Effect of Viscosity on Drug Absorption

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The effect of viscosity on the absorption rate of two model drugs (ethanol and salicylic acid) from the stomach of rats has been determined. The two drugs were administered simultaneously, dissolved in an aqueous solution containing 0, 1, or 1.5 per cent methylcellulose (Methocel 4000, 60 HG.). These solutions had a range of viscosities from about 1 to 500 cps. The osmotic pressure of the different solutions was essentially the same. The experiments were designed to allow recognition of possible effects due to complex formation and evaluation of the effect of viscosity on (*a*) the rate of movement of drug molecules to the absorbing membranes and (*b*) the rate of gastrointestinal transit of the solutions. It was found that both *a* and *b* were decreased with increasing viscosity. Correlation of ethanol and salicylic acid absorption data obtained in this study, together with consideration of the results of additional experiments with everted intestine preparations, provide an explanation for the initial lag time encountered in absorption studies with salicylic acid and certain other drugs. The results also rationalize the use of zero time shifts in the kinetic models recently developed in this laboratory for drug absorption in humans.

MANY PHARMACEUTICAL solutions are relatively viscous due to the presence of various hydrophilic polymers or other viscosity-enhancing agents added to improve taste, flow characteristics, stability, or other properties of the dosage form. These agents are, of course, used routinely in pharmaceutical suspensions for human chemotherapy and in suspensions administered to laboratory animals in pharmacologic research. A review of the literature revealed that information on the effect of viscosity on drug absorption is limited in quantity and scope. Davison *et al.* (1) found that oral administration of aqueous sodium salicylate solutions to rats yielded higher plasma and brain salicylate concentrations 30 minutes after drug administration than administration of sodium salicylate solutions containing 2% methylcellulose. Malone *et al.* (2) reported that increasing the concentration of sucrose in aqueous solutions of sodium phenobarbital (administered orally to rats) lengthened the induction time for narcosis. They concluded that the retardation of drug absorption responsible for this effect is due to the viscosity increase which accompanies increasing sucrose concentrations.

There are several possible mechanisms by which changes in viscosity might affect drug absorption rate: modification of gastric emptying rate and/or intestinal transit rate, effect on rate of movement[1] of drug molecules from the lumen center to the periphery (*i.e.*, to the absorbing membrane), and ability of intestinal content to penetrate to and come in contact with the total surface of microvilli. Since viscosity-enhancing agents contribute not only viscosity but also certain other properties intrinsic to the particular agent, one must also consider possible effects due to physicochemical interaction (complex formation), osmotic pressure [which affects gastric emptying rate (3)], and changes in pH or dielectric constant (which can change the activity of absorbable species). In the case of suspensions of drugs which are absorption rate limited by the dissolution process, increasing viscosity may decrease absorption by decreasing dissolution rate, because of (*a*) adsorption effects, (*b*) decreased diffusion rate of drug molecules through the boundary layer, and/or (*c*) lesser agitation of viscous materials in the gastrointestinal tract.

The purpose of the study described here was to investigate the effect of viscosity on drug absorption and to elucidate the mechanism or mechanisms responsible for the observed effects. Certain additional information, which has bearing upon other aspects of the kinetics of drug absorption, was obtained in the course of this investigation and also is reported.

EXPERIMENTAL

Determination of Possible Complexation of Salicylic Acid and Ethanol with Methylcellulose.—Ten-milliliter portions of a solution containing 1.5% w/v methylcellulose,[2] 2% v/v ethanol, and 100 mg.% w/v salicylic acid in 0.1 *N* hydrochloric acid were placed in bags made of Visking cellulose membrane.[3] These bags were placed in 90 ml. of 0.1 *N* hydrochloric acid contained in glass-stoppered conical flasks. The flasks were agitated in a constant-temperature bath at 37° for 4 days. Four such solutions and a control without methylcellulose were dialyzed in this experiment.

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[1] Use of the term "diffusion" is avoided purposely because gastrointestinal content is subject to agitation due to peristaltic action. Movement of drug molecules therefore involves a combination of diffusion and liquid flow.

[2] Methocel 4000 60 HG, Dow Chemical Co., Midland, Mich.

[3] Visking dialysis tubing, Visking Co., Chicago, Ill.

At the end of the equilibration period, the salicylic acid and the ethanol concentrations, both inside the bag and in the outside solution, were determined by methods similar to those described in later paragraphs.

