was not associated with any change in the venous blood sugar or NPN or the serum CO_2, Cl, Na, K, Ca, inorganic phosphorus, albumin, globulin, and uric acid. The serum total cholesterol and triglycerides and their respective α and β lipoprotein fractions remained unchanged. Serum PBI levels were not altered during therapy. The excretion of urinary 17-ketosteroids, Porter-Silber chromogens, and 11-desoxycortisol metabolites expressed in mg./day/1.0 Gm. of creatinine was essentially the same before and during ingestion of this compound. During therapy the fasting a.m. levels of plasma 17(OH) corticosteroids did not differ significantly from the values recorded during the control period.

The plasma 17(OH) corticosteroid responses to intravenous ACTH prior to and during administration of the compound were of the same order of magnitude. Therapy for 12 weeks was not accompanied by significant alteration in the hypoglycemic or hypophosphatemic effects of intravenously administered rapidly acting insulin. The urinary excretion of gonadotropins, urinary pressor activity (aortic strip assay), serum creatinine, and creatine clearances and routine urine analysis were unchanged at the end of the treatment period. Hepatic indices and the hematocrit and hemoglobin remained within the pretherapy range during ingestion of this agent.

DISCUSSION

None of the effects of this derivative of the stilbenes, variously observed in some but not all species of experimental animals tested (1), could be discerned in the trials in healthy male adults described here. Thus, anorexia, weight loss, and hypocholesterolemia (perhaps related to decreased food intake) did not occur. Also indices of pituitary, thyroid, adrenocortical, adrenomedullary, and gonadotropic and other endocrine gland function were not affected. Blood sugar responses to intravenous insulin were not changed. The compound did not alter the hepatic indices tested. In other words, effects of this stilbene derivative, observed in certain species of experimental animals, were not evident in healthy male adults receiving this compound in comparable dosages.

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Effects of Certain Preservatives on the Aging Characteristics of Acacia

By OSCAR E. ARAUJO

The rheological characteristics of acacia solutions of various concentrations were examined. Based on the results, a series of 25 per cent acacia solutions using five different preservatives and two combinations of preservatives were studied for a period of 1 year. Rheological, pH, and organoleptic data were obtained in order to describe the aging characteristics of the solutions. In each case, the results showed a reduction in pH and viscosity, which was most pronounced in the unpreserved control solution. Possible reasons for the decrease, as well as for the rate at which it occurred, are discussed.

ACACIA HAS been in use for at least 4000 years and yet comparatively little quantitative research appears in the literature (1). Curiously, in spite of the popularity enjoyed by this polysaccharide as a protective colloid and emulsifier in numerous pharmaceutical preparations, only a handful of findings pertaining to its aging characteristics are reported.

Taft and Malm (2) found that bacterial growth appeared in dilute solutions of gum arabic 36–48 hr. after preparation. Osborne and Lee (3) carried out experiments to establish the effect of aging upon preserved and unpreserved acacia mucilages. The

preservative used was 0.2% benzoic acid. These investigators reported an initial rise and an eventual decrease in the viscosity of the mucilages. They further observed a considerably greater decrease in the viscosity of the unpreserved mucilage than of the preserved one. Joslin and Sperandio (4) reported that acacia mucilages prepared with boiling water exhibited a reduction in viscosity over a period of 8 weeks. However, the authors stated that this decrease in viscosity was less pronounced than the reduction occurring in those mucilages prepared with water at room temperature. They further noted that the acacia mucilages became slightly more acidic during the aging period.

Recently, Ory and Steiger-Trippi (5) reported the changes in viscosity observed after 1 month's

storage in solutions containing 7.5% and 20% acacia. Four preservatives were used in concentrations of 2%: sorbic acid, phenyl mercuric borate, a mixture of the sodium salts of the ethyl and propyl esters of *p*-hydroxy benzoic acid, and an arylalkyl quaternary ammonium sulfate. The results indicated that a reduction in viscosity occurred in all solutions except those preserved with phenyl mercuric borate.

The above studies assumed that acacia solutions at the concentrations used behaved as Newtonian systems, and, therefore, the viscosity at only 1 rate of shear was necessary. It appeared reasonable to establish the experimental validity of such an assumption by measuring the viscosity of acacia solutions of various concentrations at several rates of shear. If the rheograms were to show Newtonian behavior, then acacia solutions of an arbitrary concentration would be studied further to determine the effect of a number of preservatives on their aging characteristics.

