Rheological Evaluation of Hog Gastric Mucin as a Model Mucus System

CHRISTOPHER MARRIOTT and IAN W. KELLAWAY ˣ

Abstract □ The rheological evaluation of preparations containing 10, 15, 20, and 25% powdered hog gastric mucin was carried out over the 13–48° temperature range using rotational and creep viscometry. The preparations were almost viscous, and no elastic behavior was demonstrated. The addition of borate ions frequently produced a slight decrease in viscosity. Tetracycline hydrochloride decreased the viscosity of the 10 and 25% materials, although the addition of this compound to fresh gastric and bronchial mucous gels markedly increased viscosity. The model is, therefore, only suitable in limited circumstances as a basis for the evaluation of the effect of drugs on gastric mucus.

Keyphrases □ Mucus system, gastric—rheological evaluation using rotational and creep viscometry, effect of borate ions and tetracycline on viscosity □ Rheological evaluation—gastric mucus system, rotational and creep viscometry □ Viscometry—rotational and creep, rheological evaluation of gastric mucus system □ Viscosity—gastric mucus system, effect of borate ions and tetracycline

Because of the heterogeneous nature of the natural mucous secretions of the body, many model systems have been suggested for use in the assessment of flow behavior and the effect of drugs *in vitro*. Some examples of studied systems are saliva (1, 2), egg white (3), alginates (4), and powdered hog stomach (5). A recently described technique uses the last material as a model system for the assessment of mucolytic drugs (6). It also has been used to evaluate the effect of mucus on the bioavailability of drugs both *in vivo* (7) and *in vitro* (8). The principal advantage of such a model is that it is freely available commercially, so it is not subject to the problems associated with the collection of mucous gels from animals.

However, previous studies with this material never established that the system produced after the addition of powdered hog stomach to water in any way resembled the mucous gel secreted in the stomach. The purposes of this study were to investigate the rheological properties of the systems produced with hog gastric mucin and to compare them with those of the gastric mucous gel obtained from fresh pig stomach.

EXPERIMENTAL

Preparation of Samples—Powdered porcine stomach mucin[1] was added to glass-distilled water and stirred to achieve dispersion. The resultant suspension was then allowed to equilibrate for 16 hr at 4°. Final concentrations of 10, 15, 20, and 25% (w/v) were used.

Sodium borate[2], when required, was dissolved in the water used for the preparation of the mucin suspension and was in contact with the mucin for the entire equilibration period.

Gastric mucous gel was obtained from the stomachs of freshly slaughtered domestic pigs (*Scrofa domestica*), which had been starved for approximately 48 hr. The stomachs were opened, and the gel was removed from the antral regions.

Human bronchial mucus was collected from hospitalized patients suffering from various chest conditions. Homogenized batches were prepared by the method reported by Marriott and Richards (9).

Tetracycline hydrochloride[3] was added to the samples 30 min prior to measurement. This short equilibration time was used because of the possibility of breakdown of the tetracycline solutions over longer periods and because it was shown previously to be adequate for any effects to be induced (10).

Rotational Viscometry—Flow curve (rheogram) determinations were carried out using an automatic rotational viscometer[4] in conjunction with an x–y recorder[5], which permitted flow curves to be plotted directly. The cup and bob measuring geometry was used, and the size of the unit was varied according to the viscosity of the sample.

The sample was loaded into the viscometer at 20°, and a sufficient time was allowed for the attainment of temperature equilibrium. The flow curve was determined using a sweep time of 120 sec and a maximum shear rate of 195 sec^{-1}. The temperature was then increased by increments of approximately 5°, and a flow curve was determined at