Determination of Temperature within the Rat Stomach.—Rats, which had been starved overnight, were anesthetized by intraperitoneal injection of an aqueous solution of urethan (50% w/v) using a dose of 0.0025-ml. solution per gram weight of the rat. The anesthetized rats were mounted on a dissection board and operated upon in the same manner as described under *Absorption Study*. The stomach was ligated tightly at the cardiac sphincter and ligated loosely at the pyloric sphincter. A small incision was made in the duodenum, and the probe of a thermistor-type temperature recorder[4] was inserted through the opening and into the stomach. Solutions were injected into the stomach with a syringe by placement of the needle alongside the probe with the ligature then tightened around the intestine, needle, and probe to prevent leakage. Five milliliters of solution warmed to 37° was injected; the needle then was withdrawn. The two types of solutions employed were: (*a*) normal saline and (*b*) 2% ethanol and 100 mg.% salicylic acid in 0.1 *N* hydrochloric acid. The abdominal incision was closed with wound clips, and the rats were allowed to remain undisturbed. The temperature in the stomach was recorded continuously for 90 minutes.

Preparation of Methylcellulose Solutions and Determination of Viscosities.—Solutions of methylcellulose were prepared by the "hot method" described in the manufacturer's product manual (4). The methylcellulose was dispersed in hot water using a Waring Blendor and the solution then allowed to cool. The necessary amounts of salicylic acid, ethanol, and hydrochloric acid were added, then sufficient distilled water was incorporated to bring the solution to the desired volume. Agitation intensity and duration, temperature, and other conditions were kept constant in order to obtain solutions of reproducible viscosities. The solutions were placed in tightly closed glass containers, which were stored in the refrigerator. The solutions containing phenol red were prepared in an identical fashion.

Rheograms were determined at 35° with an Epprecht Rheomat "15" viscometer,[5] using measuring system A. This instrument is a rotational viscometer having 15 shear rate settings for a given measuring system. To obtain data for a rheogram (Fig. 1), the viscometer cup was filled with 110 ml. of solution and the spindle immersed. Both were attached to the instrument and positioned so that the cup was immersed in a constant-temperature bath. After equilibration of temperature, the solution initially was sheared at a rate of 19.72 dyne cm.$^{-2}$ for 3 minutes, then permitted to remain undisturbed for 5 minutes. At the end of the 5-minute period, the viscometer spindle was set in motion at the lowest shear rate, and a reading was taken at the end of 60 seconds. Each rate was increased to the next setting at 60-second intervals until the highest shear rate was reached, then decreased at the same rate to the lowest shear rate. The shear stress

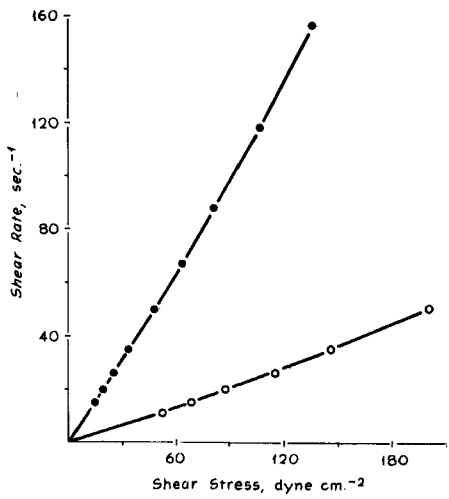

Fig. 1.—Representative rheograms (at 35°) of high and medium viscosity solutions used in the absorption studies. Both solutions contained 0.1% salicylic acid and 2% ethanol in 0.1 *N* hydrochloric acid. Key: ●, with 1.0%; O, with 1.5% methylcellulose 4000, 60 HG.

data obtained at the various shear rates were used to draw the rheograms.

Initially, viscosities were determined with both the Rheomat 15 and an R.G.I. Falling Ball viscosimeter.[6] Subsequently, the viscosities of these solutions were remeasured before each experiment with the Falling Ball viscosimeter, and only solutions with viscosities within ± 10% of the initial value (reflected by time of descent of the metal sphere) were used in the absorption and transit experiments.

Absorption Study.—Female white Wistar rats, weighing 120 to 180 Gm., were starved for 20 to 24 hours prior to the experiment and water withheld from the animals 1 hour before the operation. The animals were injected intraperitoneally with an aqueous solution of urethan (50% w/v) using a dose of 0.0025 ml. per gram weight of the rat.

