Conclusion

It was not the object of the foregoing discussion to provide a critical review of rumen physiology or bovine metabolism. Rather, information was selected which, in the judgment of the author, reflects the trends in the field. Unpublished data and speculation were included. The major purpose was to point out the dependence of bovine milk secretion on rumen activity, and to stress that the evaluation of any feeding regime must consider the reactions which occur in the rumen. Future progress in this area depends on gaining more fundamental information on the metabolism of the rumen microorganisms, the metabolism of the animal organism, and the interrelation of the two.

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SEED POLYSACCHARIDES

New Sources of Seed Mucilages

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An extensive search for potential industrial raw materials from plants not now under cultivation is under way by the U. S. Department of Agriculture (*12*). This paper reports the first phase of a survey of seeds for their content of water-soluble polysaccharides. Aside from guar and seaweed products, no industrial mucilages of consequence are produced from domestic plants. Guar gum, from the seed of a leguminous annual, has become widely accepted within the last decade; but only a minor fraction of the total consumption comes from domestic sources. Since many mucilages such as gum arabic or gum tragacanth, are imported, hand-picked plant exudates, the supply is often erratic. In this paper the terms mucilage and gum are used interchangeably.

Anderson (*1*) reported a survey of legume mucilage sources in 1949, but there has been no general search among plant seeds. The distribution of known mucilages is discussed in a recent monograph (*9*). Nearly all the seeds of the 175 species in 26 plant families examined in this study were from herbaceous annuals, mostly wild plants not cultivated in the United States or elsewhere.

Materials and Methods

Seed samples were from the same sources used by Earle *et al.* (*5*) in their search for new industrial oils. Samples

[1] Deceased.

Seeds of 175 species in 26 plant families were examined for water-soluble mucilage content. Those containing over 15% of mucilage were all from legumes. Twenty mucilages, previously unreported, are galactomannans with mannose-galactose ratios between 4 to 1 and 1 to 1.

Table I. Distribution of Seed Mucilage by Plant Family

Family	Number of Genera	Number of Species Having Indicated % Mucilage Content			
		<10	10–15	15–20	>20
Leguminosae	32	31	29	17	11
Convolvulaceae	2	..	4	..	..
Compositae	10	12	..	..	..
Malvaceae	5	8	..	..	..
Umbelliferae	7	7	..	..	..

Table II. Mucilage Content of Seeds and Mucilage Purification

Species	Mucilage Isolated from Seed		Mucilage after Chloroform–n-Butyl Alcohol Treatment		Mucilage after Copper Complex Treatment	
	% of seed[a]	Nitrogen, %	Recovery,[a] %	Nitrogen, %	Recovery,[a] %	Nitrogen, %
Alysicarpus vaginalis	15.8	6.54	61	4.86	44[b]	2.23
Astragalus cicer	15.8	4.31	69	1.83	..	..
Astragalus glycyphyllos	16.0	5.04	97	1.22	..	..
Astragalus nuttallianus	20.5	1.83	78	0.80	..	..
Astragalus sinicus	19.0	1.50	77	0.67	..	..
Astragalus tenellus	19.2	3.74	..	..	69	1.31
Cassia emarginata	27.4	0.80	46	0.40	..	..
Cassia marilandica	19.3	1.64	..	..	94	0.81
Crotalaria incana	17.5	1.05	86	0.47	..	..
Crotalaria lanceolata	18.5	4.82	49	2.88	39[b]	0.88
Crotalaria spectabilis	20.1	4.73	..	..	71	1.58
Crotalaria retusa	18.0	2.61	..	..	76	1.35
Lotus scoparius	15.2	3.99	57	1.21	..	..
Medicago hispida	19.4	3.71	80	2.34	65[b]	0.84
Medicago lupulina	17.7	4.36	53	2.43	32[b]	0.96
Medicago orbicularis	18.6	1.86	74	0.30	..	..
Melilotus indica	22.1	5.58	..	..	56	2.63
Parkinsonia aculeata[c]	28.1	4.38	54	1.74	..	..
Trifolium hirtum	19.9	5.01	41	2.12	30[b]	0.81
Trifolium resupinatum	15.6	7.72	..	..	45	3.33

[a] Protein-free basis.
[b] This treatment followed chloroform–*n*-butyl alcohol treatment; value given is based on polysaccharide present in crude mucilage.
[c] A tree, all others are herbaceous.

selected for this survey met the following criteria: The seed contained no starch (iodine test); and the sum of oil plus protein was less than 50% of the whole seed, or the sum of oil plus protein plus hull was less than 80% of the whole seed.

