strated assay coefficients of variation of <8.5% for both compounds.

In summary, this micronized assay provides a means for simultaneous analysis of carbamazepine and its 10,11-epoxide from small volumes of plasma. This is of particular value when analyzing samples from pediatric patient populations. In addition, this assay makes possible quantitation of these compounds in the biological fluids of many laboratory animals, where constraints may exist on the volume of blood that can be collected.

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OPEN FORUM

The Ulcerogenic Potential of Copper Aspirinate Seems to be More Imaginary than Real

Alich *et al.*[1] interpreted the appearance of an intravenously injected protein-bound dye in the gastric mucosa and submucosa of rats given $Cu(II)SO_4$, $Cu(II)_2$(aspirinate)$_4$, or $Cu(II)SO_4$ plus aspirin administered with or without dihydroxyaluminum glycinate and magnesium carbonate buffer as erosions. Leakage of the protein-bound dye from the vasculature was suggested as due to damaged blood vessels. Appearance of the protein-bound dye in the mucosa and submucosa in association with the above treatments does not provide evidence of either erosions or vascular damage.

Appearance of the protein-bound dye in the mucosa and submucosa of copper compound-treated rats is more likely due to leakage associated with absorption-induced hyperemia, as shown for the vehicle control, and the quantity of protein-bound dye found in these two tissues is more likely due to the amount of copper-containing compounds absorbed and their subsequent formation of quaternary dye-protein-copper complexes which produced colorations of varying intensity. Copper sulfate, which is not lipid soluble, was poorly absorbed in the time course of this experiment while more of the lipid-soluble copper aspirinate was absorbed[2]. The combination of aspirin plus copper probably produced an aspirin- and/or salicylate-copper complex as a result of the 10-min sonication, and these complexes were nearly equally well absorbed following instillation. Appearance of the dye-protein-copper complexes in the mucosa and submucosa in association with absorption of administered copper compounds is also consistent with the poor agreement between the two "lesion" grading techniques found when aspirin was administered without a copper-containing compound.

Alich *et al.*[1] suggested that, in general, the presence of buffer had no significant effect on the extent of "damage" produced under these experimental conditions. Exception is also taken to this suggestion since it seems clear that there was a statistically significant reduction in coloration in the group given the buffered copper aspirinate preparation. This reduction in coloration is consistent with a reduction in absorption of copper complex following the formation of an insoluble form of copper, $CuCO_3$, or some other nonabsorbable form of copper during the 10-min sonication of a suspension containing 27 μmol of $Cu(II)_2$(aspirinate)$_4$, 71 μmol of $MgCO_3$, and 22 μmol of dihydroxyaluminum glycinate/mL. The formation of $Cu(II)$(glycinate)$_2$, $Cu(II)$(salicylate)$_2$, and $Cu(II)_2$(aspirinate)$_4$ during the 10-min sonication of buffer, aspirin, and $CuSO_4$ may have only partially overcome the effects of $MgCO_3$ when the copper plus aspirin preparation was used. The absorption of copper when it was given as $CuSO_4$ also appeared to have been impeded by the buffer.

In their discussion of the existing literature pertaining to effects of $Cu(II)_2$(aspirinate)$_4$, Alich *et al.*[1] further reduce the credibility of their interpretation of their results as indicating gastric erosions and vascular damage. They did point out that Williams *et al.*[2] found no gastric erosions with $Cu(II)_2$(aspirinate)$_4$, but failed to mention that Williams *et al.*[2] had used doses of 115, 345, 690, and 1380 mg of $Cu(II)_2$(aspirinate)$_4$/kg of body weight, doses much greater than the 115-mg/kg dose used in their study[1].

Alich *et al.*[1] did point out that Williams *et al.*[2] reported "some evidence" of hemorrhage without gastric erosions in rats given $Cu(II)_2$(aspirinate)$_4$, but failed to mention that this hemorrhage which was observed at 345, 690, and 1380 mg/kg was not observed at 115 mg/kg. Alich *et al.*[1] offered no rationale as to how this hemorrhage, which occurred in the absence of gastric erosions, was relevant to their gastric study or in any way supported their interpretation of their results. We have determined the $LD_{50/7}$ of $Cu(II)_2$(aspirinate)$_4$ to be 895 ± 222 and 977 ± 297 mg/kg, respectively, in male and female rats using doses ranging up to 1500 mg/kg. Doses of 1000 and 1500 mg/kg did not produce gastric ulcers but did produce diarrhea. A diarrheal-inducing bolus may cause intestinal bleeding, but this cannot be mistaken for gastric bleeding, and these doses are much greater than the 115-mg/kg dose used by Alich *et al.*[1]. We have also reported results of our chronic toxicity study of $Cu(II)_2$(aspirinate)$_4$ in rats given 100 mg/kg *for 5 d/week* for 3 months[3]. No gastric histopathology was found at the light microscopic level with this more challenging treatment regimen.

