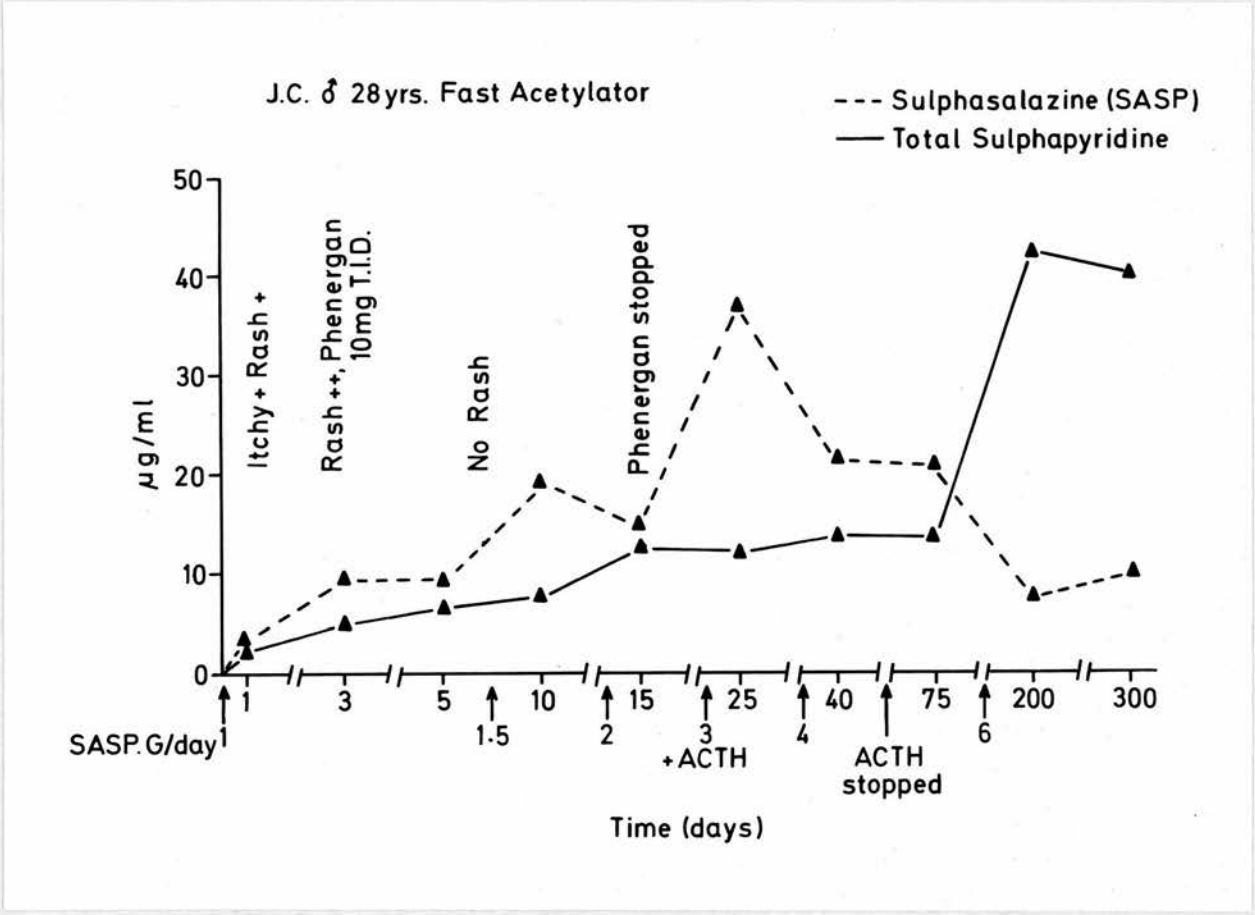


Figure illustrates the procedure adopted to reintroduce sulphasalazine (SASP) to a patient with a history of rash to SASP.

and on day ninety-two SASP was increased to 6G/day. Following this his serum total SP level went to the "therapeutic range" coincidental with the clinical improvement. The patient has been tolerating the drug well for about a year and he is in remission. The second patient with rash (L.L.) improved after stoppage of the drug. It could be reintroduced uneventfully by giving a small dose (1G/day) for a week followed by 2G/day at which it was maintained.

PATIENT WITH DRUG FEVER

One patient (J.R.) who developed fever with exanthema on the eighth day of treatment with 4G SASP/day improved following stoppage of the drug. Five days after the drug was restarted with the same dose along with Prednisolone 40 mg/day. The patient did not show any side effects subsequently. The dose was reduced to 2G/day after six months for transient reticulocytosis, without any other evidence of haemolysis.

REINTRODUCTION OF THE DRUG IN FOUR PATIENTS WITH PAST HISTORY OF TOXICITY

Table 44 shows the four patients who had different side effects related to SASP therapy one to ten years ago. SASP was discontinued in them since then. Reintroduction of the drug (SASP) could be successfully carried out in these four patients. One of them had history of rash, two had haemolysis and one had persistent sickness. The drug was started with a smaller dose then gradually built up to 2G/day. All of them were slow acetylators. Serum SASP and total sulphapyridine concentration are shown in

Table44

Re-introduction of SASP in 4 patients with a past history of SASP-toxicity 1 - 10 years ago

Name	Disease	Past Side Effects	Duration of Stoppage of SASP	SASP starting dose G/day	Serum Conc. during Maintenance dose SASP ug/ml	Total SP ug/ml	Ac. Ph
A.G.	U.C.	Skin rash	10 years	1 x 7 days 1.5 x 7 days 2 continued	2.7	24.8	Slow
A.W.	C.D.	Sickness	1 year	1 x 7 days 1.5 x 7 days 2 continued	10	22.0	Slow
M.M.	C.D.	Haemolysis	1½ years	2 continued	4.2	10.5	Slow
A.B.	C.D.	Haemolysis	2 years	2 continued	3.7	21.7	Slow

the table while they were on maintenance dose.

SUMMARY

Most of the toxic symptoms could be overcome by stopping the drug temporarily or reducing the dosage. SASP, therapy could thus be continued in twenty-six out of twenty-eight patients who exhibited side effects. The decrease in serum total SP concentration coincided with the improvement of the symptoms. Reintroduction of a therapeutic dose of SASP was possible by giving a small dose initially and then gradually increasing the dose.

CASE REPORT OF THE PATIENT WITH AGRANULOCYTOSIS DUE TO SASP

Male, 43 years, (65.5 kg.) admitted in May 1971 with three months history of diarrhoea, blood and mucous both present, 38°C, sigmoidoscopy - evidence of active proctitis.

Initial investigation showed - 24.5.1971 - Hb 13.6g%, P.C.V. 37%, M.C.H.C. 36%, E.S.R. 33 m.m. 1st hour platelets 283000/cumm., W.B.C. 10400/cumm., Neutrophils 74%, Lymphocyte 16%, Monocyte 10%, Prothrombin time 11.5 sec. (control 10.5).

Schilling test - 12.4%

Serum Folate - 6.1 ng/ml.

Examination of stool (on two different occasions) - No ova, parasite or cyst; no dysenteric group of organism.

Liver function test - All within normal limit.

Urea and electrolytes, serum calcium, magnesium and phosphate - normal.

Rectal biopsy - evidence of active proctitis.

Jejunal and liver biopsy - normal

Radiology - Barium enema showed distal ulcerative proctocolitis in acute phase.

Barium meal and follow through - normal

Acetylator phenotype (sulphadimidine test) - slow acetylator.

He was treated with - Predsol Enema, Oral Prednisolone - 30 mgm/day initially, sulphasalazine - 3G/day, Nystatin and Slow K. To this regime he improved

steadily and was discharged from the hospital after two weeks. Prednisolone was progressively reduced and then discontinued during the next four weeks. Sulphasalazine was continued in the dose of 3G/day.

Six weeks after discharge he was readmitted as an emergency with severe perineal pain for five days, no bowel movement for three days and with evidence of toxaemia. Temperature was 40°C on admission. Perineal examination revealed an ischiorectal abscess. A distended loop along the area of the transverse and descending colon could be felt. Systemic examination was otherwise negative. Chest and abdominal x-rays were negative. Sputum and M.S.U. - negative. Serum electrolytes were normal. Blood culture was negative. On admission peripheral blood picture showed W.B.C. 3,200 with 2% neutrophil (Table 42A).

SASP was immediately stopped and following treatment was started - Gentamycin 40 mg. I.M. 8 hourly, Prednisolone 60 mgm/day.

Ischio rectal abscess burst spontaneously with hot fomentation within four days of admission.

Figure 19 shows the serum sulphasalazine and total sulphapyridine concentrations during first and second admissions. Bone marrow examination immediately after second admission showed evidence of maturation arrest of W.B.C. in myelocytic stage. Erythropoiesis and megakaryocytes were normal (Figure 18a and b).

The patient improved progressively and allowed to convalescent home after ten days.

Table 42A shows the peripheral blood pictures at different times.

Table 42 (A) shows the peripheral blood pictures on different days in a patient with Agranulocytosis

1971	May 24	June 24	July 19	20	21	22	23	24	26
Hb, G/100 ml	13.6	12.3	10.1	10.5	10.5	10.2	10.2	9.4	10.1
P.C.V. %	37	37	31	29	30	28	32	29	31
Platelets X 10^3/cu.mm.	'Plenty'	-	'Plenty'	259	'Plenty'	416	-	Present	'Plenty'
WBC x 10^3/cu.mm	10	6.7	3.6	5.5	5.5	7.2	13.9	13.5	12.8
Neutrophil %	74	-	2	0	34	65	68	72	59
Lymphocyte %	16	-	53	44	56	22	20	17	20
Monocyte %	10	-	45	54	10	13	10	7	19
Eosinophil %	-	-	-	1	-	-	1	1	2
Myclocyte %	-	-	-	-	-	-	1	3	-
Retics Index	-	4.5	1.7	1.9	4.5	-	-	-	-

CHAPTER VIII

SULPHASALAZINE METABOLISM IN PATIENTS WITH ILEOSTOMY AND COLOSTOMY

SULPHASALAZINE METABOLISM IN PATIENTS WITH ILEOSTOMY AND COLOSTOMY

This chapter describes the metabolism of SASP in patients with ulcerative colitis and Crohn's disease before and after ileostomy or colostomy. The clinical details are given below.

I. Patients with ileostomy (n = 8)

Ulcerative colitis = 6 patients; Male - 4; female - 2.
Age : 20 - 50 years.

Crohn's disease = 2 patients; Male -1; female - 1.
Age : 17 & 42 years.

Study Carried out: Before and after operation - 4 patients (U.C.)

After operation only - 4 patients (2 U.C. and 2 C.D.)

Time of study following operation: For ulcerative colitis - Studied from day 1 of SASP treatment (2/3 weeks after operation) up to one month follow-up in all, up to six months follow-up in four.

For Crohn's disease - Both patients had ileostomy > 6 years ago. They were studied from day 1 after starting SASP to ten days in one and eight months in the other.

II. Patients with transverse colostomy (n = 2)

Disease: U.C. = 1 (80 years) Female) Both studied after operation.
C.D. = 1 (33 years) Male)

Patients and time of study following operation:

1. One patient (J.H.) with ulcerative colitis had defunctioning transverse colostomy for left sided colitis and diverticular disease two months prior to the study. She was studied during one to three days after starting SASP. On the fourth day SASP was discontinued for persistent vomiting.
2. The second patient (J.Cr.) had an ileo-transverse anastomosis for "regional ileitis" seven years ago and distal transverse colostomy for Crohn's colitis involving the descending colon four years prior to the study. He had been taking SASP for six months before the study.

RESULTS

Table 45 shows the concentration of SASP and its SP metabolites in the serum and urine in one representative patient (A.D.) with ulcerative colitis before and after operation. The SASP concentration in the serum and the twenty-four hour urinary excretion did not show any significant change, whereas the total SP in the serum was markedly lowered (from 37 to < 5 ug/ml) and the urinary excretion of total SP metabolites also decreased significantly (from 52% of the dose to $< 5\%$) following ileostomy.

The serum concentration and twenty-four hour urinary excretion of SASP and total SP after one and six months of operation did not show any significant change.

Table 46 shows the serum concentration and urinary excretion (mean $\pm$ S.D.) of SASP and total SP in all the four patients just prior to surgery and one to six months after subtotal or total colectomy with ileostomy. Serum SASP concentration and twenty-four hour urinary excretion of SASP did not alter significantly whereas there was a significant decrease in the total SP concentration both in serum and urine.

Table 47 shows the serum concentration and urinary excretion of SASP and total SP in the six patients with ulcerative colitis and two patients with Crohn's disease after ileostomy. The results for patients with ulcerative colitis were obtained up to six months following ileostomy. As reported earlier, the two patients with Crohn's disease

Table 45

Salicylazosulphapyridine metabolism in ulcerative colitis before and after colectomy, in one patient (A.D.)

	Time	Serum (ug/ml)		Urine (% of dose in 24 hours)	
		SASP	Total SP	SASP	Total SP
SASP 4G/day					
> 6 months					
Before surgery	15.00	10.9	36.7	4.9%	52%
Two weeks after sub-total colectomy with ileostomy SASP 4G/day					
Day 10	10.00	9.0	5.5	0.5%	3%
2 months	15.00	13.8	4.4	5.8%	3.2%
6 months	15.30	15.4	3.5	5.2%	3.4%

Table 46

Mean (± S.D.) serum concentration and twenty-four hour urinary excretion of sulphasalazine and sulphapyridine metabolites in four patients with ulcerative colitis before and after ileostomy.

	Before Ileostomy		After Ileostomy	
	Serum ug/ml	Urine % of dose in 24 hrs.	Serum ug/ml	Urine % of dose in 24 hrs.
SASP	20.2 ± 14.6	4.2 ± 2.8	15.2 ± 7.2	4.6 ± 3.0
Total Sulphapyridine	27.6 ± 5.7	42.5 ± 20.4	4.4 ± 1.4	3.5 ± 1.7

Table 47

Mean (± S.D.) Serum concentration and twenty-four hour urinary excretion of SASP and sulphapyridine metabolites in patients with ulcerative colitis or Crohn's disease following ileostomy.

