more consistent product in terms of particle size and charge. Since these properties have important effects on acid reactivity and viscosity, the achievement of homogeneous conditions during precipitation should reduce the variability in the properties of aluminum hydroxide gel. It is recommended that the precipitation system be checked to determine if homogeneous conditions exist by performing an acid titration and determining if polymerization occurs separately from precipitation.

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Omission of Pepsin from Simulated Gastric Fluid in Evaluating Activated Charcoals as Antidotes

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Abstract □ Although simulated gastric fluid USP calls for 3.2 g of pepsin/liter, most researchers omit pepsin when evaluating adsorbents. The present results show that, although pepsin adsorbs strongly to activated charcoal, it does not interfere significantly with the adsorption of a typical drug like sodium salicylate. Therefore, its omission is justified. Gastric mucin also had almost no effect on salicylate adsorption.

Keyphrases □ Pepsin—effect on adsorption of typical drugs to activated charcoal *in vitro* □ Charcoal, activated—adsorption of typical drugs, effect of pepsin *in vitro* □ Adsorption, *in vitro*—typical drugs to activated charcoal, effect of pepsin □ Gastric fluid, simulated—*in vitro* adsorption of typical drugs to activated charcoal, effect of pepsin

An adsorbent like activated charcoal can be evaluated as a potential orally administered poison antidote by dissolving a test substance in simulated gastric fluid, adding the adsorbent, and measuring the amount of drug adsorbed after equilibrium is achieved.

Although simulated gastric fluid USP contains, per liter, 3.2 g of pepsin, 2.0 g of sodium chloride, and 7.0 ml of hydrochloric acid USP (giving a pH of 1.2), it is common practice to omit the pepsin and sodium chloride in preparing simulated gastric fluids. While sodium chloride does not adsorb to materials like activated charcoal, activated charcoal strongly inactivates (1) and strongly adsorbs (2) pepsin. The possibility arises that pepsin would compete with the test substance for available adsorption sites, so its omission from simulated gastric fluid could result in a falsely high value for the amount of drug bound by the adsorbent under actual gastric conditions.

The purpose of this study was to investigate whether the inclusion or omission of pepsin from simulated gastric fluid significantly affects the measured degree of binding of a typical test substance. The omission of gastric mucin is also discussed.

BACKGROUND

The adsorbing ability of substances like activated charcoal has been assessed by dissolving the test drug in distilled water (3, 4). Since pH has a very strong influence on drug adsorption capacities (5–7), the results are not necessarily relevant to what would occur *in vivo*. For this reason, dilute hydrochloric acid solutions (usually 0.1 *M*, pH 1.0) often are used (8–13) as simulated gastric fluid. Such solutions are different from simulated gastric fluid USP in that they do not contain pepsin. Some investigators (11–13) specifically mentioned the deliberate omission of pepsin but did not comment on any possible effect. Apparently, any dilute hydrochloric acid solution of pH 1–2 is considered a valid and equivalent substitute for simulated gastric fluid USP, if not for actual gastric fluid itself.

Piper and Fenton (1) showed substantial inactivation of pepsin dissolved in a pH 1.5 potassium chloride–hydrochloric acid buffer by various adsorbents (charcoal, aluminum hydroxide, and clays). This inactivation was most likely the result of the pepsin having been adsorbed; however, direct evidence of such adsorption was not presented. Lichtwitz and Greef (2) published direct evidence of a substantial adsorption of pepsin onto various similar adsorbents. Thus, pepsin can bind well to several common adsorbents (and rather strongly to activated charcoal in particular).

Based on a molecular weight of pepsin of about 35,000, its equivalent spherical molecular radius is about 20 Å. This radius would allow pepsin to penetrate all macropores (>1000 Å) and a substantial fraction of the micropores in many adsorbent materials. For example, in most typical activated charcoals, about 50–90% of the pore volume resides in the pores of radii greater than 20 Å. Any test drug (*e.g.*, aspirin) would be much smaller in molecular size and could reach the smaller pores (<20 Å radius) where a substantial amount of the internal surface area resides. (Even if the pores of 20 Å radius or less are only 10% of the total pore volume, they would contribute a much larger proportion of the total internal surface area.)