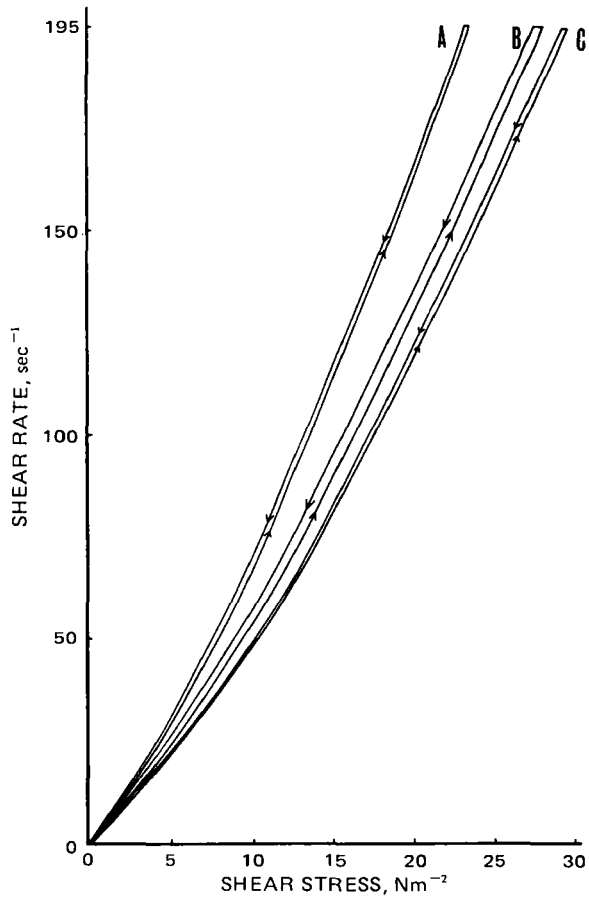

Figure 1—*Examples of flow curves obtained with the 10% mucin preparation using a sweep time of 120 sec. Key: A, 10% mucin plus 1% tetracycline hydrochloride at 33.8°; B, 10% mucin plus 1% sodium borate at 33.5°; and C, 10% mucin at 31.4°.*

[1] Crude, Type II, Sigma London Chemical Co. Ltd., London, United Kingdom.
[2] AnalaR, British Drug Houses, Poole, United Kingdom.
[3] The Boots Co. Ltd., Nottingham, United Kingdom.
[4] Rheomat 30 viscometer with Rheoscan programming unit, Contraves, A.G. Zurich, Switzerland.
[5] Farnell Instruments Ltd., Wetherby, United Kingdom.

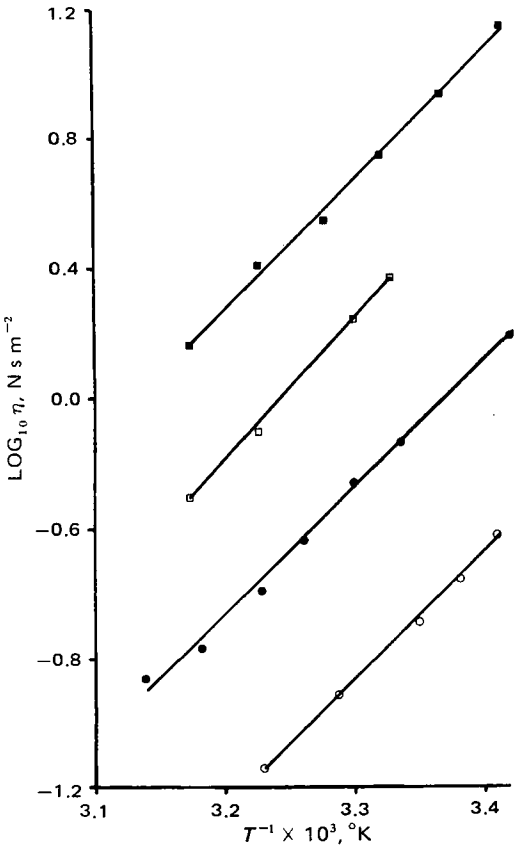

Figure 2—*Viscosity, measured by rotational viscometry, of 10, 15, 20, and 25% mucin preparations plotted logarithmically as a function of the reciprocal of the absolute temperature.*