EXPERIMENTAL

Preparation of Solutions.—All acacia solutions were prepared by placing the proper volume of distilled water previously heated to 60° into a Waring blender. The accurately weighed gum arabic was then added to the surface of the water, permitted to hydrate momentarily, and then blended for 1 min. All percentages reported are on a weight to weight basis. The preservatives used in this study were dissolved in the warm water prior to the blending procedure. Six hours were permitted to elapse after the preparation of the solutions before any measurements were made. This was done to insure complete hydration of the gum and to allow any foam created by the mixing process to subside. All solutions were examined for a period of 1 year. U.S.P. grade acacia taken from the same lot was employed in order to eliminate source variations.

pH Measurements.—A Photovolt electronic pH meter model 110 was used to measure the pH of the solutions at convenient intervals of time.

Rheological Measurements.—A Stormer viscosimeter equipped with the modified cup and bob as suggested by Fischer (6) was used in this investigation. A weight hanger with various slotted weights provided the shearing stress. Calibration curves using castor oil as a standard were constructed periodically throughout the study. The viscosity of castor oil was obtained from standard tables. An average instrumental constant, K_v, was calculated from the data collected according to the expression

$$\eta = K_v \frac{\text{Gm.}}{\text{r.p.m.}} \qquad \text{(Eq. 1)}$$

where Gm. represents the shearing stress and r.p.m. the rate of shear. Once the constant, K_v, was obtained, the apparent viscosity, η, of any system could be arrived at by means of this equation. All viscosity measurements were carried out at 20 ± 0.1°.

Organoleptic Observations.—A description of the appearance and the odor of the solutions was recorded periodically throughout the investigation.

Preservatives Used.—Five different preservatives were employed in the following concentrations: 0.2% benzoic acid, 0.2% methylparaben, 0.05%

propylparaben, 0.5% chlorobutanol, and 0.01% benzalkonium chloride. Combinations of benzoic acid–propylparaben and benzoic acid–chlorobutanol in the above percentages were also used.

RESULTS AND DISCUSSION

Verification of Newtonian Characteristics.—Solutions containing 10, 12.5, 15, 20, 25, 30, 35, 40, and 45% acacia were prepared by the previously described method. The rheological data were obtained within 24 hr. from the time of preparation, thus obviating the addition of preservatives to the solutions. Figure 1 shows representative rheograms of the results. It was clearly seen that Newtonian behavior occurred at the lower concentrations as evidenced by the linearity of the plots. At concentrations of 40% acacia and above, pseudoplastic characteristics were observed, as denoted by a decrease in viscosity with increasing shearing stress.

The power law equation (7)

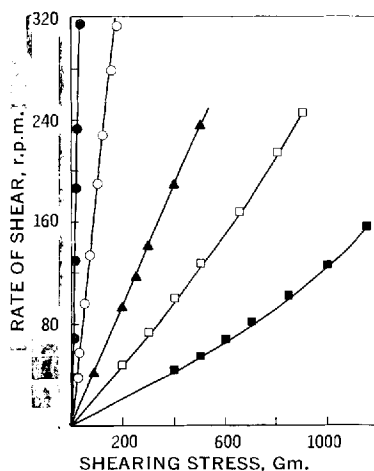$$F^N = \eta' G \qquad \text{(Eq. 2)}$$

Fig. 1.—Rheograms of unpreserved acacia solutions of various concentrations. Key: ●, 10%; ○, 25%; ▲, 35%; □, 40%; ■, 45%.
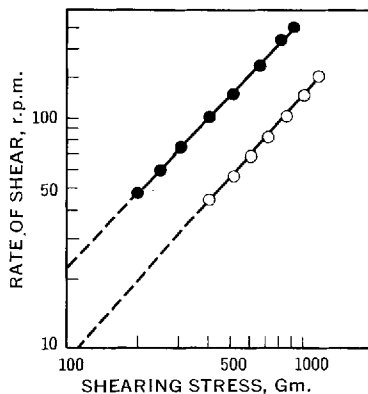

Fig. 2.—Log-log rheograms of unpreserved acacia solutions for calculation of index of pseudoplasticity. Key: ●, 40%; ○, 45%.