	Ulcerative Colitis n = 6 patients		Crohn's disease n = 2 patients	
	Serum ug/ml	Urine % of dose in 24 hrs.	Serum ug/ml	Urine % dose in 24 hrs.
SASP	15.7 ± 5.2	3.6 ± 2.8	2.6 ± 2.2	3.3 ± 1.5
Total Sulphapyridine	3.8 ± 1.5	1.5 ± 0.4	2.15 ± 0.07	1.6 ± 1.4

had ileostomy more than 6 years ago. . The table shows the serum concentration and twenty-four hour urinary excretion of SASP and total SP in these two patients after ten days (M.Mc) and eight months (C.Mc) of SASP therapy. The twenty-four hour urinary excretion of total SP was < 2% of the administered dose both in U.C. and C.D. with ileostomy. The serum concentration of total SP was also very low (< 4 ug/ml). The serum concentration and twenty-four hour urinary excretion of SASP did not differ significantly when compared to other patients with ulcerative colitis (Page 90) and C.D. (Page 112) without ileostomy. The concentrations were lower in patients with Crohn's disease and ileostomy than in the patients with ulcerative colitis and ileostomy.

5-AMINOSALICYLIC ACID

5-aminosalicylic acid concentration in the serum of the four patients studied before ileostomy ranged from 0.2 - 1.4 ug/ml but could not be detected after operation. The remaining four patients who were studied only after ileostomy had 5-ASA in the range of 0 - 0.4 ug/ml serum. The urinary excretion of 5-ASA was also low (< 1% of the dose) in patients with ileostomy compared to the patients before ileostomy (12.5 - 23.7% of the dose) (Table 48).

SASP EXCRETION IN ILEOSTOMY EFFLUENT

In four patients with subtotal colectomy who were being treated with SASP for rectal disease in situ the recovery of sulphasalazine in the twenty-four hour

Table 48

Serum concentration and twenty-four hour urinary excretion of 5-ASA in patients before and after ileostomy.

	Serum ug/ml	Urine, % of dose in 24 hours.
Before ileostomy (n = 4)	0.2 - 1.4	12.5 - 23.7
After ileostomy (same 4 patients)	0	<1%
Post-ileostomy (other 4 patients)	0 - 0.4	<1%
U.C.Patients (in steady state)	1 ± 0.9	21.2 ± 7.0

ileostomy effluent was 69.6 ± 15.5% of the administered dose. In two of these patients, effluent was collected for twenty-four hours after one month of SASP therapy and in the other two after six months of treatment. Sulphapyridine was also present in the ileostomy effluent (14.4 ± 6.9% of the dose). Splitting of SASP presumably occurred in the terminal ileum or during collection.

Table 49 shows the serum concentration and twenty-four hour urinary excretion of SASP and total SP in two patients with transverse colostomy. Both patients had a significantly higher concentration of SP in serum and urine than the ileostomy patients. The patient (J.Cr.) who had ileotransverse anastomosis and distal transverse colostomy had a lower level of serum total SP and less urinary SP than the second patient who has only transverse colostomy. The SASP concentration in these two patients did not differ significantly from the patients with ileostomy.

One patient (G.K.) had the rectum excised eight months after subtotal colectomy with ileostomy. SASP was discontinued following this operation. Six months after excision of the rectum an acute experiment with a single dose of sulphasalazine (2G) was carried out. Figure 26 shows the concentrations of SASP and total SP in serum at various time intervals up to forty-eight hours. Urinary excretion (0 - 48 hours)

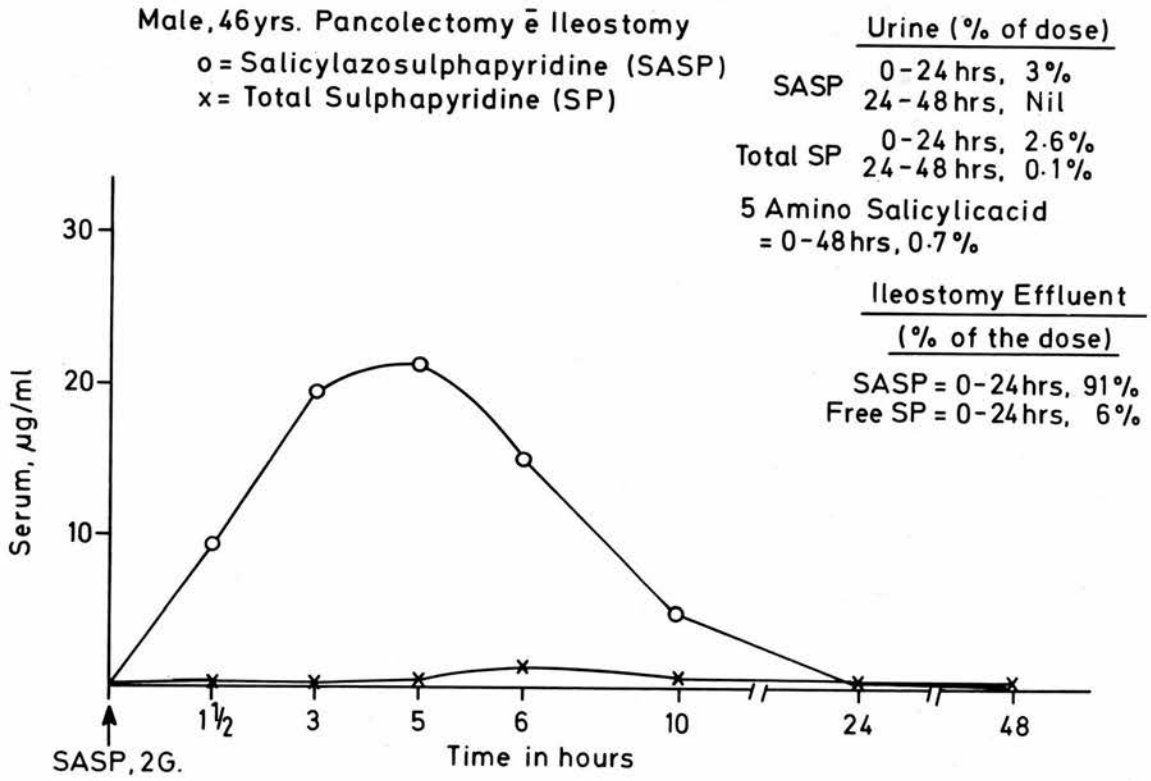
Table 49

Serum concentration and 24 hour urinary excretion of Sulphasalazine and Total Sulphapyridine in 2 patients with Transverse Colostomy

Both were taking SASP 3G/day

	Patient No. 1 (C.D.) Ileotransverse anastomosis and transverse colostomy		Patient No. 2 (U.C.) Transverse Colostomy only	
	Serum ug/ml	Urine % of dose in 24 hrs.	Serum ug/ml	Urine % of dose in 24 hrs.
SASP	8.5	2.6	7.9	3.1
Total Sulphapyridine	12.2	48.2	35.9	58.6

Figure 26



The effect of time on the serum concentration of sulphasalazine (SASP) and total sulphapyridine (total SP) in a patient with ileostomy after a single dose of SASP. The excretion of SASP and its metabolites in urine and ileostomy effluent is given as percentage of the administered dose, for SASP, % of administered dose of SASP and for SP % of administered amount of SP present in SASP.

of SASP, total SP and 5-ASA and twenty-four hour recovery of SASP and SP in ileostomy effluent are also shown in Figure 26.

SUMMARY

After ileostomy there was a significant decrease in the concentration of total sulphapyridine in serum and urine. This was accompanied by a significant decrease in urinary excretion of 5-aminosalicylic acid. There was, however, no significant difference in sulphasalazine concentration in serum and urine following ileostomy. Ileostomy effluent contained approximately 70% of the administered dose of SASP as sulphasalazine.

In the two patients with transverse colostomy the concentration of SP in serum and urine was significantly higher than the patients with ileostomy. Sulphasalazine concentration, however, did not differ significantly.

CHAPTER IX

DISCUSSION

DISCUSSION

Although the role of sulphasalazine therapy in the management of ulcerative colitis is well established, knowledge of its metabolism in man is limited (Svartz et al, 1945; Bottiger and Möllerberg, 1959; and Schröder and Campbell, 1972). Knoweledge of its mode of action was particularly lacking and this is essential for better understanding of its therapeutic role.

Measurements of serum levels of different drugs, e.g. Di-phenylhydantoin, Procainamide, Nortriptyline and Digoxin, are found to be clinically useful (Bigger et al, 1968; Koch-Weser and Klein, 1971; Asburg et al, 1971). But at the same time a large individual variation in the plasma levels is also possible, e.g. chlorpromazine and hydralazine (Curry et al, 1970: Zacest & Koch-Weser, 1972). Information of a therapeutic range of any drug is clinically helpful. As serum concentrations rise above the therapeutic range the frequency and severity of toxic effects increase progressivly though some overlapping is possible (Koch-Weser, 1972). In connection with sulphasalazine, however, one has to consider both from the therapeutic and toxicological point of view, the role of unsplit drug SASP and its two components SP and 5-ASA with their metabolites.

Hanngren et al (1963) have shown by autoradiographic studies in mice that SASP, because of its affinity for connective tissue, is fixed in the colonic wall, where they suggest it exerts therapeutic effect. However, Collins (1968) and Thayer (1970) have argued against this suggestion because ulcerative colitis is a mucosal disease.

Before studying the metabolism of SASP in the patients it was felt that the routes of SASP metabolism in healthy persons should be confirmed. It was also felt that iron, being known to "seriously interfere" with the absorption of tetracyclines in man (Neuvonen et al, 1970) might also affect SASP absorption. This was considered important as most of the patients on SASP therapy are also given iron. Comparison with another cation (Calcium) was also made.

A major part of sulphasalazine consists of sulphapyridine which might have a parallel acetylation phenotype to other drugs (Evans, 1968). Knowledge of this polymorphic acetylation in the patients which have been studied was thought to be important from both therapeutic and toxicological points of view.

A metabolic study was then carried out in patients with ulcerative colitis and Crohn's disease both during acute attacks and also in patients who were on long term therapy. Toxicity was evaluated in the light of acetylator phenotype, drug level and dosage.

To provide further information on the small intestinal absorption of the drug and the role of colon, patients with ileostomy were also studied. Fortunately four of the eight patients with ileostomy were studied before they had undergone surgery and therefore they served as their own controls.

ACUTE EXPERIMENTS IN HEALTHY VOLUNTEERS

The study of five volunteers confirmed the findings

of Schröder and Campbell (1972) that SASP absorption occurred more rapidly than sulphapyridine (Figures 5, 6 and Table 8). The appearance of SP and its metabolites in serum three to five hours after ingestion of SASP is consistent with the concept of bacterial splitting of SASP in the colon followed by its absorption (Bottiger and Möllerberg, 1959; Schröder and Campbell, 1972). The excretion of sulphapyridine is to a certain extent dictated by the acetylator phenotype (Schröder and Evans, 1972 (a)). The five volunteers had sulphapyridine in their serum after forty-eight hours and in one volunteer sulphapyridine was detected in the urine after six days (Table 10). This may be due to continued absorption of SP from the colon and also the effect of acetylator phenotype.

Iron significantly decreased the absorption of SASP but did not alter the time when the peak concentration was reached. The 'in vitro' experiments suggest that this effect is due to the precipitation of SASP as an insoluble ferric salt or by coprecipitation with ferric hydroxide (Figure 7 and Table 13). It has also been shown that ferrous sulphate interferes with the absorption of tetracyclines in man, (Neuvonen et al, 1972).

Calcium merely delayed the time of the peak serum concentration of SASP but did not affect the absorption of SASP (Figure 5). This effect could be the result of calcium delaying gastric emptying time (Hunt and Pathak, 1960). Total SP concentrations did not vary after

administration of iron or calcium along with SASP (Figure 6).

In one of the volunteers there was, in comparison to the other individuals, a rapid appearance of SP and its metabolites in the serum. This subject subsequently had a laparotomy and a single jejunal diverticulum was found. The removal of this diverticulum resulted in a more typical absorption pattern. Presumably bacterial colonisation of the diverticulum resulted in a more rapid splitting of the parent drug. This was not proven by bacteriological study as the diverticulum was unsuspected preoperatively. These studies suggest that SASP is absorbed from the small intestine where its absorption may be changed by coincidental drug therapy and SP is probably absorbed from the colon.

ACETYLATION POLYMORPHISM OF SULPHAPYRIDINE IN PATIENTS WITH ULCERATIVE COLITIS AND CROHN'S DISEASE

Certain drugs, e.g. isoniazid, sulphadimidine, hydralazine and dapsone share the same acetylation polymorphism which can influence the clearance of these drugs from the serum (Evans and White, 1964 ; Gelber et al, 1971). During SASP therapy in patients with ulcerative colitis or Crohn's disease, sulphapyridine obtained from SASP was found to share the same polymorphic acetylation as sulphadimidine (Figure 8).

Schröder and Evans (1972) has also reported parallel polymorphic acetylation of sulphapyridine and sulphadimidine in healthy volunteers.

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The bimodal distribution of acetylation phenotype in patients with ulcerative colitis or Crohn's disease was comparable both from serum and urine in patients with active disease or in remission (Figure 9). In the patients studied there were fewer fast acetylators than in the healthy volunteers (about 45%) studied by Schröder and Evans (1972). This could be due to the patients studied belonging to a separate cross section of the population, for example, they have a different age (17 - 80 years) and sex distribution compared to the healthy volunteers studied by Schröder and Evans (about 25 $\pm$ 1.5 years). They are also selected by different criteria, i.e. susceptibility to ulcerative colitis and Crohn's disease.

It was noted that more women than men were fast acetylators (Table 19). Such sex difference is worth noting. Whether this reflects susceptibility to the disease remains to be answered.