After the anesthetized animal was mounted on a dissection board, an abdominal incision was made to the left of midline, and the stomach exposed. The stomach was ligated tightly immediately proximal to the cardiac sphincter and about 0.5 cm. distally from the pyloric sphincter. Care was taken not to occlude any of the major blood vessels of the portal and mesenteric systems. A third ligature was placed loosely about the pyloric sphincter. Five milliliters of the salicylic acid–ethanol solution, previously warmed to 35°, was injected with a syringe by inserting the needle into the duodenum between the two ligatures and gently working the needle into the stomach. Before the solution was injected, the third ligature was drawn tightly around the intestine and needle to prevent leakage of the drug solution. Upon completion of this procedure, the abdominal incision was closed with wound clips, and absorption was allowed to proceed for 1.5 hours.

Five minutes prior to the end of the absorption period, a blood sample was taken from the inferior vena cava of the rat. Immediately after the stomach was excised, the abdominal incision was length-

[4] Tri-R Tempcord R, Tri-R Instruments, Jamaica, N. Y.
[5] Contraves AG, Zurich, Switzerland.
[6] Cole-Parmer Instrument and Equipment Co., Chicago, Ill.

ened in the caudal direction to expose the urinary bladder. A small needle was used to puncture the bladder and remove the urine. The bladder then was washed with 1 ml. of water, which was added to the urine.

The stomach and its contents were homogenized in a sealed microblender.[7] (This microblender is an airtight attachment of the Waring Blendor which prevents loss of ethanol by evaporation.) The homogenate was diluted suitably with 0.1 *N* hydrochloric acid and divided into two portions. One-third of the total volume was used for the ethanol assay, and the remaining two-thirds was used for the salicylic acid assay.

Assay for Salicylic Acid in Gastric Homogenate.—A modification of the method of Brodie *et al.* (5) for determination of salicylic acid in plasma was used. The two-thirds of homogenate was divided into two equal volumes, and each was extracted three times with a total of 50 ml. (20, 15, and 15 ml.) of ethylene dichloride. A suitable aliquot of each duplicate portion of ethylene dichloride was removed and shaken with an aqueous solution of 1% ferric nitrate in 0.07 *N* nitric acid. A sample of the administered solution was assayed simultaneously.

The concentration of the iron–salicylate chromophore was determined colorimetrically with a Bausch & Lomb spectronic 20 colorimeter at a wavelength of 530 mμ. Previous investigation had shown that the absorbance was proportional to the concentration of salicylic acid and had yielded a suitable standard curve and absorptivity.

Using the extraction procedure outlined above, tissue blank values were found to be negligible. Ethanol and methylcellulose were found not to interfere with the assay. Extraction efficiency was determined by homogenizing excised rat stomachs, which had been filled with a known amount of salicylic acid–ethanol solution, and extracting and assaying as described above. Recovery was consistent and averaged 93%. The amount of salicylic acid remaining unabsorbed was calculated on the basis of the concentration of the assayed solution and its dilution and fraction of total homogenate used; it was corrected for extraction efficiency.

Assay for Ethanol in Gastric Homogenate.—A modification of the method of Pawan and Hoult (6) was employed. This method entails the application of small volumes of suitably diluted homogenate to filter paper cylinders which then are mounted inside sealed conical flasks; the ethanol subsequently is distilled from these cylinders into an oxidizing medium of acid dichromate solution. The flasks are placed in an oven at 60° for 1 hour, then allowed to cool. Twenty-five milliliters of water and 3 ml. of an aqueous solution of 1% brucine, respectively, then are added to the 2 ml. of dichromate solution. The unreduced dichromate reacts with brucine to produce an orange chromophore, the concentration of which was determined with a Bausch & Lomb spectronic 20 colorimeter at a wavelength of 450 mμ. Standard curves were prepared each time using aqueous ethanol solutions of known concentrations.

It was found necessary to assay all solutions within 2 hours of one another and to adhere to a strict protocol in order to assure assay reproducibility. Tissue blank values were found to be negligible. The efficiency of the assay was determined by homogenation of excised rat stomachs containing known amounts of salicylic acid–ethanol solution. The recovery averaged 100% for five stomachs. Salicylic acid and methylcellulose were found not to interfere with the assay. However, it was important to dilute the homogenate with water, rather than 0.1 *N* hydrochloric acid, since high concentrations of the latter were found to interfere with the assay.