TABLE I.—pH OF 25% ACACIA SOLUTIONS

Preservative Used	Time Elapsed						
	0 Days	1 Wk.	2 Wk.	6 Wk.	3 Mo.	9 Mo.	1 Yr.
None	4.80	4.78	4.60	4.30	4.20	3.85	3.50
Benzoic acid	4.72	4.70	4.48	4.47	4.30	4.20	3.90
Benzoic acid–chlorobutanol	4.65	4.60	4.55	4.22	4.20	4.20	3.90
Benzoic acid–propylparaben	4.65	4.60	4.52	4.28	4.20	4.20	3.90
Propylparaben	4.79	4.60	4.65	4.35	4.32	4.25	4.00
Methylparaben	4.79	4.70	4.70	4.25	4.20	4.20	4.10
Chlorobutanol	4.80	4.78	4.62	4.34	4.30	4.25	4.00
Benzalkonium chloride	4.79	4.75	4.70	4.32	4.23	4.20	4.00

where F is the shearing stress in Gm., G is the rate of shear in r.p.m., η' is an indicator of viscosity, and N is an index of non-Newtonian behavior, has been used to describe pseudoplastic systems. This exponential expression in its logarithmic form

$$\log G = N \log F - \log \eta' \qquad \text{(Eq. 3)}$$

was applied to the rheological data of the solutions exhibiting pseudoplasticity (40 and 45% acacia). The resulting linear relationships are shown in Fig. 2. The value of N must be greater than unity if the system is pseudoplastic, and its magnitude is proportional to the degree of pseudoplasticity. The calculated value of N for the 40% acacia solution was 1.08 and that for the 45% solution was 1.15. Thus, the degree of pseudoplasticity appeared to increase with increasing concentrations of acacia. This was in line with the concept whereby linear polymers tend to orient themselves along the direction of flow when a shearing stress is applied. At low concentrations, this orientation probably occurs at minimal shearing stresses and the solution behaves as a Newtonian system, where the viscosity is constant. At higher concentrations, it is conceivable that low stresses are not sufficient to rearrange the greater number of polymers present, resulting in pseudoplastic behavior until the force is large enough to produce complete alignment.

Aging Studies—*General.*—It was apparent that it would be considerably easier to characterize a Newtonian system, which has a constant viscosity, than a pseudoplastic one. It was also felt that a solution containing enough acacia to support an appreciable amount of bacterial growth was desirable. With these criteria in mind, solutions containing 25% acacia were prepared, as before, for the aging studies conducted.

The previously mentioned preservatives were added in the amounts earlier stipulated. An unpreserved 25% acacia solution served as a control.

pH Considerations.—The change in pH of the solutions upon standing appeared to follow a definite pattern, as seen in Table I.

A sharp decrease in pH occurred in the first 6 weeks, ranging from 0.25 pH units for the benzoic acid preserved solutions to 0.54 units for the solution containing methylparaben. These values represented 30 and 78%, respectively, of the total drop in pH occurring after 1 year's storage time for each of these solutions. The mean drop in pH after 6 weeks for all solutions studied, expressed as a per cent of the decrease observed after 1 year on the shelf, was 53%.

These results seemed to indicate some manner of bacterial attack on the carbohydrate, with a consequent production of acid groups causing a pH
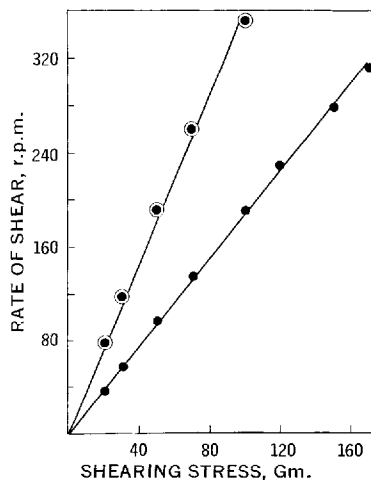

Fig. 3.—Rheograms of unpreserved 25% acacia solutions. Key: ●, 6 hr. after preparation; ◉, 1 year after preparation.
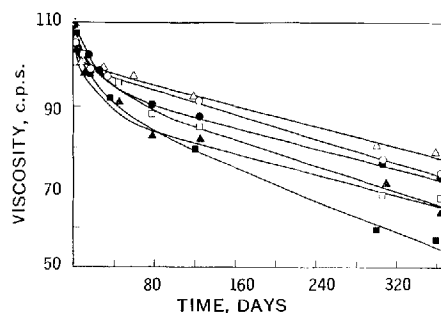

Fig. 4.—The viscosity stability of preserved and unpreserved acacia solutions. Key: △, 0.2% benzoic acid; ○, 0.2% methylparaben; ●, 0.05% propylparaben; □, 0.5% chlorobutanol; ▲, 0.01% benzalkonium chloride; ■, unpreserved.

lowering. The pH decreased rapidly at first, but after 6 weeks the medium either had become too acid to support the same rate of bacterial decomposition, or there had been a severe depletion of available substrates for bacterial action. The reduction in pH may also have resulted from spontaneous hydrolysis of certain ester linkages of the carbohydrate.