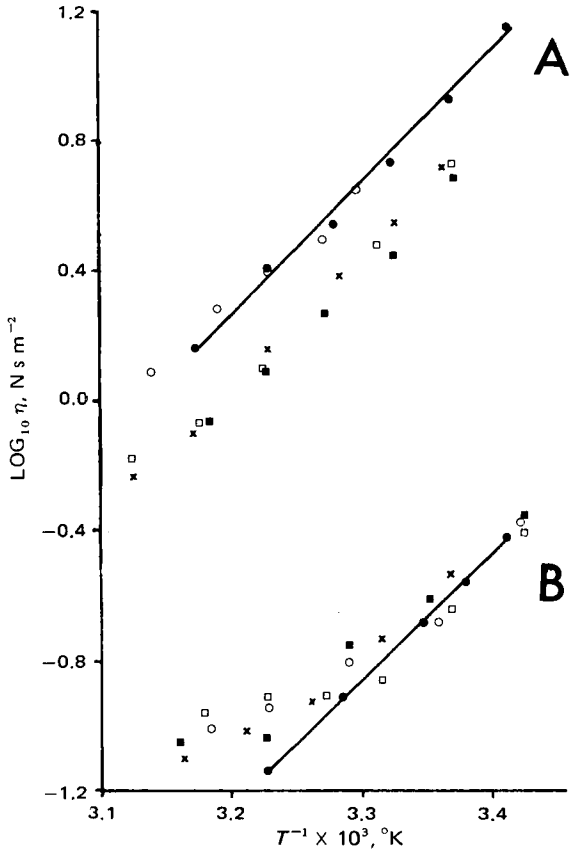

Figure 3—*Effect on viscosity, measured by rotational viscometry, of the addition of various amounts of sodium borate to (A) 25% and (B) 10% mucin preparations. In each case, the line drawn represents the mucin preparation without sodium borate. Key: ●, 0%; ○, 0.1%; ■, 0.25%; □, 0.5%; and ×, 1.0%.*

each temperature. The apparent viscosity, η, was calculated from the reciprocal of the slope of the linear portion of the curve recorded at an increasing shear rate.

Viscosity determinations were carried out with 10, 15, 20, and 25% (w/v) mucin preparations.

Creep Viscometry—This technique permits the evaluation of the viscoelastic properties of a material by measuring the compliance of the material at low shear stresses.

An air turbine viscometer with cone and plate geometry, capable of producing the low stresses, was used (11). Samples were loaded into the gap between the cone and plate, and a suitable period was allowed to attain temperature equilibrium. A small, constant stress was applied to the sample, and the resultant deformation was followed as a function of time. The temperature was then increased by approximately 5° increments, and determinations were made at several temperatures between 15 and 40°.

The residual shear viscosity, η_0, was calculated from the reciprocal of the slope of the linear strain–time plot for the mucin preparations. When creep curves typical of viscoelastic materials were produced, they were analyzed into discrete spectra using a computer program (2).

RESULTS

Some examples of the flow curves obtained with the 10% system are shown in Fig. 1. A small degree of hysteresis was apparent and was not altered by addition of either borate ions or tetracycline. In all cases, similar results were obtained when the experimental temperature was approached from a higher as well as from a lower temperature.

The derived viscosity values from the continuous shear viscometry are shown in Fig. 2, where log viscosity, η, has been plotted against the reciprocal of the absolute temperature to produce the typical Arrhenius plot. The viscosity increased with an increase in the concentration of the mucin, and the gradient of the slope was similar in each case.

When sodium borate was added to the 25% mucin system in concentrations between 0.1 and 1.0%, a slight decrease in viscosity was observed with all but the lowest concentration (Fig. 3). The gradients were again similar in all cases, in direct contrast to results obtained using the same concentrations of sodium borate with the 10% mucin preparations. Although little alteration was observed in the absolute value of the viscosity, a distinct deviation from linearity was apparent in the curves, particularly at higher temperatures.

A similar trend is demonstrated in Fig. 4, which shows the effect of tetracycline hydrochloride on the mucin preparations in the presence and absence of borate ions. Once more the additives decreased viscosity for both 10 and 25% mucin preparations, but deviation of the curves from linearity was observed in the latter case.