The amount of ethanol remaining unabsorbed was calculated from the concentration of the sample and the dilution and fraction of the initial homogenate used. The concentration of the starting solution was determined simultaneously, and the amount of ethanol absorbed was calculated on the basis of the difference between the injected and recovered amounts.

Assay of Salicylic Acid in Plasma.—Prior to removal of the stomach, a heparinized syringe and needle were used to withdraw at least 3 ml. of blood from the inferior vena cava of the rat. The blood was centrifuged at 2400 × g. for 20 minutes, and the plasma was removed and assayed for salicylic acid by means of a procedure based on that of Brodie *et al.* (5). One milliliter of plasma, acidified with 1 ml. of 3 *N* hydrochloric acid, was extracted with 25 ml. of ethylene dichloride, 20 ml. of the organic phase then was extracted with 2 ml. of a 1% ferric nitrate solution in 0.07 *N* nitric acid. Approximately 0.5 ml. of the aqueous phase was placed in a microcell and assayed colorimetrically at a wavelength of 530 mμ with a Beckman DU spectrophotometer. Blank plasma values were determined and corrected for.

Assay of Total Salicylates in Urine.—Equal volumes of the urine–bladder wash solution and concentrated hydrochloric acid were sealed in 10-ml. ampuls and heated at 100° for 16 hours to hydrolyze the salicyluric acid and salicyl-glucuronides to salicylic acid (7). The hydrolysates were treated in the same manner as the plasma to determine salicylic acid concentrations.

Determination of Gastrointestinal Transit.—The rats were trained to drink water offered from a 16-gauge ball-tipped needle attached to a 2.5-ml. syringe. Water was withheld initially from the rats, and 12 to 24 hours later the rats became thirsty and accepted water from a syringe. Subsequently, the rats were given water only by syringe for three or four feedings each day for several days. As an aid in the training, the light to the area of the cages was blocked partially, and the rats experienced full room light only at times when they were offered water or drug solution.

Prior to the experiment, the rats were deprived of food for 20 to 24 hours and deprived of water for at least 12 hours. Each rat was fed 1.5 ml. of either an aqueous solution containing 70 mg. % phenol red or a similar solution containing 1.5% methylcellulose 4000, 60 HG in addition to phenol red. These solutions had been prewarmed to 35°. The rats were decapitated exactly 30 minutes after the time of acceptance of the first drop of solution. The abdominal cavity then was exposed, and the entire gastrointestinal tract was removed. The stomach, the small intestine (divided into three portions of equal length), and the large intestine were assayed for phenol red. The large intestine

[7] Eberbach Microcontainer, Eberbach Corp., Ann Arbor, Mich.

was assayed only if phenol red was present in the ileum within 5 cm. of the caecum.

Assay for Phenol Red.—This assay procedure is a modification of the method of Reynell and Spray (8). Each tissue segment and its contents was homogenized in a Waring Blendor with 1 ml. of 1 *N* sodium hydroxide and sufficient water to yield 30 ml. of homogenate. The homogenate was filtered through a Büchner funnel and 10 ml. removed. To this aliquot was added 1 ml. of an aqueous solution of 30% w/v trichloracetic acid to precipitate the proteins. After centrifugation, part of the supernatant again was made basic by addition of sufficient 1 *N* sodium hydroxide to develop the color to maximum intensity. The solution was diluted further and, if necessary, refiltered through a Millipore filter. The solutions were assayed with a Bausch & Lomb spectronic 20 colorimeter at a wavelength of 560 mμ.

Intestinal Absorption Study.—This was performed essentially by the cannulated everted intestine procedure developed by Crane and Wilson (9), which permits repeated collection of samples from the serosal side of the intestine. The small intestine of female Wistar rats, weighing 200–250 Gm., was removed under chloroform anesthesia. The proximal segment (about 30-cm. long) was placed in an ice-water mixture and the lumen rinsed with Ringer solution. The segment then was sleeved onto a glass rod and carefully everted. The second 10 cm. (from the proximal end) was used, with the duodenal end attached to a glass cannula and the other end ligated. The everted intestinal segment was suspended in 50 ml. of Krebs-Ringer-Dextrose solution (adjusted to pH 4.0) containing 100 mg. % salicylic acid and 1% ethanol. The solution was maintained at 37° and gassed continuously with a mixture of 95% oxygen and 5% carbon dioxide. The serosal solution was 2 ml. of Krebs-Ringer-Dextrose solution (pH 7.0). At indicated times, the entire serosal solution was removed by means of a hypodermic syringe with attached polyethylene cannula and washed out with 1 ml. of Krebs-Ringer-Dextrose solution which was combined with the initially withdrawn solution. A fresh 2-ml. portion of Krebs-Ringer-Dextrose solution then was placed in the intestine segment. Serosal solutions were assayed for ethanol and salicylic acid by methods described in preceding paragraphs.

