centration, and was effected by a dose of antibody equal to twice the molar body burden of digitoxin [9]. In the current study, however, the molar dose of [^{3}H]DMI binding sites administered was more than 33 times the molar body burden of [^{3}H]DMI. The larger dose of DMI-As required to reduce [^{3}H]DMI is of potential importance because the body burden of DMI in a toxic animal or patient is quite large (>10 mg/kg) [2, 4]. Even if therapeutic use of DMI-As requires only an equimolar dose of binding sites, the dose will be substantial. If a larger dose is required, the potential problems associated with producing and administering these large doses of antiserum or antibody will be magnified. The reason for the large antibody dose required in our study is not clear. The average K_a of the high-affinity component of the antiserum is comparable to some of the antibodies used to reverse digitoxin or digoxin toxicity [10]. Possibly the binding of [^{3}H]DMI to tissues is tighter than the binding of the digitalis glycosides, and an antiserum with higher affinity for [^{3}H]DMI than used in this experiment is required. The efficiency of [^{3}H]DMI redistribution might also be improved if DMI-specific Fab fragments are used. Fab fragments distribute more extensively to extracellular fluid, and anti-digoxin Fab fragments have been shown to reverse digoxin toxicity more rapidly than an equimolar dose of anti-digoxin IgG.

In summary, DMI-As has been shown to redistribute tracer doses of [^{3}H]DMI from tissues to plasma. The rapid time course of drug redistribution and substantial reduction of radiolabel concentration in the target organs of DMI toxicity (heart and brain) support the potential clinical use of DMI-As to treat DMI toxicity. The increase in radiolabel concentration in liver and kidney after DMI-As could be due to altered distribution or metabolism of antibody–[^{3}H]DMI complexes. This possibility warrants further study because it might influence the amount of DMI-As required to effect drug redistribution and toxicity.

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Delivery of 5-aminosalicylate to the guinea pig cecum

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The effectiveness of sulfasalazine for the treatment and prophylaxis of ulcerative colitis [1] is considered to be mediated by 5-aminosalicylate (5-ASA), which is released by the intestinal flora [2–5]. That 5-ASA is the active component of sulfasalazine is consistent with its distribution to the colon [2–5] as well as its effectiveness when administered rectally to treat ulcerative diseases of the distal colon [6–10]. It appears likely, therefore, that sulfasalazine is merely a pro-drug which delivers 5-ASA to the colon where it is released by the intestinal bacteria [11].

In the search for an animal model of human ulcerative colitis, there has been particular interest in the observation that such rodents as the guinea pig respond to degraded carrageenan with a lesion in the lower bowel that resembles the human disease [12–14]. Unfortunately, it is not clear whether 5-ASA is effective in this guinea pig model [15–17]. Thus, while Jensen *et al.* [18] found that sulfasalazine administered by gastric fistula was quite effective in suppressing carrageenan-induced ulcers in the guinea pig, they could not demonstrate the effectiveness of a slow release preparation of 5-ASA. A possible explanation of this finding is that the preparation did not provide an adequate concentration of 5-ASA in the lower bowel. Previous work had shown that oral 5-ASA does not provide as high a concentration of 5-ASA in the colon of a rat as an equimolar dose of sulfasalazine [3].

In an attempt to determine whether 5-ASA can be administered more reliably in high concentrations to the cecum

and other sites of the carrageenan-induced ulcers, we have administered 5-ASA solutions directly into the cecum of the guinea pig by means of an implantable osmotic pump. We report the distribution of 5-ASA and its metabolite 5-acetamidosalicylate (Ac-5-ASA) in cecal contents as well as urine and feces following this form of administration.

Materials and methods

5-ASA was a gift from Pharmacia Inc. (Piscataway, NJ) and Rowell Laboratories (Baudette, MN). Ac-5-ASA and *N*-propionyl-5-ASA (Pro-5-ASA) were synthesized as previously described [2] and purified by recrystallization from equal parts of ethanol and H_2O. Male Hartley guinea pigs were purchased from Charles River Breeding Laboratories (Wilmington, MA), maintained on guinea pig chow (Ralston Purina Co., St. Louis, MO) and housed for these experiments in rat metabolism cages (Acme Research Products, Cleveland, OH).