The evaluation of the samples by creep viscometry produced the type of creep compliance curve shown in Fig. 5. The mucin systems did not exhibit any marked degree of viscoelasticity since the curves are linear at times greater than 240 sec and not the exponential curves characteristic of viscoelastic materials. Such behavior approaches that of purely viscous systems, which would produce a straight line passing through the origin. The experimentally obtained curves (Fig. 5) may not be analyzed by means of the usual discrete spectral analysis (12), and the only information readily obtainable is the residual viscosity, η_0, calculated from the reciprocal of the slope.

The Arrhenius plot constructed from these results is shown in Fig. 6 for 15, 20, and 25% preparations. No results could be obtained with the 10% system because of the insensitivity of the technique when applied to such materials. However, linear curves with similar slopes were obtained for the three concentrations investigated, and once more it is apparent that the addition of 1% tetracycline hydrochloride produced a decrease in viscosity of the 25% mucin preparation.

A creep compliance curve obtained from gastric mucous gel obtained from the pig stomach is shown in Fig. 7. The curve is typical of those obtained with uncross-linked polymer systems that are viscoelastic (12), and obvious differences exist between this curve and those shown in Fig. 5. When 1% tetracycline hydrochloride was added,

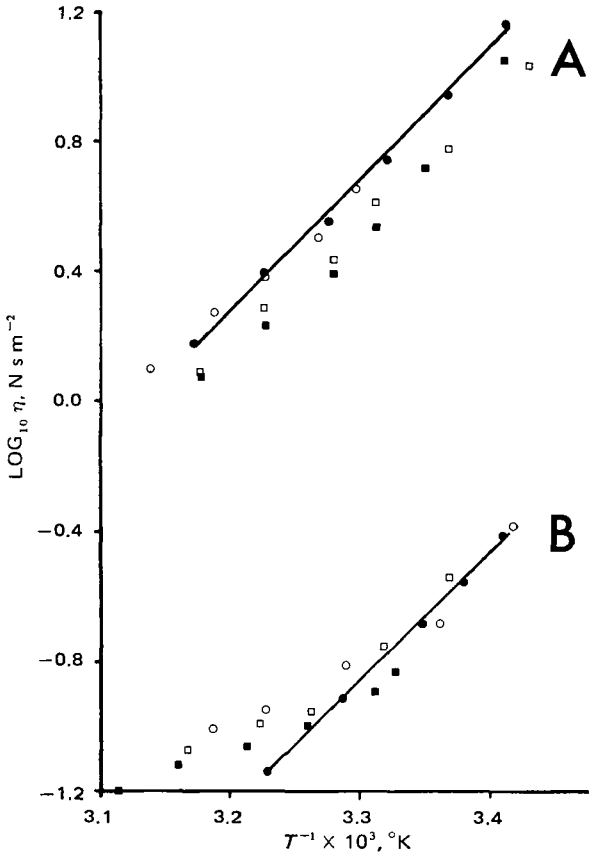

Figure 4—*Effect of tetracycline hydrochloride (1%) on the viscosity measured by rotational viscometry of (A) 25% and (B) 10% mucin preparations. In each case, the lines drawn represent the untreated samples. Key: ●, no additive; ○, 0.1% sodium borate; ■, 1% tetracycline hydrochloride; and □, 0.1% sodium borate and 1% tetracycline hydrochloride.*

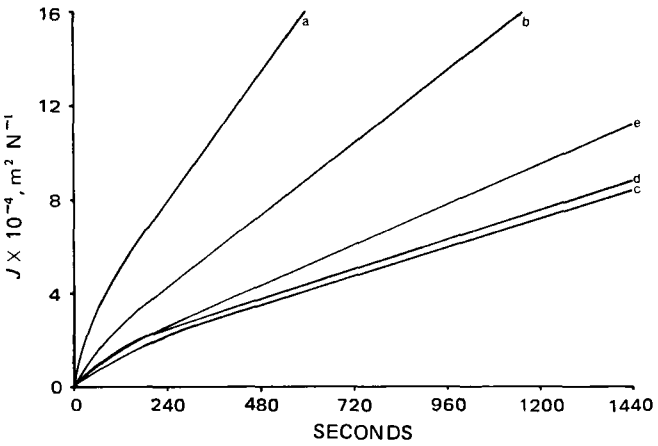

Figure 5—*Creep compliance curves for 15, 20, and 25% mucin preparations. The residual viscosity, η_0, was calculated from the reciprocal of the slope of the linear portions. Key: a, 15% mucin; b, 20% mucin; c, 25% mucin; d, 25% mucin plus 1% sodium borate; and e, 25% mucin plus 1% tetracycline hydrochloride.*