As the acetylation phenotype is genetically controlled it is expected that this should be constant to a certain extent in any individual. As the information of knowing the acetylator phenotype may be of some importance in therapy (Harris, 1961) it is convenient to know if one determination of the acetylator phenotype is sufficient in the absence of severe liver damage (Levi et al, 1968). There are few studies (Hughes et al, 1954; White and Evans, 1968) in which this has been followed over a period of

time and particularly during the transition from active disease to remission state. In this study a constancy of acetylation of sulphapyridine obtained from SASP in patients with ulcerative colitis or Crohn's disease was noted during the period of one year (Table 17). This constancy was unaffected by activity of the disease. Phenotyping from SP concentration following SASP therapy was possible from urine measurements after day 2 of therapy and from day 5 from serum.

In the steady state an increase or a decrease of the dose of SASP (between 2 - 8 G/day) did not alter the percentage of acetylation of SP in serum and urine to a significant degree, although the concentration of total SP in the serum and urine increased or decreased respectively. This was found to be true for the concentration range of 10 to 80 ug/ml. (Table 18). Possibly slow and fast acetylators have different enzyme systems which are not saturated within this concentration range.

Although many of these patients were taking other drugs including corticosteroids, diazepam, nitrazepam, iron, codeine and sometimes barbiturates, acetylation was apparently not influenced by these drugs. Levi, Sherlock and Walker (1968) showed that acetylation of isoniazid was undisturbed in patients with liver damage provided the bilirubin level was less than 2 mg/100 ml. of serum.

HYDROXYLATION OF SP AND CONJUGATION WITH GLUCURONIC ACID

In addition to N-^{4} acetylation, sulphapyridine undergoes hydroxylation in position five of the pyridine ring

followed by conjugation with glucuronic acid (Schröder and Campbell, 1972). These transformations are produced by induceable hepatic microsomal enzymes (Cucinell *et al*, 1965; Conney, 1967; Evans, 1970). Unlike acetylation polymorphism there was no correlation between percentage hydroxylation (expressed as percent glucuronidised out of total SP) and serum total SP concentration. Similar observations were reported by Schröder and Evans (1972 (a)). But in patients who were slow acetylators and poor hydroxylators the serum total SP concentrations were high (page 87). This has also been reported in normal volunteers (Schröder and Campbell, 1972). Hydroxylation was not as constant as acetylation (Page 91). This may be due to the effect of coincidental administration of other drugs such as corticosteroids and sometimes barbiturates, compounds known to induce hydroxylation in the liver (Catz and Taffe, 1962; Conney, 1967) or of the disease state. Older patients appear to be poor hydroxylators and six out of fifteen patients over 60 years exhibited toxicity (21% for whole groups, Page 127). No correlation was found between total SP glucuronide concentration in the urine and the acetylator phenotype. Schröder and Evans (1972 (a) and (b)) reported similar findings in normal volunteers.

ULCERATIVE COLITIS

INPATIENT STUDY

The steady state serum concentration of SASP was achieved within three days and the mean serum concentration was 10 - 15 ug/ml of serum (Figure 10). Between 1 and 13% of the drug was recovered unchanged in the urine during the first ten days of therapy and also in urine from patients on long term therapy. Svartz, et al 1945; Bottiger et al, 1959; and Schröder and Campbell, 1972, also reported similar findings in healthy persons.

Sulphapyridine and its metabolites were only demonstrable in the serum about three to five hours after the ingestion of the first dose of SASP in patients with ulcerative colitis. Since slphapyridine if administered alone can be mostly absorbed from the small intestine (Goodman and Gillman, 1970) this delay may be accounted for by the time taken for bacterial metabolism of SASP to SP and 5-ASA in the colon (Peppercorn and Goldman, 1972) and subsequent absorption through the colonic mucosa. This possibility was confirmed by the ileostomy study (vide later) in which situation very little sulphapyridine appeared in the systemic circulation (Tables 45 and 46).

It was found that these patients (11) who improved clinically within ten days of therapy had a significantly higher concentration of serum total SP than those (2) who did not (Figure 11).

A steady state serum concentration of total SP was achieved at a level of about 40 - 45 ug/ml within five

days (Figure 11) in the patients who responded to therapy. The serum concentration subsequently was lower as most patients had their dose regime reduced during or shortly after discharge.

When the patients who failed to improve were given an increased dose (6G/day from 4G/day) of SASP they improved clinically and their serum total SP concentration increased to the range of patients who responded initially. This suggests that there is a relationship between serum total SP concentration and the clinical state. This was further suggested by the results of the three patients admitted with relapse (Table 21). There was no relationship between serum SASP concentration and clinical state.

Urinary excretion of SASP was steady from day 1 and was low in all patients, whereas in patients with remission, the total SP excretion was significantly higher (57.6% of the administered dose (Table 24) than the two patients who did not initially improve (13% of the dose), but was lower than that reported for normal volunteers (80% of the dose) (Schröder and Campbell, 1972).

After absorption sulphapyridine is distributed throughout the whole body (Frisk, 1941; Hanngren et al, 1963). Further metabolism of SP is determined by acetylation polymorphism. Of the sixteen patients in this study, ten were slow acetylators and six were fast acetylators. No relationship between acetylator phenotype and clinical response was obtained.

There was a significant difference in the distribution of individual metabolites of SP in the urine among the slow and fast acetylators, but the total SP excretion did not show a significant change between the two groups during ten days of SASP therapy. However, the serum concentration of total SP tended to slowly increase when the patient is a slow acetylator and particularly if he is also a poor hydroxylator (Figure 12). There appears to be a relationship between toxicity, concentration of total SP and slow acetylators. Nine of the sixteen patients studied had unwanted side effects of the drug and of these, seven were slow acetylators. All seven patients had serum total SP concentration of over 50 ug/ml (Page 95).

It was found that clinical remission free from toxic effects was associated with a serum concentration of total SP of between 20 - 50 ug/ml.

The serum concentration of 5-ASA was found to be very low (1 ug/ml $\pm$ 0.9) though urinary excretion was about 22% of the dose. In healthy persons a 33% urinary excretion with serum concentration of $<$ 2 ug/ml have been reported (Schröder and Campbell, 1972). In view of the low serum concentration with wide overlapping of 5-ASA both in active and remission states, there was no correlation between serum concentration and clinical state. 5-ASA was found in the serum almost entirely in the free form, whereas in the urine it was mostly present as acetylated 5-ASA both in slow or fast acetylators. Schröder and Campbell (1972) reported similar observations

in healthy persons.

Though clinical remission equates with serum SP concentration, molar equivalent amount of 5-amino salicylic acid is liberated and remains in the colon, where it could exert some therapeutic effect.

From the follow-up study it seems that a maintenance dosage between 2 - 3G SASP/day was adequate. Fast acetylators required at least 3G SASP to reach such a concentration range, whereas slow acetylators may achieve this with 2G/day.

ULCERATIVE COLITIS

OUTPATIENT STUDY

It has been suggested that SASP is more effective in preventing relapse rather than in the treatment of acute episodes (Misiewicz et al, 1965). The serum concentrations of the drug were measured in patients on long term therapy (from six months to fifteen years) and related to the clinical situation.

Serum SASP concentration in the sixty-four outpatients were distributed over a wide range both during active and remission state and could not be correlated with disease activity (Table 27). Schröder and Campbell, 1972 suggested that such interindividual variation was due to differences in absorption. Another explanation suggested by the current study is that, as SASP has a shorter serum half-life (three to five hours), the serum concentration of SASP varies according to when the patient took the last dose before collection of blood. Many of

the patients in this study were taking the tablet twice daily, usually in the morning and late evening and the collection of serum was made in the outpatient clinic between 2 and 5 p.m.

This similarity in concentrations of SASP between patients with active disease or in remission could be due to the small bowel being free from disease and hence absorption will be comparable. This aspect was studied in patients with ileostomy and is discussed later.

In all but three (95%) of the fifty-one samples taken from patients in remission the serum total SP concentration exceeded 20 ug/ml, whereas in twenty-four of the thirty-one samples (77%) collected from patients during the active phase, serum total SP concentration was less than 20 ug/ml (Figure 13). About one third of both slow and fast acetylators were in active state of the disease. The serum total SP concentration reflects the rate of SP absorption from the colon and its elimination. Low serum values for total SP concentration in patients with active disease were found consistently in both studies, inpatient and outpatient irrespective of slow or fast acetylator, but it is not known whether this is the cause of the continued activity or an effect of it. From the inpatient study, however, it appears that absorption of SP occurs in the presence of active colitis (Figure 11).

The difference in the serum values of free SP compared in active and remission states may be due to the fact that the majority of the patients (two-thirds) were slow

acetylators and the main component of total SP in slow acetylators was free SP. Fast acetylators had a higher concentration of AcSP in the serum than free SP, irrespective of the clinical state of the disease, and the reverse was the case for slow acetylators (Tables 28 and 22). Free SP concentration in fast acetylators in remission was lower than that of slow acetylators in the active state (Table 28). Therefore, from the results of both the groups it was found that only total SP differed significantly during active and remission state irrespective of acetylator phenotype.

Nevertheless free SP may have a systemic effect in the alleviation of colitis (Svartz and Kallner, 1940(a) and Svartz, 1942). There have been no reports since then of patients being treated with sulphapyridine only, though other absorbable or nonabsorbable sulphonamides have been used with varying success (Gaspar, 1945; Trier, 1948; Mœrtel and Bargen, 1959).

The levels of 5-ASA in the serum of twenty-five patients with colitis, both with active disease and remission, were very low (0 - 2.8 ug/ml.). No correlation could be found between the disease state and the serum concentration of 5-ASA (Table 27).

The results provided guidance for the dose regime for SASP to be used in long term management of ulcerative colitis. Over half of the patients with active disease were taking 2G or less SASP per day resulting in a serum

total SP of less than 20 ug/ml (Figure 14), 80% of the patients in remission were taking 3G or more. However, seven of the nine patients who had side effects due to SASP (Page 127) were taking 4G or more of SASP/day and serum concentration of total SP in these patients was more than 50 ug/ml.

The serum concentration of SASP showed little relationship to dosage (Table 29) or to activity of the disease (Table 28). This might as well be the reflection of poor absorption of SASP from small intestine and also lack of significant difference in the amount of absorption after giving a higher dose (Müller-Wieland et al, 1970). Serum total SP concentrations varied significantly with dose (Table 29) and with the state of activity of the disease (Figure 14 and Table 28). Fast acetylators had lower serum total SP concentration than slow acetylators (Figure 13). There was, however, no relationship between the serum concentration of total SP and the dosage of SASP in terms of mg/kg of body weight. This was also shown by Schröder and Campbell (1972). This further indicates the colonic absorption of SP after SASP being split in the colon. The net amount of SP available in the colon will therefore depend on the dosage administered. The lack of relationship between the clinical state and serum SASP concentration, but a significant relationship between clinical state and serum total SP suggests the effect of the drug from the luminal side rather than the serosal (by SASP) side.

From this data it appears that 3G SASP/day is the most effective dose for therapeutic benefit with least chance of having side effects. Slow acetylators sometimes may have "adequate" serum concentrations with 2G SASP/day, and may show toxic effect with 3G/day, illustrating the value of knowing the acetylator phenotype.

Seven patients had active colitis, despite serum concentration of the drug and its metabolites in the same range of the patients in remission (Figure 13). They were also receiving corticosteroids and in four cases azathioprine. These patients may represent a different form of ulcerative colitis who show resistance to all medical therapy and in whom serum concentrations of the drug or its metabolites bear no relationship to the disease state. Three of these patients (A.D., C.S. and E.C.) subsequently required colonic surgery (vide ileostomy study, Page 161).

The response to treatment did not appear to be influenced by the type of tablet (plain or enteric coated) used. The only difference observed between plain and enteric coated tablets was the slightly lower serum concentration of SASP with the latter, but this did not apply to the SP metabolites (Page 103). The contents of enteric coated tablets are probably not so readily adsorbed as those of the plain tablets. When SASP reaches the colon the type of tablet is of little importance.

CROHN'S DISEASE

INPATIENT STUDY

In the seven patients studied with Crohn's disease

interpretation was difficult because the number of patients was small, the dosage was variable, the anatomical extent of the disease differed and three patients had surgical intervention. With the limited data available, it appears that the metabolism of SASP is similar to that in ulcerative colitis.

In these patients the dose of SASP varied from 2G to 6G/day and the results (Page 112) would suggest that there is less absorption of SASP from the small intestine and SP from the colon. This is probably due to functional loss of ileum and colon due to disease and surgical intervention (Page 110). Four of the seven patients were in remission up to one year of follow-up and their SASP concentration at the steady state ranged from 0.4 to 10 ug/ml and total SP exceeded 20 ug/ml. Although in ulcerative colitis serum concentration of total SP over 20 ug/ml equated with clinical remission, in Crohn's disease this relationship was not so obvious. These biochemical findings are perhaps in keeping with the conflicting clinical reports of the value of SASP therapy in Crohn's disease (Lennard-Jones, 1970; Meeuwisse and Hansing, 1970; Goldstein and Murdock, 1971; Cooke, 1972).

OUTPATIENT STUDY

In the twenty-nine outpatients studied there was no significant relationship between serum SASP concentration and the disease state (Figure 15). Serum total SP concentration, however, correlated with the clinical state but to a lesser degree than in ulcerative colitis.

Although 74% of the patients in remission had serum total SP concentration greater than 20 ug/ml, 40% of the patients with active disease had serum total SP concentration in the same range. These findings are further in keeping with the inpatient study and reflect the conflicting clinical impressions as mentioned earlier. But some benefit with SASP therapy can be suggested from these results.

It would appear that patients with an ileotransverse anastomosis (of at least two years) metabolised SASP similarly to patients with an intact gastro-intestinal tract (Table 34).

Increased serum concentrations of total SP in patients with both ileal and colon disease compared to those with only ileal disease (Table 35) may be due to enhanced splitting of SASP by changes in colonic flora or increased absorption of SP resulting from increased colonic mucosal premeability (Harris et al, 1972).

The effect of dosage was reflected in the serum total SP but not in the SASP concentration (Table 36). These differences are similar to those discussed in the ulcerative colitis outpatient study.