RESULTS AND DISCUSSION

Effect of Viscosity on Gastric Absorption.—The effect of viscosity on the rate of absorption of salicylic acid and ethanol from the rat stomach is shown in Table I. Both salicylic acid and ethanol absorption from the low viscosity solution was significantly greater ($p < 0.05$) than from the high viscosity solution. There was no statistically significant difference between drug absorption from medium and high viscosity solutions, but absorption of both ethanol and salicylic acid from the three solutions showed perfect rank-order correlation with the reciprocal of viscosity. Similarly, plasma salicylate levels 90 minutes after drug administration showed perfect rank-order correlation with absorption and with the reciprocal of viscosity; mean plasma salicylate levels were 9.3, 7.2, and 7.0 mg. % for the low, medium, and high viscosity solutions, respectively. However, precision of measurements was poor due to the small blood samples, and the plasma level data are presented only as supplementary evidence. No measureable quantities of of salicylic acid or its metabolites were found in the urine at 90 minutes, evidently because of the small amount absorbed (less than 3 mg.) and also because general anesthetic agents (probably the anesthetic state as such) decrease renal blood flow, filtration rate, and excretion (10).

Table I.—Effect of Viscosity on Absorption of Salicylic Acid and Ethanol from Rat Stomach

	% Absorbed[a]			
	Salicylic Acid		Ethanol	
Soln.	Mean[b]	S.D.	Mean[b]	S.D.
Low viscosity	59	11	48	7
Medium viscosity	36	10	33	12
High viscosity	22	7	29	6

[a] In 90 minutes. [b] Ten animals each.

It should be pointed out that, by the experimental method used in this study, absorption is measured as the amount (or per cent) of drug which has actually entered the circulation. This is distinct from the methodology used by Schanker *et al.* (11), who do not determine the amount of drug remaining in the gastric wall. The latter method can suggest absorption even when no drug has actually entered the general circulation.

The viscosities of the drug–methylcellulose solutions used in this study were determined at 35°, which was the average intragastric temperature recorded during a period of 90 minutes in two rats which had been subjected to the same procedures (anesthesia, surgery, and gastric administration of drug solution) as were used in the absorption experiment. The flow characteristics of the solutions were found to be pseudoplastic, with no indication of yield points, but there was only minor deviation from Newtonian flow at low shear rates (Fig. 1). Based on the initial linear portion of the respective rheograms, solutions containing 1.5% methylcellulose had a viscosity of 460 cps., while solutions containing 1.0% methylcellulose had a viscosity of 95 cps. The solutions without methylcellulose were Newtonian, of course, with a viscosity somewhat less than 1 cps. Since the solutions were introduced into the stomach by means of a hypodermic needle and syringe (which involves considerable shear) and since polymers can undergo depolymerization and changes in rheologic characteristics when their dispersions are subjected to high shear rates (12), viscosities were determined before and after passage of the methylcellulose containing solutions through a hypodermic needle and syringe. There was no measurable quantitative or qualitative rheologic change.

Several other effects must be ruled out before the observed differences in absorption rate can be ascribed to the difference in the viscosity of the respective solutions. Complex formation between drugs and nonabsorbable macromolecules can reduce absorption rate (13), but this was not a factor in the present study since equilibrium dialysis of the ethanol–salicylic acid–methylcellulose solutions against pure solvent revealed no evidence of complex formation. Possible effects of viscosity on gastric emptying rate were excluded by gastric ligation. The surface tensions of 1.0 and 1.5%

solutions of methylcellulose are essentially the same, and contributions of methylcellulose to the osmotic pressure of the solutions can be neglected in view of the nonionic character and high molecular weight of the polymer. Some grades of methylcellulose may contain a number of residual carboxyl groups which might cause an increase in the pH of the gastric content (and thereby affect the absorption rate of salicylic acid), but such effects were prevented by using 0.1 *N* hydrochloric acid as the solvent. Therefore, the observed decrease of absorption rate with increasing viscosity is clearly due to the slower rate of movement of drug molecules to the absorbing membranes. These observations indicate also that, under the experimental conditions, movement of drug to absorption sites (rather than membrane permeation) is the absorption rate-limiting process in the case of ethanol and salicylic acid. The viscosity effect will not be evident when absorption is rate limited by the membrane permeation process.