an increase in the structure of the gel was produced. This effect is similar to that described previously for homogenized bronchial mucous gels (Fig. 8) (10). Since in all cases the curves are of the shape expected for viscoelastic materials, they can be analyzed in terms of a mechanical model (Table I).

DISCUSSION

The nature of the material produced when dried hog gastric mucin is added to water is difficult to describe. It does possess some characteristic stickiness of mucous gels and does to some extent exhibit a "rubbery" consistency. However, it is obvious from the results that the material does not possess any innate elastic behavior and is almost purely viscous (Fig. 5). Also, although the viscosity of the preparation increases with increasing mucin concentration, the fact that the slopes of the Arrhenius plots remain parallel indicates that no increase in interaction between the molecules has occurred.

It has been suggested that the lack of a coherent gel structure may be overcome by the inclusion of borate ions in the mucin preparations (6). This suggestion could not be substantiated, since the addition of such ions usually produced a decrease in viscosity (Figs. 3 and 4). Since borate ions are universal cross-linking agents, it is tempting to assume that the mucin does not contain glycoprotein, which is generally considered to be responsible for the gel-like nature of mucus (13). However, Deman *et al.* (5) demonstrated a reduction in viscosity after incubation of a mucin preparation with neuramidase, indicating the presence of a sialic acid containing glycoprotein. Other workers (14) separated a glycoprotein from this material, and it appears that the reason why no gel is produced is due to the low percentage of glycoprotein present and/or to the high concentration of cellular contaminants in such a preparation.

However, the presence of some glycoprotein in the mucin, albeit at low concentration, may permit the use of the model system in the investigation of the effect of drugs on the physical properties of gastric mucus and the evaluation of the effect of mucus as a barrier to drug absorption. Davis *et al.* (6) claimed that the action of mucolytic drugs, which decrease viscosity, can be demonstrated using 10% hog gastric mucin. However, such a model also should be capable of demonstrating the effect of compounds that increase viscosity of mucous gels.

It was reported recently that the tetracycline antibiotics, particularly tetracycline and oxytetracycline, are capable of producing large increases in the viscosity of bronchial mucus (8). The addition of 1% tetracycline hydrochloride produced no increase in the viscosity of the mucin systems. Indeed, in all instances a small decrease in viscosity was apparent. This result is obviously not the true tetracycline effect, since a vast increase in structure was produced when a similar concentration was added to gastric mucus (Table I). This effect is substantiated by results obtained with bronchial mucus, indicating that it is not a function of the source of the mucous gel.

It is interesting to compare the viscosities of the natural gastric mucus and mucin preparations. Even the 25% material is 10 times less viscous than gastric mucous gel (Fig. 6 and Table I), necessitating the use of unreasonably high concentrations of mucin if the model is to

Table I—Values of Parameters Derived from the Creep Curves for Gastric and Bronchial Mucus before and after the Addition of 1% Tetracycline Hydrochloride[a]

Material	Additive	J_0, $m^2 N^{-1}$	η_0, $N s m^{-2}$	J_1, $m^2 N^{-1}$	J_2, $m^2 N^{-1}$	J_3, $m^2 N^{-1}$	τ_1, sec	τ_2, sec	τ_3, sec
Gastric mucus	—	0.0129	42,837	0.0115	0.0040	0.0071	831	201	31
Gastric mucus	1% Tetracycline hydrochloride	0.0038	543,819	0.0030	0.0028	0.0011	573	123	30
Bronchial mucus	—	0.0145	44,164	0.0323	0.0017		648	10	
Bronchial mucus	1% Tetracycline hydrochloride	0.0059	438,222	0.0103	0.0023		441	30	

[a] J_0 is the instantaneous compliance; η_0 is the residual shear viscosity; and J_1, τ_1, *etc.*, are the compliance and retardation times of the Voigt units necessary to fit the exponential portion of the curves.