The type of tablet, plain or enteric coated, had no significant effect on serum total SP concentration or serum SASP concentration.

ADVERSE REACTIONS DURING SASP THERAPY

During SASP therapy twenty-eight of the one hundred and thirty-three patients studied had various side effects (Table 38). These included nausea and vomiting, skin

rash, fever, "cyanosis" and different blood dyscrasias. Various side effects have been reported (Svartz, 1942, 1948, 1954; Lagercrantz, 1949; Morrison, 1953; Moertel and Bargen, 1959; Lennard-Jones et al, 1960; Truelove and Watkinson, 1962; Baron et al, 1962; Dick et al, 1964; Misiewicz et al, 1965; Collins, 1968; Wallace, 1970 and Hilditch, 1972; Schröder and Evans, 1972 (b)). In the present study the overall incidence of side effects was 21% (including four patients who were included after exhibition of side effects) which compared favourably with previous reports (9 to 55%). Although in patients on long term therapy the incidence was lower (12%). However, the relationship of these side effects to serum concentration of SASP and its metabolites had not previously been investigated. From the present study, serum SASP concentration was not related to side effects but there was a significant association with serum total SP concentration of more than 50 ug/ml and toxic symptoms. These side effects due to SASP therapy occurred mainly during the introduction of the drug (first six to eight weeks). Similar observations were also made by Baron et al (1962); Dick et al (1964) and Misiewicz et al (1965). The greater incidence of toxicity during the first six to eight weeks of therapy is probably due to initial use of higher doses of SASP. Whereas the low incidence on long term therapy may be related to lower dose and also the development of a steady state tolerated by the tissue. Side effects in patients on long term therapy mainly

occurred after an increase in the dose of SASP.

The nausea and vomiting which occurred from the onset of therapy which could be overcome by perseverance or by changing to an enteric coated tablet (Page 148) may be due to a gastric irritant effect. However, nausea and vomiting of later onset could only be overcome by stopping the drug (Figure 20) and is probably due to the high total serum SP concentration (Table 39, Figure 21). Sulphapyridine therapy has also been shown to cause nausea and vomiting but no serum values of SP were quoted (Hawking and Lawrence, 1950). In one of the patients vomiting started again four weeks after starting the second course of SASP therapy with a reduced dose. Symptom correlated with a high serum total SP concentration (50 ug/ml) although this level was lower than the first course of therapy. This may be related to physical exertion which may enhance side effects (Evans, 1969; Schröder and Evans, 1972 (b)) and the patient was back to her normal work during the second course of therapy.

Bluish discolouration of the skin and mucosa in patients on SASP therapy has been described previously (Svartz, 1942). However, the cause of this apparent "cyanosis" is not yet clear. In the present study "cyanosis" occurred in ten patients, six of whom were outpatients and were on long term therapy. Although cyanosis was related to the high serum total sulphapyridine concentration ($>$ 55 ug/ml) (Table 40) sulph-

haemoglobin and methaemoglobin were not detected in any of these patients. This was also observed by Svartz and Kallner (1940 (b)) and Svartz (1942). The oxygen combining power of blood in their study was normal. This was not measured in this study. They suggested that cyanosis is due to the formation of a compound (not sulphhaemoglobin) which was thought to be a combination of the amide component of the sulphonamide and the porphyrin moiety of the haemoglobin.

Haemolytic anaemia due to SASP (sometimes with Heinz bodies) (Table 41; Figure 17) has previously been reported in the literature (Spriggs et al, 1958; Böttiger et al, 1963; Gardner et al, 1964; Dick et al, 1964). Dyer (1972) suggested the possibility of Glucose-6 Phosphatedehydrogenase (G6PD) deficiency related to SASP-induced anaemia. G6PD deficiency anaemia with sulphonamides is well known (Meyler, 1966). G6PD estimations were not carried out in the present study. Six other patients had a transient reticulocytosis with no alteration in the Hb (Table 41). A similar finding was reported by Dyer (1972).

Leucopaenia and agranulocytosis during SASP therapy have been reported (Thirkettle et al, 1963; Collins, 1968). The mechanism of these side effects in sulphasalazine therapy is not clear. In the present study both occurred during the first six to eight weeks of therapy (Table 42). A similar finding was reported by Ritz and Fisher (1960). Moeschlin et al, (1960) and Evans et al (1958) reported

the formation of leukoagglutinin in patients treated with sulphapyridine and sulphasalazine respectively. However, the relationship of these toxic reactions with blood level of SASP and SP has not been previously investigated. In the patient with leucopaenia the drug was reintroduced in a smaller dose (1G/day increasing to 2G/day after seven days) without complication. No attempt was made to reintroduce the drug in the patient with agranulocytosis.

Skin rashes which appeared within twelve to forty-eight hours of starting treatment (1G/day) in two patients could not be related to the serum concentration of SASP and SP and were probably due to a tissue sensitivity to sulphonamides. The occurrence of skin rashes has been reported in different series (Svartz, 1942; 1948; Lennard-Jones et al, 1960; Truelove et al, 1968; Collins, 1968).

Fever and exanthema were reported by Svartz (1942) to be "common toxic manifestations" during the seventh to ninth day of SASP therapy. In the present study, however, only one patient had this toxic symtpom which developed on the eighth day of therapy, coincidental with a very high serum total SP (over more than 90 ug/ml).

In the present study the side effects were associated with both the serum total SP concentration and the dosage of SASP but not with the serum concentration of SASP, AcSP or SP-glucuronides or 5-ASA (Table 43). In 1967, Van der Grient (1967) stated that high plasma concentrations of sulphonamides were associated with toxic symptoms.

Unfortunately no plasma values were given. A relationship between the side effects of SASP with dosage was previously reported (Bargen, 1962; Baron et al, 1962; Dick et al, 1964). Most of the patients with side effects in the current study were taking 4G SASP or more a day.

Of the twenty-eight patients with side effects twenty-four were slow acetylators, suggesting that acetylator phenotype is important in relation to the development of toxicity of SASP. This is probably due to the fact that slow acetylators accumulate SP in the blood and increased frequency of toxicity for slow acetylators has been reported for isoniazid phenelzine and hydralazine (Hughes et al, 1954; Devadatta et al, 1960; Evans et al, 1965; Perry et al, 1967). Of the four fast acetylators exhibiting toxic symptoms, two were nauseated from the beginning of therapy, one had "cyanosis" and one had skin rash. None of these complications except "cyanosis" could be correlated with serum total SP concentration.

PROCEDURES ADOPTED TO OVERCOME SASP TOXICITY

In two of the twenty-eight patients who exhibited side effects, SASP therapy was stopped permanently (Page 144).

Reduction of dosage to 2G/day, temporary stoppage of the drug followed by reintroduction of SASP in a smaller dose (1G/day) with gradual increase to 2G/day, but not beyond 3G/day was found to be equally useful. Serial blood counts, specially in patients with previous haematological complications and administration of antihistamines for sensitivity rashes which were independent of dosage

were also successful procedures in overcoming the side effects (Figure 20 to 25 and Table 44). One patient (fast acetylator) who had skin rash with 1G SASP/day has been tolerating 6G/day for over one year (Figure 25). Svartz, 1942, also reported successful reintroduction of the drug after "desensitisation" with a small dose and "injection of "liver extracts".

SASP METABOLITES IN PATIENTS WITH ILEOSTOMY AND COLOSTOMY

There is controversy regarding the amount of SASP absorbed from the small intestine. Svartz et al (1945) suggested that the drug is largely absorbed from the small intestine, whereas Müller-Weiland et al (1970) found poor absorption at this site.

Schröder and Campbell (1972) have more recently reported that "one third" of the administered dose of SASP is absorbed from the small intestine, whether the drug is excreted in the bile is unknown.

The results of the present study suggest that -

1. Small intestinal absorption of SASP is up to about 10% of the administered dose. This absorption of SASP is not modified by colectomy and hence SASP as such is not absorbed from the colon. This was demonstrated by the results of the four patients who were studied before and after ileostomy (Tables 45 and 46).
2. Most SASP is split in the colon to SP and 5-ASA probably by bacteria. Peppercorn and Goldman (1972) have recently shown that after giving an oral dose of SASP to germ-free rats only 1 - 2% of the drug could be

recovered in the urine and most of it was in the stool as unsplit SASP. There were no split products obtained in the urine which were, however, exclusively recovered after giving SASP to conventional rats. Walker (1970) and Soleim and Schelkine (1972) reported that most of the strains of gut bacteria can split various azo compounds for example food colouring agents. Historically important antibacterials like Prontosil and Neoprontosil exert their therapeutic effect due to their active metabolite sulphanilamide which may be liberated by azoreduction with the help of gut flora (Gingell et al, 1969).

The current study has shown that in patients with ileostomy receiving SASP for up to eleven months, the ileostomy effluent contains 70% unchanged SASP and 14% free SP. Further evidence of the role of the colon was provided by the fact that one patient with ileo-transverse anastomosis and transverse colostomy absorbed less SP than one with transverse colostomy (Table 49), probably because of a reduced colonic absorbing surface. The latter patient with a transverse colostomy had the same concentration range of SASP and total SP both in serum and urine compared to the patients with intact colon. It has been reported that colostomy flora is similar to normal faecal flora (Scarpino et al, 1969; Finegold et al, 1970) and can presumably split adequate amount of SASP.

3. A small amount ($< 5\%$ of the administered dose) of SP metabolites is excreted in the urine following ileostomy.

This SP may be derived from SASP split by liver azoreductase (Fouts et al, 1957) or bacterial colonisation of the terminal ileum, although two patients with Crohn's disease who had ileostomy for more than six years had very low serum and urinary SP concentrations (Table 47). This may be due to low splitting of SASP as a result of difference in bacterial flora. Gorbach et al, 1967 reported that the bacteria flora in ileostomy effluent is different both quantitatively and qualitatively when compared to normal faecal flora. The bacteriological study of the ileostomy effluent in the present study was however not carried out. The low SP may also be due to inadequate time of absorption and ileal involvement, particularly in Crohn's disease.

Figure 27 summarises the absorption metabolism and excretion of SASP and its metabolites in patients with ulcerative colitis.

The present study extends knowledge of sulphasalazine metabolism in man particularly in patients with ulcerative colitis and Crohn's disease. The ileostomy study further establishes that only a small portion of SASP is absorbed from the small intestine and presence of the colon is essential for splitting of SASP into its components SP and 5-ASA. Most of the SP, but only a small proportion of 5-ASA is absorbed from the colon. Serum total sulphapyridine correlated to dosage in patients with ulcerative colitis and Crohn's disease and a serum concentration of total SP over 20 ug/ml equated with

Figure 27

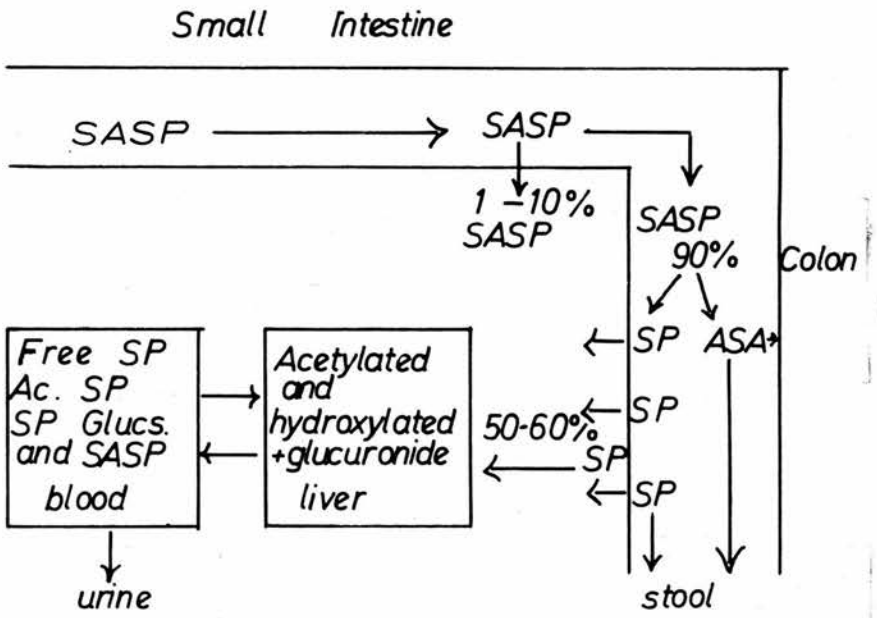

Figure illustrates the absorption, distribution and excretion of sulphasalazine and its metabolites in patients with ulcerative colitis

the therapeutic success particularly in patients with ulcerative colitis. Serum concentration of total SP in excess of 50 ug/ml produced various side effects. The serum concentration was related to acetylator phenotype and to a lesser extent hydroxylation capacity. These side effects could be overcome by temporary stoppage or reduction of the dosage. It is important that all patients treated with SASP should be screened for acetylator phenotype to determine the optimum maintenance dosage.

Unlike serum total SP concentration, the concentrations of SASP or 5-ASA in the serum could not be associated with therapeutic success or toxic symptoms. It is possible that SASP is serving as a vehicle which allows either or both of its metabolites to reach the site of inflammation. There has been no study on the therapeutic efficacy of 5-ASA. Further investigation regarding its role is necessary, particularly because of the known anti-inflammatory effects of salicylic acid and its different derivatives. It is also possible that the therapeutic effect is achieved during the passage of SP through the mucosa of the colon. Further study to evaluate the effect of sulphapyridine when administered alone, orally, (and possibly by enema) in patients with inflammatory bowel disease is also indicated.

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APPENDICES

APPENDIX I

The chemicals which were used for the different extraction procedures are listed below.