Absorption rate was not directly proportional to the reciprocal of viscosity. This is to be expected since the Einstein equation relating diffusion coefficient to viscosity (14) applies primarily to colloidal noninteracting particles of spherical shape. Dullien (15) has discussed reasons why the product of diffusion coefficient in solutions and viscosity, in general, is not constant. Also, the gross viscosity measured with Couette-type viscometers (and other types of viscometers) does not necessarily reflect the microscopic viscosity encountered by diffusing drug molecules. In addition to viscosity, hydration and friction effects must be considered. Most important, however, the stomach cannot be equated to a diffusion chamber since there is movement of gastric contents due to breathing and peristalsis and mixing effects due to continuous secretion of gastric fluids.

Relation Between Ethanol and Salicylic Acid Absorption.—Since absorption of ethanol and salicylic acid was determined simultaneously, it is informative to consider the relationship of one to the other. Figure 2 shows a plot of per cent salicylic acid absorbed *versus* per cent ethanol absorbed for each of 30 animals. A regression line fitted to the data shows a positive intercept on the ethanol axis. This suggests that an appreciable amount of ethanol entered the general circulation before any salicylic acid appeared. The cannulated everted intestine procedure of Crane and Wilson (9) was used for a direct determination of a possible lag time in the absorption of salicylic acid. Definite evidence for such a lag phase was obtained (Fig. 3). Similar experiments with ethanol yielded a smaller lag time. The most informative experimental approach was the simultaneous determination, by the everted intestine procedure, of ethanol and salicylic acid absorption as a function of time. Figure 4 shows the ethanol–salicylic acid ratio of absorption in the time periods 0–7.5, 7.5–15, 15–30, and 30–45 minutes. The first ratio was significantly greater ($p < 0.05$) than the subsequent ratios. The third and fourth ratios reflect equilibrium conditions. The magnitude of the numerical ratio at equilibrium is a function of the respective concentration of the drugs in the mucosal (outer) solution, but the *change* in ratio with time prior to equilibrium reflects the different lag times and clearly parallels the gastric absorption data and supports the conclusions derived from them.

Lag time effects with *in vitro* intestinal preparations have been observed previously (16, 17), but there has been some question of whether these effects, which occur in the physiologically somewhat unrealistic mucosa-to-serosa transfer (*i.e.*, diffusion across the wall), would occur also during physiologic absorption (*i.e.*, diffusion from mucosa to capillaries within the gastrointestinal wall). The experimental data provide evidence that this is so. Apparently, drug initially accumulates in the gut wall (16, 18) and begins to appear in the

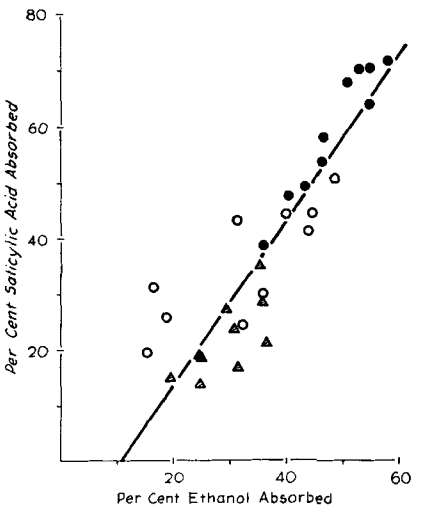

Fig. 2.—Relation between gastric absorption of ethanol and salicylic acid by rats from solutions of different viscosities. Key: ●, low viscosity; O, medium viscosity; ▲, high viscosity solution.

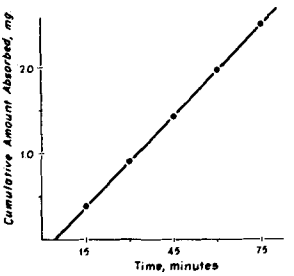

Fig. 3.—Intestinal absorption of salicylic acid determined by the cannulated everted intestine technique. Note the apparent lag time before absorption is evident. (Average of four determinations.)