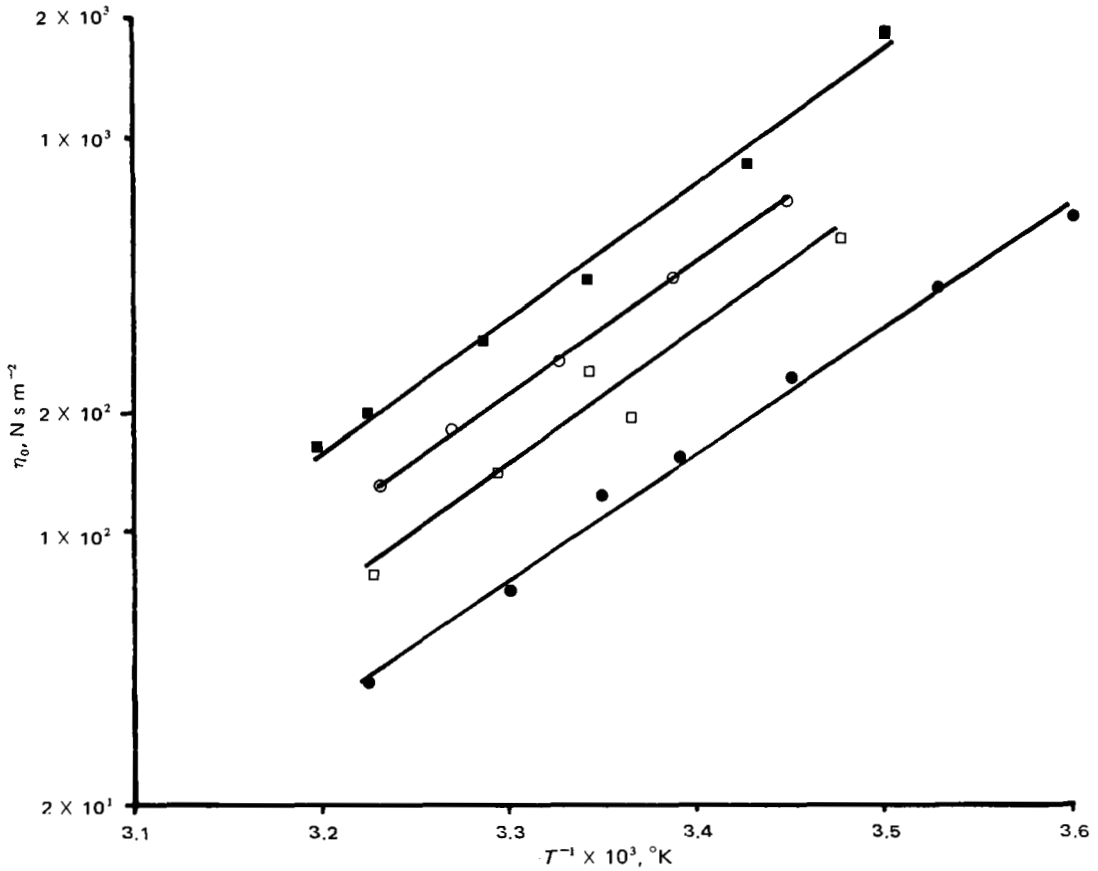

Figure 6—*Effect of temperature on the residual viscosity, η_0, calculated from the creep compliance curves for 15, 20, and 25% mucin preparations. Key: ■, 25% mucin; ○, 25% mucin plus 1% tetracycline hydrochloride; □, 20% mucin; and ●, 15% mucin.*