Alich *et al.*[1] incorrectly indicated that we had reported that aspirin was an active antiulcer agent in the Shay rat[4]. They also failed to notice that we reported a reduction in gastric acid secretion in response to treatment of the Shay rat with copper complexes and not an accumulation of gastric acid following pyloric ligation as they indicated. The antisecretory effects of copper complexes have been confirmed and extended by others[5-8]. These results support the suggestion that antisecretory activity, in part, accounts for their mechanism of antiulcer activity. Alich *et al.*[1] also overlooked the report by Hayden *et al.*[9] that $Cu(II)_2$(aspirinate)$_4$ prevents aspirin-potentiated ulcers in the Shay rat.

Alich *et al.*[1] questioned the significance of data obtained in nonfasted rats in comparison with their data, but failed to recognize that our Shay rat preparation, like theirs, includes fasting.

Alich *et al.*[1] did point out that Rainsford and Whitehouse[10] reported the production of ulcers in rats with $Cu(II)_2$(aspirinate)$_4$ (200 mg/kg), but overlooked the response to that report which pointed out that those studies were flawed by the use of an inappropriate suspending agent, which has the potential of removing the copper from the administered complex, and the solution of copper complexes in 20 mM hydrochloric acid, which has the potential of destroying the "dissolved" complex and freeing the ulcerogenic ligand[11].

Alich *et al.*[1] did point out that Boyle *et al.*[12] found an antiulcer effect for $Cu(II)_2$(aspirinate)$_4$, but failed to mention that the dose was 345 mg/kg, a dose 3 times as large as their 115-mg/kg dose, and dismissed their results for specious reasons, the inclusion of both number and size of lesions in their assessment of the ulcerogenicity of aspirin.

Alich *et al.*[1] also pointed out that the results reported by Lewis[13,14] and Brown *et al.*[15] were "about the same for free aspirin and $Cu(II)_2$(aspirinate)$_4$" and indicated that the method used for quantitation of was less than state of the art, but failed to mention that doses of 300 mg/kg were used.

The significance of $Cu(II)_2$(aspirinate)$_4$-induced gastric irritation, even if it did occur with doses of 100 mg/kg and higher, remains obscure since the antiulcer dose of this compound is 5–10 mg/kg[4,9,13]. The 10-mg/kg dose has also been shown to markedly increase the rate of interstitial tissue repair in the surgically induced ulcer using histochemical techniques and light microscopy[13].

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Alich *et al.*[1] claimed that many possible variables introduced by others had been eliminated from their studies, but failed to recognize that they had introduced new variables that others had not introduced and that their results are subject to an alternative interpretation. As it turns out, this is the second paper coauthored by Alich and Wittmers on the subject of $Cu(II)_2(aspirinate)_4$ wherein conclusions were not supported by the evidence provided. In an earlier paper[14] these authors contended that salicylic acid was a contaminant of $Cu(II)_2(aspirinate)_4$, but failed to provide evidence in support of this contention[15]. The present contention by Alich *et al.* that $Cu(II)_2(aspirinate)_4$ causes erosions and vascular leakage at a dose of 100 mg/kg is not supported by evidence and it is also clearly inconsistent with results reported by ourselves and others they cited. Results obtained by Alich *et al.*[1] may be better interpreted in terms of absorption of copper compounds.

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Response to: "The Ulcerogenic Potential of Copper Aspirinate Seems to be More Imaginary than Real"

Dr. John Sorenson has responded to a paper in which we showed that when intact rat stomachs were subjected to aspirin or copper aspirinate under normal conditions of absorption, and when mucosal damage was highlighted with a protein-binding dye, observations differed from those made on Shay-prepared rats by Sorenson[1]. Our data indicated that copper aspirinate gave much larger superficial lesion indices and slightly smaller deep lesion indices than aspirin alone[2]. Dr. Sorenson's article contains several comments deserving of our response.

1. Dr. Sorenson, in taking exception to our results, makes frequent reference to studies which employed the Shay rat as a model. As we indicated in our paper, the Shay rat is quite a different model from ours. We attempted to approximate normal conditions under which drugs are presented to and absorbed from the stomach. We wish to reiterate that our model is different from the Shay model, and appears to produce different results. Since the Shay rat has frequently been used by other laboratories, an effort was made to refer to such data where appropriate.