Polysaccharide material was isolated from whole seeds ground to pass a 30-mesh screen. A preliminary extraction with hexane was tried on a few samples with high oil content, but abandoned as unnecessary for a general survey. The ground material was extracted two or three times with 10 to 20 volumes of hot (80° C.) water. For each extraction, the slurry was stirred for 30 minutes and then pressed through cheesecloth. Combined extracts were centrifuged at 1800× gravity to remove debris. Mucilage was precipitated by addition of four volumes of 95% ethanol. This crude mucilage was dehydrated with ethanol, filtered under vacuum, dried, and weighed. Correction was made for proteinaceous material (N × 6.25) remaining with the mucilage; the nitrogen content ranged from 0.8 to 6.5%.

Nitrogen content of the crude mucilages was lowered by one of the following methods: precipitation of protein with chloroform–*n*-butyl alcohol (*2*), modified by omission of pH 4.8 buffer and inclusion of only one solvent treatment; or precipitation of polysaccharide as the copper complex (*3*).

Mucilages were hydrolyzed by heating 2% solutions in 1*N* hydrochloric acid 6 hours in a steam bath. Occasionally it was necessary to heat the solutions for a few hours longer to complete hydrolysis.

Hydrolyzates were chromatographed without removal of hydrochloric acid, using Whatman No. 4 paper (developed 4 hours) or Whatman No. 1 paper (developed 16 hours). The developing solvent was ethyl acetate–pyridine–water (12:5:4); the color reagent, applied by dipping, was aniline–diphenylamine–phosphoric acid (*11*). Sugars were identified by comparison with standards chromatographed simultaneously.

Quantitative estimation of the sugars present was made by a modification of the phenol–sulfuric acid method of Smith (*4, 7, 8*). Mucilage hydrolyzate equivalent to 200 μg. of polysaccharide was spotted on Whatman No. 1 paper and developed for 16 hours. Areas of the chromatogram containing sugars were found by comparison with guide strips treated with the color reagent. These areas (5 × 5 cm.) were cut from the paper and eluted with 4 ml. of water. A 2-ml. aliquot was placed in a test tube. Then 0.5 ml. of 8% phenol (w./v.) and 6 ml. of concentrated sulfuric acid were added with mixing. After the solution cooled, the color was read in a spectrophotometer at 490 mμ.

Optical activity of the polysaccharides was measured in 1*N* sodium hydroxide. The mucilage solutions were cloudy and required centrifugation before reading in the polarimeter. Polysaccharide concentrations in the supernatant were determined by the phenol–sulfuric acid method.

Results and Discussion

Water-soluble seed mucilages are distributed rather unevenly among the different plant families examined (Table I). The Leguminosae are high in mucilage, although this observation may reflect the make-up of the seed collection; 88 of the 175 species surveyed were legumes. Convolvulaceae is the only other family that contains over 10% of mucilage in the members examined (four species). Other plant families appear to be less favorable mucilage sources, but a survey of additional members would determine whether this inference is correct.

Where the species examined duplicated those of Anderson (*1*), agreement in mucilage content was good, with two exceptions. Anderson reported 14% of mucilage from *Parkinsonia aculeata;* we found 28% (protein-free basis). The respective figures for *Crotalaria intermedia* were 9 and 23%.