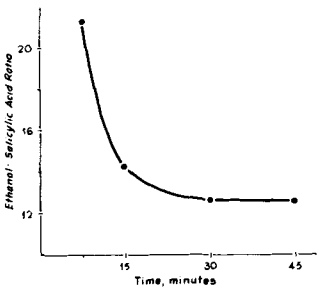

Fig. 4.—Relative intestinal absorption of ethanol and salicylic acid as a function of time, studied by the cannulated everted intestine technique. Outer solution contained 0.1% salicylic acid and 1.0% ethanol in Krebs-Ringer-Dextrose solution adjusted to pH 4.0. (Average of six determinations.)

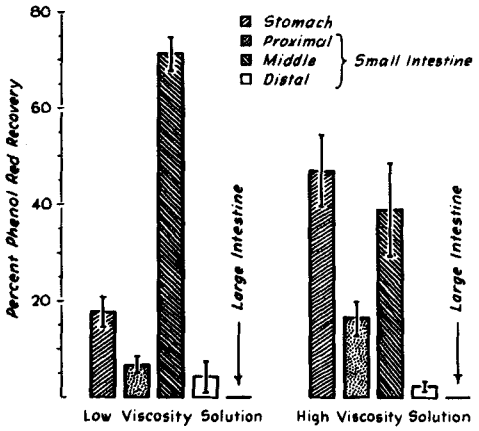

Fig. 5.—Phenol red recovery from various regions of the rat gastrointestinal tract 30 minutes after voluntary ingestion of phenol red solutions of low and high viscosity, respectively, illustrating the effect of viscosity on gastrointestinal transit. Each set of bars represents the average of 10 animals; vertical lines are standard deviations.

general circulation only after the gut wall is relatively saturated. Human absorption data recently reported have shown similar evidence of an initial lag phase: a linear plot of aspirin absorption by humans *versus* aspirin dissolution (from tablets) shows an intercept on the dissolution axis (19), and a kinetic model for absorption of aspirin (based on 30 experiments with 15 subjects) required inclusion of a zero time shift to account for an initial absorption lag phase (20). The animal data reported here constitute a rationale for the use of zero time shifts in kinetic models for drug absorption (even if appreciable absorption occurs already in the stomach). These time shifts are usually small (several minutes) compared with time shifts related to gastric emptying, which are usually several times larger.[8]

Effect of Viscosity on Gastrointestinal Transit.—Several necessary precautions were observed in determining the effect of viscosity on gastrointestinal transit. The animals were trained to drink the solutions voluntarily from the blunt tip of a hypodermic needle. Experimental procedures used by others, involving forced gastric intubation with or without anesthesia, are likely to superimpose stress effects on the effects due to viscosity and may decrease the sensitivity of the determination. Also, preliminary experiments were carried out to determine the optimum time for assessing gastrointestinal transit. Methylcellulose, rather than sucrose or glucose, was used as the viscosity enhancing agent because the latter contribute not only viscosity but also osmotic pressure and specific effects on gastric emptying (3). Phenol red was used as a marker because it is poorly absorbed (21). The slight absorption occurs by passive diffusion (22) and, due to its low rate, will be rate limited by the membrane permeation process and, therefore, will not be affected by viscosity. Average phenol red recovery, when the substance was added to tissue homogenates, was 93%. Phenol red recovery in the actual experiments was the same with low and high viscosity solutions and averaged 81%. Reynell and Spray (8) found that phenol red recovery from rats killed immediately after administration of the substance was always somewhat less than recovery of phenol red when added *in vitro* to homogenates. On the basis of their initial *in vivo* recovery of 90% and the recovery here of 81% after 30 minutes, it can be concluded that phenol red absorption was about 10%.

Figure 5 shows the amount of phenol red (expressed as the per cent of the total recovered amount) found in various sections of the rat gastrointestinal tract 30 minutes after administration of a low viscosity (aqueous) and high viscosity (1.5% methylcellulose) solution, respectively. Differences between respective gastric phenol red contents and between respective combined gastric and proximal intestine contents were statistically significant ($p < 0.05$) as such and also upon logarithmic transformation. There is some theoretical justification for presenting the average values as geometric means, but differences between these and arithmetic means were small.