1. Pure sulphasalazine - supplied by Pharmacia, Sweden
2. Pure sulphapyridine - supplied by Pharmacia, Sweden
3. Pure acetylsulphapyridine - supplied by Pharmacia, Sweden.
4. Pure acetyl 5-aminosalicylic acid - supplied by Pharmacia, Sweden.
5. Hydrochloric acid (analar) specific gravity 1.16, BDH, England.
6. Amylacetate (analar), BDH, England.
7. Sodium hydroxyde (analar), BDH, England
8. β -glucuronidase/arylsulphatase, Boehringer, Mannheim, 15427E GAF.
9. Limpet acetone powder, Sigma.
10. Acetate buffer (A) acetic acid (analar), BDH, England.
11. Sodium chloride (analar), BDH, England.
12. IsoButyl methyl ketone, May and Baker, Ltd., England.
13. Sodium nitrite (analar), BDH, England.
14. Ammonium sulphamate, BDH, England.
15. N-1-Naphthylethylenediamine dihydrochloride, BDH, England.
16. Titanium trichloride solution, 15% W/V TiCl3, BDH, England.
17. Acetic alhydride (analar), BDH, England.

18. Sodium dihydrogen orthophosphate (analar), BDH, England.

19. Disodium hydrogen orthophosphate anhydrous (analar), BDH, England.

Some abbreviations used for the results in the appendices:

Day	day of Sulphasalazine Therapy
ASP	Acetyl sulphapyridine
SPG	Sulphapyridine glucuronide
ASPG	Acetyl sulphapyridine glucuronide
% Ac.	Percent of acetylated sulphapyridine
O.P.	Outpatient follow-up
B.D.	Twice daily
T.I.D.	Three times a day
Q.I.D.	Four times a day

Bracketed figures underneath Urinary (mg/24 hours) SASP and total SP recovery indicate % of the administered dose excreted in 24 hours.

Figures after Sulphadimidine indicate % Acetylated in serum

APPENDIX 2

INPATIENT STUDY

Ulcerative Colitis - 23 patients

Crohn's Disease - 10 patients

ANN PEARSON - SULPHADIMIDINE (9.6%) - DISTAL PROCTOCOLITIS - SLOW ACETYLATOR

SASP 1G Q.I.D.

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
1	14	15.1	36.4	15	0	0.7	52.1								
	16.30	13.5	36.2	12.2	0	2.6	51.0	46.4	157.7	287.4	51.8	77.4	574.3		
	21	10.4	30.6	10.3	9	4.1	54.0	(1.2%)					(23%)		
5	9.30	18.4	64	10.4	7.7	3.4	85.5	16							
	14.45	10.8	59	7.5	0.7	3.7	70.9	15	124.5	514	364.7	441.4	119.1	1439.2	34
	21	10.7	60	9.1	6.1	2.6	77.8	15	(3.1%)					(58%)	
9	9.30	17.3	63.6	10.4	3.2	3.4	80.6	17	108.0	593	379.5	404	195.8	1572.3	36.5
	16.30	10.4	64.5	8.5	1.1	5.3	79.4	17	(2.7%)					(63%)	
10	Discharged from hospital														
11	Nausea + + + at home														

ANN PEARSON (Contd.)

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% AC.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
12	11	20.8	69.3	6.7	3.5	7.6	87.1	16							
Drug Stopped															
13	9.30	3.9	35.6	8.8	7	6.0	57.4								
Course 2:	4 days after Course 1SASP 1G T.I.D. for 3 days then 0.5G Q.I.D.														
30	16	6.1	20.9	2.8	2.3	1.8	27.8	17							
42	9.30	3.8	37.0	8.5	0.2	4.2	49.9	25							
Nausea															
43	9.30	3.4	39.6	6.9	0.4	2.4	49.6	18							
Drug Stopped															
Course 3:	14 days after Course 2 SASP 0.5G T.I.D. x 7 days then 0.5G Q.I.D.														
15	9.30	6.4	23.4	3.9	0.5	2.5	30.3	21							
142	10	3.8	25.2	1.9	0	2.1	29.2	14							
342	10	5.2	29.5	3.8	0.2	3.6	37.1	20							

WILLIAM REID (22 years) - SULPHADIMIDINE (18.6%)
DISTAL PROCTOCOLITIS - SLOW ACETYLATOR - SASP 1G QID

Serum ug/ml								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac
0		0.6	1.1	0.5	0			
1	09	2.3	1.1	0.5	0.8	0.1	4.8	19
	12	15.8	1.1	0.7	0.6	0	2.4	
	20	26.1	10.0	2.6	1.0	0	13.6	
3	08	53.3	31.3	4.8	3.5	1.5	41.1	16
	12	56.8	19.1	6.1	0.7	0.4	26.3	24
	20	49.1	21.8	6.0	0.8	0	28.6	20
5	09	48.9	29.1	8.7	1.2	0	39	22
	16	77.9	31.9	5.9	0	0.6	38.4	16.9
	20	43.5	31.4	5.4	0	0.7	37.5	16
7	10	49.9	28.2	8.5	1.9	0	38.6	22
	16	21.1	29.2	8.0	0	0	37.2	22
12	09	51.2	32.7	4.2	2.7	0.7	40.3	24
	16	44.4	35.1	6.4	0	8	49.5	26
19	10	60.2	42.3	7.3	0.5	0.6	50.7	17
	16	47.4	35.1	9.9	5.9	0	50.9	19
	Dose 0.5G Q.I.D. Nausea ++							
20	10	30.6	37.6	6.4	4.3	0	48.3	13
	Nausea - little							
27	10	19.2	18.0	3.0	0.4	1.2	22.6	18.5
36	15	13.0	23.7	3.5	0	3.0	30.2	21
89	11	18.7	17.9	3.5	4.2	2.3	27.9	20
399	16	75.3	28.5	11.8	0.2	0.1	40.6	27

WILLIAM REID (contd.)

Urine mg/24 hrs								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
1		325 (8.1%)	117.4	66.6	29	2.8	215.8 (9%)	32
3		778 (19%)	578.4	182.7	191.5	69.4	1022 (41%)	24.7
5		226 (5.7%)	373.4	273.5	158.3	13.3	818.5 (33%)	35
12		525 (13%)	565.4	226.2	480.1	198.4	1470 (59%)	29
19		361 (14%)	319.6	157	239.7	114.3	830.6 (54%)	32.6

JESSIE ROBERTSON (25 years). SLOW ACETYLATOR (SULPHADIMIDINE 16%) - DISTAL PROCTO-COLITIS
SASP - 1G Q.I.D.

		Serum ug/ml.							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
0		0.6	1.2	0.4											
1	09.00	11.5	12.8	7.8	0	0	20.6		73.7 (1.8%)	187	251	59	45	542 (22%)	
	17.00	4.7	35.5	9.8	0	0	45.3								
3	09.00	26.5	38.1	5.3	0	0.1	43.5	12	140	372	235	102	15	724	35
	17.00	15.6	49.8	2.0	0	3.3	55.1	10	(3.5%)					(29%)	
8	09.00	17.8	102.5	0.9	0	2.5	105.9		128	619	303	150	9	1081	29
	19.00	17.2	93.1	2.9	0.2	4.0	100.2	7	(3.2%)					(44%)	
	Rash started														
10	09.00	23.9	91.7	4.7	1.3	0.2	97.9	6	242	815	269	295	32	1411	22
	17.00	24.1	91.2	2.6	0.5	1.3	95.6		(6%)					(57%)	
	Drug stopped														

JESSIE ROBERTSON (contd.)

Serum ug/ml									Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
Second Course of Therapy with Steroids and SASP 1G Q.I.D.															
0		0.6	1.9	1.1											
2	10.00	28.3	21.6	2.8	0.2	0	24.6	11							
	16.00	22.3	28.4	3.3	0.1	3.8	35.6	19							
3	09.00	32.0	37.1	8.2	0	0	45.3	18	167	535	146	145	121	947	28
	16.00	12.7	43.2	4.3	0	1.2	48.7	11	(4.2%)					(38%)	
	HOME														
7	11.00	33	65	6.2	0	0	71.2	9	129	567	230	160	75	1032	30
									(3.2%)					(42%)	
15	11.00	12.1	62	10.4	0.3	0.2	72.9	14	113	746	209	153	88	1196	25
									(2.8%)					(48%)	
32	15.00	7.4	71	4.7	0.1	0.1	75.9	7							

JESSIE ROBERTSON (contd.)

Serum ug/ml									Urine mg/24 hrs.						
Day	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% AC.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% AC.
150	14.00	14.0	81.9	4.6	1.1	2.1	89.7	7	193.8 (4.8%)	624	246	501	65	1436 (58%)	22
160	SASP ↓ 0.5G Q.I.D.														
345	14.00	13.5	29.4	4.3	1.0	9.8	44.5								

JANE WHYTE (72 years) - SULPHADIMIDINE (81%)
TOTAL ULCERATIVE PROCTOCOLITIS - FAST ACETYLATOR
SASP 1G T.I.D.

Serum ug/ml								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
0		0.6	0.9	0.9	0	0		
1	12	31.7	1.0	0.6	0	0	1.6	
	20	20.1	7.1	10.5	0	0.8	18.4	
3	10	19.1	9.4	24.0	0.1	1.3	34.8	75
	17.30	5.1	9.1	22.0	0.1	4.3	35.5	74
5	16	6.7	8.3	23.3	0.9	4.2	36.6	75
	20	14.2	8.6	25.1	0	2.5	36.2	76
6	14	29.1	6.3	20.8	0	1.6	28.8	78
7	09	8.7	6.7	22.4	1.2	11.9	42.3	81
	18	14.2	4.7	17.7	0	1.6	24	80
10	9.30	23.2	8.7	23.4	0.2	5.0	37.4	76
	18.30	10.5	8.0	22.2	0	3.7	33.9	76
37	O.P.	33.3	12.7	25.7	0	2.9	41.3	69
39	Readmission Leucopaenia							
42	09	38.5	14.1	16.6	0	7.6	38.3	64
	21	28.0	17.2	19.0	0	13.8	50.5	66
	Drug stopped SASP restarted 0.5G Q.I.D. after 4 weeks							
92	15	12.6	8.1	12.5	0.2	1.6	22.4	63
126	15.30	9.5	7.9	14.2	0.1	0.2	22.4	64

JANE WHYTE (Contd.)

Urine, mg/24 hrs.								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
1		142 (4.7%)	55.5	264	4.6	28	352.1 (19%)	83
3		54 (1.8%)	94	593	23	98	808 (43%)	85
6		96 (3.2%)	71	450	12.9	125	658.9 (35%)	87
10		119 (3.9%)	94.6	532	31	121	778.6 (42%)	84
42		73 (3.6%)	88	278	0.7	132	498.7 (40%)	82

PHILLIP TAYLOR (20 years) - SULPHADIMIDINE (29%) - ULCERATIVE COLITIS - SLOW ACETYLATOR
SASP 1G Q.I.D.

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
1	14	7.9	1.3	0.7	0.2	1.3	3.5								
3	10	5.1	4.3	2.0	0	0.2	5.5	40	42	24	41.1	35	1.2	101.2	41
5	14	6.4	4.0	2.5	0	0	6.5	38	(1.04%)					(4.3%)	
	20	8.9	4.9	0	0	3	7.9	38							
9	12	10.8	5.2	3.3	0.3	0.2	9.0	38	62	45.8	56.6	24.7	31.3	158.5	
	17.30	8.1	5.5	1.6	0	1.2	8.3	34	(1.5%)					(6.4%)	
15	SASP increased to 1.5G Q.I.D.														
20	10	42.3	27.3	7.5	0	0.2	35.0								
	16	20	26.6	7.8	0	3.4	37.8	30							
31	09	18.3	42.3	7.0	0	8.0	57.3	27	164	532.4	516.4	812.2	129.5	1990.5	33
	21	14.8	52.2	8.1	0	12.9	73.2	29	(2.7%)					(54%)	
36	11	11.4	69.2	7	2	1.2	79.4		149.8	1255	423.5	144	407.8	2230	37
	21	23.7	53.9	17.4	0	0	71.3	24	(2.4%)					(60%)	

PHILLIP TAYLOR (contd.)

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
46	9.30	14.9	69.6	17.4	2.7	4	93.7	23	94	1031	392.5	97	317.4	1838	39
	21	13.3	69.8	12.4	2.5	4.7	89.4	19	(1.6%)					(50%)	
Home - drug discontinued by himself. 8 weeks after, readmission with a severe relapse															
SASP 2G Q.I.D.															
10	9	27.4	53	12	3.5	0	68.5	18	144	303	190	177	35	705	32
	17	12.4	50.1	15.9	5.3	0.5	71.8	23	(1.8%)					(14%)	
15	Cyanosis followed by haemolysis														
16	SASP reduced to 1G Q.I.D.														
23	16.30	5.3	31.3	10.8	2	0	44.1	25							
	SASP stopped - haemolysis not improving enough														
27	9	0	0.6	0.7	1.5	2.5									
27	SASP restarted 0.5G B.D.														

PHILLIP TAYLOR (contd.)

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
30	13	6.8	13.5	2.1	0.5	2.5	18.6	25	2.2	75	43	13	15	146	39
	16.30	4.8	10.1	4.2	2.7	0.8	17.8	28	(0.2%)						
32	SASP increased to 0.5G Q.I.D.														
38	10	0.9	12.8	7.4	2.1	0	22.3	33	17.8	43	41	'35	15	134	41
	16.30	4.1	10.5	5.5	1.6	0	17.6	31	(0.9%)					(11%)	
	21	6.3	15.3	5.1	0	0.3	20.7	26							
48	Home - SASP 0.5G Q.I.D.														
75	15	1.6	0.2	1.1	1.2	0	2.5								
O.P. ? patient not taking the drug properly															
4 months after, patient readmitted with another relapse															

ESTHER ADELMAN (63 years)

LEFTSIDED PROCTO-COLITIS - SLOW ACETYLATOR - (Sulphadimidine - 7%) - SASP, 1G Q.I.D.