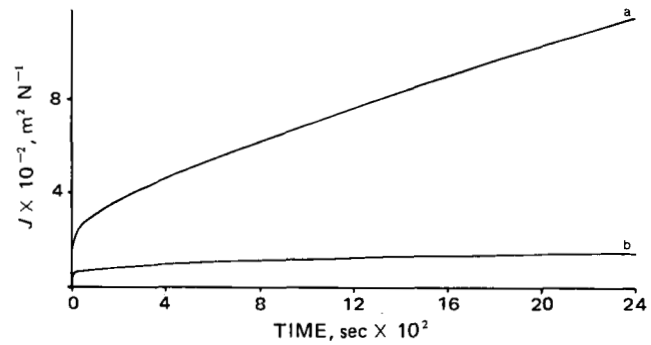

Figure 7—*Creep compliance curves for (a) fresh gastric mucous gel and (b) after the addition of 1% tetracycline hydrochloride.*

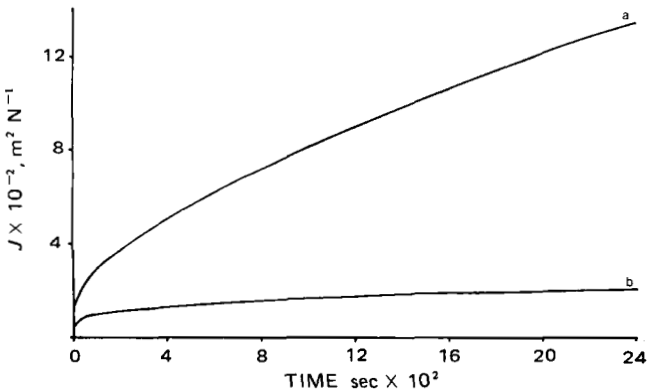

Figure 8—*Creep compliance curves for (a) homogenized bronchial mucous gel and (b) after the addition of 1% tetracycline hydrochloride.*

be realistic. However, the viscosity of human gastric aspirate was found to possess a similar viscosity to that of the 10% mucin when measured by rotational viscometry (15).

Therefore, the use of preparations containing dried hog gastric mucin as model systems for gastric mucous gels apparently is justified only when investigating compounds that decrease viscosity. The marked inability of the model to demonstrate any increase in viscosity with compounds such as tetracycline may limit its usefulness in screening tests. Indeed, with this model such compounds may even appear to possess mucolytic properties. However, if these limitations are considered, the mucin preparation may serve as a useful model for gastric mucus, although it is unlikely that its use in a screening test for agents that reduce the viscosity of bronchial mucus is justified.

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Absorption and Distribution of Radioactivity from Suppositories Containing ^{3}H-Benzocaine in Rats

JAMES W. AYRES [x], DUANGCHIT LORSKULSINT, ALBERT LOCK, LYNDA KUHL, and PAUL A. LASKAR

Abstract □ The effects of the suppository vehicle, drug concentration, and nonionic surfactants on *in vitro* benzocaine dialysis through a cellulose membrane and on rectal absorption in rats of total radioactivity following administration of ^{3}H-benzocaine were investigated. *In vitro* dialysis correlated quite well with *in vivo* absorption, and drug release was greater from water-soluble vehicles than from oleaginous vehicles. Inclusion of a nonionic hydrophilic or lipophilic surfactant in cocoa butter resulted in a statistically significant increase for *in vitro* drug release, while a lipophilic surfactant showed little effect *in vivo* and a hydrophilic surfactant depressed release *in vivo*. Both types of surfactant had small effects on release from polyethylene glycol. *In vitro* release of benzocaine from some commercially available suppositories was compared with experimental preparations. Variation in blood radioactivity following administration of the same concentration of ^{3}H-benzocaine in the same dosage form in male and female rats is reported.