Table III. Composition and Optical Rotation of Galactomannans

Species	Sugars Recovered from Polysaccharide, % of Theory	Sugars in Hydrolyzate, %		Specific Rotation in N NaOH	
		Mannose	Galactose	$[\alpha]_D$	G./100 ml.
Cassia marilandica	71	79	21	+27	0.52
Cassia emarginata	56	73	27	+21	1.20
Crotalaria spectabilis	72	74	26	+20	0.68
Crotalaria retusa	83	74	26	+29	0.62
Crotalaria incana	66	73	27	+23	0.84
Crotalaria lanceolata	85	72	28	+22	0.85
Parkinsonia aculeata	80	73	27	−10	0.70
Cyamopsis tetragonolobus (guar)	79	63	37	+53	0.68
Astragalus sinicus	74	62	38	+74	0.71
Astragalus tenellus	78	58	42	+80	1.20
Astragalus nuttallianus	79	58	42	+60	0.79
Astragalus cicer	70	57	43	+64	0.66
Astragalus glycyphyllos	72	55	45	+72	0.79
Alysicarpus vaginalis	84	59	41	+57	0.70
Medicago orbicularis	74	61	39	+55	0.87
Medicago hispida	85	55	45	+76	0.63
Medicago lupulina	80	53	47	+85	0.38
Lotus scoparius	74	53	47	+79	0.58
Melilotus indica	83	51	49	+89	0.73
Trifolium hirtum	78	51	49	+88	0.50
Trifolium resupinatum[a]	65	48	46	+84	0.37

[a] Also contains about 6% glucose.

Amaryllidaceae	1(1)	Euphorbiaceae	3(3)	Onagraceae	3(2)
Anacardiaceae	3(1)	Hydrophyllaceae	3(2)	Ranunculaceae	3(3)
Aquifoliaceae	2(1)	Iridaceae	1(1)	Rosaceae	3(3)
Boraginaceae	3(2)	Labiatae	3(2)	Scrophulariaceae	4(3)
Capparidaceae	3(2)	Liliaceae	4(3)	Solanaceae	5(4)
Celastraceae	2(2)	Limnanthaceae	1(1)	Verbenaceae	2(2)
Cruciferae	3(3)	Linaceae	1(1)	Vitaceae	3(2)

Seeds from the above families contain less than 10% of mucilage; each family was represented by one to five species (one to four genera) shown above (number of genera shown in parentheses):

The composition of mucilages from 20 of the species containing 15% or more seed mucilage on a protein-free basis has not been previously reported, although the mucilage content of a few had been estimated. Constituent sugars were determined after purification to lower the nitrogen content (Table II). With one exception, each polysaccharide on hydrolysis gives rise to only two sugars, galactose and mannose (Table III). The mannose content of these galactomannans ranges from 79 to 51% corresponding to mannose-galactose ratios of between 4 to 1 and 1 to 1.

Trifolium resupinatum mucilage contains galactose and mannose in predominant proportions, about 6% of glucose, and a trace of uronic acid. It was not determined whether these are minor constituents of the mucilage or arise from an associated polysaccharide. Absence of minor amounts of other sugars in the remaining legume species suggests an associated polysaccharide.

Mucilage isolated from seeds of *Cyamopsis tetragonolobus* (guar) is included for comparison. Its values are in agreement with those previously reported (*6*). Although the ratio varied widely among the species examined, the variation within a given genus was not great.

Specific optical rotations of the mucilages increase regularly, with few exceptions, as mannose content decreases. Smith and Montgomery (*10*) noted this effect among a smaller group of galactomannans. This may indicate the essential similarity in the mode of linkage in the various galactomannans. Structural differences in the mucilage from *Parkinsonia aculeata* are indicated by its negative rotation. Other legume mucilages of similar composition have positive rotations. A smooth curve drawn through a plot of mannose content *vs.* rotation suggested that mucilages from *Astragalus sinicus* and *A. tenellus* had slightly higher rotations than were expected. These discrepancies might be caused by accumulation of small errors in determination of composition and optical activity, rather than by structural differences.

These results indicate that a number of previously uninvestigated legumes contain appreciable amounts of galactomannan mucilages of widely differing chemical composition. Those species that appear favorable as industrial mucilages from the standpoint of mucilage content and agronomic characteristics are undergoing further study.

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