Day	Time	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac.
			Serum ug/ml					
1	18	6.2	14.6	5.5	0	0.2	26.5	
3	9	10.9	25.9	3.3	0	0	29.2	11
	14	8.3	22.1	-1.0	0	0.9	24.0	8
	17	19.7	16.8	0.9	0	0.4	18.1	7
4	10	7.3	27.6	1.8	0.2	0	29.7	6
5	10	6.0	27.0	2.5	0	1.0	30.5	11
8	10	8.6	32.0	4.5	0.2	0.3	37.0	12
	14	11.6	36.7	1.9	0	1.3	39.9	8
10	10	9.5	34.2	1.3	0.4	4.6	40.5	
	12	5.2	29.8	2.5	0	4.4	36.7	17
	17	2.7	28.0	2.0	1	4.6	35.6	
SASP - 0.5G Q.I.D.								
151	15	2.2	19.6	6.2	0.2	1.0	27.0	
			Urine mg/24 hrs.					
3		136 (3.4%)	392	260	265	121	1038 (41.9%)	37
5		142 (3.6%)	634	330	189	156	1309 (52.8%)	37
10		169 (4.2%)	796	312	281	145	1534 (62.8%)	30

ROBINA BANKS (39 years) - SULPHADIMIDINE (14%)
DISTAL PROCTOCOLITIS - SLOW ACETYLATOR - SASP 1G Q.I.D.

Serum ug/ml								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
0		0.1	1.9	1	0			
1	09	5.2	5.6	4.1	0	0	9.7	45
	17	8.5	11.8	2.2	0	7.7	21.7	
3	09	13.5	3.1	6.8	0	13.1	50.9	40
	17	14.8	31.1	5.5	0	13.6	50.2	38
8	09	17.6	57.3	8.2	0	12.3	77.8	26
Dose missed at 18.00								
	19	3.8	49.7	7.8	0	14.5	72	31
10	09	10.7	52.9	4.7	0	12.3	70	24
	17	8.5	52.6	9.4	0	7.0	69	24
11	Home - SASP 0.5G Q.I.D.							
338	10	3.9	24	4.3	1	9.9	39.2	36
Urine mg/24 hrs.								
1		56 (1.4%)	75	71.4	77.4	35.1	258.9 (10.4%)	41
3		105 (2.6%)	243	167	205.2	145.5	760.7 (30.7%)	41
8		79 (2.0%)	403.5	288.4	347.5	114.4	1153 (46.5%)	35
10		80 (2%)	475.6	228.4	347.4	138.4	1189.8 (48%)	31

THOMAS FINLAY (47 years) - DISTAL PROCTO-COLITIS
SLOW ACETYLATOR (Sulphadimidine 32%) - Agranulocytosis after 7 weeks of therapy - SASP 1G T.I.D.

Day	Time	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
			Serum ug/ml					
4	9.30	14.7	8	5.3	4	0	17.3	31
	17.00	13.8	8.7	6.9	3	0	18.6	37
	20.00	11.6	9.3	8.3	0.7	0.4	18.7	
6	9.30	8.8	19.9	12.3	8.6	0.1	40.8	30
	17.00	6.3	16.1	11.5	3.6	0.8	32.0	38
	20.00	4.5	14.4	10.3	4.5	0	29.2	35
8	9.00	9.9	16.9	9.4	3.1	0	29.4	33
	17.00	13.3	13.5	13.1	2.6	0	29.2	40
	Discharged							
51	Readmission - Ischiorectal Abscess							
52	Agranulocytosis - drug stopped after 6 a.m. dose							
52	11.00	19.1	33.5	13.0	2.5	0	50.0	26
	16.00	17.9	32.9	12.5	4.1	0	49.5	26
53	9.30	6.9	24.9	13.8	5.8	0	44.5	31
54	14.00	1.1	7.3	9.1	4.1	0	20.5	44
58	12.00	0.5	1.9	1.6	0	0	3.5	46
			Urine mg/24 hrs.					
4		98 (3.3%)	229	234	148	113	724 (39%)	48
6		114 (3.8%)	185	218	157	142	702 (38%)	51
8	Home							

JOHN COLLIE (28 years) SULPHADIMIDINE (67%) - FAST ACETYLATOR - ULCERATIVE COLITIS

8 months ago, rashes and fever with SASP for 5 days. Drug discontinued, temporary remission, reintroduction of SASP during active state

SASP 0.5G B.D. not on steroids

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
1	11	3.1	0.8	1.9	0	0	2.7		1.8	17.3	94.6	12.8	42.5	167.2	82
	17	4.5	2.1	2.2	0	0	4.3	51	(0.81%)						
10 - 24 hours - itchiness, flushing of skin 10 hours after starting the drug															
3	10	8.7	1.8	2.6	0.1	1	4.5								
	17	10.3	1.6	2.7	1.1	0.3	5.7	53							
2 - 3, obvious rashes, exposed area (Phenergan) 10 mg. T.I.D. - improvement noted from day 4															
6	10	9.4	1.9	3.8	0	0	5.7		52.2	51	122	9.8	119.9	302.7	80
	17	9.4	2.7	3.5	0.1	1.5	7.8	64	(5.2%)						
8 Rashes totally disappeared. SASP 0.5G T.I.D.															
10	9.30	14.6	2.8	2.9	0	2.3	8.0		171	42.4	137.3	17.1	158.6	355.4	83
	17	24.7	2.3	2.9	0	2.4	7.6	69	(11.4%)					(38.2%)	

JOHN COLLIE (Contd.)

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
14	SASP 0.5G Q.I.D.														
16	10	13.3	5.3	2.9	0	2.4	13.8		201	95.4	252.1	22.6	307.7	677.8	82.6
	15	16.2	6	5.5	0.3	2.7	12.9	64	(10%)					(54.6%)	
22	10	15	3.6	3.8	0	3.3	10.7	66	92.9	64.3	176.1	25.7	216.9	483	81.4
									(4.6%)					(39%)	
23	SASP 1G T.I.D.														
27	11	37.8	3.9	4.9	0	3.4	12.2	68							
28	SASP 1G Q.I.D.														
31	11	39.3	3.2	5.6	0.1	2.2	11.1	70	292.4	63.4	178.8	29.9	214.3	486	80.8
									(7.3%)					(19.6%)	

JOHN COLLIE (Contd.)

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
38	11	26.4	5.2	7.9	0	0.4	13.5	62	335.5 (8.4%)	64	231.4	35.7	224.2	555.3 (22.4%)	82
71	11	25.6	4.7	7	0.5	1.3	13.5	62							
92	SASP 1.5G Q.I.D.														
215	10	6.2	13.3	15.6	0.5	13.0	42.4	67	399.8 (6.7%)	195.6	698.6	112	685	1691.2 (45.5%)	82
296	15	9.5	15.5	16.1	0.9	6.4	38.9	58							

LINDA LONNIE (19 years) - SLOW ACETYLATOR (SULPHADIMIDINE 5%) - ULCERATIVE COLITIS - RASH ON SASP 1 year ago, stopped. SASP Reintroduced. Initial dose 0.5G B.D.

DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
Serum ug/ml.								
2	9.30	13	7.8	3	0.2	4.0	15.0	46
9	9.30	9.2	7.4	3.4	0.2	3.4	14.4	46
10	SASP 0.5G T.I.D.							
11	14	16.3	11.1	5.4	2.1	2.3	20.9	37
16	9	9.8	17.2	6.7	2.0	4.0	29.9	36
17	SASP 0.5G Q.I.D.							
40	17	15.9	21.6	5.7	1.4	5.0	33.7	32
100	11	9.0	16.0	6.7	1.1	3.9	27.7	38
127	15	16.7	24.4	8.0	2.0	9.6	40.0	44
Urine mg/24 hrs.								
9		55.6 (2.8%)	94.4 (15.2%)	107.3 (17.3%)	112.2 (18.1%)	102.5 (16.5%)	416.4 (67%)	50

MARION NICOL - SULPHADIMIDINE (68%) - DISTAL PROCTOCOLITIS - FAST ACETYLATOR

SASP - 1G Q.I.D.

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
2	09	10	0.2	1.0	0	0									
3	11	4.5	0.3	0.9	0	0									
	12.15	8.0	0.5	0.6	0	0			24.3	1.4	3.8	1.4	1.0	7.6	
	20	11.5	0.4	0.7	0	0			(0.6%)						
4	12	19	1.3	1.2	0	0	2.5		47.3	1.9	5.8	7.9	47.3	62.9	84
									(1.2%)					(2.5%)	
5	10	16.0	1 .9	0.8	0	0	2.7								
	12	14.0	1.9	0.9	0	1.4	3.2	55							
11	15	11.0	5.1	8.2	3.0	3.4	19.7	59	110.5	159	112.2	56.5	315	642.7	66
	20	32.0	8.0	11.0	1.7	3.7	24.4	60	(2.8%)					(26%)	

MARION NICOL (Contd.)

Serum ug/ml									Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
12	10	11.0	5.2	2.7	4.0	12.4	24.3	62							
	12.30	13.5	5.2	4.8	6.1	12.0	28.1	59	48.2	143	99.3	107	331	680.3	64
	15	15.0	8.5	4.1	0	7.3	19.9	57	(1.2%)					(27.7%)	
13	09	13.0	7.1	4.6	0.6	6.4	18.7	59	44.9	188	138	37.8	269	632.8	68
									(1.3%)					(25.5%)	
16	Home SASP 1G B.D.														
61	14	6.5	7.8	6.2	1.7	6.7	22.4	58	25.3	79	84.6	64.7	348.1	576.4	75
	16	4.1	9.0	7.4	0.4	5.5	22.3	58	(1.3%)					(46.5%)	
86	15	7.4	Insufficient Sample												
312	15	6.8	10.2	8.0	0.6	2.8	21.6	50							

T. BROWNING (36 years) - Sulphadimidine (75%)
ULCERATIVE COLITIS - FAST ACETYLATOR - SASP 1G T.I.D.

Day	Time	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac.
		Serum ug/ml						
1	16.00	6.6	4.9	5.3	3.5	0	13.7	
	21.00	6.7	4.9	5.4	2.0	0.3	12.6	45
4	10.00	10.6	9.5	15.8	1.6	2	28.9	61
	21.00	11.1	10.2	15.3	3.9	0.4	29.8	53
5	Home							
9	10.00	11.7	13	17.2	3.2	3.7	37.1	56
44	15.00	10.5	13.9	15.5	2.6	4.7	36.7	55
		Urine mg/24 hrs						
1		53 (1.8%)	32.3	190.5	46.2	5.5	274.5 (15%)	71.4
4		104.3 (3.5%)	132.3	535.5	289.8	170.5	1128 (60.6%)	63

J.H. (80 years) - Sulphadimidine (38%)
DISTAL PROCTOCOLITIS WITH TRANSVERSE COLOSTOMY
SLOW ACETYLATOR - SASP 1G T.I.D.

DAY	TIME	SASP	TOTAL SP	% Ac
	Serum ug/ml			
3	15.00	7.9	35.9	27
4	Nausea - Drug stopped			
5	10.00	2.9	40.7	29
	Urine mg/24 hrs.			
3		93.2 (3.1%)	1089.9 (58.6%)	31
5		5.3 (0.2%)	854 (46%)	33

DAVID FERGUSON (35 years), Sulphadimidine (34%), slow acetylator, Left sided Ulcerative Colitis

SASP 1G T.I.D.

Day	Time	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac
1	10	12.5	4.6	2.1	0.1	0.2	7.0								
	14	8.9	15.5	6.5	0.2	0.1	22.3								
3	10	13.6	45.1	13.1	4.7	9.1	72.0	31	61 (2%)	381	364	193	274	1212 (65%)	53
	Nausea + + +														
	Drug changed to Enteric Coated tablet														
	Nausea persisting														
4	Drug stopped after 6 a.m. dose														
	11	8.6	46.2	14.7	3.8	9.0	73.7								
5	10	1.9	38.1	10.6	2.7	8.5	59.9	32							
6	11	1.3	15.1	7.8	0.9	3.3	27.1	40							

2nd course of therapy, SASP 0.5G B.D.

MARGARET DAVIDSON (47 years)
ULCERATIVE PROCTOCOLITIS - FAST ACETYLATOR -
SULPHADIMIDINE (62%) - SASP 1G B.D. for 1 year

Serum ug/ml								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
On admission - before steroids (prednisolone)								
	14	9.6	8.8	8.7	0	3.3	20.8	57
2	steroids 40 mgm prednisolone/day on admission							
	12	8.1	10.4	8.2	1.2	2.2	22	
	16	12.4	9.9	6.7	0.5	5.3	22.4	
	20	8.9	10.8	7.3	0.2	3.6	21.9	50
10	10	9.3	10.8	9.9	0	3.1	23.8	
	20	6.9	10.6	10.9	0.6	2.1	24.2	49
Urine mg/24 hrs.								
10		56 (2.8%)	176	493	181	441	1291 (83%)	72

WILLIAM COCHRANE (17 years)
CHRONIC ULCERATIVE COLITIS - SLOW ACETYLATOR
SASP - 1G T.I.D. for 2 years

Serum ug/ml								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
On Admission		4.2	11.2	-0.7	1.1	4	17.0	28
SASP 1G Q.I.D. (Plain)								
5	12	12.1	30.8	4.7	7.5	3.8	46.8	18
	21	11.4	35.9	4.4	9	5.7	55	18
10	SASP 1G Q.I.D. (Enteric coated)							
15	9	2.6	29.4	5.5	3.2	2	40.1	18.7
	12	4.8	33.6	5.0	4.3	3.9	46.8	19
	21	4.7	35.1	3.6	8.8	8	55	20.9
Urine mg/24 hrs								
5		191 (4.8%)	474.8	646.8	603.2	241.5	1968 (79%)	45
15		3.6 (0.1%)	469.2	540.5	592.7	608.2	2210.6 (89.1%)	51

MARY BUCHANAN (27 years) - SULPHADIMIDINE (63%) - PANCOLITIS - FAST ACETYLATOR

SASP 1G T.I.D. for 2 months

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
On Admission															
1G Q.I.D.		20.5	8.5	9.1	0.2	0.5	18.3	52							
65	9.30	36	11.6	12.3	4.2	3.6	31.7	50	295.2 (7.4%)	183.0	456.9	197.3	543.1	1380.3 (55.6%)	72
70	9.30	25.2	14.5	16.6	1.2	2.9	35.2	55							

JOHN WALLACE (46 years) - Sulphadimidine (25%)