To obtain an indication of the effect of viscosity on transit rate in each segment of the gastrointestinal tract, transit was calculated by the method of Reynell and Spray (8), who define it as "that percentage of the amount of phenol red entering the segment during the time since intubation which has moved to the next segment during the same period of time." Results are shown in Table II and indicate that the major effect of viscosity is on gastric emptying and that the effect of viscosity on gastrointestinal transit tends to become dissipated in the distal segment of the intestinal tract. This indicates that assessing gastrointestinal motility on the basis of the time of appearance in the feces of a charcoal meal is not a particularly sensitive method. The same applies for measuring the per cent of small intestine traversed in a given time. Stickney and Northup (23) were unable to detect effects due to viscosity (using methylcellulose) or sedation (with pentobarbital) by this method for reasons which are obvious from the results of the present study. The dissipation of viscosity effects (on gastrointestinal transit) in the distal direction is not due to intestinal absorption of methylcellulose, since the latter is not absorbed (24). However, there may be hydration effects which may retard inspissation of intestinal contents and thus diminish the difference in the viscosities of distal intestinal content with and without methylcellulose.

The data presented in Table II are in agreement with the conclusion reached by Reynell and Spray (8) that "propulsive movement of small intestinal

Table II.—Effect of Viscosity on Gastrointestinal Transit

Site	% Transit[a] Low Viscosity Soln.	% Transit[a] High Viscosity Soln.	Ratio
Stomach	82	53	1.5
Proximal small intestine	92	69	1.3
Middle small intestine	6	7	
Distal small intestine	0	0	

[a] Mean of 10 animals.

[8] Time shifts related to gastric emptying may be necessary in the case of weakly basic drugs, dosage forms requiring the higher pH or enzymic milieu found in the intestine for drug dissolution or release to occur, and drugs absorbed solely by active intestinal transport.

contents becomes progressively slower as the caecum is approached." The effect of viscosity on gastric emptying is important for drug absorption, since the large surface area available for absorption makes the small intestine the optimum absorption site, even for weakly acidic drugs which show greater intrinsic absorption rates in the acidic stomach. This is evident from both human (25) and animal (8) absorption data.

In conclusion, increasing viscosity can decrease the absorption rate of drugs from the gastrointestinal tract by retarding the movement of drug molecules to the absorbing membranes and by slowing gastrointestinal transit. In addition, there may be specific effects due to complex formation, osmotic pressure, and other factors mentioned in preceding sections of this paper. The results of the present study have direct application to the use of viscous solutions or suspensions in pharmacologic studies on animals. The magnitude of effects encountered in humans with therapeutically realistic volumes of viscous solutions is presently under study and will be reported in a subsequent communication.

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Potential Radiation Protective Agents III

Mercapto Analogs Related to Ephedrine

By K. VENKATRAMANA BHAT and WALTER C. MCCARTHY

Reaction of chlorodeoxypseudoephedrine hydrochloride with sodium thioacetate gave 1-phenyl-1-mercapto-2-(*N*-methylacetamido) propane. This product was resistant to hydrolysis by aqueous acid or base, but the corresponding disulfide was reduced by lithium aluminum hydride to 1-phenyl-1-mercapto-2-(*N*-methyl-*N*-ethylamino) propane. Condensation of chlorodeoxypseudoephedrine hydrochloride with potassium ethyl xanthate gave 3,4-dimethyl-5-phenylthiazolidine-2-thione, which was also resistant to hydrolysis, but was reduced to 1-phenyl-1-mercapto-2-(*N,N*-dimethylamino) propane.

IN RECENT YEARS, there has been much interest in compounds containing mercapto and amino groups on adjacent carbon atoms as radiation protective agents. Mercapto analogs of known pharmaceutical agents that contain hydroxy and amino groups on adjacent carbon atoms would be of much interest for investigation as antiradiation compounds, not only because they possess a structural moiety associated with high radiation protective activity, but also because the parent hydroxy compounds are known to be absorbed and transported to many widely distributed tissue sites in the body. Since certain sympathomimetic amines have been demonstrated to possess radiation protective activity, the mercapto analogs related to ephedrine were selected early for investigation. In a previous paper (1), it was shown that chlorodeoxypseudoephedrine hydrochloride reacted with thiourea to give, instead of the expected isothiuronium salt, a cyclization product, 3,4-dimethyl-5-phenyl-2-iminothiazolidine hydrochloride. Because of the

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