Therefore, pepsin and a typical drug might compete strongly in the

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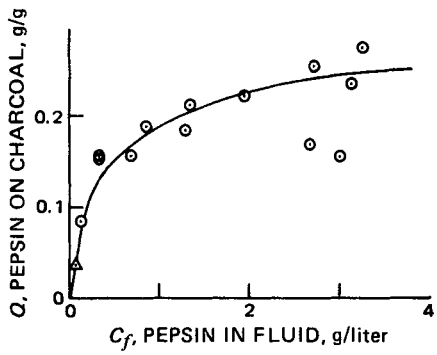

Figure 1—*Isotherm for the binding of pepsin from simulated gastric fluid onto powdered activated charcoal.*

larger pores but not in the smaller pores. The overall degree of competition is uncertain and can only be determined by experiments. To determine the degree to which pepsin adsorption can interfere with, and decrease the extent of, adsorption of a typical drug, the following experiments were conducted.

EXPERIMENTAL

Studies with Pepsin—Pepsin[1], 4 g/liter, was dissolved in a pH 1.2 potassium chloride–hydrochloric acid buffer to give a solution nearly identical to simulated gastric fluid USP. The adsorbent chosen was powdered activated charcoal[2], prepared by washing twice with 6 *M* HCl (to remove leachable inorganic ash), washing six times with distilled water, and drying at 120° for 24 hr.

Aliquots of 10 ml of the pepsin solution were combined with known amounts of the charcoal in glass vials. The vials were then capped and shaken for about 24 hr (prior tests showed that 24 hr is enough to assure nearly complete equilibration). After allowing another 24 hr for the charcoal to settle, the supernates were filtered through 0.45-μm microporous membranes[3]. One milliliter of each of the clear filtrates was combined with 10 ml of a biuret reagent prepared from 10% (w/v) potassium hydroxide–0.5% (w/v) $CuSO_4 \cdot 5H_2O$ (10:1) in distilled water. By reaction with the peptide linkages in the enzyme, this solution produced stable blue-colored complexes, which were assayed colorimetrically at 550 nm. A relation between absorbance and pepsin concentration was established by treating solutions of known pepsin concentrations according to the same procedure (shaking, settling, and filtering). Beer's law was obeyed by this system.

Although these experiments established the adsorption isotherm for pepsin binding to charcoal, the central question is not the extent to which pepsin will adsorb to the charcoal, but whether the adsorption of pepsin would interfere with the adsorption of a typical drug. Solutions of reagent grade sodium salicylate[4] (1 g/liter) in the pH 1.2 buffer were prepared and contacted with the charcoal in the same way as the pepsin solution (as already described) to establish the degree to which salicylate adsorbs to the charcoal. The clear filtrates from the samples were analyzed for salicylate by the colorimetric method (14) involving Trinder's reagent in which the intensity of a purple-colored complex is measured at 540 nm.

Pepsin was then added, at 4 g/liter, to samples containing the salicylate and powdered charcoal. Absorbances were measured at 540 nm after filtration of the supernates and addition of Trinder's reagent to the filtrates. From these measurements, the degree of interference of pepsin with sodium salicylate adsorption was determined.

Studies with Gastric Mucin—Based on the work of Gudiksen (15), Decker *et al.* (16) studied drug adsorption on activated charcoal using a simulated gastric fluid containing 0.5 g of gastric mucin/liter rather than pepsin. To determine if omission or inclusion of gastric mucin in simulated gastric fluid would have any effect on drug adsorption, the experiments with pepsin were repeated with gastric mucin. It was impossible to obtain an adsorption isotherm for the gastric mucin[5] on charcoal because the microporous membranes filtered out the mucin from the supernate. Thus, there appears to be no way of removing the charcoal from the supernate while leaving the mucin behind at its original concentration. It was possible, however, to repeat the interference tests successfully.