SEGMENTAL COLITIS (CROHN'S) SLOW ACETYLATOR, SASP 3G/day

		Serum ug/ml.					Total	
Day	Time	SASP	SP	ASP	SPG	ASPG	SP	% Ac.
5	12.30	9.6	29.9	4.8	3.5	0.5	38.7	14
	21.00	3.8	24.1	4.7	0	3.0	31.8	
7	9.30	6.9	24.4	4.7	0.1	2.3	31.5	
	15.00	4.8	23.5	4.5	2.4	0.1	30.5	15
	20.00	4.2	22.9	2.8	0.2	5.3	31.2	
11	12.00	13.5	24.4	7.5	0	0.8	32.7	15
	20.00	9.3	24.7	3.6	1.1	1.0	30.4	
22	10.00	7.4	33.1	4.3	0	5.4	42.8	
	14.00	7.0	31.2	5.5	3.6	0.4	40.7	14
	20.00	6.7	35.2	7.3	0	3.6	46.1	
145	14.30	7.4	40	4.3	0.8	3.5	48.6	16
410	15.00	9.5	39.7	4.2	1.0	1.2	46.1	12
		Urine mg/24 hrs						
5		98 (3.2%)	391	202	88	77	758 (41%)	37
7		74 (2.5%)	377	605	469	55	1506 (81%)	44
11		112 (5.6%)	410	254	424	241	1329 (71%)	37
22		94 (3.1%)	433	209	446	606	1694 (91%)	48

ROSS McLELLAN (24 years) SLOW ACETYLATOR
CROHN'S DISEASE, (both small and large intestine)
SASP - 6G/Day and steroids

Serum ug/ml								
Day	Time	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
4	12	6.8	25.1	4.9	0.2	1.8	32.0	21
*7	10	6.2	41.1	9.2	0.5	1.2	52.0	20
	21	5.4	42.2	10.4	0.1	0.8	53.5	21
14	17	8.2	47.3	13.4	0.6	1.5	62.8	24
'Cyanosis' followed by haemolysis, Heinz bodies present SASP stopped, SASP restarted after 8 days, 0.5G B.D.								
3		2.6	7.5	2.3	0.2	0.8	10.8	28
4	SASP 0.5G Q.I.D.							
**7	21	4.6	14.9	4.5	0.3	1.0	20.7	26
200	16	0.4	17.1	2.5	0.3	1.3	21.2	18
12 months clinical follow up - in remission								
Urine, mg/24 hrs.								
* 7		106 (18%)	615	385.6	640.2	363	2003.8 (53.9%)	37
** 7		37 (1.9%)	210	139.4	196.8	131.2	677.4 (55%)	40

* 7th day of initial SASP therapy

** 7th day during 2nd course of therapy

MARY McCONNELL (23 years) - SLOW ACETYLATOR (SULPHADIMIDINE 12%) CROHN'S DISEASE WITH PROTEIN LOOSING ENTEROPATHY
SASP - periodically; 1969 until January, 1971 when Haemolysis with 4G, drug stopped
SASP - reintroduced September, 1971, 0.5G Q.I.D.
no steroid

Serum ug/ml								
Day	Time	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac.
1	16.30	3.6	9.6	5.7	0.9	3.2	19.4	45
5	16.30	4.2	5.9	3.8	0	0.8	10.5	44
43	16.30	3.5	7.2	4.2	0.5	0.9	12.8	40
Urine mg/24 hrs.								
1		57.6 (2.8%)	40	62.8	62	100.4	265.2 (21%)	61
5		55.2 (2.7%)	42.5	58.9	67.3	110.2	278.9 (22.5%)	60

6 months clinical follow up - no evidence of haemolysis, clinical state - 'no change'

AGNES BELL (28 years) SLOW ACETYLATOR (SULPHADIMIDINE 15%) CROHN'S DISEASE WITH PROTEIN LOOSING ENTEROPATHY - SASP 2G/day from 1966 until January, 1970 dose increased to 3G/day January, 1970. February 1970, haemolysis, drug stopped. Reintroduced September, 1971 SASP 0.5G Q.I.D. no steroid

DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
			Serum ug/ml					
1	16.30	4.8	5.4	4	0	0.5	9.9	
5	10.	4.8	17.7	7.4	0	1.4	26.5	
	16.30	4.3	19.7	6.2	0	1.5	27.4	28
13	14.30	3.9	22.4	3.9	0	0.7	27	
	21.30	3.7	16.7	3.0	0.1	1.9	21.7	23
			Urine mg/24 hrs.					
1		84.3 (4.2%)	87.3	161.8	83.2	75	407.3 (33%)	58
5		86.9 (4.3%)	158.4	272.6	128.3	227.0	786.3 (63%)	63
13		57.4 (2.9%)	192	267.4	162.2	254.5	876.1 (71%)	60

12 months clinical follow up - no evidence of haemolysis

clinical state - Mild Activity

MAUREEN CLARK (23 years) - CROHN'S DISEASE
ILEOTRANSVERSE ANASTOMOSIS (INTESTINO-VESICAL FISTULA)
SASP - 1G Q.I.D.

Serum ug/ml				
DAY	TIME	SASP	TOTAL SP	% Ac.
6 months*	10	5.7	45.2	15
" + 7	21	4.3	54.5	10
Ileotransverse anastomosis on 10.8.71 SASP restarted from 25.8.71, 0.5G Q.I.D.				
2	9.30	2.3	16.8	20
	15	2.2	20.7	20
15	11.30	5.7	18.2	22
22	11.30	7.0	19.7	19
218	16.30	5.0	22.3	20
Urine mg/24 hrs.				
6 months* after operation		9.5 (0.2%)	980.3 (39.5%)	22
2/day		8.5 (0.4%)	410.5 (33%)	28
22/day		10.4 (0.5%)	439.5 (35%)	40

M. YOUNG (41 years) - SLOW ACETYLATOR (SULPHADIMIDINE - 16.8%)
CROHN'S DISEASE WITH RIGHT HEMICOLECTOMY (1966)
SASP, 1G Q.I.D. for 6 days then 1G T.I.D. and Steroid

Day	Time	SASP	SP	ACSP	SPG	ACSPG	Total SP	% Ac.
			Serum ug/ml					
1	9	3	3.2	1.3	0.6	0.8	5.9	
	17	5.7	3.7	1.4	0.6	0	5.7	
	20	2.4	3.4	1.1	0.0	0.2	4.7	
3	9	7.3	6.2	1.0	0.0	0.3	7.5	17
	17	4.0	5.3	1.3	0.4	0.2	7.2	
5	9	6.3	5.6	1.0	0.1	0.0	6.8	
	17	2.0	5.1	3.4	0.0	0.6	9.1	20
	20	2.0	4.1	2.3	0.0	0.2	6.6	
7	9	5.6	9.4	1.7	0.2	0	11.3	15
	20	2.4	12.9	0.9	0.1	2	15.9	
10	9	12.1	12.8	2.1	0.1	0	15.0	14
	17	4.2	12.8	2.5	0.0	0.3	15.2	
	20	3.9	12.1	2.0	0.0	0	14.6	17
56	15	3.0	17.4	6.7	1.1	0	25.2	
			Urine mg/24 hrs					
1		9.8 (0.2%)	68.5	138.8	26.8	23.6	257.7 (10.4%)	
3		34.4 (0.9%)	75.1	45.1	76.8	71.1	268.1 (10.8%)	43
5		14.0 (0.4%)	59.0	32.7	63.4	59	214.1 (8.6%)	42.8
7		3.3 (0.1%)	187.3	29.3	115.3	178.4	510.3 (27.4%)	41
10		6.5 (0.2%)	140.6	100.3	79.4	80.7	401 (21.6%)	45

GEORGE FISHER (42 years) - SLOW ACETYLATOR - SD (26%)
CROHN'S DISEASE, RIGHT HEMICOLECTOMY - SASP, 4G/day,
NO STEROID

Serum ug/ml								
Day	Time	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac.
1	10	17	0.8	0.3	0.8	0.4	2.3	
10	10	15.9	18.3	0.8	0	3.5	22.6	20
63	16	9.8	16.9	2.9	0.2	1.7	21.7	21
Urine mg/24 hrs.								
1		163.6 (4%)	140	11	3.5	2.8	157.3 (6%)	
10		223.4 (5.5%)	534	140	38	133	845 (34%)	32

J. CRUICKSHANK (33 years)
CROHN'S DISEASE (S.I. + L.I.) - SASP 1G T.I.D.
ILEOTRANSVERSE ANASTOMOSIS AND TRANSVERSE COLOSTOMY

Serum ug/ml				Urine mg/24 hrs.	
DAY	TIME	SASP	TOTAL SP	SASP	TOTAL SP
6 months	16.00	9.4	12.2	78.7 (2.6%)	903.1 (48.5%)

ALISON DUDLEY (21 years) - ULCERATIVE COLITIS - SLOW ACETYLATOR - SASP 4.5G/day for about 1 year then 4G/day

		Serum ug/ml							Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
Aug.'71 4G/day															
	15	10.9	24.3	9	2	1.4	36.7	28	220.5 (4.9%)	377	417	328	326	1448 (58%)	51
12.11.71 (on admission)															
Admission with relapse															
	12	22.6	16	3.2	0.8	0	20.0								
15.11.71															
	15	23.0	16	5.0	0.1	4.2	25.3	36							
21.11.71 Subtotal colectomy															
SASP restarted 10 days after operation 0.5G Q.I.D.															
10	10	9	2.2	1	0	2.3	5.5		3 (0.5%)	3.7	17.1	11.5	5.1	37.4 (3%)	59

ALISON DUDLEY (Contd.)

Serum ug/ml									Urine mg/24 hrs.						
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
15	SASP 1G Q.I.D.														
21	12	21.6	1.3	1.1	0	2.7	5.1								
60	15	13.8	1.2	1.9	0.3	1,0	4.4		235.2 (5.8%)	7.7	35.5	18.7	18	80 (3.2%)	66
192	15.30	15.4	1.4	1.1	0.4	0.6	3.5		208.4 (5.2%)	8.2	30.4	20.1	25.6	84.3 (3.4%)	66

CAROL STEEL (24 years)

LEFTSIDED PROCTO-COLITIS - FAST ACETYLATOR

SASP, 1G T.I.D. for 6 months (from January, 1971)

Serum ug/ml								
Day	Time	SASP	SP	ASP	SPG	ASPG	Total SP	% Ac
July O.P.	15	8.5	6.5	19.7	3.7	3.2	33.1	69
Aug O.P.	15	8.6	6.0	21.6	2.0	1.1	30.7	73
Sept O.P.	16	6.6	8.4	23.0	2.7	8.0	42.1	74
Oct O.P.	15	9.4	11.3	12.9	0.4	9.5	34.1	66
Nov O.P.	14	30.5	8.9	8.3	0	6.2	23.4	62
Feb. 1972 - admitted for continued symptoms - elective surgery								
On Admission*		29.2	10.1	7.5	0.3	1.4	19.3	
Proctocolectomy with Ileostomy SASP restarted 2 weeks after operation 1G T.I.D.								
5	11	6.6	0.9	0.9	0.3	0.4	2.5	52
35	11	7.6	0.9	1.5	0.3	0	2.7	56
Urine mg/24 hrs.								
On admission*		108.2 (3.5%)	68.6	402.8	190.4	376.1	1037.9 (55.8%)	75
5	After Ileos-tomy	158.4 (5.2%)	2	11.7	5.6	10.9	30.2 (1.6%)	75
35		108.9 (3.6%)	5	12.2	14.9	21.4	53.5 (2.8%)	63

WILLIAM MARTIN (50 years) - SLOW ACETYLATOR
LEFTSIDED ULCERATIVE COLITIS - SASP 1 G T.I.D.

DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
		Serum ug/ml						
38	9.30	62.9	21	2.3	1.6	0	24.9	
39	10	42.5	27.2	1.6	1.4	0	30.2	
13th September, 1971 - Subtotal Colectomy with Ileostomy SASP 1G T.I.D. From 30th September, 1971								
7	9.30	14.8	1.7	0.7	0	0.2	2.6	
	17	10.6	2.2	1.5	0	0	3.7	
10	10	35.5	4.6	0.7	0	0	5.3	
	17	24.9	5.3	1.3	0.2	0	6.7	
6 mths O.P.	14	2.5	2.4	0.1	0.1	0.1	2.7	
11 mths O.P.	14	2.9	2.9	1.1	0.4	0.2	4.6	
		Urine mg/24 hrs.						
38		234.5 (5.8%)	410.2	332.4	242.1	276.2	1260.9 (50.8%)	48
7		175.8 (5.9%)	49.1	34.6	0.4	0.2	84.3 (4.5%)	41.3
10		261.8 (8.7%)	31.9	21.8	24.6	33.7	112 (6%)	49
11 mths		172.4 (5.8%)	12.9	19.0	35.6	36.2	103.7 (5.6%)	53

G. KIDD (45 years) - ULCERATIVE PROCTOCOLITIS - SUBTOTAL COLECTOMY WITH ILEOSTOMY - 2 weeks after ileostomy - SASP 1G T.I.D.

Serum ug/ml								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac
1	14	2.4	0.5	0.2	0.2	2.1	3.0	
6	14	3.5	1.5	1.2	0.2	0.8	3.7	54
10	14	8.7	1.5	2.0	0.3	0.5	4.3	58
63	15 O.P.	7.6	0.2	0.3	0	0.7	1.2	
172	14.30 O.P.	15.4	1.2	1.6	0.1	0	2.9	55
Urine mg/24 hrs								
1		25 (0.8%)	6.2	11.3	1.5	4.8	23.8 (1.3%)	67.6
6		38 (1.3%)	7.2	37.8	13.8	8.8	67.7 (3.6%)	69
10		55 (1.8%)	11.5	23.9	15.1	19.4	69.9 (3.7%)	62

GEORGE MCBEAN (47 years) - ULCERATIVE PROCTOCOLITIS - SUBTOTAL COLECTOMY WITH ILEOSTOMY - 2 weeks after ileostomy - SASP 1 G T.I.D.