Animal experiments. To implant the osmotic pumps for the infusion of 5-ASA, male guinea pigs (450–500 g) were pre-anesthetized by subcutaneous ketamine (35 mg/kg) and atropine sulfate (0.3 mg/kg); full anesthesia was then obtained with 2% halothane in oxygen. Through a midline incision in the lower abdomen the cecum was exposed and its wall pierced by a catheter (0.3 × 0.9 mm tygon microbore tubing, Horton Co., Akron, OH) which was secured with a purse string suture (6:0 Tevdek, Deknatel, Queens Village, NY). The catheter was attached to a 2-ml capacity 2 ML1 Alzet Osmotic Pump (Alza Corp., Palo Alto, CA), and the pump, catheter and cecum were replaced in the peritoneal cavity; the peritoneum was then closed with 3:0 chromic gut and the skin with 3:0 silk (Ethicon, Queens Village, NY). We are indebted to Dr. John Fara of the Alza Corp. for providing us with a video tape demonstrating this procedure and to Dr. Nils Lausen of the Harvard Animal Research Center for additional guidance in performing the animal surgery.

The pumps, in batches that were rated to deliver either 9.76, 10.40 or 11.63 μl/hr, had been charged previously with approximately 2.2 g of a solution that contained 67 mg of 5-ASA per g of 1 M glycine buffer, pH 10.5. In some cases, the pump charge also contained a tracer amount of [^{14}C]acetate (52,000 cpm/g; 2.36 mCi/mmole) that enabled the delivery of drug to be estimated from the proportion of radiolabel that remained in the pump at the end of the experiment. When the pumps used in a group of animals had different rates of delivery, results with all pumps were normalized to the delivery rate of a single batch of pumps for purposes of comparison.

Urine and feces were collected for a period that started 1 day prior to implantation of the pump, continued during the period that the pump was in place (7 days), and ended 4 days after the pump had been removed. The pump and its catheter were removed, as they had been implanted, a purse string suture being used to close the incision in the cecal wall. In another experiment to determine the fate of 5-ASA, four guinea pigs (225–250 g) were killed 4 days after the pumps were implanted, their ceca were weighed and a 2-g portion of the contents was assayed for 5-ASA and Ac-5-ASA.

Analytical methods. Urine samples were prepared for analyses by filtration through 0.45 μm Millex filters (Millipore, Bedford, MA) and diluted from 10- to 500-fold with water. The daily collection of feces was homogenized in a Waring blender, and a 2-g aliquot was extracted three times with 8 ml of 0.1 M KH_2PO_4, pH 6.0. The extracts were pooled, diluted to 25 ml, and filtered.

5-ASA in urine and feces was assayed after formation of its *N*-propionyl derivative. To form the propionyl derivative of 5-ASA, a 1-ml sample of a fecal extract or urine, while stirring vigorously, was treated successively with additions of 25 μl of propionic anhydride (Aldrich, Milwaukee, WI), followed at 5 min by 10 N NaOH (150 μl) and 5 min later by 10 N HCl (150 μl). Such samples were then analyzed as described below. Recoveries of 5-ASA and Ac-5-ASA, which were added to feces and extracted by these methods, were, respectively, 75% and greater than 95%.

HPLC was carried out with a Hewlett–Packard (Palo Alto, CA) model 1080A instrument using a 5 μ C-18 reverse phase column (25 cm by 4.6 mm, Supelco, Bellefonte, PA) at room temperature, unless otherwise stated. Two solvent systems were suitable. System I was isocratic for 5 min, the eluate consisting of 15% acetonitrile in 0.1 M KH_2PO_4 at a flow rate of 2 ml/min. A linear gradient was then applied that brought the concentration of acetonitrile to 40% over a period of 5 min; Ac-5-ASA and Pro-5-ASA eluted at 3.5 and 6.1 min respectively. To regenerate the column a linear gradient was applied that returned the eluent to 15% acetonitrile over a period of 2 min, followed by an isocratic elution with 15% acetonitrile for 5 min. System II, which was used to assay Pro-5-ASA, was isocratic 20% methanol in 0.1 M KH_2PO_4 for 7 min and then consisted of a linear gradient to 50% methanol over 7 min. In this system, which had a flow rate of 1.5 ml/min at 40°, Ac-5-ASA eluted at 7.0 min and Pro-5-ASA at 11.2 min. The column was then regenerated by a linear gradient that returned to 20% methanol over 3 min, followed by an isocratic elution with 20% methanol for an additional 5 min.

Ac-5-ASA and Pro-5-ASA in the HPLC eluate were detected and quantified in comparison with standards by their absorbance at 245 nm using a Hewlett–Packard 1040A Diode Array Detector. This detector also enabled the identity of compounds to be confirmed by comparing ultraviolet spectra in the eluate to those of authentic standards, such data having been normalized for plotting with a Hewlett–Packard 85 personal computer.