Keyphrases □ Absorption—benzocaine from suppositories, effect of vehicle, drug concentration, and nonionic surfactants, rats □ Distribution—benzocaine from suppositories, effect of vehicle, drug concentration, and nonionic surfactants, rats □ Benzocaine—absorption and distribution from suppositories, rats □ Suppositories—absorption and distribution of benzocaine, rats □ Dosage forms—suppositories, absorption and distribution of benzocaine, rats

It is well recognized that formulation factors can influence the availability of a drug from a dosage form. Surface-active agents included in dosage forms may exert their effects on the active ingredient, the dosage form itself, or the membrane at the absorption site. Surfactants have been reported to increase and to decrease the absorption of drugs (1). Moreover, varying the concentrations of a surfactant can enhance or retard drug absorption, depending on the type of surfactant and whether or not micelle formation occurs (1).

The complex mechanisms of surfactant effects on drug absorption were reviewed previously (2). The *in vitro* release of benzocaine from ointment vehicles was reported (3) and compared (4) to the rate of absorption and resulting total blood level radioactivity following rectal administration of 20% ^{3}H-benzocaine (ethyl *p*-aminobenzoate) from ointment vehicles in rats. This paper reports the effects of suppository vehicles, variations in drug concentration, and the presence of a nonionic hydrophilic or lipophilic surfactant on the *in vitro* dialysis of benzocaine and the absorption of ^{3}H-benzocaine in rats.

EXPERIMENTAL

Dosage Form Preparation—All suppositories were prepared by the fusion method, and commercial products were used as received. The reagents and equipment used were similar to those reported previously (3, 4). Additional materials used in the present experiment were: dialysis membrane, available as a 2.54-cm (1-in.) × 30.5-m (100-ft) roll[1]; cocoa butter[2]; polysorbate 80[3]; and sorbitan monooleate[4].

For *in vivo* studies, ^{3}H-benzocaine was dissolved in the polyethylene glycol vehicle (75% polyethylene glycol 1000 and 25% polyethylene glycol 4000) or suspended in the cocoa butter vehicle. Suppository vehicles containing ^{3}H-benzocaine were poured into plastic, disposable, U-80 insulin syringes, which were refrigerated until completely congealed. The tips of the syringes were cut off, and the excess semisolid was removed.

A suppository volume of 0.5 ml was used for the experiment. The amount of surfactant used was too small to weigh directly, and the aliquot method was used for preparation.

***In Vitro* Dialysis**—Dialysis tubing was cut into 10-cm lengths and soaked for at least 24 hr in distilled water. At the time of the test, the tubing was closed and weighted at one end by tying with a thin strip of the dialysis tubing to a glass stopper. The suppository was introduced into the tubing followed by 2.5 ml of distilled water. The top was tied to form a container, which was as nearly full as possible without loss of water.

The sample was then placed in a 600-ml beaker containing 500 ml of distilled water maintained at 37.5°. It floated upward, being held near the center of the container by the glass stopper weight. At the appropriate time periods, 5-ml samples were pipetted from the beaker and 5 ml of distilled water (37.5°) was returned to the beaker. Care was taken to draw each sample from as close to the same place in the beaker as possible and to avoid stirring.

Analytical Method—The analysis of the benzocaine released during the *in vitro* test was carried out by the method of Matsumoto *et al.* (5). Aliquot portions of a sample solution were pipetted into a test tube followed by 2 *N* HCl (2 ml) and 0.2% $NaNO_2$ (0.4 ml), and the mixture was shaken for 5 min. Then 0.5% $NH_4SO_3NH_2$ (0.4 ml) was added, and the mixture was shaken for 3 min. *N*-(2-Diethylaminoethyl)-1-naphthylamine hydrochloride (1.0 ml of 0.5%) was then added with shaking.

After 30 min of intermittent shaking, the percent transmittance was measured at 550 nm and the concentration of benzocaine was determined from a standard curve.

***In Vivo* Studies**—Female Sprague–Dawley rats were used for all experiments except the male *versus* female study. Animal weights varied between 100 and 280 g. Surgical preparation, cannulation,

[1] Seamless regenerated cellulose dialysis tubing, Catalog No. 25225-226, VWR Scientific Supplies, Portland, Ore.
[2] Hershey Food Corp., Hershey, Pa.
[3] Tween 80, J. T. Baker, Phillipsburg, N.J.
[4] Span 80, J. T. Baker, Phillipsburg, N.J.