Serum ug/ml								
DAY	TIME	SASP	SP	ASP	SPG	ASPG	TOTAL SP	% Ac.
1	16	1.5	0.3	0.9	1.2	0.2	2.6	
5	16	2.8	1.3	1.2	0.2	0.7	3.4	41
10	16	3	0.8	0.7	1.1	0. 8	3.4	44
36	16 O.P.	14.5	1.7	0.7	0.1	0	2.5	25
55	16.30 O.P.	27.5	1.5	0.5	0.2	0	2.2	23
197	16 O.P.	16.6	1.6	0.3	0	0	1.9	16
Urine mg/24 hrs.								
1		28 (0.9%)	2.3	9.1	10.2	4.7	26.3 (1.4%)	52
5		72 (2.3%)	19.4	20	7.5	6.6	53.5 (2.9%)	49.7
10		41 (1.4%)	11.2	9.5	9.9	8.9	39.5 (2.1%)	46

CHARLES MCNAB (42 years) - CROHN'S DISEASE
ILEOSTOMY for 11 years - SASP, 1G T.I.D.

Serum ug/ml							
Day	Time	SASP	SP	ASP	SPG	ASPG	TOTAL SP
1	14.00	0.3	0.5	1	0.4	0	1.9
3	10.00	4	0.1	1.0	0.2	0	1.3
	15.00	1.3	0.5	0.2	0.1	0	0.8
86	15.00	3.6	0.8	0.9	0.2	0.1	2.0
240	10.30	4.2	0.8	1.1	0.1	0.1	2.1
Urine mg/24 hrs							
3		6.1 (0.2%)	0.5	0.4	0.1	0.6	1.6 (0.1%)
86		48 (1.6%)	0.2	1.3	0.2	0.6	2.3 (0.1%)
240		50 (1.7%)	0.3	6.1	0.6	2.8	9.8 (0.6%)

M. McIntosh (17 years)

CROHN'S DISEASE - ILEOSTOMY - SASP 1G T.I.D.

Serum ug/ml				Urine mg/24 hours	
DAY	TIME	SASP	TOTAL SP	SASP	TOTAL SP
1	4 p.m.	2.3	2.2	13	13.8
	8 p.m.	2.6	3.0	(0.4%)	
3	12 noon	1.6	2.7	7	18.7
	5 p.m.	1.6	2.6	(0.2%)	(1.0%)
	9 p.m.	2.3	2.6		
5	9 a.m.	2.1	2.1	13	33.0
	3 p.m.	1.0	2.1	(0.4%)	(1.8%)
	9 p.m.	2.3	2.0		
10	3 p.m.	0.8	2.0	9	42.1
	8 p.m.	1.0	2.2	(0.3%)	(2.3%)

EDWARD CARR (50 years) - TOTAL PROCTOCOLITIS, CHRONIC PANCREATITIS, FAST ACETYLATOR

SASP 1G Q.I.D. from 23/3/71 and also on Pancrex 10G Q.I.D.

		Serum ug/ml			Urine mg/24 hours		
DAY	TIME	SASP	TOTAL SP	% AC	SASP	TOTAL SP	% AC
4.5.71	11	13.3	11.4	58			
19.5.71	10	9.6	13.7	65	31 (0.8%)	291.7 (12%)	75.8
	21	6.8	14	65			
3.6.71	14 (O.P.)	3.2	19.1	64.4			
29.7.71	14.30 (O.P.)	2.2	17.3	69.9			
12.8.71	14.30 (O.P.)	11.2	25.9	70			
9.9.71	14.30 (O.P.)	14.0	20.9	66.5			
7.3.72	Pancolectomy with Ileostomy - SASP restarted from 24.3.72 1G Q.I.D.						
29.3.72	09	15.8	2.4	66.6	28 (0.9%)	39.9 (2%)	84.5

EDWARD CARR (Contd.)

Serum ug/ml					Urine mg/24 hours		
DAY	TIME	SASP	TOTAL SP	% AC	SASP	TOTAL SP	% AC
5.4.72	12	14.2	5.9	66.5			
	No Pancrex for 2 days						
7.4.72	09	14.2	6.1	72	101 (3.3%)	39.4 (2%)	89
27.4.72	14.30	10.5	5.8	74			

J. H. (80 years) - SULPHADIMIDINE (38%) - DISTAL PROCTOCOLITIS with TRANSVERSE COLOSTOMY
SLOW ACETYLATOR

SASP 1G T.I.D.

Serum ug/ml					Urine mg/24 hours		
DAY	TIME	SASP	TOTAL SP	% AC	SASP	TOTAL SP	% AC
3	15	7.9	35.9	27	93.2 (3.1%)	1089.9 (58.6%)	31
4	Nausea - drug stopped						
5	10	2.9	40.7	29	5.3 (0.2%)	854 (46%)	33

APPENDIX 3

OUTPATIENT STUDY

Ulcerative Colitis - 64 patients

Crohn's Disease - 29 patients

Ulcerative Colitis

Name	Date	SASP ug/ml Serum	TOTAL SP ug/ml Serum	% Ac
ARNOLD, H.	4.11.71	5.6	47.3	41
BAYNE, R.	15. 7.71	3.3	18.5	32
BERNARD, C.	28. 1.71	15.3	63.6	23.5
	12. 8.71	17.7	46.9	28.3
	30. 9.71	20.2	28.2	28.7
BELL, J.	22. 7.71	9.2	25.1	24
BOYLE, M.	22. 7.71	8.6	36.4	21
BRIGGS, M.	9. 9.71	9	31.6	68
BRODIE, G.	19. 8.71	30	23.7	71.7
BROWN, I.	25. 2.71	4.4	21.6	48
	26. 8.71	21.9	21.1	55
BURNS, T	10. 8.72	12.6	70.4	26
CALDER, R.	18. 3.71	10.7	29.6	38
CAMERON, I.	22. 7.71	4.7	30.6	23
CAMPBELL, W.	29. 7.71	12.2	37.3	29
	26. 8.71	29.5	54.3	38
	7.10.71	15.4	38.8	34.7
CARR, E.	3. 6.71	3.2	19.1	64.4
	29. 7.71	2.2	17.3	69.9
	9. 9.71	14	20.9	66.5
CARR, R.	29. 7.71	18.7	27.7	29.6
	28.10.71	9.8	29.7	35
COOPER, E.	9. 9.71	16	90	16
	14.10.71	57.7	91	15.7
CRAUNSTON, C.	3. 6.71	7.9	28.5	18
CROCK, V.	27.5 .71	3.2	18.7	21
CUNNINGHAM, R.	29. 7.71	5.8	22.5	65

Ulcerative Colitis (contd.)

Name	Date	SASP ug/ml Serum	TOTAL SP ug/ml Serum	% Ac
DALZEIL, H.	22. 7.71	5	9.1	33
DOWD, J.	30. 9.71	18.6	51.2	9.2
DUDLEY, AL.	12. 8.71	24.3	36.7	28
ENWIN, J.	13. 1.71	6.3	48.8	40
FLET, M.	8. 7.71	3.1	29.9	54
FRASER, D.	5. 8.71	19.7	58.6	78
GIBSON, A.	25. 2.71	46.8	10.6	78
	5. 8.71	60	16.3	71
GOULD, J.	8. 7.71	10.8	10.7	10
GRANT, A.	30. 3.72	1.7	24.8	41.5
GRANT, C.	9. 9.71	35	33.8	25.4
GRANT, J.	3. 6. 71	6.1	10.1	26
HALDANE, J.	26. 8.71	34.3	28.8	42
HOGG, C.	25. 2.71	8.9	17	34
	26. 8.72	7.7	6.3	42
HOPKINSON, J.	4. 3.71	18.9	18.3	27
	2. 9.71	7.0	33.5	25
KNOWLES, C.	16.12.71	4	17.9	24.6
LEWIS, A.	29. 7.71	5.4	28.1	31
LOCKE, M.	16. 9.71	15.8	42.3	41
	16. 3.72	18.3	63.5	30.6
	6. 4.72	12.2	42.5	40
LUNDIE, J.	13. 5.71	7.7	22.6	52
MCKAY, R.	16. 9.71	7	32.4	19
MCDONALD, D.	13. 5.71	7	20.5	38.5
MCGLIP, M.	5. 8.71	10.4	53.9	25
MCINNES, E.	13. 5.71	4.7	28.9	41
	9. 9.71	9.4	18.2	46

Ulcerative Colitis (contd.)

Name	Date	SASP ug/ml Serum	TOTAL SP ug/ml Serum	% Ac
MARSHALL, J.	26. 8.71	33.3	52.6	19.5
METACALFE, E.	13. 4.72	30	37.8	20
	10. 8.72	8	20.0	30
MILLS, R.	8. 7.71	2.8	13.8	26.8
MONK, R.	30. 3.72	2.6	29.3	31.7
NICOL, R.	9. 3.72	4.9	18.7	34
NISBET, A.	8. 7.71	2.4	46.8	18.4
ORR, A.	1. 5.71	2	19.9	69
	1. 9.71	3.9	32.3	61.6
ORR, I.	25. 3.71	25.2	7.7	67
	20. 5.71	34.6	18.5	55
OVENSTONE, A.	16. 9.71	55.8	25.8	53
PEARCE, P.	3. 6.71	8.9	22.9	64
RING, S.	2. 9.71	13.5	31.2	56.7
RITCHIE, A.	3. 6.71	15.4	28.9	25.6
	7.10.71	25	23.5	27
RUSSELL, R.	4. 3.71	11.4	29.6	32
	3. 6.71	7.5	14.9	32
	7.10.71	0.3	8.5	37
SIMPSON,J.	20. 5.71	4.9	12	44
SMITH, B.	4. 3.71	9.1	43.1	47
SMITH, J.	3. 6.71	5.7	24.4	15.6
STEELE, C.	15. 7.71	8.5	33	69
	12. 8.71	8.6	30.7	73
STEVENSON, M.	11. 3.71	0.7	6.9	51
	9. 9.71	5.7	29.4	55

Ulcerative Colitis (contd.)

Name	Date	SASP ug/ml Serum	TOTAL SP ug/ml Serum	% Ac
TALBOT, W.	30. 9.71	19.5	30.8	54.5
TAYLOR, R.	6. 3.72	8.2	47.2	17
	20. 3.72	3.1	48.1	21
THROWER, C.	5. 4.71	0.7	7.7	13
WALKER, A.	25. 2.71	6.9	28.5	28
	26. 8.71	2.3	31.5	27.6
WALKER, D.	3. 6.71	2.4	24.6	10.6
WATSON, A.	11. 4.72	10	15.6	20.5
WILKS, H.	20. 5.71	4.4	20	23
	16. 9.71	5.1	18.2	18.6

Crohn's Disease

Name	Date	SASP ug/ml Serum	TOTAL SP ug/ml Serum	% Ac
BANKS, J.	15. 7.71	2.6	59.4	9.4
	22. 6.72	12.7	33	23
BRIGHTMAN, D.	14.10.71	8.8	35.5	27
BUCHAN, S.	25. 2.71	2.3	30.5	35
CAIRNS, D.	26. 8.71	3.7	25.6	21.5
CORNER, R.	29. 7.71	7.6	22.6	34
CRAIG, M.	26. 8.71	0	9.7	46.4
DEWAR, I.	18.11.71	8.3	13.4	44
DICK, W.	30. 9.71	0	43.5	39.6
	18. 1.72	6.7	94.5	29
	20. 1.72	7	64.1	23
DROZDOWSKI, A.	28. 1.71	5.3	67.3	19.8
	7.10.71	0.4	23	26
ELLIOT, G.	19. 8.71	1.8	24.7	37.6
GALBRAITH, J.	13. 5.71	3	35.4	32
	30. 9.71	4.1	41.9	42
GILHOOLEY, M.	29. 7.71	2.7	10.1	59
GUTHRIE, E.	9. 9.71	4.2	25.3	16
HOGG, D.	13. 5.71	3.4	17.2	45
	9.9 .71	0.2	32.8	53
KAYE, R.	22. 7.71	4.6	16.9	29.5
KNOWLES, C.	16.12.71	5.2	29.6	15.5
MCKERRACHER, S.	25. 2.71	4.2	8.4	29.8
	26. 8.71	4.4	13.9	44
MCLEOD, J.	2. 9.71	35.2	29.5	50
MARSHALL, A.	3. 6.71	2.1	12.3	19.5
MILLAR, D.	9.10.71	3.1	17.3	17

Crohn's Disease (contd.)

Name	Date	SASP ug/ml Serum	TOTAL SP ug/ml Serum	% Ac
MONCUR, M.	8. 7.71	3.7	13.1	77
MORRISON, A.	26. 8.71	13.3	18.1	79.5
PHILP, J.	8. 7.81	2.5	29.8	24.5
REID, W.	25. 3.71	8.9	32.2	42
REYNOLDS, C.	20. 5.71	24.6	39.3	26.7
	5. 8.71	22.4	20.3	39
ROSS, D.	26. 8.71	4.1	15	31
	30. 9.71	3.5	13.6	23.5
SHARP, A.	4. 3.71	3.3	30	72
	2. 3.72	36.7	14.5	62.1
SHEPHERD, J	30. 9.71	2	24.7	34.8
WILLS, E.	11. 3.71	5.9	35.9	55

Thesis: Salicylazosulphapyridine Metabolism in Clinical Practice

X_1^2 values (X_1^2 = 3.84 significant at 5%)

1. Sex vs. Acetylator Phenotypes, i.e. slow and rapid

   a) for patients with Ulcerative Colitis

   X_1^2 = 2.65 N.S.

   b) for patients with Crohn's Disease

   X_1^2 = 2.16 N.S.

   c) for total number of patients

   X_1^2 = 5.51 Significant at 2.5%

2. Side effects vs. slow and rapid acetylators

   X_1^2 = 2.83 N.S.

3. Age (>60 or <60 years) vs. Side effects

   X_1^2 = 1.82 N.S.

4. Serum total sulphapyridine concentration (>20 or <20 ug/ml) vs. remission or active disease

   X_1^2 = 41.50 Significant at 0.1%

N.S. = not significant