Results and discussion

The time course of the distribution of 5-ASA and Ac-5-ASA in the excreta of guinea pigs receiving 5-ASA by the cecal route is shown in Table 1. It is of particular interest that the recovery of Ac-5-ASA (20.1%) in the feces was six times greater than that of 5-ASA (3.4%). This result was similar to that observed previously when sulfasalazine was administered orally to guinea pigs for 4 days. Under those conditions, the recovery of Ac-5-ASA and 5-ASA in the feces were, respectively, 16.0 and 2.7%.*

To confirm that 5-ASA was acetylated in the ceca, four additional guinea pigs were killed after pumps had been in place for 4 days, a time when the data of Table 1 indicate that a steady state had been reached. At that time, the concentrations of 5-ASA and Ac-5-ASA were, respectively, 140 ± 35 and 163 ± 36 μg/g (mean ± SD, expressed in terms of the weight of 5-ASA per weight of cecal contents). The relatively greater proportion of Ac-5-ASA in the feces than in the cecum suggests that 5-ASA is progressively acetylated as it proceeds down the lower gastrointestinal tract. This acetylation reaction can be attributed to the flora, which has been demonstrated to carry out this reaction *in vitro*.*

We compared our data on the recovery of 5-ASA and Ac-5-ASA in the guinea pig cecum with that of Jensen *et al.* [18], who had instilled an average dose of 130 mg/kg of 5-ASA daily by means of a gastric fistula. Although their experiments yielded concentrations of 5-ASA that were approximately twenty times greater (and a concentration of Ac-5-ASA that was approximately twenty times less) than ours, there are several differences in the experiments. First of all, we administered the 5-ASA by continuous infusion whereas Jensen *et al.* administered it by a daily bolus. Furthermore, our administration of 5-ASA, approximately 4.2 mg/day/kg, was considerably less than theirs. Nevertheless, the use of osmotic pumps enabled much

* B. J. Dull, K. Salata and P. Goldman, unpublished observations.

Table 1. Recovery of 5-ASA and Ac-5-ASA when 5-ASA was delivered to the ceca of guinea pigs by osmotic pumps*

Day	Urine Ac-5-ASA	Feces 5-ASA	Feces Ac-5-ASA
1	2.7 ± 1.0	0.1 ± 0.1	0.4 ± 0.2
2	9.5 ± 2.3	0.8 ± 0.4	3.1 ± 0.6
3	9.5 ± 2.6	0.7 ± 0.4	4.0 ± 1.1
4	9.1 ± 1.7	0.7 ± 0.3	3.8 ± 0.5
5	8.3 ± 1.6	0.7 ± 0.1	4.2 ± 0.6
6	11.6 ± 4.1	0.6 ± 0.5	4.0 ± 1.4
7	9.0 ± 3.4	0.5 ± 0.3	3.4 ± 1.5
8	1.3 ± 1.9	0	0.4 ± 0.3
9	1.0 ± 1.4	0	0.5 ± 0.5
10	0.2 ± 0.4	0	0
11	0.2 ± 0.4	0	0
Recovery (%)	53.0 ± 5.3	3.4 ± 1.6	20.1 ± 3.0

* Osmotic pumps containing a solution of 5-ASA (see Materials and Methods) were implanted in the ceca of six guinea pigs on day 0 and removed on day 7. All pumps except one were rated to deliver 9.76 μl/hr, the other being rated at 11.73 μl/hr. Daily recoveries are expressed in terms of the mean ± SD of 5-ASA content (mg).

Table 2. Effect of osmotic pumps on cecal weight*

Treatment	Cecal weight (g) Including contents	Cecal weight (g) Empty
Pump (glycine buffer)	9.3, 9.0	2.9, 2.5
Pump (empty)	17.0, 16.2	2.5, 2.6
No operation	23.2, 27.0	2.7, 2.4

* Guinea pigs (225–250 g) in groups of two had installed 2 ML1 Alzet Osmotic Pumps that were either charged with 1 M glycine buffer, pH 10.5, or remained empty. Another two guinea pigs were untreated. At 4 days all animals were killed, and their ceca were weighed both with contents and then after the ceca had been slit to remove contents, rinsed and then gently blotted dry.

higher concentrations of 5-ASA to be achieved and to do so with much less variability.

Attention must also be called to the effect of implanting the osmotic pump on the weight of the cecum. Table 2 shows that the weight of the ceca was greatly diminished in guinea pigs killed 4 days after pumps had been implanted. This diminished weight can be attributed to a diminished cecal content and occurred to some extent even when empty pumps were implanted. Diminished weight of intestinal contents is a consequence of diminished food intake [19], and a decreased food intake was observed following surgery in these animals. The weight of the cecum itself, however, did not appear to change as the result of the implantation of the pump. It remains to be seen, therefore, whether pumps are suitable as a means of administering drugs directly to the cecum, for example, as a means of testing their effectiveness against carrageenan-induced ulcerative colitis.

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