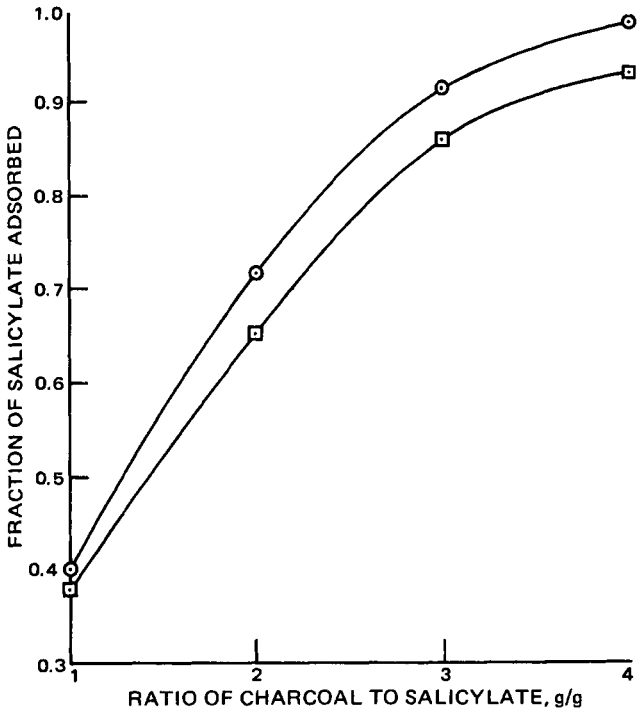

Figure 2—*Salicylate adsorption by charcoal from a 1 g of sodium salicylate/liter solution in the absence of pepsin (○) and in the presence of 4 g of pepsin/liter (□).*

RESULTS AND DISCUSSION

In the experiments designed to determine the degree of pepsin adsorption to charcoal, the amounts were computed for each sample using the mass balance equation $Q = V(C_0 - C_f)/W$, where V is the volume of the aliquot in liters, W is the weight of the charcoal in grams, Q is the weight of pepsin adsorbed on the charcoal (grams per gram), and C_0 and C_f are the initial and final fluid phase pepsin concentrations (grams per liter), respectively. Figure 1 shows the adsorption data obtained, expressed in terms of the quantities Q and C_f. The triangular data point near the origin is the data point of Piper and Fenton (1), computed assuming that the pepsin inactivation observed was due to adsorption and was directly proportional to the extent of such adsorption. While this assumption may not be entirely correct, their data point does fit in reasonably well with the present data.

The scatter in the neighborhood of $C_f = 3$ g/liter occurs because the stock pepsin solution concentration, C_0, was 3.48 g/liter. (The stock pepsin solution was prepared at a nominal 4-g/liter concentration, but the source pepsin was, by the supplier's own assay, only 87% pure.) Thus, to obtain adsorption data at concentrations near 3 g/liter, it was necessary to use very little charcoal and to measure a relatively small change in concentration, $(C_0 - C_f)$, due to adsorption. The greater relative errors in W and $(C_0 - C_f)$ result in larger errors in the Q values in this region.

Studies of the degree of salicylate binding to charcoal and of the extent to which pepsin (at 4-g/liter concentration) interferes with salicylate binding are summarized in Fig. 2. Over the range of the ratio of charcoal to salicylate shown (from 1 to 4 g of charcoal/g of salicylate), the fraction of salicylate adsorbed in the presence of pepsin ranged from about 5 to 9% lower than the fraction of salicylate bound in the absence of pepsin. Clearly, the effect of 4 g of pepsin/liter was not large.

The present studies of the effects of gastric mucin (at 0.5 g/liter) on the binding of salicylate can be summarized as follows. For samples having charcoal to salicylate ratios of 1, 2, 3, and 4, the fractions of salicylate adsorbed in the absence of added mucin were 0.401, 0.717, 0.914, and 0.984, respectively (these data points are shown along the upper line in Fig. 2). With the addition of 0.5 g of gastric mucin/liter to samples

[1] Product P 7012 (twice crystallized), Sigma Chemical Co., St. Louis, Mo.
[2] Norit A, American Norit Co., Jacksonville, Fla.
[3] Amicon Corp., Lexington, Mass.
[4] J. T. Baker Chemical Co., Phillipsburg, N.J.
[5] ICN Pharmaceuticals, Cleveland, Ohio.

having the same charcoal to salicylate ratios, the fractions of salicylate adsorbed were 0.401, 0.711, 0.920, and 0.984, respectively. Clearly, these results show either zero effect, a very slight interference, or a very slight enhancement of adsorption. Overall, no effect of significance can be identified.

CONCLUSIONS

Whether results similar to those obtained using sodium salicylate would be found with other kinds of drugs is, of course, unproved. However, if simple adsorption of the test drug and of the pepsin or gastric mucin is all that occurs, results with other drugs should not be radically different.