The over-all mean decrease in pH after 1 year for all the preserved solutions was 0.75 units, while that for the unpreserved solution was 1.30. This was expected since the latter should have supported the

greatest amount of bacterial decomposition and, consequently, the largest reduction in pH.

Organoleptic Qualities.—None of the solutions showed any visible growth in the first month of storage. Each initially exhibited its characteristic chemical odor, depending on the preservative used. The unpreserved solution had a typical musty odor of gum, which grew unpleasant after 10 days. The solution containing benzalkonium chloride, after only 1 week, gave rise to a pungent, disagreeable odor, which became increasingly offensive as time elapsed. Two months after preparation, mold growth appeared on both the unpreserved solution and the one containing benzalkonium chloride. There appeared to be gas released as the containers were opened, a common occurrence in many types of bacterial action.

After 1 year of shelf life, all the solutions exhibited a slight degree of turbidity and, as before, the unpreserved and benzalkonium chloride solutions gave evidence of the greatest amount of growth and most offensive odors. The reduction in pH of the solutions, due to the aforementioned bacterial attack, provided a good medium for mold growth most apparent in the unpreserved solution where the drop in pH was the greatest.

Rheological Observations.—It was assumed that 25% acacia solutions behaved as Newtonian systems throughout the entire study. This premise was verified by rheograms of the solutions constructed at various storage times. Figure 3 shows representative plots where the linear characteristics can be seen.

The changes in viscosity of the various solutions during the period studied are shown graphically in Fig 4. In order to avoid confusion, the plots for the solutions containing the combinations of preservatives were not included. The data collected, however, would place these graphs immediately below the plot for the solution containing benzoic acid.

A comparison of the rheological results with the pH observations previously discussed revealed certain similarities. First, a definite reduction in viscosity was observed in all solutions. Second, as expected, the unpreserved solution showed the greatest decrease in viscosity at the end of 1 year. Finally, a sharp decrease in viscosity occurred during the first 6 weeks on the shelf. The same rationale used in explaining the reduction in pH would apply here.

It is interesting to note that, unlike the pH observations, the viscosity drop after 6 weeks, expressed as a per cent of the total reduction over a 1-year period, ranged only from 30% for the solution containing methylparaben to 42% for the propylparaben preserved solution. Therefore, at least from a viscosity stability standpoint, it makes little difference whether the acacia solutions are preserved.

It is obvious, however, as can be seen from the organoleptic qualities described, that other factors have to be considered.

The rate of reduction in viscosity became essentially linear for all solutions following the pronounced drop seen in the first 6 weeks. The degradation of most linear polymers has been reported to be primarily first order (8), while that of tragacanth has been described as zero order (9).

It was apparent from the results of this investigation that the viscosity stability curves for the initial 6-week period fit neither a first nor a zero-order process. It could be argued, however, that after 6 weeks the essentially linear nature of the data throughout the remaining shelf life suggests a zero-order degradation.

The results of this investigation appeared to indicate that, when all aging characteristics were considered, benzoic acid was the most effective preservative used. Furthermore, when benzoic acid was combined with two of the other preservatives used, little difference was seen in the behavior of the solutions.

SUMMARY

1. The rheograms of a series of acacia solutions ranging in concentration from 10–45% were constructed. Newtonian behavior was observed in all except the 40–45% solutions, which showed pseudoplastic characteristics.

2. A series of 25% acacia solutions using various preservatives were studied for a period of 1 year.

3. The pH of all these solutions decreased rapidly in the first 6 weeks, then tapered off. The largest reduction in pH occurred in the unpreserved control solution.

4. The appearance and odor of the solutions during a 1-year period were noted and various degrees of growth and turbidity were observed, along with a variety of fermented and unpleasant odors.

5. The viscosity of the solutions was calculated at various intervals of time, and viscosity–stability curves were shown. All the solutions revealed a decrease in viscosity with age. The benzoic acid preserved solution exhibited the smallest reduction, while the unpreserved solution revealed the greatest drop in viscosity.

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