2. Dr. Sorenson interprets the lesion index graphs incorrectly. The lesion index does not measure depth of color, but sums over the number of lesions of each diameter within one of the two color groups. Therefore, the index gives a number related to total area of mucosa damaged for each kind of lesion. Our paper indicates that the two color groups were independently verified by microscopic examination to be deep and superficial erosions, the deep erosions being a very dark blue, the superficial a royal blue. Dr. Sorenson hypothesizes that the color in the mucosa is caused by a dye-protein-copper complex formed when the copper is absorbed into the mucosa and the dye-protein complex leaves the vascular system. While this is an interesting hypothesis, it does not account for all of our results portrayed in Fig. 1A and B of our paper[2]. Dr. Sorenson's hypothesis may be possible or appropriate in A, for the sets of data containing Cu(II), but does not account for the lesion index in the controls or in those rats treated with aspirin. Likewise, in B of Fig. 1, the hypothesis may fit the last two sets of bars, but not the copper sulfate control or the rats treated with aspirin. Our observations from past work indicate that Cu(II) is absorbed into the mucosa whether complexed or not. In 1- or 2-h experimental periods similar to those described in our paper, but in which no dye was used, mucosal tissue exposed to copper-containing compounds, became a uniform grey-blue color and yielded extremely high values for copper analysis indicating that copper was absorbed into the tissue[3].

In any case, the amounts of copper present in our experiments would be too small to account for the extent of the coloration of the lesions. Likewise the hypothesis does not explain why there are two distinct shades of blue. Finally, the hypothesis cannot be applied to all the data presented in the paper.

3. Sorenson states that appearance of the protein-dye complex in the mucosa under the conditions of our experiment "does not provide evidence of either erosions or vascular damage." He attributes the leakage to absorption-induced hyperemia. Under normal physiological conditions, Pontamine Sky Blue 6BX, and other protein-binding dyes, do not leak from the vascular system in any significant amount[4]. The protein movement from the vascular volume into the interstitial space is very slow, as indicated by the small permeability coefficients for molecules of this size range. All animals in our experiments were sacrificed 10 min post dye injection. The discussion in our paper details the damage to blood vessels which allows leakage of the protein–dye complex into the tissue. Use of a protein-bound dye to aid assessment of mucosal damage is not unique with us. Dr. Sorenson is aware of the papers of West and co-workers[5,6] who also used this method.

4. Dr. Sorenson, commenting on our observations of hemorrhage in the absence of visible erosions, claims we ". . . offered no rationale as to how this hemorrhage which occurred in the absence of erosions was relevant to their gastric study or in any way supported their interpretation of their results." Sorenson appears not to have understood the description of Robins' work[7]. Robins observed (electron microscope) that prior to the appearance of visible mucosal erosions, and within minutes of being placed in the stomach, aspirin caused small blood vessels to develop fenestrations and later to break down completely[7]. These observations lend support to our suggestion that bleeding occurs prior to the appearance of visible lesions. Our paper describes samples which were found, on microscopic examination, to have no lesions, but by the dye method to present superficial erosions. The extravascular accumulation of the protein-dye complex in these cases indicated that the capillary endothelium was damaged prior to the development of the lesions. We also observed complete ischemia in the centers of a number of erosions, suggesting that the circulation was severely disrupted in these areas within the 2-h exposure to the drug.

5. Dr. Sorenson refers to a reduction in gastric acid secretion in response to the treatment of the Shay rat with copper complexes. The Sorenson reference given[8] contains no mention of the measurement of acid secretion. Further, other references described inhibition of acid secretion in Shay rats by copper–amino acid complexes[9,10]. Since controls were also Shay rats, it is not possible to determine whether the acid secretion was more or less than that in a non-Shay rat. It does not appear legitimate to extrapolate such results, in Shay rats, treated with copper–amino acid complexes, to non-Shay rats treated with copper aspirinate. West and co-workers (whose work Sorenson uses to buttress his arguments in this discussion) did not find the Shay-prepared rat to be the perfect model for testing activities of drugs[6].

6. Dr. Sorenson proposes that the complex of copper aspirinate is formed by the 10-min sonication of the Cu^{2+} and aspirin mixture to be placed in the stomach. He also proposes that $CuCO_3$ or "some other nonabsorbable form of copper" is formed from copper aspirinate during the sonication in the buffered system. This is an interesting proposal, the decomposition appearing more plausible than the formation of the complex (although in our thermal analysis experiments decomposition is not observed below 265°C). In any case, it does not seem to be legitimate to propose two opposite effects by the same sonication.

7. Dr. Sorenson states that we "incorrectly indicated" that he had reported that aspirin was an active antiulcer agent in the Shay rat. Our reference is to a specific paper[1], in which the statement is made (on p. 139): "Aspirin and anthranilic acid were active at 225 mg/kg ig. Again, if this is real activity in this model then these two compounds lack potency. The question as to whether or not these are irritant-induced or false positive results remains to be answered."

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