However, the question of whether pepsin and/or mucin should be added to simulated gastric fluid to make evaluations more realistic is overshadowed by the implications of data obtained by Andersen (17). He carried out adsorption tests in actual gastric contents (pH 1.5, 60% solids) obtained by aspiration of the stomachs of human volunteers after test meals had been consumed. Values for the amounts of mercuric chloride, barbital, and strychnine adsorbed on charcoal were only about 50–60% of the amounts adsorbed from pH 1.5 hydrochloric acid solutions.

Since the reductions in adsorption were quite large and probably depend on a whole complex array of solid and dissolved materials present in the stomach, it appears that absolute similarity between simulated gastric fluid and actual gastric contents is not achieved simply by adding pepsin and/or mucin to the simulated gastric fluid recipe. Therefore, it is probably just as well to omit both pepsin and mucin in tests involving simulated gastric fluid and to figure, as a rule of thumb, that the amount of drug adsorbed *in vivo* will be roughly half of that adsorbed *in vitro*. The experimental results reported in this paper give one a feeling for the influence of two specific important constituents of actual gastric fluid and indicate that they, at least, are not crucial in affecting adsorption.

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Synthesis and Antiulcerogenic Evaluation of 2-(Substituted Phenylimino)-2*H*-quinolizines

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Received July 1, 1977, from the *Chemical Research Division and the Biological Research Division, Norwich-Eaton Pharmaceuticals, Division of Morton-Norwich Products, Inc., Norwich, NY 13815.* Accepted for publication November 22, 1977.

Abstract □ The synthesis and antiulcerogenic evaluation of a series of 2-(substituted phenylimino)-2*H*-quinolizines are described. The most active compound, the 4-chlorophenylimido derivative, inhibited ulcer formation by 98%.

Keyphrases □ Quinolizines, substituted phenylimino—synthesized, evaluated for antiulcerogenic activity □ Antiulcerogenic activity—various 2-(substituted phenylimino)-2*H*-quinolizines evaluated □ Structure–activity relationships—various 2-(substituted phenylimino)-2*H*-quinolizines evaluated for antiulcerogenic activity

The synthesis and preliminary pharmacological evaluation of a series of 2-(substituted amino)quinolizinium bromides were reported previously (1, 2). As a continuing part of this investigation, the synthesis and antiulcerogenic testing of a series of 2-(substituted phenylimino)-2*H*-quinolizines (II*a*–II*f*, Table I) are now described. Compounds II*a*–II*f*, when examined for antiulcerogenic action by the modified Shay procedure (2), inhibited ulcer formation as much as 98%.

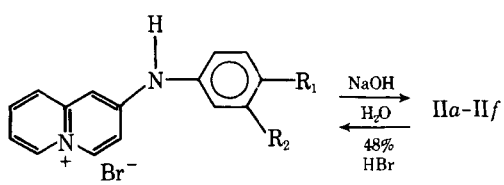
DISCUSSION

The syntheses of the intermediate compounds, I*a*–I*f*, were described previously (1–3). Their reaction with aqueous sodium hydroxide provided the phenylimino-2*H*-quinolizines (II*a*–II*f*) in high yield (Scheme I). Compounds II*a*–II*f* are the first reported examples of this class of compounds. The elemental analyses and IR and NMR spectra of II*a*–II*f* are consistent with the assigned structures.

For II*a* as an example, the IR spectrum showed strong absorption at 1640, 1560, and 1490 cm^{-1}. These absorptions are characteristic of all other phenylimino-2*H*-quinolizines as well.

The NMR spectral assignment of II*a* was made by use of homonuclear decoupling experiments. The NMR spectrum (dimethyl sulfoxide-d_6) of II*a* showed a *meta* split doublet at 6.22 ppm integrating for one proton, assigned to the proton at position 1. The proton at position 3 appeared as a split doublet (*ortho* and *meta* coupling) at 6.57 ppm. The proton at the 4-position of the quinolizine ring appeared as an *ortho* coupled doublet at 7.89 ppm. The proton at the 6-position appeared as a doublet at 7.85 ppm and showed *ortho* and *meta* splitting.

The proton at the 7-position of the quinolizine ring appeared as a split



I*a*–I*f*

Scheme I